

European Commission



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

INDOXACARB

**Volume 3 – B.9 (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.9. ECOTOXICOLOGY DATA AND ASSESSMENT OF RISKS FOR NON-TARGET SPECIES

Ecotoxicological studies described in this document address data requirements specified in Commission Regulation (EU) No 284/2013 of 1 March 2013 setting out the data requirements for plant protection products, in accordance with Regulation (EC) No 1107/2009 concerning the placing of plant protection products on the market.

This document includes summaries of studies conducted with Indoxacarb 150 g/L EC and risk assessments conducted with indoxacarb (DPX-KN128), Indoxacarb 150 g/L EC, and all significant metabolites.

Indoxacarb 150 g/L EC has not been previously evaluated at the EU level. During Annex I inclusion of indoxacarb, DPX-MP062 30WG was the representative formulation containing the insecticidally active isomer DPX-KN128 and the non-insecticidal isomer IN-KN127 (75:25). The use of DPX-MP062 was an improvement over the previous technical material, DPX-JW062, which was a 50:50 mixture of the two isomers (DPX-KN128:IN-KN127). Processes were subsequently developed during the 2000s that allowed for the commercial production of >99% indoxacarb (DPX-KN128) technical containing ≤1% IN-KN127. Today, DPX-KN128 is the primary technical material used as basis for formulation of end-user products.

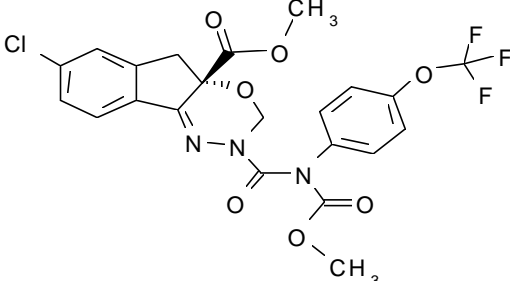
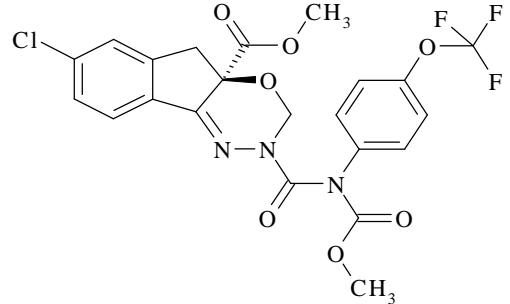
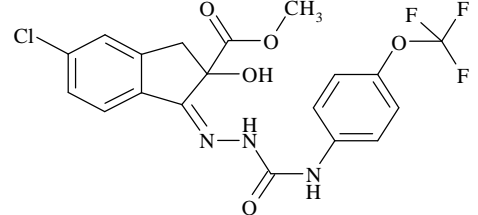
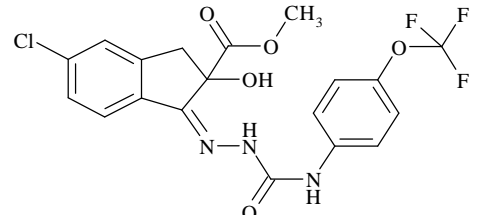
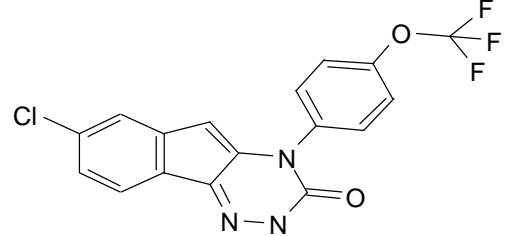
Table 1
Test material composition

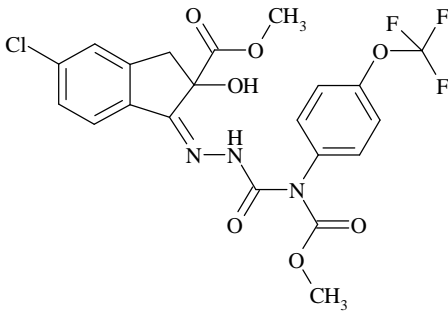
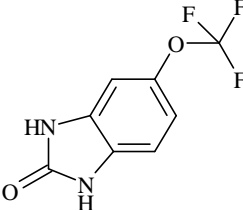
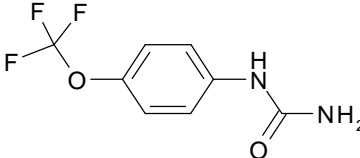
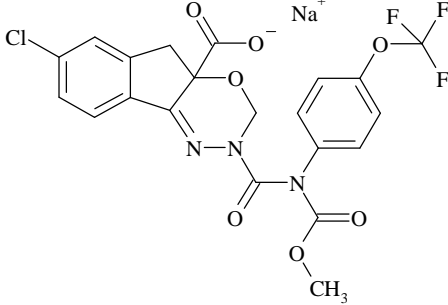
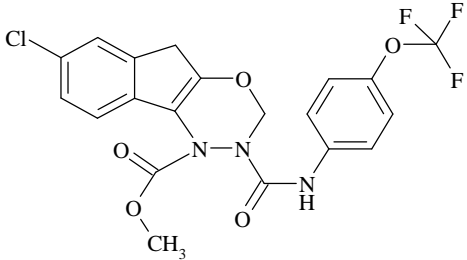
Test material	DPX-KN128 (active isomer)	IN-KN127 (inactive isomer)	Description
DPX-MP062	75%	25%	Enriched technical material
DPX-KN128	>99%	<1%	Pure technical material

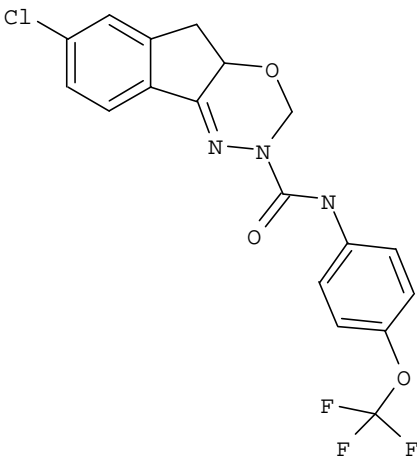
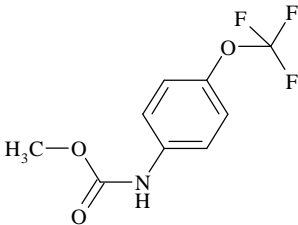
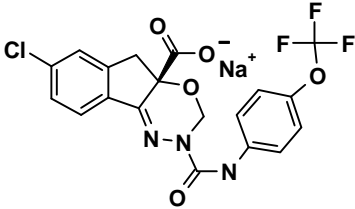
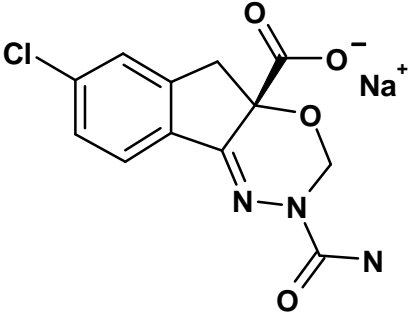
Consideration of metabolites

The occurrence and risk from potentially ecotoxicologically relevant metabolites of indoxacarb (DPX-KN128) have been considered. The metabolites to which non-target organisms could be exposed are presented in Table 2.

Table 2
Indoxacarb: Active substance and metabolites addressed in this document

Compound / Codes	Chemical Structure	Considered in ecotoxicological section for
DPX-KN128		Soil, surface water, sediment
IN-KN127		-
IN-JT333		Soil, surface water, sediment
IN-JU873		Soil, surface water
IN-ML438		Soil, surface water

IN-KG433		Soil, surface water, sediment
IN-MK643		Soil, surface water
IN-MK638		Soil, surface water
IN-KT413		Soil, surface water, sediment
IN-MP819		Surface water, sediment

IN-MS775		Surface water, sediment
IN-KB687		Soil, surface water
IN-U8E24		Soil, surface water, sediment
IN-UYG24		Surface water

B.9.1. EFFECTS ON BIRDS AND OTHER TERRESTRIAL VERTEBRATES

B.9.1.1. Effects on birds

Report: [REDACTED] (2006); Indoxacarb (DPX-KN128) 150 g/L EC: An acute oral toxicity study with the northern bobwhite

DuPont Report No.: DuPont-18923

Guidelines: OPPTS 850.2100 (1996), U.S. EPA 71-1 (1982) **Deviations:** None

Testing Facility: [REDACTED]

Testing Facility Report No.: 112-577

GLP: Yes

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

Executive summary:

Indoxacarb 150 g/L EC was administered to fasted northern bobwhite quail (*Colinus virginianus*) in an acute oral toxicity study. The study was conducted according to guidelines U.S. EPA OPPTS 850.2100 (1996) and U.S. EPA FIFRA 71-1 (1982). Five quail/sex/dose received single oral doses of either 0, 12, 32, 54, 90, 150, and 250 mg a.s./kg bw at a dose volume of 4 mL/kg bw in water. Birds were observed for clinical signs of toxicity, body weight effects and mortality for 14 days after dosing. There were no mortalities in the control and at 12 mg a.s./kg bw treatment. Ten percent mortality was observed in the 32 and 54 mg a.s./kg bw treatments, 50% at 90 mg a.s./kg bw, 80% at 150 mg a.s./kg bw and 100% at 250 mg a.s./kg bw treatments. Clinical signs of toxicity were observed at all doses but surviving birds had recovered by the end of the test. There were no test-item related body weight effects noted at 12 mg a.s./kg bw; however, general dose-dependent effects were apparent at higher doses. When compared to the control group, there were no apparent treatment related effects upon feed consumption for surviving males or females at any of the dosage levels tested at the end of the study. The acute oral LD₅₀ value for northern bobwhite quail exposed to Indoxacarb 150 g/L EC by single oral dose was 89 mg a.s./kg bw. The no mortality dosage was 12 mg a.s./kg bw.

I. MATERIALS AND METHODS

A. MATERIALS

- | | |
|---------------------------------------|---|
| 1. Test material: | Indoxacarb 150 g/L EC |
| Lot/Batch #: | KN128-150 |
| Purity: | 150 g a.s./L |
| CAS#: | None for the formulation
173584-44-6 for indoxacarb active substance |
| Description: | Liquid |
| Stability of test compound: | Not applicable for a single oral dose test |
| 2. Test vehicle and positive control: | Deionised water |
| 3. Test organism: | Northern bobwhite |
| Species: | <i>Colinus virginianus</i> |
| Age at dosing: | 29 weeks |
| Weight at dosing: | 204 to 226 g |
| Source: | |
| Acclimation period: | 11 weeks |
| Diet: | <i>Ad libitum</i> , except for fasting period at least 17 hours before dosing; game bird ration formulated according to specification |
| Water: | <i>Ad libitum</i> , tap water |
| Housing: | Pen with 78 × 51 cm floor space and ceiling height 20 to 25 cm.
External walls, ceilings and floors were constructed of wire mesh and/or galvanized sheeting |
| 4. Environmental conditions | |
| Temperature: | Average 24 ± 0.5°C |
| Relative humidity: | Average 74 ± 4% |
| Photoperiod: | 8 hour photoperiod (approximately 176 lux) |

B. STUDY DESIGN AND METHODS

1. Experimental start/completed
25-July-2006 to 08-August-2006

2. Experimental treatments

In an acute toxicity study, northern bobwhite quail (*Colinus virginianus*) were exposed to Indoxacarb 150 g/L EC. Indoxacarb 150 g/L EC was administered in water by single oral dose to fasted northern bobwhite quail. Five quail/sex/dose received doses of 0, 12, 32, 54, 90, 150, and 250 mg a.s./kg bw at a dose volume of 4 mL/kg bw. There were 5 birds per pen.

3. Observations

Birds were observed for clinical signs of toxicity, body weight effects, and mortality for 14 days after dosing. Average feed consumption was determined on Days 0-3, 4-7, and 8-14.

4. Statistics

Mortality data were analysed using probit analysis to calculate the LD₅₀ value and the 95% confidence intervals. No statistical analyses were applied to separate mean responses among treatment groups for the endpoints of food consumption and body weight.

II. RESULTS AND DISCUSSION

A. FINDINGS

No mortalities were observed in the control group. No clinical signs of toxicity were observed in the control group. There were no test item-related body weight or food intake effects noted. Results are summarised in Table 3 and Table 4.

Table 3
Acute oral toxicity of Indoxacarb 150 g/L EC to northern bobwhite quail

Dose (mg a.s./ kg body wt)	Sex	Toxicological results ^a	Duration of clinical signs ^b	Time to death after application ^b
0	M	0/0/5	—	—
	F	0/0/5	—	—
12	M	0/3/5	1 hour	—
	F	0/2/5	<1 day	—
32	M	0/4/5	1 day	—
	F	1/1/5	4 days	1 hour
54	M	0/5/5	1 day	—
	F	1/1/5	3 days	1–1.5 hour
90	M	1/3/5	9 days	2 days
	F	4/1/5	3 days	0.5 to 2 hours
150	M	3/2/5	14 days	1 to 4 days
	F	5/0/5	—	1 h to 2 days
250	M	5/0/5	—	2 h to 7 days
	F	5/0/5	—	2 h to 2 days

^a Number of animals which died/number of animals with clinical signs/number of animals used

^b — indicates not applicable

Table 4
Acute oral toxicity to northern bobwhite quail - Summary of endpoints

Test item	Indoxacarb 150 g/L EC
Test object	Northern bobwhite quail
LD ₅₀	89 mg a.s./kg bw
Lowest observed effect level (LOEL)	12 mg a.s./kg bw
Highest tested dose without mortality	12 mg a.s./kg bw

III. CONCLUSIONS

The acute oral LD₅₀ value for northern bobwhite quail was 89 mg indoxacarb/kg bw. The no mortality dosage was 12 mg a.s./kg bw.

([REDACTED] 2006)

RMS comment

Study submitted to the EU for the first time in this submission.

This study was conducted in compliance with the current guideline. The acute oral LD₅₀ value for northern bobwhite quail was 89 mg indoxacarb/kg bw. This study is acceptable.

B.9.1.2. Effects on terrestrial vertebrates other than birds

For mammalian studies with the formulated product Indoxacarb 150 g/L EC, please see section CP 6.

B.9.2. RISK ASSESSMENT FOR BIRDS AND OTHER TERRESTRIAL VERTEBRATES

RISK ASSESSMENT FOR BIRDS

Application conditions and exposure scenario

Based on the use of Indoxacarb 150 g/L EC, the following exposure scenarios will be addressed:

- Screening assessment crops: use on maize (representing field maize and sweet corn) and leafy vegetables

For the purposes of calculations presented here, birds are assumed to feed exclusively on contaminated material.

The following assumptions are used in the avian risk assessment:

- Application rate: 37.5 g indoxacarb/ha
- Minimum application interval: 20 days (maize) and 7 days (lettuce)
- Number of applications: 2 applications (maize) and 4 applications (lettuce)
- BBCH 13-49, seed crops: BBCH 13-59 for lettuce, BBCH 34-77 for maize

Guidelines

The avian risk assessment is based on the EFSA guidance document; Risk Assessment for Birds and Mammals, European Food Safety Authority (EFSA), Parma, Italy. EFSA Journal 2009: 7(12):1438.

Avian toxicity endpoints

A summary of the toxicity endpoints of indoxacarb (DPX-KN128) technical, Indoxacarb 150 g/L EC, and major metabolites to birds is provided in Table 5. In keeping with EU Directive 2010/63/EU Article 4 for the reduction of the use of animals to obtain information in a project, vertebrate endpoints were utilized from the studies with DPX-MP062. The endpoints for use in the risk assessment will be lowered to reflect the amount of indoxacarb (DPX-KN128) technical in DPX-MP062 (75%). Studies were conducted with IN-JT333, as it is considered a toxicologically significant metabolite. Endpoints selected for use in the risk assessment are summarised in Table 6.

Table 5
Summary of avian toxicity endpoints for indoxacarb and its metabolites

Toxicity study (species)	Test substance	LD₅₀ or LC₅₀ (mg a.s./kg bw/day)	Lowest lethal dose (mg a.s./kg bw/day)	NOEL or NOEC (mg a.s./kg bw/day)	Reference
Acute oral (northern bobwhite)	DPX-MP062 technical	98 mg DPX-MP062/kg bw 73.5 mg DPX-KN128/kg bw	63 mg DPX-MP062/kg bw	37.8 mg DPX-MP062/kg bw	AMR 3940-96, Revision No. 2 ^a
Acute oral (northern bobwhite)	IN-JT333	1750 mg IN-JT333/kg bw	292 mg IN-JT333/kg bw	≤292 mg IN-JT333/kg bw	AMR 3890-96 ^a
Acute oral (northern bobwhite)	Indoxacarb 150 g/L EC	89 mg DPX-KN128/kg bw	12 mg DPX-KN128/kg bw	-	DuPont-18923 ^b
Short-term dietary (northern bobwhite)	DPX-MP062 technical	808 mg DPX-MP062/kg feed (340 mg DPX-MP062/kg bw/d)	-	-	AMR 4094-96 ^a
Short-term dietary (Mallard)	DPX-MP062 technical	>5620 DPX-MP062/kg feed (>1803 mg/kg bw/d)	-	-	AMR 4093-96 ^a
Subchronic and reproductive (northern bobwhite)	DPX-MP062 technical	-	-	144 mg/kg feed (75.7 mg DPX-MP062/kg bw/d)	AMR 4096-96, Revision No. 1 ^a
Subchronic and reproductive (mallard)	DPX-MP062 technical	-	-	720 mg/kg feed (105 mg DPX-MP062/kg bw/d)	AMR 4095-96 ^a

^a Summarised in Volume 3_CA_B9

^b Summarised in this document.

Table 6
Avian toxicity endpoints used in risk assessment for indoxacarb and its metabolites

Study	Test substance	Test species	EU agreed endpoints	Endpoints used in risk assessment
Acute toxicity	DPX-MP062 technical	Northern Bobwhite	LD ₅₀ = 98 mg DPX-MP062/kg bw	LD ₅₀ = 73.5 mg DPX-KN128/kg bw
Acute toxicity	IN-JT333	Northern Bobwhite	LD ₅₀ = 1750 mg IN-JT333/kg bw	LD ₅₀ = 1750 mg IN-JT333/kg bw
Acute toxicity	Indoxacarb 150 g/L EC	Northern Bobwhite	-	LD ₅₀ = 89 mg DPX-KN128/kg bw ^a
Dietary toxicity (short-term)	DPX-MP062 technical	Northern Bobwhite	LD ₅₀ = 340 mg DPX-MP062/kg bw/d	-
Reproductive toxicity (long-term)	DPX-MP062 technical	Northern Bobwhite	NOEL = 75.7 mg DPX-MP062/kg bw/d	NOEL = 7.35 mg DPX-KN128/kg bw/d (LD ₅₀ /10)

^a Summarised in Volume 3_CA_B9

The acute risk to birds of indoxacarb was assessed by calculating toxicity exposure ratios (TER_a) given in Table 7.

Table 7
Screening level acute TER_a for birds exposed to Indoxacarb 150 g/L EC in maize and leafy vegetables

Scenario	Species	LD ₅₀ (dietary) (mg a.s./kg bw/d)	SV (90 th %) ^a	Rate applied (kg a.s./ha)	MAF ^b	DDD ^c	TER ^d	Regulation (EC) 546/2011 trigger
Maize	Small omnivorous bird	73.5	158.8	0.0375	1.1	6.55	11.2	10
Leafy vegetables	Small omnivorous bird	73.5	158.8	0.0375	1.8	10.72	6.9	10

^a Shortcut value for 90th percentile residue per unit dose

^b The applicant proposed MAF values recalculated according to EFSA:1438 (2009), Appendix H, however RMS preferred the values calculated with the EFSA excel tool (even if the differences are slight, values in the table are corrected)

^c Daily dietary dose (DDD = RUD_{SV} × Rate × MAF)

^d Toxicity/exposure ratio (TER_a = LD₅₀ (mg a.s./kg bw/day)/DDD)

Note: Bold values are below the trigger value

The TER_a value for maize is greater than the TER trigger of 10, indicating acceptable risk to birds from Indoxacarb 150 g/L EC, when used in accordance with the proposed label. The TER_a value for leafy vegetables is below the Regulation (EC) 546/2011 trigger of 10, indicating a need for refinement. A refined risk assessment is provided in Table 8.

Table 8
Tier 1 TER_a for birds exposed to Indoxacarb 150 g/L EC in leafy vegetables

Scenario	Species	LD ₅₀ (dietary) (mg a.s./kg bw/d)	SV (90 th %) ^a	Rate applied (kg a.s./ha)	MAF ^b	DDD ^c	TER ^d	Regulation (EC) 546/2011 trigger
Leafy vegetables BBCH 10-49	Small granivorous bird “finch”	73.5	27.4	0.0375	1.8	1.9	39.7	10
Leafy vegetables BBCH ≥50	Small granivorous bird “finch”	73.5	8.2	0.0375	1.8	0.6	132.8	10
Leafy vegetables BBCH 10-49	Small omnivorous bird “lark”	73.5	24.0	0.0375	1.8	1.6	45.4	10
Leafy vegetables BBCH ≥50	Small omnivorous bird “lark”	73.5	7.2	0.0375	1.8	0.5	151.2	10
Leafy vegetables BBCH 10-19	Medium herbivorous/granivorous bird “pigeon”	73.5	90.6	0.0375	1.8	6.1	12.0	10
Leafy vegetables BBCH 10-19	Small insectivorous bird “wagtail”	73.5	26.8	0.0375	1.8	1.8	40.6	10
Leafy vegetables BBCH ≥20	Small insectivorous bird “wagtail”	73.5	25.2	0.0375	1.8	1.7	43.2	10

^a Shortcut value for 90th percentile residue per unit dose

^b The applicant proposed MAF values recalculated according to EFSA:1438 (2009), Appendix H, however RMS preferred the values calculated with the EFSA excel tool (even if the differences are slight, values in the table are corrected)

^c Daily dietary dose (DDD = RUD_{SV} × Rate × MAF)

^d Toxicity/exposure ratio (TER_a = LD₅₀ (mg a.s./kg bw/day)/DDD)

The acute risk was also assessed for IN-JT333, as it is considered to be a toxicologically significant metabolite.

Table 9
Screening level acute TER_a for birds exposed to IN-JT333 in maize and leafy vegetables

Scenario	Species	LD ₅₀ (dietary) (mg a.s./kg bw/d)	SV (90 th %) ^a	Rate applied (kg a.s./ha)	MAF ^b	DDD ^c	TER ^d	Regulation (EC) 546/2011 trigger
Maize	Small omnivorous bird	1750	158.8	0.0375	1.1	6.55	267.2	10
Leafy vegetables	Small omnivorous bird	1750	158.8	0.0375	1.8	10.72	163.3	10

^a Shortcut value for 90th percentile residue per unit dose

^b The applicant proposed MAF values recalculated according to EFSA:1438 (2009), Appendix H, however RMS preferred the values calculated with the EFSA excel tool (even if the differences are slight, values in the table are corrected)

^c Daily dietary dose (DDD = RUD_{SV} × Rate × MAF)

^d Toxicity/exposure ratio (TER_a = LD₅₀ (mg a.s./kg bw/day)/DDD)

The toxicity/exposure ratio (TER) calculated in respect of the acute exposure of birds feeding on a range of possible food sources, assuming worst-case exposure (feeding exclusively on contaminated material), demonstrates that there is no significant practical risk to avian species.

The TER_a values are greater than the Regulation (EC) 546/2011 trigger of 10, indicating acceptable acute risk to birds from indoxacarb and IN-JT333 following application of Indoxacarb 150 g/L EC at the proposed label rates.

Exposure *via* Drinking Water

Acute drinking water risk assessment

Selection of relevant scenarios

Two scenarios were identified as relevant for assessing the risk of pesticides *via* drinking water to birds:

- Leaf scenario (use on lettuce only): Birds taking water that is collected in leaf whorls after application of a pesticide to a crop and subsequent rainfall or irrigation.
- Puddle scenario: Birds taking water from puddles formed on the soil surface of a field when a (heavy) rainfall event follows the application of a pesticide to a crop or bare soil.

Leaf Scenario: Indoxacarb (DPX-KN128) is intended to be applied by spray equipment to leafy vegetables at growth stage 13 until harvest, thus a leaf scenario acute drinking water assessment for birds is required. The PEC_{pool} is calculated as a 5 fold dilution of the maximum concentration in the spray tank:

$$PEC_{pool} = C_{spray}/5$$

Table 10
Tier 1 avian acute drinking water TER_a for Indoxacarb 150 g/L EC–Leaf scenario

	LD₅₀ (mg a.s./kg bw/day)	DWR^a for small granivorous bird (15.3 g bw) in L/kg bw/d	PEC_{pool} (mg a.s./L)	TER	Regulation (EC) 546/2011 Trigger
Leaf scenario (PEC _{pool})	73.5	0.46	37.5	4.3	10

^a Drinking water rates

Note: Bold values are below the trigger value.

Table 11
Tier 1 avian acute drinking water TER_a for IN-JT333–Leaf scenario

	LD₅₀ (mg/kg bw/day)	DWR^a for small granivorous bird (15.3 g bw) in L/kg bw/d	PEC_{pool} (mg a.s./L)	TER	Regulation (EC) 546/2011 Trigger
Leaf scenario (PEC _{pool})	1750	0.46	37.5	101.4	10

^a Drinking water rates

The TER for exposure to birds from the consumption of pooled spray applications of Indoxacarb 150 g/L EC is below the relevant Commission Regulation (EU) 546/2011 criterion of 10.

The applicant proposed the following refinement:

The exposure estimate is unrealistic for 4 reasons. First, the calculation of water flux is determined for a 24-hour period of time and does not include metabolic water produced from the diet. Second, to compare to the acute oral gavage toxicity value, the exposure component of the TER_a must assume that an individual bird will drink the equivalent of 46% of its body weight in water in one bout. This is not realistic. A simple refinement of the exposure assumption would be to assume there are 3 equal bouts of water consumption during the day (15.3% of body mass), which results in $TER_a = 13.1$. Third, according to Nolting (2010)¹, it can be assumed that food uptake by birds can realistically be reduced to 50% within treated fields ($PT = 0.5$), because birds, due to their mobility, take up food items from different sources, which results in $TER_a = 26.2$. Fourth, the current calculation of exposure rates in leaf whorls represent an extreme worst-case, which is not likely to occur at all. Pools formed in whorls require a specific combination of leaf morphology and weather conditions, i.e. rainfall or irrigation events shortly after application followed by a period of extreme drought, which prevents the formation of puddles that will most likely not affect birds on population relevant level. With regard to the behaviour of the product on leaf surfaces, it was shown that the product is rapidly bound to the waxy surface of leaves and absorbed by the plant material. In a plant metabolism study, foliar material was treated with a single application of formulated [¹⁴C]DPX-JW062, labelled in the indanone ring or the trifluoromethoxyphenyl ring (AMR 2730-93, found in Indoxacarb EU Renewal Dossier, Document M-CA, Section 6, DuPont-41110 EU, Revision No. 1). Even on Day 0, less than half of the residue was on the surface and the percentage on the surface decreased further with time after treatment. Therefore, it can be concluded that there is a sufficient margin of safety, and exposure to indoxacarb or IN-JT333 via drinking water will not pose an acute risk to birds.

However, in order to reduce the acute risk to birds the following mitigation measure could be applied as proposed by the EFSA Bird and Mammal Guidance document:

- *Avoid sprinkling/irrigation of the crop until one day after application*

RMS comment

- **The refinement provided, based on several assumptions (PT value, dissipation, behavior of the bird,...), is not acceptable. None of these assumptions allow to discard an acute risk at the time of application. The applicant was asked to provide a refinement based on other parameters. No further refinement was proposed but the mitigation measure reported above “Avoid sprinkling/irrigation of the crop until one day after application”. RMS considers that this mitigation measure is not sufficient as rainfall may occur. The EFSA guidance provides other measures to mitigate the risk such as:**
 - **apply only at early stages of crop development and**
 - **provide bird netting on the crop after application.**

RMS would recommend to limit the applications at earlier growth stages (before BBCH 19 in the case of lettuce). If bird netting is used, RMS would recommend to ensure that no water remains on the lettuce, however this should be considered a Member State issue.

Puddle Scenario:

Birds may be exposed to Indoxacarb 150 g/L EC through drinking water by taking up water from puddles formed on the soil surface following heavy rainfall events after the application of pesticides. No specific calculations of exposure and TER are required when the ratio of effective application rate (in g/ha) to the relevant endpoint (in mg/kg bw/day) does not exceed 50 in the case of less sorptive substances ($K_{oc} < 500$ L/kg) or 3000 in the case of more sorptive substances ($K_{oc} \geq 500$ L/kg).

The HQ values are lower than the EFSA Journal 2009: 7(12):1438 trigger value, indicating acceptable acute risk to birds from indoxacarb following application of Indoxacarb 150 g/L EC at all proposed label rates.

¹ Nolting (2010). Bundesamt für Verbrauchersicherheit und Lebensmittelsicherheit. Bekanntmachung über die Umsetzung des EFSA-Guidance Document zur Risikobewertung für Vögel und Säuger (BVL 10/02/14). 1. Juni 2010. Bundesanzeiger. Nummer 94. Pp 2228-2229.

Table 12
Drinking water risk assessment - ratio of effective application rate to acute toxicity endpoint for birds
potentially exposed to indoxacarb-puddle scenario

Crop	LD ₅₀ (mg a.s./kg bw/d)	Rate applied (g a.s./ha)	MAF ₉₀ ^a	AR _{eff} ^b	HQ ^c	Trigger value ^d
Maize	73.5	37.5	1.7	64.0	0.87	3000
Leafy vegetables	73.5	37.5	3.4	126.1	1.72	3000

^a Mean multiple application factor (based on a DT₅₀, soil of 39.9 day for DPX-MP062)

^b Effective application rate (AR × MAF)

^c Hazard quotient (ratio of effective application rate to relevant endpoint)

^d Trigger based on a K_{FOC} of 5125 mL/g (mean)

Table 13
Drinking water risk assessment - ratio of effective application rate to acute toxicity endpoint for birds
potentially exposed to IN-JT333-puddle scenario

Crop	LD ₅₀ (mg/kg bw/d)	Rate applied (g a.s./ha)	MAF ₉₀ ^a	AR _{eff} ^b	HQ ^c	Trigger value ^d
Maize	1750	37.5	1.9	70.6	0.04	3000
Leafy vegetables	1750	37.5	3.8	140.6	0.08	3000

^a Mean multiple application factor (based on a DT₅₀, soil of 111 day for IN-JT333, considered over-conservative by RMS)

^b Effective application rate (AR × MAF)

^c Hazard quotient (ratio of effective application rate to relevant endpoint)

^d Trigger based on a K_{OC} of 17300 mL/g (mean)

Short-term toxicity exposure ratio (TER_{st})

In cases where the dietary LD₅₀ is lower than the acute LD₅₀, the dietary LD₅₀ is to be used in the acute risk assessment. This is not the case for indoxacarb (DPX-KN128). Therefore, as it is already covered by the acute risk assessment, no specific short-term risk assessment was conducted.

Long-term toxicity exposure ratio (TER_{lt})

The long-term avian risk assessment for the screening step TER calculation is given in Table 14.

Table 14
Screening Step TER_{lt} for birds exposed to Indoxacarb 150 g/L EC in maize and leafy vegetables

Scenario	Species	LD ₅₀ /10 ^a (avian repro) (mg a.s./kg bw/d)	SV (mean) ^b	Rate applied (kg a.s./ha)	MAF ^c	TWA	DDD ^d	TER ^e	Regulation (EC) 546/2011 trigger
Maize	Small omnivorous bird	7.35	64.8	0.0375	1.3	0.53	1.67	4.4	5
Leafy vegetables	Small omnivorous bird	7.35	64.8	0.0375	2.2	0.53	2.83	2.6	5

^a The reproductive toxicity (long-term) evaluation was based on the 1/10th the LD₅₀ instead of the chronic NOEL as the NOEL exceeds 1/10th of the LD₅₀.

^b Residue per unit dose short cut value is based on the 50th percentile residue unit dose for avian indicator species

^c The applicant proposed MAF values recalculated according to EFSA:1438 (2009), Appendix H, however RMS preferred the values calculated with the EFSA excel tool (even if the differences are slight, values in the table are corrected)

^d Daily dietary dose (DDD = RUD × Rate × f_{twa} × MAF), (assumes an f_{twa} of 0.53)

^e Toxicity/exposure ratio (TER_{lt} = LD₅₀/10 (mg a.s./kg bw/day)/DDD)

Note: Bold values are below the trigger value

The TER_{lt} values are below the Regulation (EC) 546/2011 trigger of 5, indicating a need for refinement. A refined risk assessment is provided in Table 15.

Table 15
Tier 1 reproductive TER_{it} for birds exposed to Indoxacarb 150 g/L EC in maize and leafy vegetables

Scenario	Species	LD ₅₀ /10 ^a (mg a.s./kg bw/d)	SV (mean) ^b	Rate applied (kg a.s./ha)	MAF ^c	TWA	DDD ^d	TER ^e	Trigger value
Maize BBCH 30-39	Medium granivorous bird "game bird"	7.35	1.5	0.0375	1.3	0.53	0.04	189.6	5
Maize BBCH >40	Medium granivorous bird "game bird"	7.35	0.8	0.0375	1.3	0.53	0.02	355.6	5
Maize BBCH 30-39	Small omnivorous bird "lark"	7.35	5.4	0.0375	1.3	0.53	0.14	52.7	5
Maize BBCH >40	Small omnivorous bird "lark"	7.35	2.7	0.0375	1.3	0.53	0.07	105.4	5
Maize BBCH 30-39	Medium herbivorous/granivorous bird "pigeon"	7.35	11.4	0.0375	1.3	0.53	0.29	25.0	5
Maize BBCH >40	Medium herbivorous/granivorous bird "pigeon"	7.35	5.7	0.0375	1.3	0.53	0.15	49.9	5
Maize BBCH ≥20	Small insectivorous bird "wagtail"	7.35	4.8	0.0375	1.3	0.53	0.12	59.3	5
Leafy vegetables BBCH 10-49	Small granivorous bird "finch"	7.35	12.6	0.0375	2.2	0.53	0.55	13.3	5
Leafy vegetables BBCH >50	Small granivorous bird "finch"	7.35	3.8	0.0375	2.2	0.53	0.17	44.2	5
Leafy vegetables BBCH 10-49	Small omnivorous bird "lark"	7.35	10.9	0.0375	2.2	0.53	0.48	15.4	5
Leafy vegetables BBCH >50	Small omnivorous bird "lark"	7.35	3.3	0.0375	2.2	0.53	0.14	50.9	5
Leafy vegetables Leaf development BBCH 10-19	Medium herbivorous/granivorous bird "pigeon"	7.35	22.7 ^f	0.0375	2.2	0.53	1.00	7.3	5
Leafy vegetables BBCH 10-19	Small insectivorous bird "wagtail"	7.35	11.3	0.0375	2.2	0.53	0.49	14.9	5
Leafy vegetables BBCH >20	Small insectivorous bird "wagtail"	7.35	9.7	0.0375	2.2	0.53	0.42	17.3	5

- ^a The reproductive toxicity (long-term) evaluation was based on the 1/10th the LD₅₀ instead of the chronic NOEL as the NOEL exceeds 1/10th of the LD₅₀.
- ^b Shortcut value for 90th percentile residue per unit dose
- ^c The applicant proposed MAF values recalculated according to EFSA:1438 (2009), Appendix H, however RMS preferred the values calculated with the EFSA excel tool (even if the differences are slight, values in the table are corrected)
- ^d Daily dietary dose (DDD = RUD × Rate × ftwa × MAF), (assumes an ftwa of 0.53)
- ^e Toxicity/exposure ratio (TER_{it} = NOEL (mg a.s./kg bw/day)/DDD)
- ^f Applicant notes that EFSA:1438 (2009) lists an incorrect FIR/bw for the generic focal bird “medium herbivorous/granivorous bird ‘pigeon’”. The correct FIR/bw calculated according to Appendix F and Appendix G of EFSA:1438 (2009) is 0.79, resulting in a median RUD shortcut value of 22.7, instead of 37.0. RMS agrees with the recalculated Shortcut Value for the “leafy vegetables leaf development BBCH 10-19.

The TER_{it} exceed the Regulation (EC) 546/2011 trigger of 5 for all maize and leafy vegetables scenarios, indicating safe use of the product.

Reproductive drinking water risk assessment

In line with the drinking water risk assessment for acute exposure, a ratio of effective application rate to relevant endpoint was calculated:

The HQ_{it} values are below the trigger value proposed by EFSA:1438 (2009), indicating acceptable chronic risk to birds from indoxacarb (DPX-KN128) following application of Indoxacarb 150 g/L EC at the proposed label rates (Table 16).

Table 16
Drinking water risk assessment-ratio of effective application rate to reproductive toxicity endpoint for birds potentially exposed to indoxacarb-puddle scenario

Crop	LD ₅₀ /10 (mg a.s./kg bw/d) ^a	Rate applied (g a.s./ha)	MAF _m ^b	AR _{eff} ^c	HQ ^d	Trigger value ^e
Maize	7.35	37.5	1.7	64.0	8.71	3000
Leafy vegetables	7.35	37.5	3.4	126.1	17.16	3000

- ^a The reproductive toxicity (long-term) evaluation was based on the 1/10th the LD₅₀ instead of the chronic NOEL as the NOEL exceeds 1/10th of the LD₅₀.
- ^b Mean multiple application factor (based on a DT₅₀, soil of 39.9 day for DPX-MP062)
- ^c Effective application rate (AR × MAF)
- ^d Hazard quotient (ratio of effective application rate to relevant endpoint)
- ^e Trigger based on a K_{FOC} of 5125 mL/g (mean)

Effects of secondary poisoning

Indoxacarb and its metabolites

Bioaccumulation and food chain behaviour

The log K_{ow} of indoxacarb is greater than 3. Therefore, potential risks from exposure through consumption of earthworms and fish are warranted.

The applicant was asked to provide a risk assessment for metabolites as well. The applicant proposed the following risk assessment:

The log K_{ow} values for the metabolites were estimated using EPIWIN 4.1. Additionally, for the metabolites where log Kow is ≥3 the bioconcentration factor fish was estimated as well with EPIWIN 4.1. Estimated log K_{ow} and BCF values are summarised in Table 17.

Table 17
Estimated Log K_{ow} , K_{oc} and BCF values for indoxacarb metabolites using EPIWIN 4.1

<i>Substance</i>	<i>Log K_{ow}^a</i>	<i>K_{oc}</i>	<i>BCF^a</i>
IN-JT333	5.06	17300	3.01
IN-KG433	4.09	314	2.37
IN-KB687	2.80	-	-
IN-MK643	2.25	-	-
IN-KT413	0.51	-	-
IN-JU873	4.55	13167	2.67
IN-ML438	3.65	19601	1.95
IN-MK638	1.75	-	-
IN-MP819	4.56	19601	2.68
IN-MS775	5.56	19601	3.34
IN-U8E24	0.97	-	-
IN-UYG24	-2.34	-	-

^a Estimated using EPIWIN 4.1

Consequently, a risk assessment for secondary poisoning will be performed for IN-JT333, IN-KG433, IN-JU873, IN-ML438, IN-MP819 and IN-MS775.

RMS comment

RMS does not consider the estimated values above sufficiently robust for a use in risk assessment. These estimations are obtained from only one model (EPIWIN 4.1). It would have been useful to have other estimations from other models to lower the uncertainty on these values. It would also have been useful to have an estimated value for the parent compound to make a comparison with the experimental data. Besides it is not known if this model is adequate for this active substance.

The risk assessment proposed by the applicant is reported thereafter:

Exposure from earthworm to earthworm-eating birds

Results are summarised in Table 18. The TER_{it} values, based on the dry soil approach, exceed the Regulation (EC) 546/2011 criterion of 5, indicating safe use.

Table 18
Tier 1 food-chain from earthworms to earthworm-eating birds risk assessment for Indoxacarb 150 g/L EC

Substance	Crop	PEC plateau soil mg/kg	BCF ^a dry soil	DDD ^b dry soil mg/kg/d	TER ^c dry soil	TER Trigger
Indoxacarb	Maize	0.042	5.24	0.23	31.8	5
	Leafy vegetables	0.169	5.24	0.93	7.9	5

^a Soil to earthworm bioconcentration factor calculated for dry soil (K_{ow} : 44668.36; K_{oc} : 5125 for indoxacarb)

^b Daily dietary dose calculated for dry soil

^c Toxicity/exposure ratio calculated for dry soil

RMS comment

RMS considers the estimated values of Log Kow and BCF for all metabolites not sufficiently robust (see comment above) and consequently that the risk assessment for metabolites is not fully reliable. The TER calculated by the applicant for metabolites were therefore deleted. For the parent compound indoxacarb,

reliable values (based on valid BCF) are available. The TER calculated for the active substance show acceptable risk for earthworm-eating birds.

Exposure from fish to fish-eating birds

Results are summarised in Table 19. The TER_{it} values exceed the Regulation (EC) 546/2011 criterion of 5, indicating safe use.

Table 19
Tier 1 food-chain from fish to fish-eating birds risk assessment for Indoxacarb 150 g/L EC and metabolites

Substance	Crop	PEC water mg/L	Fish BCF ^a L/kg	PEC fish mg/kg	DDD ^b mg/kg/d	TER ^c	TER Trigger
Indoxacarb	Maize	0.00014	77.3 ^a	0.01	0.00	8059	5
	Leafy vegetables	0.00053	77.3 ^a	0.04	0.00	2128	5

^a Fish bioconcentration factor (measured value)

^b Daily dietary dose from fish consumption

^c Toxicity/exposure ratio ((LD₅₀/10)/DDD), LD₅₀/10 = 7.35 mg a.s./kg bw/d

RMS comment

RMS considers the estimated values of Log Kow and BCF for all metabolites not sufficiently robust (see comment above) and consequently that the risk assessment for metabolites is not fully reliable. The TER calculated by the applicant for metabolites were therefore deleted. For the parent compound indoxacarb, reliable values (based on valid BCF) are available. The TER calculated for the active substance show acceptable risk for fish-eating birds.

RISK ASSESSMENT FOR MAMMALS

Based on the use of Indoxacarb 150 g/L EC, the following exposure scenarios will be addressed:

- Screening assessment crop: Maize (representing sweet corn and field maize) and leafy vegetables

For the purposes of calculations presented here, mammals are assumed to feed exclusively on contaminated material.

The following assumptions are used in the mammalian risk assessment:

- Application rate: 37.5g indoxacarb/ha
- Minimum application interval: 20 days (maize) and 7 days (lettuce)
- Number of applications: 2 applications (maize) and 4 applications (lettuce)
- BBCH 13-49, seed crops: BBCH 13-59 for lettuce, BBCH 34-77 for maize

Guidelines

The mammalian risk assessment is based on the EFSA guidance document: Risk Assessment for Birds and Mammals, European Food Safety Authority (EFSA), Parma, Italy. EFSA Journal 2009: 7(12):1438.

Mammalian toxicity endpoints

A summary of the toxicity endpoints of indoxacarb (DPX-KN128) technical, Indoxacarb 150 g/L EC, and major metabolites to mammals is provided in Table 20. In keeping with EU Directive 2010/63/EU Article 4 for the reduction of the use of animals, vertebrate endpoints were utilized from the studies with DPX-JW062. The endpoints for the use in the risk assessment were lowered to reflect the amount of indoxacarb (DPX-KN128) technical in DPX-JW062 (50%). Studies were conducted with IN-JT333, as it is considered a toxicologically significant metabolite. Details of mammalian studies are provided in the section Toxicology. Endpoints selected for use in the risk assessment are summarised in Table 20.

Table 20
Mammalian toxicity endpoints of indoxacarb

Study	Test substance	Test species	Endpoints	Reference
Acute toxicity	DPX-KN128 technical	Rat	LD ₅₀ = 843 mg/kg bw (males) LD ₅₀ = 179 mg/kg bw (females) ^a	HLO-1997-00055 ^a
Acute toxicity	Indoxacarb 150 g/L EC	Rat	LD ₅₀ = 976.8 mg product/kg bw (LD ₅₀ = 146.4 mg DPX-KN128 /kg bw) ^a	DuPont-13455 ^a
Acute toxicity	IN-JT333	Rat	LD ₅₀ = 52 mg/kg bw (males) LD ₅₀ = 39 mg/kg bw (females)	HLR 927-96 ^a
Reproductive toxicity (long-term)	DPX-JW062 ^b	Rat	NOAEL = 1.2 mg DPX-JW062 /kg bw/d ^b Applicant proposal: NOAEL = 4.6 mg DPX-KN128 /kg bw/d ^c RMS proposal: NOAEL = 0.68 mg DPX-KN128 /kg bw/d	HLO 115-96, Revision No. 1 ^a

^aSummarised in section Toxicology.

^bDPX-JW062 is a racemic mixture of DPX-KN128 and IN-KN127

^c Proposed for ecotoxicological risk assessment.

The acute risk to wild mammals was assessed by calculation of toxicity exposure ratios (TER_a) given in Table 21.

Table 21
Screening step TER_a for mammals exposed to Indoxacarb 150 g/L EC in maize and leafy vegetables

Scenario	Species	LD ₅₀ (acute) (mg a.s./kg bw/d)	SV (90 th %) ^a	Rate applied (kg a.s./ha)	MAF ^b	DDD ^c	TER ^d	Trigger value
Maize	Small herbivorous mammal	146.4	136.4	0.0375	1.1	5.63	26.0	10
Leafy vegetables	Small herbivorous mammal	146.4	136.4	0.0375	1.8	9.21	15.9	10

^a Short cut value is based on the 90th percentile residue unit dose

^b Multiple application factor

^c Daily dietary dose (DDD = RUD × Rate × MAF)

^d Toxicity/exposure ratio (TER_a = LD₅₀ (mg a.s./kg bw/day)/DDD)

The acute risk was also assessed for IN-JT333, as it is considered to be a toxicologically significant metabolite.

Table 22
Screening step TER_a for mammals exposed to IN-JT333 in maize and leafy vegetables

Scenario	Species	LD ₅₀ (acute) (mg/kg bw/d)	SV (90 th %) ^a	Rate applied (kg a.s./ha)	MAF ^b	DDD ^c	TER ^d	Trigger value
Maize	Small herbivorous mammal	39	136.4	0.0375	1.1	5.63	6.9	10
Leafy vegetables	Small herbivorous mammal	39	136.4	0.0375	1.8	9.21	4.2	10

^a Shortcut value for 90th percentile residue per unit dose

^b Multiple application factor

^c Daily dietary dose (DDD = RUD_{SV} × Rate × MAF)

^d Toxicity/exposure ratio (TER_a = LD₅₀ (mg a.s./kg bw/day)/DDD)

Note: Bold values are below the trigger value

The TER_a values are above the trigger value of 10, indicating acceptable risk to mammals from indoxacarb when used in accordance with the proposed label for Indoxacarb 150 g/L EC. The metabolite IN-JT333 TER_a values are below the trigger of 10, indicating a need for refinement. A refined risk assessment is provided in Table 23.

Table 23
Tier 1 TER_a for mammals exposed to IN-JT333 in maize and leafy vegetables

Scenario	Species	LD ₅₀ (acute) (mg/kg bw/d)	SV (90 th %) ^a	Rate applied (kg a.s./ha)	MAF ^b	DDD ^c	TER ^d	Trigger value
Maize BBCH ≥20	Small insectivorous mammal “shrew”	39	5.4	0.0375	1.15	0.22	175.1	10
Maize BBCH 30-39	Small herbivorous mammal “vole”	39	68.2	0.0375	1.15	2.81	13.9	10
Maize BBCH >40	Small herbivorous mammal “vole”	39	34.1	0.0375	1.15	1.41	27.7	10
Maize BBCH 30-39	Small omnivorous mammal “mouse”	39	8.6	0.0375	1.15	0.35	109.9	10
Maize BBCH >40	Small omnivorous mammal “mouse”	39	4.3	0.0375	1.15	0.18	219.9	10
Leafy vegetables BBCH 10-19	Small insectivorous mammal “shrew”	39	7.6	0.0375	1.77	0.51	76.0	10
Leafy vegetables BBCH >20	Small insectivorous mammal “shrew”	39	5.4	0.0375	1.77	0.36	107.0	10
Leafy vegetables BBCH 40-49	Small herbivorous mammal “vole”	39	136.4	0.0375	1.77	9.29	4.2	10
Leafy vegetables BBCH >50	Small herbivorous mammal “vole”	39	40.9	0.0375	1.77	2.77	14.1	10
Leafy vegetables BBCH 13-59	Large herbivorous mammal “Lagomorph”	39	35.1	0.0375	1.77	2.36	16.5	10
Leafy vegetables BBCH 10-49	Small omnivorous mammal “mouse”	39	17.2	0.0375	1.77	1.16	33.6	10
Leafy vegetables BBCH >50	Small omnivorous mammal “mouse”	39	5.2	0.0375	1.77	0.35	111.1	10

^a Shortcut value for 90th percentile residue per unit dose

^b Multiple application factor

^c Daily dietary dose (DDD = RUD_{SV} × Rate × MAF)

^d Toxicity/exposure ratio (TER_a = LD₅₀ (mg a.s./kg bw/day)/DDD)

Note: Bold values are below the trigger value

The TER_a for IN-JT333 is below the relevant Commission Regulation (EU) 546/2011 trigger of 10 for voles foraging in leafy vegetables. This suggests that the metabolite IN-JT333 may pose a risk to small herbivorous mammals and therefore a refined risk assessment was conducted.

Refined risk assessment for IN-JT333 – leafy vegetables

For simplicity, the Tier 1 risk to wild mammals from dietary exposure to IN-JT333 residues was assessed by assuming that Indoxacarb 150 g/L EC degraded 100% to IN-JT333 and therefore residues of IN-JT333 were equivalent to Indoxacarb 150 g/L EC. However, plant metabolism studies indicate that IN-JT333 is not a major plant metabolite, which indicates that the exposure assumed in the Tier 1 assessments presented here by far exceeds potential exposure to field residues of IN-JT333. This suggests that the maximum residue level of IN-JT333 in foliage within the treated field is likely to be <10%. Refined daily dietary dose levels based on the

assumption that IN-JT333 occurs in plants at a maximum of 10% were used to determine acute TERs for IN-JT333 based on more realistic dietary exposure to residues. The revised acute TERs exceed the Commission Regulation (EU) 546/2011 trigger of 10 (Table 24) and demonstrate that the actual risk IN-JT333 posed to small herbivores from applications of Indoxacarb 150 g/L EC is acceptable.

Table 24
Refined acute TER_a for small herbivorous mammals and dietary exposure to IN-JT333 from applications of Indoxacarb 150 g/L EC

Crop/focal Species	BBCH growth stage	Rate Applied (kg a.s./ha)	Short Cut Value ^a	MAF ^b	DDD _{refined} (mg/kg bw/d) ^c	LD ₅₀ (mg/kg bw/d)	TER ^d	Trigger value
Leafy vegetable - Small herbivorous mammal "vole"	40-49	0.0375	136.4	1.8	0.93	39	42.0	10

a Short cut value is based on the 90th percentile residue unit dose and FIR/bw for mammal generic focal species

b Multiple application factor

c Refined daily dietary dose based on 10% formation in plant tissue (multiple)
(DDD = SV × Application rate × 0.1 × MAF) (90th percentile MAF)

d Toxicity/exposure ratio = LD₅₀/DDD

Conclusions

The TER_a values for all proposed uses of Indoxacarb 150 g/L EC are greater than the Commission Regulation (EU) 546/2011 trigger of 10, indicating that Indoxacarb 150 g/L EC will not pose an acute risk to wild mammals. IN-JT333 poses a minimal risk to all focal species except small herbivores. However, IN-JT333 is not a major metabolite in plants and therefore dietary exposure and risk can be considered negligible for this metabolite. It may therefore be concluded that applications of Indoxacarb 150 g/L EC will pose minimal acute risks to mammals at all proposed label rates.

Acute drinking water risk assessment – puddle scenario

Mammals may be exposed to Indoxacarb 150 g/L EC through drinking water by taking up water from puddles formed on the soil surface following events of heavy rainfall after the application of pesticides. According to the EFSA guidance document, no specific calculations of exposure and TER are required when the ratio of effective application rate (in g/ha) to the relevant endpoint (in mg/kg bw/day) does not exceed 50 in the case of less sorptive substances ($K_{oc} < 500$ L/kg) or 3000 in the case of more sorptive substances ($K_{oc} \geq 500$ L/kg).

The HQ values are lower than the EFSA Journal 2009: 7(12):1438 trigger value, indicating acceptable acute risk to mammals from indoxacarb (DPX-KN128) following application of Indoxacarb 150 g/L EC at the proposed label rates.

Table 25
Drinking water risk assessment - ratio of effective application rate to acute toxicity endpoint for
mammals potentially exposed to Indoxacarb 150 g/L EC

Crop	LD ₅₀ (mg a.s./kg bw/d)	Rate applied (g a.s./ha)	MAF ₉₀ ^a	AR _{eff} ^b	HQ ^c	Trigger value ^d
Maize	146.4	37.5	1.7	64.0	0.43	3000
Leafy vegetables	146.4	37.5	3.4	126.1	0.86	3000

^a Mean multiple application factor (based on a DT₅₀, soil of 39.9 day for DPX-MP062)

^b Effective application rate (AR × MAF)

^c Hazard quotient (ratio of effective application rate to relevant endpoint)

^d Trigger based on a K_{FOC} of 5125 mL/g (mean)

Table 26
Drinking water risk assessment - ratio of effective application rate to acute toxicity endpoint for
mammals potentially exposed to IN-JT333

Crop	LD ₅₀ (mg a.s./kg bw/d)	Rate applied (g a.s./ha)	MAF ₉₀ ^a	AR _{eff} ^b	HQ ^c	Trigger value ^d
Maize	39	37.5	1.9	70.6	1.81	3000
Leafy vegetables	39	37.5	3.8	140.6	3.60	3000

^a Mean multiple application factor (based on a DT₅₀, soil of 111 day for IN-JT333, considered over-conservative by RMS)

^b Effective application rate (AR × MAF)

^c Hazard quotient (ratio of effective application rate to relevant endpoint)

^d Trigger based on a K_{FOC} of 17300 mL/g (mean)

Short-term toxicity exposure ratio (TER_{st}) for mammals

The short-term toxicity exposure ratio for mammals is currently not a data requirement.

Long-term toxicity exposure ratio (TER_{lt}) for mammals

The following text describes and justifies the selection of the most appropriate endpoint for use in long-term risk assessment for wild mammals in line with the “Guidance of EFSA: Risk Assessment for Birds and Mammals, European Food Safety Authority (EFSA), Parma, Italy. EFSA Journal 2009: 7(12):1438”, which is, according to the applicant, the 2-generation NOAEL 4.6 mg indoxacarb/kg bw/d instead of the 2-generation NOAEL 3.8 mg DPX-JW062/kg bw/d presented in the Indoxacarb DAR, Volume 3, Addendum 3, B.9.3.1 (2005).

The Tier 1 toxicology NOEL for the rat reproduction study with DPX-JW062 is 20 ppm (equivalent to 1.2 mg DPX-JW062./kg bw/d) for adults and offspring based on minimal body weight effects in F₀ females and their F₁ pups.

RMS comment:

RMS agrees with the choice of the NOEC of 20 ppm (equivalent to 1.2 mg DPX-JW062./kg bw/d, see Volume 3 CA_B6). However, as the compound DPX-JW062 is a 50:50 mixture of the two isomers (DPX-KN128:IN-KN127), it is proposed to express the endpoint in DPX-KN128 by dividing the value of 1.2 mg/kg bw/d obtained for DPX-JW062 by a factor 2*. This results in a NOAEL value of 0.6 mg DPX-KN128/kg bw/d. This endpoint is used at screening step.

***RMS notes that there is no available toxicity study on mammal for IN-KN127. It is therefore not known if IN-KN127 is toxic or not to mammals. This calculation (using factor 2) conservatively assumes that all the toxicity is carried by DPX-KN128.**

Long-term toxicity exposure ratio (TER_{lt}) for indoxacarb (DPX-KN128) following application of Indoxacarb 150 g/L EC is given in Table 27.

Table 27
Screening step TER_{lt} for mammals exposed to Indoxacarb 150 g/L EC in maize and leafy vegetables

Scenario	Species	NOEL mammal repro. (mg a.s./kg bw/d)	RUD _{SV} (mean) ^a	Rate applied (kg a.s./ha)	MAF ^b	DDD ^c	TER ^d	Trigger Value
Maize	Small herbivorous mammal	0.6	72.3	0.0375	1.25	1.87	0.32	5
Leafy vegetables	Small herbivorous mammal	0.6	72.3	0.0375	2.23	3.16	0.19	5

a Short cut value is based on mean residue unit dose

b Multiple application factor calculated according to EFSA:1438 (2009) Appendix H.

c Daily dietary dose (DDD = RUD × Rate × f_{twa} × MAF), (assumes an f_{twa} of 0.53)

d Toxicity/exposure ratio (TER_{lt} = NOAEL (mg a.s./kg bw/day)/DDD)

Note: Bold values are below the trigger value

The TER_{lt} values are below the Regulation (EC) 546/2011 trigger of 5, indicating a need for a Tier 1 risk assessment.

According to the applicant, for the wild mammal risk assessment, the relevant Tier 2 NOEL is 100 ppm based on the “Guidance of EFSA: Risk Assessment for Birds and Mammals, European Food Safety Authority (EFSA). EFSA Journal 2009: 7(12):1438” for birds and mammals risk assessment, concerning relevance of endpoints in long-term toxicity tests:

- No reproductive effects at 20 ppm, 60 ppm and 100 ppm
- No effects on developmental outcomes at 20 ppm, 60 ppm and 100 ppm
- Effects on body weight in F₀ adult females and their F₁ offspring at 60 ppm and 100 ppm were minimal, transient, and known to be reversible and did not affect reproduction. It should be noted that all females gained weight during the study so the parameter observed in this study was reduction in weight gain, not weight loss. There were no effects on the pups of the F₁ generation so body weights were fully restored in the population.

Since the ecologically relevant NOEL of 100 ppm is based on findings in F₀ females and their F₁ offspring, the endpoint in units of daily dose (mg/kg bw/d) for the wild mammals risk assessment is based on compound consumption in this group. Using the overall average food intake for F₀ females fed a dietary concentration of 100 ppm DPX-JW062, the endpoint is recalculated to be 4.6 mg a.s./kg bw/d. Therefore, the most relevant Tier 2 NOEL from the rat reproduction study for use in ecological risk assessments of wild rodents is 4.6 mg indoxacarb/kg bw/day.

RMS comment:

RMS does not agree with the proposal.

The mean body weights of F₁ pups in the 60 and 100 ppm groups were decreased in a dose-related manner throughout lactation. These decreases were consistent with the effects on P₁ maternal body weight during the premating and gestation periods, were dose-related, and therefore were attributed to treatment (see study summary in the toxicology section).

Mean body weights of F₂ pups in the 100 ppm group were slightly reduced at birth and remained reduced throughout the lactation period. The difference was slightly above 10 % at day 4 however, these differences were not statistically significant. No effects were noted at 20 or 60 ppm.

Table 28
Two-generation reproduction study: Litter and pup general observations P₁ and F₁ rats

Parameter	Litter and pup data											
	0 ppm			20 ppm			60 ppm			100 ppm		
	Mean	N	SD	Mean	N	SD	Mean	N	SD	Mean	N	SD
P₁												
Pup weight/litter (g)												
At birth	6.48	22	0.71	6.40 (98.8%)	24	0.54	6.51 (100.5%)	23	0.64	6.14 (94.8%)	22	0.63
Day 4 preculling	10.21	22	1.27	9.95 (97.5%)	24	1.26	9.70 ^a (95.0%)	23	1.39	8.62 ^b (84.4%)	21	1.08
Day 4 postculling	10.24	22	1.24	9.92 (96.9%)	24	1.35	9.83 (96.0%)	23	1.51	8.68 ^b (84.8%)	20	1.12
Day 7	16.79	22	1.48	16.31 (97.1%)	24	1.98	15.21 ^a (90.6%)	23	2.32	13.72 ^b (81.7%)	19	1.59
Day 14	33.98	22	2.82	33.62 (98.9%)	24	3.30	31.92 (93.9%)	23	5.55	30.25 ^b (89.0%)	19	2.08
Day 21	55.05	22	3.93	54.85 (99.6%)	24	5.38	53.71 (97.6%)	22	5.03	50.53 ^b (91.8%)	19	3.76
F₁												
Pup weight/litter (g)												
At birth	6.48	22	0.77	6.26 (96.6%)	18	0.64	6.47 (99.8%)	21	0.61	5.89 (90.9%)	14	0.56
Day 4 preculling	10.13	22	1.30	9.71 (95.9%)	17	1.38	9.95 (98.2%)	20	1.83	9.02 (89.0%)	15	1.22
Day 4 postculling	10.13	22	1.31	9.76 (96.3%)	17	1.37	10.00 (98.7%)	20	1.83	9.09 (89.7%)	15	1.18
Day 7	16.22	22	1.40	16.01 (98.7%)	17	1.78	16.31 (100.6%)	20	3.23	15.20 (93.7%)	15	1.61
Day 14	32.74	22	2.81	33.88 (103.5%)	17	3.17	33.19 (101.4%)	20	6.10	31.74 (96.9%)	15	3.27
Day 21	54.62	22	4.22	55.13 (100.9%)	17	5.30	55.19 (101.0%)	20	9.18	53.56 (98.1%)	15	5.41

^a Significantly different from control by the one-way analysis of covariance (ANCOVA), p <0.05.

^b Significantly different from control by the one-way analysis of covariance (ANCOVA), p <0.01.

Due to the effects on the mean body weight of F1 pups in the 60 and 100 ppm, RMS would consider the NOAEL of 20 ppm relevant for the ecological risk assessment. Effects on bodyweight (even if transitory) have to be taken into account in the choice of the relevant endpoint to be used in the risk assessment (according to EFSA guidance). It is agreed that reproduction parameters were not affected under laboratory conditions but RMS considers that a diminution of the bodyweight at the earliest stages of life might be an adverse effect in natural conditions. Besides RMS notes that decrease of the foetal weight was one of the most sensitive parameter observed in all developmental and multigeneration available toxicity studies.

A conversion of the relevant endpoint (20 ppm) is necessary for the TER calculations (expressed as daily intakes in mg/kg bw/day). It is to be noted that the above mentioned mean daily intakes of 0.6 mg DPX-KN128 (also corresponding to 20 ppm) that was used for the calculations at screening step, corresponds to mean daily intake during premating period for F0 males as a worst case assumption (see Volume 3 CA_B6).

A more relevant endpoint is proposed by RMS for the ecological risk assessment purpose. The mean daily intakes were taken from the toxicology section and are reported in the table below for each concentration at different stages.

Table B.6.6.1-12
Summary of mean consumption values (mg/kg/day) for DPX-JW062 rat reproduction study

	20 ppm	60 ppm	100 ppm
F0 males, premating (test weeks 1-10)	1.234	3.678	6.079
F0 females, premating (test days 1-71)	1.482	4.302	6.743
F0 females, gestation (GD 7-21)	1.361	4.090	6.769
F0 females, lactation (LD 7-14)	3.041	8.741	14.815
F1 males, premating (test Days 29-148)	1.521	4.584	7.817
F1 females, premating (test Days 29-141)	1.797	5.440	9.337
F1 females, gestation (GD 7-21)	1.434	4.324	6.961
F1 females, lactation (LD 7-14)	2.727	7.920	14.266

As the reduced mean pup weights in F₁ pups during lactation at 60 ppm and above were likely secondary to altered growth and nutrition in the dams, RMS proposes to consider the effects on the F0 females bodyweight during gestation.

Considering the NOAEL of 20 ppm and the food consumption of the females during gestation, RMS proposes a converted NOAEL of 1.361 mg DPX-JW062/kg bw/d. This endpoint expressed in DPX-JW062 has to be converted in DPX-KN128 by using a factor 2 (see previous comment).

The NOAEL retained by RMS to be used in the risk assessment is therefore of 0.68 mg DPX-KN128/kg bw/d.

The NOAEL of 0.68 mg DPX-KN128/kg bw/d covers the other NOAEL values issued from developmental studies (a NOAEL of 1.5 mg DPX-KN128/kg bw/d was obtained in a developmental neurotoxicity study and a NOAEL of 2 mg DPX-MP062/kg bw/d equivalent to 1.5 mg DPX-KN128/kg bw/d was obtained in an other developmental toxicity study).

Finally it is also noted that the parental NOAEL (reproduction study) is also of 20 ppm based on decreased bodyweight gains on P1. This effect is considered of lesser relevance for the purpose of ecological risk assessment.

As stated above, for the Tier 1 risk assessment, the ecotoxicological relevant endpoint was used in line with EFSA:15438 (2009).

As the NOAEL proposed by the applicant is not accepted by RMS, the TER values were recalculated by RMS using the NOAEL value of 0.68 DPX-KN128/kg bw/d.

Table 29
Tier 1 TER_{It} for mammals exposed to Indoxacarb 150 g/L EC in maize and leafy vegetables

Scenario	Species	NOAEL mammal repro. (mg a.s./kg bw/d)	RUD _{SV} (mean) ^a	Rate applied (kg a.s./ha)	MAF ^b	DDD ^c	TER ^d	Regulation (EC) 546/2011 trigger
Maize BBCH ≥20	Small insectivorous mammal “shrew”	0.68	1.9	0.0375	1.3	0.05	13.9	5
Maize BBCH 30-39	Small herbivorous mammal “vole”	0.68	36.1	0.0375	1.3	0.94	0.7	5
Maize BBCH ≥40	Small herbivorous mammal “vole”	0.68	18.1	0.0375	1.3	0.47	1.5	5
Maize BBCH 30-39	Small omnivorous mammal “mouse”	0.68	3.9	0.0375	1.3	0.10	6.7	5
Maize BBCH ≥40	Small omnivorous mammal “mouse”	0.68	1.9	0.0375	1.3	0.05	13.9	5
Leafy vegetables BBCH 10-19	Small insectivorous mammal “shrew”	0.68	4.2	0.0375	2.2	0.19	3.7	5
Leafy vegetables BBCH ≥20	Small insectivorous mammal “shrew”	0.68	1.9	0.0375	2.2	0.08	8.2	5
Leafy vegetables BBCH 40-49	Small herbivorous mammal “vole”	0.68	72.3	0.0375	2.2	3.1	0.2	5
Leafy vegetables BBCH ≥50	Small herbivorous mammal “vole”	0.68	21.7	0.0375	2.2	0.96	0.7	5
Leafy vegetables all seasons	Large herbivorous mammal “Lagomorph”	0.68	14.3	0.0375	2.2	0.63	1.1	5
Leafy vegetables BBCH 10-49	Small omnivorous mammal “mouse”	0.68	7.8	0.0375	2.2	0.35	2.0	5
Leafy vegetables BBCH ≥50	Small omnivorous mammal “mouse”	0.68	2.3	0.0375	2.2	0.10	6.8	5

^a Shortcut value for mean residue per unit dose

^b Multiple application factor calculated according to EFSA:1438 (2009) Appendix H.

^c Daily dietary dose (DDD = RUD_{SV} × Rate × MAF × TWA)

^d Toxicity/exposure ratio (TER_{It} = NOAEL (mg a.s./kg bw/day)/DDD)

Note: Bold values are below the trigger value

For use on maize:

The TER_{It} values for Indoxacarb 150 g/L EC are higher than the Commission Regulation (EU) 546/2011 trigger value of 5 for all wild mammal indicator species with the exception of voles in maize. For voles, TER_{It} values are below the trigger value of 5. A refined long-term risk assessment is therefore triggered for voles in maize.

No refinement was proposed by the applicant for this use as the endpoint proposed by the applicant for the TER calculations (4.6 mg/kg bw/d) led to acceptable TER values at Tier 1 for all indicator species. Therefore the long-term risk assessment for small herbivorous mammals is not finalized.

For use on lettuce:

The TER_{It} values for Indoxacarb 150 g/L EC are higher than the Commission Regulation (EU) 546/2011 trigger value of 5 for only two indicator species and at later growth stages (BBCH ≥ 50 for small omnivorous and BBCH ≥ 20 for small insectivorous). The TER_{It} values for small and large herbivorous (at all intended growth stages), for small omnivorous (at early growth stage) and small insectivorous mammal (at early growth stage) are below the trigger value of 5.

No refinement was proposed by the applicant for large herbivorous, small omnivorous and small insectivorous mammals for this use as the endpoint proposed by the applicant for the TER calculations (4.6 mg/kg bw/d) led to acceptable TER values at Tier 1 for these indicator species. Therefore the long-term risk assessment for large herbivorous, small omnivorous and small insectivorous mammals is not finalized.

For voles, TER_{It} values are also below the trigger value of 5. A refined long-term risk assessment is therefore triggered for voles in maize. The risk refinement proposed by the applicant is based on several parameters (refined toxicity endpoint, PD values, relevance of the vole). None of these options were accepted by RMS (see below). Therefore the long-term risk assessment for small herbivorous mammals is not finalized.

The risk refinement proposed by the applicant was reported below for detailed information. However, as these refinements are not accepted by RMS, the final conclusions are based on the Tier 1 TER calculations above.

Refined risk assessment for small herbivore mammals (applicant proposal)

Common vole (*Microtus arvalis*)

The current Guidance Document (EFSA:1438, 2009²) proposes the common vole (Microtus arvalis) as representative small herbivorous focal species in leafy vegetables and maize.

The common vole is distributed homogeneously in large parts of Europe, from the Atlantic coast of France to Central Russia. It is absent from the British Isles, most of Mediterranean and Fennoscandia (Mitchell-Jones et al., 1999³; Grimmberger & Rudloff, 2009⁴).

² EFSA (2009). "Guidance of EFSA - Risk Assessment for Birds and Mammals." EFSA Journal 7(12): 1-139.

³ Mitchell-Jones AJ, Amori G, Bogdanowicz W, Kryštufek B, Reijnders PJH, Spitzenberger F et al (1999). The Atlas of European Mammals, Poyser: London.

⁴ Grimmberger, E. & Rudloff, K. 2009. Atlas der Säugetiere Europas, Nordafrikas und Vorderasiens. Natur und Tier - Verlag GmbH.

Table 30
Body weight of common vole (*Microtus arvalis*) reported in Niethammer & Krapp (1982)

Area	Sex	Body weight (g)	Mean (g)
Halle (Eastern Germany)	Male	26.2	26.0
Rhineland (Western Germany)	Male	25.7	
Halle (Eastern Germany)	Female	25.6	29.6
Rhineland (Western Germany)	Female	33.5	
Geomean			27.7

In two studies on body weight of common voles from Germany, the mean body weights for females are 25.6 and 33.5 g and for males 26.2 and 25.7 g (Niethammer & Krapp, 1982⁵). The calculated geometric mean of the body weight for the species is 27.7 g. Relationship between body weight (b.w. in grams) and daily energy expenditure (DEE in kJ) can be described by the equation: $\log DEE = \log a + b \times \log b.w.$, using the relevant constants for the species group (mammals) from Appendix G of the wildlife Guidance Document. The energy expenditure of common vole of 27.7 g b.w. results in a DEE of 70.0 kJ/day.

RMS comment

The applicant was asked to provide the publications used for the refinement of the weight and the diet of the vole. A summary of each publication was also requested to allow each Member State to check the relevance of these parameters. However as these studies are part of the common literature on voles and not specific to indoxacarb only references were provided. Neither summary nor any statement on the relevance of the parameters was provided. The parameters used for the refinement could not be checked by RMS.

Refinement of Portion of Food Type in the Diet (PD)

The natural food of the common vole consists of green vegetation such as grasses (i.e. monocots), clover and other dicotyledonous herbs and crops. In addition, it feeds on seeds, bark, and subterranean plant parts, roots, and occasionally on arthropods (Niethammer & Krapp, 1982).

On monoculture arable fields options of food choice are limited. In contrast, on grassland habitats a large number of different plant species is available as potential food for voles. To assess the influence of wildflower strips on the spatio-temporal behaviour of voles and their impact on adjacent crop fields, a common vole population was monitored by automatic radio tracking during the summer and early autumn. The wildflower strip (130 m long, 6 m wide) was located within an agricultural landscape in Switzerland. The adjacent crops were maize and wheat in 2000, maize and sugar beet in 2001. The wildflower strip was in a late succession stage and was dominated by tansies, teasels, wild parsnip and grasses (Briner et al., 2005⁶).

Voies showed small home ranges with a median size of 125 m² located almost exclusively within the wildflower strip. 90% of the total home range area and almost 100% of all core areas (30 m² at centre of home range polygons) were situated within the wildflower strip. Home ranges were stable with a median overlap of 90% for two consecutive days (Briner et al., 2005).

Voies developed high population densities of up to 650 individuals/ha within a wildflower strip, and these high densities occurred without the population expanding into adjacent crop areas. Even sugar beets as adjacent

⁵ Niethammer, J. & Krapp, F. 1982. *Microtus arvalis* (Pallas, 1779) - Feldmaus. In: Handbuch der Säugetiere Europas (Ed. by Niethammer, J. & Krapp, F.), pp. 285 - 318. Wiesbaden: Aula-Verlag.

⁶ Briner, T., Nentwig, W. & Airoldi, J.-P. 2005. Habitat quality of wildflower strips for common voles (*Microtus arvalis*) and its relevance for agriculture. *Agriculture Ecosystems & Environment*, 105, 173-179.

field, which are known to be palatable to voles (Balmelli et al., 1999⁷), did not attract the voles (Briner et al., 2005⁶).

It can therefore be concluded that field margins and wildflower strips are high-quality habitats for voles, able to support high-population densities without increasing the tendency of voles to disperse into adjacent agricultural fields. Because vole populations living in grass habitats near to crop fields did not expand into those fields, it is reasonable to assume that the diet of such voles is comparable to the diet of voles living in grassland. In the diet of the common voles inhabiting a meadow in central Germany, dicotyledonous plant species predominated in spring and summer, while in autumn the proportion of monocotyledons increased in the voles' diet. The average number of different plant species was 4.3 per stomach (range: 1-9). Comparing the biomass available (roughly 70% monocotyledons and 30% dicotyledons) with the biomass consumed by common voles (roughly 36% monocotyledons and 64% dicotyledons) during the study period it was evident that the common vole has a selective food intake and preferred dicotyledons (Rinke, 1991⁸).

Table 31
Diet of common voles (% volume) in a meadow in central Germany by Rinke (1991)

<i>Season</i>	<i>Monocotyledons (% volume)</i>	<i>Dicotyledons (% volume)</i>	<i>No. of voles</i>
<i>Spring</i>	24	76	23
<i>Summer</i>	25	75	152
<i>Autumn</i>	48	52	188
<i>Total</i>	36	64	363

Thus, for the risk assessment a PD of 24% monocotyledons and 76% dicotyledons in the summer, for the diet of common voles is considered. This value reflects a conservative assumption regarding the diet of common voles during the spring/summer period.

RMS comment

The applicant was asked to provide the publications used for the refinement of the weight and the diet of the vole. A summary of each publication was also requested to allow each Member State to check the relevance of these parameters. However as these studies are part of the common literature on voles and not specific to indoxacarb only references were provided. Neither summary nor any statement on the relevance of the parameters was provided. The acceptability of the publication cited above could not be checked by RMS except for Rinke (1991). RMS previously considered this publication acceptable at national level. However the relevance of the PD values for spring is not justified. RMS considers that these values might not be conservative as the RUD value for monocotyledons is higher than for dicotyledons (according to the EFSA guidance).

Food intake rate (FIR) of common vole

The notifier proposed to reconsider FIR and FIR/bw values. As the parameters used for the calculation of the FIR/bw could not be checked, RMS considered this calculation not reliable and didn't present it.

⁷ Balmelli, L., Nentwig, W. & Airoidi, J.-P. 1999. Nahrungspräferenzen der Feldmaus *Microtus arvalis* in der Agrarlandschaft unter Berücksichtigung der Pflanzeninhaltsstoffe. Zeitschrift für Säugetierkunde, 64, 154-168.

⁸ Rinke, T. 1991. Percentage of volume versus number of species: availability and intake of grasses and forbs in *Microtus arvalis*. Folia Zoologica, 40, 143-151.

Residues in food items of common vole

For the vole as the representative small herbivorous focal species in crops where indoxacarb will be applied, the RUD values for grass (monocots) and weeds (dicots) will be taken from Appendix F of the Guidance Document (EFSA, 2009).

Refinement of proportion of diet obtained from the treated area (PT)

In a conservative approach, the PT will not be refined. Hence, for the assessment of the long-term risk it is conservatively assumed that common voles forage exclusively in the area contaminated by spray drift.

Relevance of voles in agricultural landscapes (Notifier's proposal)

*The environmental risk assessment for wild mammals evaluates the potential impact of pesticide application in agricultural landscapes, on a 'representative' species likely to be present in the crop at the time of application. As a worst-case assumption the representative species will have a low bodyweight combined with a high food intake rate (FIR) and will consume a relevant food type potentially carrying residues. According to the current guidance document, the common vole (*Microtus arvalis*) is the representative small herbivorous species.*

The use of the common vole in mammalian environmental risk assessment under consideration of population dynamics, habitat and food preferences, their potential as agricultural pests were reviewed by Jacob et al. (2014⁹):

*Common voles (*Microtus arvalis*) are common small mammals with a widespread European distribution ranging from Northern Spain to the Middle East and Central Russia. In these agroecosystems, the common vole forms an important component of the food web and also provides ecosystem services such as soil aeration, with their extensive burrow system providing shelter for different species. However, the common vole is also considered to be an important vertebrate pest species in different crops types across European agricultural landscapes, where it consumes plant material above and below ground whilst also disrupting crop plant roots through nesting and burrowing activity that resulting in significant crop damage/reduced yields.*

The common vole is primarily a grassland species that is well adapted to steppe habitats. Primary habitats are meadows, set-aside land, flower strips, grassy field verges, alfalfa and clover fields. They prefer to inhabit undisturbed short vegetation and can be found in grass leys in forests after clear cuts and other grassy habitats. This habitat preference is of advantage for voles as survival of voles is greater in primary habitats where refuges are more abundant than in secondary habitats (agricultural areas). Furthermore, it must also be considered that secondary habitats cannot maintain common vole populations for long periods given the seasonal nature of farming. Populations in secondary/agricultural habitats will be regularly disrupted by harvest and tilling.

*Primary habitats provide a higher quality habitat and the amount of food required to satisfy the energy budgets of stable populations in off-crop areas sufficient for maintaining essential body functions including reproduction. In general, common voles in primary habitats often feed on grasses because grasses are widely available but prefer to consume protein-rich herbaceous perennial plants where possible. Agricultural areas (secondary habitats) are colonised when the carrying capacity of primary grassland habitats are exceeded. This occurs during multi-annual outbreaks (every 2-5 years), when population sizes can exceed 1000 individuals per hectare. When colonizing secondary habitats, rodents damage crops directly by feeding on shoots and leaves, which results in yield losses or to decreased crop quality. In addition, secondary damage results in plants becoming more susceptible to attack by viral, bacterial and fungal diseases. This is particularly problematic in perennial crops such as orchards and vine. Moreover, indirect costs associated with rodent management can be substantial. During vole population outbreaks to agricultural grassland grazing livestock may have to be transferred early from pastures to stables, resulting in additional husbandry costs for fodder. In such cases, in-crop common vole population control management is commonly practised to avoid significant crop damage. However, even with extensive direct action during outbreaks, *Microtus* populations are seen to recover relatively quickly although data to confirm recovery are scarce. These findings,*

⁹ Jacob, J., Manson, P., Barfknecht, R. Fredricks, T. (2014): Common voles (*Microtus arvalis*) ecology and management: implications for risk assessment of plant protection products. Pest Manag. Sci. 70(6): 869-878.

along with the exceptional reproductive potential of common voles, indicate that common voles will overcome adverse effects of in-crop following application of plant protection products at the landscape level.

When considering benefits and damage caused by the common vole during periodic outbreaks, the associated crop losses and management cost suggest that this species is the most serious vertebrate pest in European agriculture.

The species' status as a crop pest, its high fecundity, resilience to disturbance and intermittent colonisation of crop habitats are important characteristics that should be reflected in risk assessment. Based on the information provided in the scientific literature, it seems justified to modify elements of the current risk assessment scheme for plant protection products including the use of realistic food intake rates, reduced assessment trigger values (already applied in certain EU member states e.g. Germany, or to consider the use of alternate focal rodent species in European risk assessment:

- The common vole is a model species that exists in cropped areas and, given body weight and food intake rates, does represent a worst-case exposure model. It seems therefore reasonable to consider an adjustment in the trigger values to account for reduced uncertainty, associated with the evaluation of derived TER values from acute and reproduction dietary risk assessments (e.g. Germany already accepts trigger values of ≥ 5 in the acute and ≥ 2 in the long-term risk assessment)¹⁰.
- The EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009) indicates that a 25 g vole must consume 1.33 times its own bodyweight (the default food intake rate/bodyweight (FIR/bw)) to satisfy the theoretical daily energy expenditure (DEE). However, in laboratory studies, common voles have been found to only consume about a third of their body weight per day and values as low as 10% based on the uptake of dry matter have been reported. As shown in laboratory studies, even at low temperatures when food uptake is highest, an amount of food equivalent to about 50% of the body weight is eaten, although this was not verified under field conditions.

Some of these pragmatic modifications to the approaches used in risk assessment are already applied in some EU Member States. Therefore, it is reasonable to consistently apply such pragmatic and realistic approaches in risk assessments for plant protection products across the EU.

When considering the modifications proposed by Jacob et al. (2014) for the presented long-term risk assessment for Indoxcarb 150 g/l EC the assessment results in the following conclusions:

- When considering that a 25 g vole must consume 0.5 times its own bodyweight to satisfy the theoretical daily energy expenditure (DEE), the above presented refined risk assessment would result in a TER of 6.0.
- When considering a reduced trigger value of 2 for the long-term risk assessment presented above, acceptable risk could be already shown at Tier 1 for BBCH >50 and in the refined risk assessment for BBCH 40-49.
- The above presented long-term risk assessment shows that for the other considered small mammalian species, the omnivorous wood mouse, safe uses could be demonstrated with a high margin of safety.

Overall, a high margin of safety was demonstrated for small herbivorous mammals and it seems highly unlikely, that small herbivorous mammals will be at risk in agricultural landscape or that abundances of populations will be affected on the long-term scale.

RMS comment

The applicant was asked to provide the publications used for the refinement of the weight and the diet of the vole. A summary of each publication was also requested to allow each Member State to check the relevance of these parameters. However as these studies are part of the common literature on voles and

¹⁰ Adoption of the EFSA-Guidance Document on Risk Assessment for Birds and Mammals. BVL notification no. 10/02/14 of 1 June 2010, German Federal Gazette ('Bundesanzeiger'), 2228-2229.

not specific to indoxacarb only references were provided. Neither summary nor any statement on the relevance of the parameters was provided. The parameters and assumptions used for the refinement could not be checked by RMS.

Besides, the applicant assumes that the vole consumes only 0.5 times its own bodyweight to satisfy the theoretical daily energy expenditure (DEE). This assumption was not justified.

RMS does not agree to lower the trigger value.

The long-term risk assessment for the small herbivorous provided by the applicant is not considered acceptable.

It could be considered at national level whether voles are pests or protected species.

Reproductive drinking water risk assessment

In line with the drinking water risk assessment for acute exposure, a ratio of effective application rate to relevant endpoint was calculated with reproduction endpoints.

The HQ_{it} values are below the trigger value proposed by EFSA Journal 2009: 7(12):1438, indicating acceptable chronic risk to mammals from indoxacarb (DPX-KN128) following application of Indoxacarb 150 g/L EC at all proposed label rates.

Table 32
Drinking water risk assessment - ratio of effective application rate to reproductive toxicity endpoint for mammals potentially exposed to Indoxacarb 150 g/L EC - puddle scenario

Crop	NOAEL (mg a.s./kg bw/d)	Rate applied (g a.s./ha)	MAF _m ^a	AR _{eff} ^b	HQ ^c	Trigger value ^d
Maize	0.68	37.5	1.7	64.0	94.1	3000
Leafy vegetables	0.68	37.5	3.4	126.1	185.4	3000

^a Mean multiple application factor (based on a DT₅₀ soil of 39.9 day for DPX-MP062)

^b Effective application rate (AR × MAF)

^c Hazard quotient (ratio of effective application rate to relevant endpoint)

^d Trigger based on a K_{FOC} of 5125 mL/g (mean)

Effects of secondary poisoning

Bioaccumulation and food chain behaviour

The log K_{ow} of indoxacarb is greater than 3. Therefore, potential risks from exposure through consumption of earthworms, fish, and terrestrial vertebrate prey are warranted.

The applicant was asked to provide a risk assessment for metabolites as well. A risk assessment for secondary poisoning was performed for IN-JT333, IN-KG433, IN-JU873, IN-ML438, IN-MP819 and IN-MS775 since estimated log K_{ow} values are greater than 3. However RMS does not consider these estimated values reliable (see RMS comments in birds part) and the TER provided for these metabolites were deleted. The risk assessment from exposure through consumption of earthworms and fish needs to be addressed for all relevant metabolites. Further information is required.

Exposure from earthworm to earthworm-eating mammals

Table 33
Tier 1 food-chain from earthworms to earthworm-eating mammals risk assessment for
Indoxacarb 150 g/L EC

Substance	Crop	PEC soil mg/kg	BCF ^a dry soil	DDD ^b dry soil mg/kg/d	TER ^c dry soil	TER Trigger
Indoxacarb	Maize	0.042	5.24	0.28	2.41	5
	Leafy vegetables	0.169	5.24	1.13	0.60	5

Note: Bold values are below the trigger value

^a Soil to earthworm bioconcentration factor calculated for dry soil (K_{ow} : 44668.36; K_{oc} : 5125 for indoxacarb)

^b Daily dietary dose calculated for dry soil

^c Toxicity/exposure ratio calculated for dry soil

The TER are below the trigger of 5. The risk for earthworms eating-mammals needs to be refined. The applicant proposed the following refinement:

Refinement of Portion of Food Type in the Diet (PD)

According to the EFSA guidance the appropriate species of concern for earthworm-eating mammals is the common shrew (Sorex araneus). The risk assessment is based on the worst-case assumption of a 10-g shrew entirely feeding on earthworms.

Common shrews are predators feeding on a wide range of invertebrates such as earthworm, woodlice, spiders, slugs, snails and insect larvae as well as small amounts of plant material including seeds (Gurney et al. 1998¹¹).

When looking at the composition of diet of the common shrew, the proportion of Lumbricidae ranges between 4% and 80% based on the data from several field studies given in Gurney et al. (1998). In order to refine the PD the 90th percentile was calculated based on the data given in Gurney et al. (1998). The resulting 90th percentile of 73% is considered in the risk assessment. This reflects a conservative assumption regarding the diet of the common shrew and is therefore still applying a high margin of safety.

RMS comment

RMS considers the estimated values of Log K_{ow} and BCF for all metabolites not sufficiently robust (see comment in birds part) and consequently that the risk assessment for metabolites is not reliable. The TER calculated by the applicant for metabolites were therefore deleted. For the parent compound indoxacarb, reliable values (based on valid BCF) are available at Tier 1 only. The TER value calculated at Tier 1 was however below the trigger of 5 for the uses on maize and lettuce. A refinement was therefore required for these uses. Concerning the PD value proposed by the applicant, neither publication, nor summary, or any statement on the relevance of this parameter were provided. The refined risk assessment based on the PD value is not accepted by RMS. Besides the chronic toxicity endpoint was not considered valid by RMS. In the absence of further data, the risk assessment for effects via secondary poisoning is considered not finalized. The refined TER proposed by the applicant for the active substance were therefore deleted.

Exposure from fish to fish-eating mammals

Results are summarised in Table 34. The TER_{it} exceed the Regulation (EC) 546/2011 criterion of 5, indicating safe use.

¹¹ Gurney, J.E., Peretta, J., Crocker, D.R. and Pascual, J.A. 1998. Mammal bible. Contract PN0910.

Table 34
Tier 1 food-chain from fish to fish-eating mammal risk assessment for Indoxacarb 150 g/L EC

Substance	Crop	PEC water mg /L	fish BCF L/kg	PEC fish mg /kg	DDD ^b mg/kg/d	TER ^c	TER Trigger
Indoxacarb	Maize	0.00014	77.3 ^a	0.01	0.00	835	5
	Leafy vegetables	0.00053	77.3 ^a	0.04	0.00	220	5

^a Fish bioconcentration factor (measured value)

^b Daily dietary dose from fish consumption

^c Toxicity/exposure ratio

RMS comment

RMS considers the estimated values of Log Kow and BCF for all metabolites not sufficiently robust (see comment in birds part) and consequently that the risk assessment for metabolites is not reliable. The TER calculated by the applicant for metabolites were therefore deleted. For the parent compound indoxacarb, reliable values (based on valid BCF) are available. The TER calculated for the active substance show acceptable risk for fish-eating mammals.

B.9.3. EFFECTS ON AQUATIC ORGANISMS

B.9.3.1. Acute toxicity to fish, aquatic invertebrates, or effects on aquatic algae and macrophytes

Report: [REDACTED] (2006); Indoxacarb (DPX-KN128) 150 g/L EC: Static, acute, 96-hour toxicity test to rainbow trout, *Oncorhynchus mykiss*

DuPont Report No.: DuPont-18927

Guidelines: U.S. EPA 72-1 (1985), OECD 203 (1992) **Deviations:** None

Testing Facility: [REDACTED]

Testing Facility Report No.: DuPont-18927

GLP: Yes

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

Executive summary:

The acute toxicity of Indoxacarb 150 g/L EC to unfed fingerling rainbow trout, *Oncorhynchus mykiss*, a cold-water fish, was determined in an unaerated, static, 96-hour dose-response test. The test was conducted in accordance with U.S. EPA Pesticide Assessment Guidelines, Subdivision E, Section 72-1 (1985), and OECD Guideline for the Testing of Chemicals Section 2 (Part 203) (1992). Treatments consisted of a dilution water control, and five nominal concentrations of 1.8, 3.5, 7.0, 14, and 28 mg formulated product/L. The corresponding mean, measured concentrations of indoxacarb (DPX-KN128) were 0.203, 0.399, 0.671, 2.18, and 4.15 mg a.s./L. The 96-hour LC₅₀ for *Oncorhynchus mykiss* based on mortality and nominal total formulation concentrations was 7.0 mg formulated product/L (equivalent to 0.84 mg a.s./L based on mean, measured concentrations).

I. MATERIALS AND METHODS

A. MATERIALS

- | | |
|--|---|
| 1. Test material: | Indoxacarb 150 g/L EC |
| Lot/Batch #: | KN128-150 |
| Purity: | 150 g a.s./L |
| CAS#: | None for the formulation
173584-44-6 for indoxacarb active substance |
| Description: | Liquid |
| Stability of test compound: | Shown to be stable in the test system by analysis |
| 2. Control: | Dilution (laboratory well water) water |
| Test vehicle: | Dilution (laboratory well water) water |
| Toxic reference: | None |
| 3. Test organism: | Rainbow trout |
| Species: | <i>Oncorhynchus mykiss</i> |
| Age at dosing: | Life stage: fingerling |
| Weight at dosing: | 0.347 to 0.811 g in wet weight |
| Initial population: | 5 fish per test chamber |
| Source: | |
| Acclimation period: | 38 days |
| Diet: | Pre-test (25 hr): unfed
Test period: unfed |
| Test chamber: | Test chambers were stainless steel aquaria [30 (length) × 30 (width) × 30 (height) cm] which held approximately 20 L of test solution (26-L maximum volume; approximately 22.5-cm test solution depth). |
| Test medium: | well water |
| 4. Environmental conditions (in-life period) | |
| Temperature: | 12.8°C with a range of 11.9 to 13.4°C |
| Photoperiod: | A photoperiod of 16 hours light (approximately 207-381 Lux) and 8 hours darkness was employed which included 30 minutes of transitional light (6-120 Lux) preceding and following the 16-hour light interval. |

B. STUDY DESIGN AND METHODS

1. Experimental start/completed
05-June-2006 to 09-June-2006
2. Experimental treatments
The acute toxicity of Indoxacarb 150 g/L EC to unfed fingerling rainbow trout, *Oncorhynchus mykiss*, a cold-water freshwater fish, was determined in an unaerated, static, 96-hour dose-response test. Treatments consisted of a dilution water control and five nominal concentrations of 1.8, 3.5, 7.0, 14, and 28 mg formulated product/L. Two replicate control test chambers and two replicate test concentration chambers containing 5 fish each were exposed to each treatment concentration and control.
3. Observations
Mortality and behavioural observations were made every 24 hours. Dead fish were removed from the test chambers when observed.
4. Statistics
The 24-, 48-, 72-, and 96-hour LC₅₀ values were calculated by the moving average angle method based on nominal, total formulation concentrations of Indoxacarb 150 g/L EC and mortality.

The highest nominal, total formulation concentration causing no mortality at test end and the lowest nominal, total formulation concentration causing 100% mortality at test end were assessed by visual observation.

II. RESULTS AND DISCUSSION

A. FINDINGS

Nominal test concentrations were 1.8, 3.5, 7.0, 14, and 28 mg formulated product/L. The corresponding mean, measured concentrations of indoxacarb were 0.203, 0.399, 0.671, 2.18, and 4.15 mg a.s./L and ranged from 60 to 98% of nominal concentrations. All measured values of indoxacarb were within 1.5× of the lowest value for all samples within a concentration with the exception of the Day 4 nominal 3.5 and 7 mg formulated product/L concentrations. All validation criteria were met for the study. A summary of cumulative mortality and sublethal effects is presented in Table 35 and Table 36, respectively.

Table 35
Observed mortality of rainbow trout, *Oncorhynchus mykiss*, exposed to Indoxacarb 150 g/L EC for 96 hours in an unaerated, static, acute test

Nominal Indoxacarb 150 g/L EC concentration (mg/L)	Cumulative mortality (No. dead/No. at test start) ^a							
	24 hour		48 hour		72 hour		96 hour	
	A	B	A	B	A	B	A	B
Water Control (0.0)	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
1.8	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
3.5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
7.0	1/5	1/5	2/5	3/5	2/5	3/5	2/5	3/5
14	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
28	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5

^a A and B represent replicates; each replicate contained 5 fish (total 10 fish per test concentration) at test start.

Table 36
Observed sublethal effects of rainbow trout, *Oncorhynchus mykiss*, exposed to Indoxacarb 150 g/L EC for 96 hours in an unaerated, static, acute test

Nominal Indoxacarb 150 g/L EC concentration (mg/L)	Sublethal effects (Number affected/Number alive) ^a							
	24 hour		48 hour		72 hour		96 hour	
	A	B	A	B	A	B	A	B
Water Control (0.0)	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
1.8	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
3.5	1 ^f /5	1 ^e /5	1 ^{ef} /5	3 ^f /5	0/5	1 ^e /5	0/5	0/5
7.0	1 ^{cdeg} 1 ^{ef} 2 ^{bf} /4	4 ^{cdeg} /4	1 ^e 1 ^{bf} 1 ^{ef} /3	2 ^{cdeg} /2	2 ^e /3	2 ^{cdeg} /2	1 ^e /3	1 ^{cdeg} /2
14	L	L	L	L	L	L	L	L
28	L	L	L	L	L	L	L	L

^a A and B represent replicates; each replicate contained 5 fish (total 10 fish per test concentration) at test start.

^b Erratic swimming

^c Laboured respiration

^d Lying on the bottom

^e Lethargic

^f Rapid respiration

^g Partial loss of equilibrium

L Total mortality

III. CONCLUSION

The 96-hour LC₅₀ for *Oncorhynchus mykiss* based on mortality and nominal total formulation concentrations was 7 mg formulated product/L (equivalent to 0.84 mg a.s./L).

2006)

RMS comment

Study submitted to the EU for the first time in this dossier.

This study was conducted in compliance with the current guideline. The 96-hour LC₅₀ for *Oncorhynchus mykiss* based on nominal total formulation concentrations was 7 mg formulated product/L (equivalent to 0.84 mg a.s./L). Nominal concentrations can be used as initial measured concentrations were above 80% of nominals. This study is acceptable.

Report: Turner, J.T. (2006); Indoxacarb (DPX-KN128) 150 g/L EC: Static, acute, 48-hour toxicity test with *Daphnia magna*

DuPont Report No.: DuPont-18928

Guidelines: U.S. EPS 72-2 (1985), OECD 202 (2004) **Deviations:** None

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

Testing Facility Report No.: DuPont-18928

GLP: Yes

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

Executive summary:

The acute toxicity of Indoxacarb 150 g/L EC to unfed *Daphnia magna* was determined in an unaerated, static, 48-hour test. The test was conducted in accordance with U.S. EPA Pesticide Assessment Guidelines, Subdivision E, Section 72-1 (1985), and OECD Guideline for the Testing of Chemicals Section 2 (Part 202) (2004). Treatments consisted of a dilution water control, and five nominal concentrations of 0.2, 0.4, 0.8, 1.5, and 3.0 mg formulated product/L. The corresponding mean, measured concentrations of indoxacarb (DPX-KN128) were 0.029, 0.047, 0.095, 0.215, and 0.552 mg a.s./L. The 48-hour EC₅₀ for *Daphnia magna* based on immobility and nominal formulation concentrations was 1.67 mg formulated product/L (equivalent to 0.256 mg a.s./L).

I. MATERIALS AND METHODS

A. MATERIALS

- | | |
|---|---|
| 1. Test material: | Indoxacarb 150 g/L EC |
| Lot/Batch #: | KN128-150 |
| Purity: | 150 g a.s./L |
| CAS#: | None for the formulation
173584-44-6 for indoxacarb active substance |
| Description: | Liquid |
| Stability of test compound: | Shown to be stable in the test system by analysis |
| 2. Control: | Dilution (laboratory well water) water |
| Test vehicle: | Dilution (laboratory well water) water |
| Toxic reference: | None |
| 3. Test organism: | |
| Species: | <i>Daphnia magna</i> |
| Age at dosing: | <24 hours |
| Initial population: | 5 daphnids per test chamber |
| Source: | Haskell Laboratory, in-house culture |
| Diet: | Unfed during test |
| Test chamber: | 250-mL Pyrex beaker containing 200 mL of test solution
(6.5-cm test solution depth), covered with a glass plate |
| 4. Environmental conditions
(in-life period) | |
| Temperature: | 20 to 20.1°C |
| Photoperiod: | 16 hr light (227 to 448 lux) and 8 hr darkness which included
30 min transitional light (18 to 37 lux) preceding and
following the 16-hr light interval |

B. STUDY DESIGN AND METHODS

1. Experimental start/completed
06-June-2006 to 08-June-2006
2. Experimental treatments
The acute toxicity of Indoxacarb 150 g/L EC to unfed *Daphnia magna* (<24-hour old) was determined in an unaerated, static, 48-hour test. Treatments consisted of a dilution water control, and five nominal concentrations of 0.2, 0.4, 0.8, 1.5, and 3.0 mg formulated product/L. Five daphnids were used per replicate with four replicates per test concentration and control.

3. Observations

Immobility and behavioural observations were made every 24 hours.

4. Statistics

The 24- and 48-hour EC₅₀ values were calculated by probit analysis based on mean measured indoxacarb concentrations as well as nominal, total formulation concentrations of Indoxacarb 150 g/L EC and immobility. The highest nominal, total formulation concentration causing no immobility at test end was assessed by visual observation.

II. RESULTS AND DISCUSSION

A. FINDINGS

Mean, measured concentrations of indoxacarb were 0.029, 0.047, 0.095, 0.215, and 0.552 mg a.s./L and ranged from 74 to 116% of nominal concentrations. All validation criteria were met for the study. A summary of observed immobility and sublethal effects is presented in the tables below.

Table 37

Summary of observed immobility of unfed *Daphnia magna* exposed to Indoxacarb 150 g/L EC for 48 hours in an unaerated, static, acute test

Nominal Indoxacarb 150 g/L EC concentration (mg/L)	Immobility (No. immobile/No. at test start) ^a							
	24 hours				48 hours			
	A	B	C	D	A	B	C	D
Dilution water control (0.0)	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
0.2	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
0.4	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
0.8	0/5	0/5	2/5	0/5	0/5	0/5	2/5	0/5
1.5	2/5	3/5	2/5	3/5	2/5	4/5	4/5	4/5
3.0	2/5	0/5	1/5	2/5	3/5	3/5	3/5	4/5

^a A–D represent replicate test chambers containing 5 daphnids each at test start

Table 38

Summary of sublethal effects of *Daphnia magna* exposed to Indoxacarb 150 g/L EC for 48 hours in an unaerated, static, acute test

Nominal Indoxacarb 150 g/L EC concentration (mg/L)	Sublethal effects (Number affected/Number alive) ^a							
	24 hours				48 hours			
	A	B	C	D	A	B	C	D
Dilution Water Control (0.0)	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
0.2	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
0.4	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
0.8	3 ^b /5	3 ^b /5	2 ^b /3	2 ^b /5	3 ^b /5	5 ^b /5	2 ^b /3	5 ^b /5
1.5	3 ^b /3	2 ^b /2	3 ^b /3	2 ^b /2	3 ^b /3	1 ^b /1	1 ^b /1	1 ^b /1
3.0	3 ^b /3	5 ^b /5	4 ^b /4	3 ^b /3	2 ^b /2	2 ^b /2	2 ^b /2	1 ^b /1

^a A–D represent replicate test chambers containing 5 daphnids each at test start

^b Daphnids were lethargic

III. CONCLUSION

The 48-hour EC₅₀ for *Daphnia magna* based on immobility and nominal total formulation concentrations was 1.67 mg formulated product/L, (equivalent to 0.256 mg a.s./L).

(Turner, J.T., 2006)

RMS comment

Study submitted to the EU for the first time in this dossier.

This study was conducted in compliance with the current guideline. The applicant was asked to provide another EC₅₀ value based on initial measured concentration as the initial concentration was below 80% of nominals in one tested dose. No recalculated EC₅₀ was provided. Besides there was more than 50% effect on immobility at concentrations lower than the EC₅₀ calculated by the applicant. RMS calculated an EC₅₀ of 0.22 mg DPX-KN128/L (95% confidence intervals: 0.16-0.31). This is equivalent to a concentration of 1.38 mg/L of Indoxacarb 150 g/L EC. EC₅₀ = 1.38 mg/L of Indoxacarb 150 g/L EC

Report: Dinehart, S. (2014); Indoxacarb (DPX-KN128) 150 g/L EC: Acute toxicity with the Mysid shrimp, *Americamysis bahia*, determined under static test conditions

DuPont Report No.: DuPont-38348

Guidelines: OPPTS 850.1035 (1996) **Deviations:** None

Testing Facility: ABC Laboratories, Inc. (Missouri), Columbia, Missouri, USA

Testing Facility Report No.: 80401

GLP: Yes

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

Executive summary:

The acute toxicity of Indoxacarb 150 g/L EC with the mysid shrimp, *Americamysis bahia*, was determined in a 96-hour static test. The test was conducted in accordance with the U.S. EPA Ecological Effects Test guidelines, OPPTS 850.1035.

The test substance, Indoxacarb 150 g/L EC, contains 143.0 g indoxacarb (DPX-KN128)/L with a density of 0.950 g/mL. The study was conducted with five nominal concentrations of Indoxacarb 150 g/L EC (1.26, 2.5, 5.0, 10, and 20 mg formulated product/L; equivalent to 0.19, 0.38, 0.75, 1.5, and 3.0 mg a.s./L) and a dilution water control at a temperature range of 24.2 to 26.0°C. Five mysids were used per test substance concentration and dilution water control replicate, for a total of 20 mysids per treatment. The treatment mean, measured concentrations of indoxacarb during the 96-hour exposure were 0.0684, 0.182, 0.381, 0.808, and 1.98 mg a.s./L, or 36 to 66% of the nominal concentrations. After 96 hours of exposure, mortality was 5, 5, 5, 40, 100, and 100% in the 0 (control), 1.26, 2.5, 5.0, 10 and 20 mg formulated product/L treatments (corresponding to <LOD, 0.0684, 0.182, 0.381, 0.808, and 1.98 mg a.s./L based on mean, measured concentrations of indoxacarb). The highest nominal concentration causing 5% mortality (equivalent to control mortality) at test end was 2.50 mg formulated product/L (corresponding to 0.182 mg a.s./L based on mean, measured concentrations of indoxacarb). The lowest nominal concentration causing 100% mortality at test end was 10 mg formulated product/L (corresponding to 0.808 mg a.s./L based on mean, measured concentrations of indoxacarb). The 96-hour LC₅₀ was estimated to be 5.48 mg formulated product/L (95% confidence limits of 4.72 and 6.36 mg formulated product/L) and 0.422 mg a.s./L (95% confidence intervals of 0.359 and 0.495 mg a.s./L) based on nominal formulation and mean, measured indoxacarb concentrations.

I. MATERIALS AND METHODS

A. MATERIALS

- | | |
|--|--|
| 1. Test material: | Indoxacarb 150 g/L EC |
| Lot/Batch #: | KN128-311 |
| Purity: | 150 g a.s./L |
| Description: | Emulsified concentrate |
| CAS#: | None for the formulation;
173584-44-6 for the active substance indoxacarb |
| Stability of test compound: | Not stable in the test system |
| 2. Control: | Dilution (laboratory saltwater) water |
| Solvent control: | None |
| Test vehicle: | Dilution (laboratory saltwater) water |
| Toxic reference: | None |
| 3. Test organism: | Mysid Shrimp |
| Species: | <i>Americamysis bahia</i> |
| Age at dosing: | <24 hours |
| Initial population: | 5 mysids per test chamber, 4 replicates per treatment for a total of 20 mysids per treatment |
| Source: | ABC Laboratories, in-house culture |
| Diet: | Fed <i>ad libitum</i> during test |
| Test chambers: | 500-mL glass jars with 250-mL solution volume |
| 4. Environmental conditions (in-life period) | |
| Temperature: | 24.2 to 26.0°C (of test chambers) |
| Photoperiod: | 14 hr photoperiod (506 lux) and 10 hr darkness which included two-30 min transitional light periods. |

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
14-November-2013 to 18-November-2013
2. Experimental treatments
The acute toxicity of indoxacarb to *Americamysis bahia* was determined in a static, 96-hour test. Treatments consisted of a dilution water control and five nominal concentrations of 1.26, 2.5, 5.0, 10, and 20 mg formulated product/L, equivalent to 0.19, 0.38, 0.75, 1.5, and 3.0 mg a.s./L. Five mysids were used per test concentration and control replicate for a total of 20 mysids per treatment.
3. Observations
Mortality and behavioural observations were made at 24, 48, 72, and 96 hours. Dead mysids were removed from the test chambers when observed.
4. Statistics
All statistical analyses were performed with SAS software (version 9.3). Estimates of LC₅₀ values and their 95% confidence limits were calculated using the probit method and Trimmed or Untrimmed Spearman-Kärber method. When the P value for Goodness of Fit was >0.05 and there was no other evidence of questionable convergence, the probit method was selected for reporting. When this criterion was not achieved, the Trimmed or Untrimmed Spearman-Kärber method was selected for reporting.

II. RESULTS AND DISCUSSION

A. FINDINGS

Nominal concentrations were 1.26, 2.5, 5.0, 10, and 20 mg formulated product/L, equivalent to 0.19, 0.38, 0.75, 1.5, and 3.0 mg a.s./L. The treatment mean, measured concentrations of indoxacarb during the

96-hour exposure were 0.0684, 0.182, 0.381, 0.808, and 1.98 mg a.s./L, or 36 to 66% of the nominal concentrations. Recoveries from the indoxacarb QC samples ranged from 102 to 107% of the nominal concentrations throughout the test.

All results from biological responses were based on mean measured concentrations of indoxacarb. A summary of cumulative mortality and sublethal effects are presented in Table 39.

Table 39
Observed mortality and sublethal effects of mysid shrimp, *Americamysis bahia*, exposed to Indoxacarb 150 g/L EC for 96 hours in a static acute test

Nominal Indoxacarb 150 g/L EC/L concentration (mg FP/L) ^a	Mean, measured Indoxacarb concentration (mg a.s./L) ^a	Cumulative mortality/Number at test start																Mean % mortality at 96 hours
		24 Hours				48 Hours				72 Hours				96 Hours				
		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	
0 (Control)	0 (Control)	0/5	0/5	0/5 ^b	0/5	0/5	0/5	0/5 ^b	0/5	0/5	0/5	0/5 ^b	0/5	0/5	0/5	1/5	0/5	5
1.26	0.0684	0/5	0/5	0/5	0/5 ^b	0/5	0/5	0/5	0/5 ^b	0/5	0/5	0/5	0/5 ^b	0/5	0/5	0/5	1/5	5
2.5	0.182	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5	5
5.0	0.381	0/5 ^b	1/5	0/5	0/5	0/5 ^{b,c}	3/5	3/5	1/5 ^d	0/5 ^{b,d}	3/5	3/5	1/5	1/5	3/5	3/5	1/5	40
10	0.808	2/5	1/5 ^b	0/5	0/5 ^b	5/5	4/5 ^b	5/5	4/5 ^b	5/5	4/5 ^b	5/5	4/5 ^b	5/5	5/5	5/5	5/5	100
20	1.98	5/5	3/5 ^c	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	100

^a Nominal Concentration of a.s = Nominal concentration of FP x (143.0 g a.s./L/density, 0.950 g/mL/1000 mL/L).

FP = Formulated Product, Indoxacarb 150 g/L EC

^b One mysid shrimp not found. Not Found organisms were considered dead at study termination.

^c Two mysid shrimp were lethargic.

^d One mysid shrimp was lethargic.

Notes: Test chambers contained five mysid shrimp each at test initiation.

III. CONCLUSION

Indoxacarb 150 g/L EC was assessed for acute toxicity with the mysid shrimp, *Americamysis bahia*, in a 96-hour static test. After 96 hours of exposure, mortality was 5, 5, 5, 40, 100, and 100% in the 0 (control), 1.26, 2.5, 5.0, 10, and 20 mg formulated product/L nominal treatments (corresponding to <LOD, 0.0684, 0.182, 0.381, 0.808, and 1.98 mg a.s./L based on mean measured concentrations of indoxacarb). The highest nominal concentration causing 5% mortality (equivalent to control mortality) at test end was 2.50 mg formulated product/L (corresponding to 0.182 mg a.s./L based on mean measured concentrations of indoxacarb). The lowest nominal concentration causing 100% mortality at test end was 10 mg formulated product/L (corresponding to 0.808 mg a.s./L based on mean measured concentrations of indoxacarb). The 96-hour LC₅₀ was estimated to be 5.48 mg formulated product/L (95% confidence limits of 4.72 and 6.36 mg formulated product/L) and 0.422 mg a.s./L (95% confidence intervals of 0.359 and 0.495 mg a.s./L) based on nominal formulation and mean, measured indoxacarb concentrations, respectively.

(Dinehart, S., 2014)

RMS comment

Study submitted to the EU for the first time in this dossier.

This study was conducted in compliance with the current guideline. The applicant was asked to provide another EC50 value based on initial measured concentration as the initial concentration was below 80% of nominals in one tested dose. No recalculated EC50 was provided. RMS calculated an EC50 of 0.75 mg DPX-KN128/L. This is equivalent to a concentration of 5.0 mg/L of Indoxacarb 150 g/L EC.

EC50 =5.0 mg/L of Indoxacarb 150 g/L EC

Report: Sloman, T.L. (2006); Indoxacarb (DPX-KN128) 150 g/L EC: Static, 72-hour growth inhibition toxicity test to the green alga, *Pseudokirchneriella subcapitata*

DuPont Report No.: DuPont-18926

Guidelines: OECD 201 (2006) **Deviations:** None

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

Testing Facility Report No.: DuPont-18926

GLP: Yes

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

Executive summary:

The effect of Indoxacarb 150 g/L EC on the growth and growth rate of the green alga *Pseudokirchneriella subcapitata* (formerly known as *Selenastrum capricornutum*) was determined in a 72-hour test without test medium renewal. The test was conducted in accordance with OECD Guidelines for the Testing of Chemicals Section 2 (Part 201) (2006). Treatments consisted of five nominal concentrations of 0.20, 0.59, 1.8, 5.3, 16 mg formulated product/L (equivalent to 0.0318, 0.0938, 0.286, 0.843, and 2.54 mg a.s./L), a blank control, and an abiotic (stability) control. The E_bC₅₀ (0-72 hr) for cell count was 12.5 mg formulated product/L and the NOEC was 1.8 mg formulated product/L, based on nominal concentrations. The E_rC₅₀ (0-72 hr) for growth rate was >16 mg formulated product/L and the NOEC was 1.8 mg formulated product/L, based on nominal concentrations.

I. MATERIALS AND METHODS

A. MATERIALS

- | | |
|-----------------------------|--|
| 1. Test material: | Indoxacarb 150 g/L EC |
| Lot/Batch #: | KN128-150 |
| Purity: | 150 g a.s./L |
| CAS#: | None for the formulation
173584-44-6 for indoxacarb active substance |
| Description: | Liquid |
| Stability of test compound: | Shown to be unstable under the conditions of the test |
| 2. Control: | AAP nutrient medium |
| Test vehicle: | AAP nutrient medium |
| Toxic reference: | Not applicable |
| 3. Test organism: | Green alga |
| Species: | <i>Pseudokirchneriella subcapitata</i> (formerly known as <i>Selenastrum capricornutum</i>) |
| Initial population: | Approximately 10000 cells/mL |
| Source: | Department of Botany - Culture Collection of Algae - The University of Texas at Austin - Austin, Texas |
| Growth medium: | AAP nutrient medium |
| Test chamber: | 250-mL Erlenmeyer flask containing 50 mL of test solution and fitted with a foam stopper |
| 4. Environmental conditions | |
| Temperature: | 24.0 to 24.1°C (Environmental growth chamber) |
| Photoperiod: | 24 hour photoperiod (7030 to 7720 lux) |

B. STUDY DESIGN AND METHODS

1. Experimental start/completed
16-May-2006 to 19-May-2006
2. Experimental treatments
A study was conducted to determine the effect of Indoxacarb 150 g/L EC on the growth and growth rate of the green alga *Pseudokirchneriella subcapitata* (formerly known as *Selenastrum capricornutum*). The algae were exposed to an untreated control and nominal concentrations of 0.20, 0.59, 1.8, 5.3, and 16 mg formulated product/L (equivalent to 0.0318, 0.0938, 0.286, 0.843, and 2.54 mg a.s./ha) in AAP nutrient medium for 72 hours, without test medium renewal. Each test concentration and the untreated control were tested as 3 replicates. The abiotic (stability) control was tested as a single replicate.
3. Observations
Test concentrations were measured on Day 3 to verify stability of the test item. Healthy cell counts were recorded approximately 0, 24, 48, and 72 hours after test initiation. Healthy cell count (cell density), area under the growth curve, and growth rate were recorded and expressed as percent inhibition relative to the blank control following exposure to Indoxacarb 150 g/L EC for 72 hours.

4 Statistics

The following statistical procedures were used to determine the EC₅₀ and significant differences between the control and the test item concentrations in determination of the NOEC.

Parameter	72-Hour EC ₅₀	72-Hour NOEC
Healthy Cell Count:	Bruce-Versteeg	Jonckheere test ^a
Area under the Growth Curve	Bruce-Versteeg	Jonckheere test ^a
Growth Rate	MAXSD (Maximum Safe Dose)	t-test ^a

^a alpha = 0.05

II. RESULTS AND DISCUSSION

A. FINDINGS

The mean, measured concentration of indoxacarb and the 16 mg formulated product/L abiotic control on Day 0 ranged from 88 to 112% of nominal concentrations (nominal indoxacarb concentrations corrected for 15.9% purity by analysis), indicating accuracy of the abiotic and test concentration solutions. After 3 days, the mean, measured concentrations of the test item were ND, <LOQ, <LOQ, 0.0642, and 0.186 mg a.s./L, and the concentration of the abiotic control was 0.487 mg formulated product/L. The untreated control solutions contained no detectable concentrations of indoxacarb on both Day 0 and Day 3. Indoxacarb 150 g/L EC was determined to be unstable over the course of the test.

A summary of algal growth inhibition following exposure of *Pseudokirchneriella subcapitata* to Indoxacarb 150 g/L EC for 72 hours is presented in the table that follows.

Table 40
Summary of algal growth inhibition following exposure of
***Pseudokirchneriella subcapitata* to Indoxacarb 150 g/L EC for 72 hours**

Nominal Indoxacarb 150 g/L EC concentration (mg/L)	Mean cell density (cells/mL)	% Inhibition relative to control ^a		
		Cell density	Area under the growth curve	Growth rate
Untreated control (0.0)	2.87×10^6	—	—	—
0.20	3.53×10^6	-23	-20	-4
0.59	3.27×10^6	-14	-16	-2
1.8	2.54×10^6	12	5	2
5.3	2.11×10^6	26	23	6
16	1.51×10^6	48	49	12

^a Positive values indicate stimulation; negative values indicate inhibition

III. CONCLUSIONS

The effects of Indoxacarb 150 g/L EC expressed as mg formulated product/L were as follows:

Healthy Cell Count (density):	E_bC_{50} (0-72 hr) = 12.5 mg formulated product/L 72-hr NOEC = 1.8 mg formulated product/L
Area Under the Growth Curve:	E_bC_{50} (0-72 hr) = 12.8 mg formulated product/L 72-hr NOEC = 1.8 mg formulated product/L
Growth Rate:	E_rC_{50} (0-72 hr) = >16 mg formulated product/L 72-hr NOEC = 1.8 mg formulated product/L

(Sloman, T.L., 2006)

RMS comment

Study submitted to the EU for the first time in this dossier.

This study was conducted in compliance with the current guideline. Initial measured concentrations were between 80-120% of nominals. Endpoints based on nominals are acceptable.

Healthy Cell Count (density):	E_bC_{50} (0-72 hr) = 12.5 mg formulated product/L 72-hr NOEC = 1.8 mg formulated product/L
Area Under the Growth Curve:	E_bC_{50} (0-72 hr) = 12.8 mg formulated product/L 72-hr NOEC = 1.8 mg formulated product/L
Growth Rate:	E_rC_{50} (0-72 hr) = >16 mg formulated product/L 72-hr NOEC = 1.8 mg formulated product/L

B.9.3.2. Additional long-term and chronic toxicity studies on fish, aquatic invertebrates and sediment dwelling organisms

Residue data in fish (long-term)

Residues in fish were not measured for Indoxacarb 150 g/L EC since the potential for bioconcentration is low for indoxacarb (DPX-KN128) (see Indoxacarb Volume 3CA).

Chronic toxicity to fish

Chronic toxicity (28-day exposure) to juvenile fish

The studies conducted with indoxacarb can be used to predict the toxicity of the formulated product (see Indoxacarb Volume 3CA).

Fish early life stage toxicity test

The studies conducted with indoxacarb can be used to predict the toxicity of the formulated product (see Indoxacarb Volume 3CA).

Fish life cycle test

The studies conducted with indoxacarb can be used to predict the toxicity of the formulated product (see Indoxacarb Volume 3CA).

Chronic toxicity to aquatic invertebrates**Chronic toxicity to *Daphnia magna* (21-day)**

The studies conducted with indoxacarb (DPX-KN128) can be used to predict the toxicity of the formulated product (see Indoxacarb Volume 3CA).

Chronic toxicity for a representative species of aquatic insects

The studies conducted with indoxacarb can be used to predict the toxicity of the formulated product (see Indoxacarb Volume 3CA).

B.9.3.3. Further testing on aquatic organisms

TERs for the respective sensitive organisms are higher than the respective trigger of 100 or 10 so there is no need for further testing.

B.9.4. RISK ASSESSMENT FOR AQUATIC ORGANISMS**Aquatic application conditions, exposure scenario, and risk assessment assumptions**

The aquatic risk assessment will consider the worst-case exposure scenario of Indoxacarb 150 g/L EC applied at 250 mL product/ha (37.5 g indoxacarb (DPX-KN128)/ha) to maize (representing field and sweet corn) and lettuce.

Exposure assessment (predicted environmental concentrations [PEC])

Predicted environmental concentrations in surface water (PEC_{sw}) and sediment (PEC_{sed}) were calculated for indoxacarb (DPX-KN128) and its major metabolites (aquatic and soil) based on the maximum application rate of 37.5 g a.s./ha in maize and lettuce using the latest FOCUS surface water exposure assessment tools [FOCUS, 2001].

Details of the predicted environmental concentrations for Indoxacarb 150 g/L EC in surface water, arising as a consequence of over-spraying, drift and run-off, are provided in Volume 3CP B-8.

A summary of the ecotoxicological endpoints for indoxacarb technical, Indoxacarb 150 g/L EC and its major metabolites is provided below in Table 41.

Table 41
Ecotoxicological endpoints for aquatic species

Group	Test substance	Time-scale (Test type)	End point	Toxicity ¹
Laboratory tests				
Fish				
<i>Oncorhynchus mykiss</i>	DPX-KN128	Acute 96 hr (flow-through)	Mortality, LC ₅₀	>0.17 mg a.s./L (mm)
<i>Lepomis macrochirus</i>	DPX-MP062 (79:21 mixture DPX-KN128/IN- KN127)	Acute 96 hr (flow-through)	Mortality, LC ₅₀	0.90 mg DPX- MP062/L (mm)
<i>Oncorhynchus mykiss</i>	Indoxacarb 150 g/L EC	Acute 96 hr (static)	Mortality, LC ₅₀	7 mg prep./L (0.84 mg a.s./L) (nom)
<i>Pimephales promelas</i>	DPX-KN128	Chronic 28 days (flow- through)	NOEC (ELS)	0.0675 mg/L (mm)
<i>Oncorhynchus mykiss</i>	DPX-MP062 (79:21 mixture DPX-KN128/IN- KN127)	Chronic 90 days (flow- through)	NOEC (ELS)	0.15 mg DPX- MP062/L (mm)
<i>Pimephales promelas</i>	IN-JT333	Chronic 28 days (flow- through)	NOEC (ELS) EC10	0.00242 mg/L (mm) 0.00249 mg/L (mm)
<i>Oncorhynchus mykiss</i>	IN-JT333	96 hr (flow- through)	Mortality, LC ₅₀	0.029 mg/L (mm)
<i>Oncorhynchus mykiss</i>	IN-KN124	96 hr (semi- static)	Mortality, LC ₅₀	>0.0931 mg/L (mm)
<i>Oncorhynchus mykiss</i>	IN-KN125	96 hr (semi- static)	Mortality, LC ₅₀	0.0105 mg/L (mm)
<i>Oncorhynchus mykiss</i>	IN-JU873	96 hr (semi- static)	Mortality, LC ₅₀	>0.441 mg/L (mm)
<i>Oncorhynchus mykiss</i>	IN-KG433	96 hr (flow- through)	Mortality, LC ₅₀	>0.22 mg/L (mm)
<i>Oncorhynchus mykiss</i>	IN-KT413	96 hr (static)	Mortality, LC ₅₀	>1.06 mg/L (mm)
<i>Oncorhynchus mykiss</i>	IN-MK638	96 hr (static)	Mortality, LC ₅₀	28 mg/L (mm)
<i>Oncorhynchus mykiss</i>	IN-MK643	96 hr (static)	Mortality, LC ₅₀	6.99 mg/L (mm)
<i>Oncorhynchus mykiss</i>	IN-KB687	96 hr (static)	Mortality, LC ₅₀	11.9 mg/L (mm)
<i>Oncorhynchus mykiss</i>	IN-MP819	96 hr (flow- through)	Mortality, LC ₅₀	>0.368 mg/L (mm)

<i>Oncorhynchus mykiss</i>	IN-MS775	96 hr (static)	Mortality, LC ₅₀	>0.00396 mg/L (mm)
<i>Oncorhynchus mykiss</i>	IN-U8E24	96 hr (static)	Mortality, LC ₅₀	46.5 mg/L (mm)
<i>Oncorhynchus mykiss</i>	IN-UYG24	96 hr (static)	Mortality, LC ₅₀	>115 mg/L (mm)
Aquatic invertebrates				
<i>Daphnia magna</i>	DPX-KN128	48 h (flow-through)	Mortality, EC ₅₀	>0.17 mg/L (mm)
<i>Mysidopsis bahia</i>	DPX-KN128	96 h (flow-through)	Mortality, EC ₅₀	>0.126 mg/L (mm)
<i>Daphnia magna</i>	Indoxacarb 150 g/L EC	48 h (static)	Mortality, EC ₅₀	1.38 prod./L (0.22 mg a.s./L) (ini)
<i>Mysidopsis bahia</i>	Indoxacarb 150 g/L EC	96 hr (static)	Mortality, LC ₅₀	5.0 mg prep./L (0.75 mg a.s./L) (ini)
<i>Daphnia magna</i>	DPX-KN128	21 d (semi-static)	Reproduction and development, NOEC	0.0351 mg/L (mm)
<i>Mysidopsis bahia</i>	DPX-MP062 (79:21 mixture DPX-KN128/IN-KN127)	28 days (flow-through)	Reproduction and development, NOEC	0.0184 mg DPX-MP062/L (mm) (equivalent to 0.0145 mg DPX-KN128/L)
<i>Daphnia magna</i>	IN-KT413	21 d (semi-static)	Reproduction and development, NOEC	3.9 mg/L (mm)
<i>Daphnia magna</i>	IN-JT333	48 h (semi-static)	Mortality, EC ₅₀	>0.029 mg/L (mm)
<i>Mysidopsis bahia</i>	IN-JT333	96 h (semi-static)	Mortality, EC ₅₀	0.07 mg/L (mm)
<i>Daphnia magna</i>	IN-KN124	48 h (semi-static)	Mortality, EC ₅₀	>0.106 mg/L (mm)
<i>Daphnia magna</i>	IN-KN125	48 h (semi-static)	Mortality, EC ₅₀	>0.121 mg/L (mm)
<i>Daphnia magna</i>	IN-JU873	48 h (semi-static)	Mortality, EC ₅₀	0.379 mg/L (mm)
<i>Mysidopsis bahia</i>	IN-JU873	96 h (semi-static)	Mortality, EC ₅₀	>1.47 mg/L (mm)
<i>Daphnia magna</i>	IN-KG433	48 h (static)	Mortality, EC ₅₀	>0.23 mg/L (mm)
<i>Daphnia magna</i>	IN-KT413	48 h (static)	Mortality, EC ₅₀	>0.967 mg/L (mm)
<i>Mysidopsis bahia</i>	IN-KT413	96 h (static)	Mortality, EC ₅₀	2.8 mg/L (mm)
<i>Daphnia magna</i>	IN-MK638	48 h (static)	Mortality, EC ₅₀	80 mg/L (mm)
<i>Mysidopsis bahia</i>	IN-MK638	96 h (static)	Mortality, EC ₅₀	41.1 mg/L (mm)

<i>Daphnia magna</i>	IN-MK643	48 h (static)	Mortality, EC ₅₀	34.1 mg/L (mm)
<i>Mysidopsis bahia</i>	IN-MK643	96 h (static)	Mortality, EC ₅₀	16.4 mg/L (mm)
<i>Daphnia magna</i>	IN-KB687	48 h (static)	Mortality, EC ₅₀	7.83 mg/L (mm)
<i>Mysidopsis bahia</i>	IN-KB687	96 h (semi-static)	Mortality, EC ₅₀	7.2 mg/L (mm)
<i>Daphnia magna</i>	IN-MP819	48 h (flow-through)	Mortality, EC ₅₀	0.06 mg/L (mm)
<i>Daphnia magna</i>	IN-MS775	48 h (static)	Mortality, EC ₅₀	>0.00567 mg/L (mm)
<i>Daphnia magna</i>	IN-U8E24	48 h (static)	Mortality, EC ₅₀	>12 mg/L (nom)
<i>Daphnia magna</i>	IN-UYG24	48 h (static)	Mortality, EC ₅₀	>120 mg/L (nom)
Sediment-dwelling organisms				
<i>Chironomus riparius</i> (spiked water)	DPX-KN128	28 d (static)	EC10	0.00168 mg/L (mm)
<i>Chironomus riparius</i> (spiked water)	DPX-KN128	28 d (static)	NOEC development rate	0.00292 mg a.s./kg dry sediment (mm) 0.0018 mg a.s./L (mm)
<i>Chironomus riparius</i> (spiked water)	IN-KT413	28 d (static)	NOEC development rate EC10 (development time)	0.024 mg/L (mm) 0.088 mg/L (mm)
<i>Chironomus riparius</i> (spiked sediment)	IN-JT333	28 d (static)	NOEC development rate	0.096 mg/kg dry sediment (mm)
<i>Chironomus riparius</i> (spiked sediment)	IN-KG433	28 d (static)	NOEC emergence ratio	0.17 mg/kg dry sediment (ini)
<i>Chironomus riparius</i> (spiked sediment)	IN-KT413	28 d (static)	NOEC development rate	7.5 mg/kg dry sediment (mm)
<i>Chironomus riparius</i> (spiked sediment)	IN-MP819	28 d (static)	NOEC emergence, development rate	86.2 mg/kg dry sediment (mm)
<i>Chironomus riparius</i> (spiked sediment)	IN-MS775	28 d (static)	NOEC emergence ratio	2.2 mg/kg dry sediment (mm)
Algae				
<i>Pseudokirchneriella subcapitata</i>	DPX-KN128	72/96 h (static)	Growth rate: 72 h E _r C ₅₀ NOEC _r Biomass: 72 h E _b C ₅₀ NOEC _b Yield: 72 h E _y C ₅₀ NOEC _y	>0.0793 mg/L (mm) 0.0793 mg/L (mm) >0.0793 mg/L (mm) 0.0793 mg/L (mm) >0.0793 mg/L (mm) 0.0793 mg/L (mm)

<i>Pseudokirchneriella subcapitata</i>	Indoxacarb 150 g/L EC	72 h (static)	Growth rate: 72 h E_rC_{50} NOECr Biomass: 72 h E_bC_{50} NOECb	>16 mg/L (nom) 1.8 mg/L (nom) 12.5 mg/L (nom) 1.8 mg/L (nom)
<i>Pseudokirchneriella subcapitata</i>	IN-JT333	72/96/120 h (static)	Growth rate: 72 h E_rC_{50} NOECr Biomass: 72 h E_bC_{50} NOECb	>0.0075 mg/L (mm) 0.0075 mg/L (mm) >0.0075 mg/L (mm) 0.0075 mg/L (mm)
<i>Pseudokirchneriella subcapitata</i>	IN-KN124	72/96 h (static)	Growth rate: 72 h E_rC_{50} NOECr Biomass: 72 h E_bC_{50} NOECb Yield: 72 h E_yC_{50} NOECy	>0.0478 mg/L (mm) 0.0478 mg/L (mm) >0.0478 mg/L (mm) 0.0478 mg/L (mm) >0.0478 mg/L (mm) 0.0478 mg/L (mm)
<i>Pseudokirchneriella subcapitata</i>	IN-KN125	72/96 h (static)	Growth rate: 72 h E_rC_{50} NOECr Biomass: 72 h E_bC_{50} NOECb Yield: 72 h E_yC_{50} NOECy	>0.0508 mg/L (mm) 0.0508 mg/L (mm) >0.0508 mg/L (mm) 0.0508 mg/L (mm) >0.0508 mg/L (mm) 0.0508 mg/L (mm)
<i>Pseudokirchneriella subcapitata</i>	IN-JU873	72 h (static)	Growth rate: 72 h E_rC_{50} NOECr Biomass: 72 h E_bC_{50} NOECb Yield: 72 h E_yC_{50} NOECy	>0.265 mg/L (mm) 0.0332 mg/L (mm) >0.265 mg/L (mm) 0.0332 mg/L (mm) >0.265 mg/L (mm) 0.0332 mg/L (mm)
<i>Pseudokirchneriella subcapitata</i>	IN-KT413	72 h (static)	Growth rate: 72 h E_rC_{50}	>105 mg/L (mm)

<i>Pseudokirchneriella subcapitata</i>	IN-MK638	72 h (static)	Growth rate: 72 h E_rC_{50} NOECr Biomass: 72 h E_bC_{50} NOECb Yield: 72 h E_yC_{50} NOECy	37.2 mg/L (mm) 0.641 mg/L (mm) 7.55 mg/L (mm) 0.641 mg/L (mm) 7.45 mg/L (mm) 0.641 mg/L (mm)
<i>Pseudokirchneriella subcapitata</i>	IN-MK643	72 h (static)	Growth rate: 72 h E_rC_{50} NOECr Biomass: 72 h E_bC_{50} NOECb Yield: 72 h E_yC_{50} NOECy	59.7 mg/L (mm) 3.40 mg/L (mm) 31.8 mg/L (mm) 3.40 mg/L (mm) 31.8 mg/L (mm) 3.40 mg/L (mm)
<i>Pseudokirchneriella subcapitata</i>	IN-KB687	72 h (static)	Growth rate: 72 h E_rC_{50} NOECr Biomass: 72 h E_bC_{50} NOECb Yield: 72 h E_yC_{50} NOECy	>2.26 mg/L (mm) 0.0679 mg/L (mm) 1.41 mg/L (mm) 0.0227 mg/L (mm) 1.39 mg/L (mm) 0.0227 mg/L (mm)
<i>Pseudokirchneriella subcapitata</i>	IN-MP819	72 h (static)	Growth rate: 72 h E_rC_{50} NOECr Biomass: 72 h E_bC_{50} NOECb Yield: 72 h E_yC_{50} NOECy	>0.358 mg/L (mm) 0.358 mg/L (mm) >0.358 mg/L (mm) 0.358 mg/L (mm) >0.358 mg/L (mm) 0.358 mg/L (mm)
<i>Pseudokirchneriella subcapitata</i>	IN-MS775	72 h (static)	Growth rate: 72 h E_rC_{50} NOECr Biomass: 72 h E_bC_{50} NOECb Yield: 72 h E_yC_{50} NOECy	>0.052 mg/L (mm) 0.052 mg/L (mm) >0.052 mg/L (mm) 0.052 mg/L (mm) >0.052 mg/L (mm) 0.052 mg/L (mm)
<i>Pseudokirchneriella subcapitata</i>	IN-U8E24	72/96 h (static)	Growth rate: 72 h E_rC_{50} NOECr Biomass: 72 h E_bC_{50} NOECb Yield: 72 h E_yC_{50} NOECy	55.2 mg/L (mm) 6.1 mg/L (mm) 31.3 mg/L (mm) 6.1 mg/L (mm) 32.6 mg/L (mm) 6.1 mg/L (mm)

<i>Pseudokirchneriella subcapitata</i>	IN-UYG24	72/96 h (static)	Growth rate: 72 h E _r C ₅₀ NOECr Biomass: 72 h E _b C ₅₀ NOECb Yield: 72 h E _y C ₅₀ NOECy	>106 mg/L (mm) 27.4 mg/L (mm) 74.5 mg/L (mm) 6.64 mg/L (mm) 73.0 mg/L (mm) 6.64 mg/L (mm)
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¹ (nom) nominal concentration; (mm) mean measured concentration; (ini) initial measured concentration; prep.: preparation; a.s.: active substance

The toxicity endpoints available for the preparation Indoxacarb 150 g/L EC do not indicate a higher toxicity of the formulated product. No TER calculation was therefore considered necessary for the formulation.

Toxicity/exposure ratios for the most sensitive aquatic organisms (Regulation (EU) N° 284/2013, Annex Part A, point 10.2)

The relevant guidance was the Guidance Document on Aquatic Ecotoxicology¹² and the updated guidance of EFSA (2013)¹³.

Use on maize:

Table 42 FOCUS_{sw} step 1-3 - TERs for indoxacarb – Maize at 37.5 g a.s./ha [2 applications]

Scenario	PEC global max (µg L)	fish acute	fish chronic	Aquatic invertebrate s	Aquatic invertebrate s-prolonged	Algae	Sed. dweller prolonged
		<i>Oncorhynchus mykiss</i>	<i>Pimephales promelas</i>	<i>Mysidopsis bahia</i>	<i>Mysidopsis bahia</i>	<i>Pseudokirchneriella subcapitata</i>	<i>Chironomus riparius</i>
		LC ₅₀	NOEC	EC ₅₀	NOEC	EC ₅₀	EC10
		>170 µg/L	67.5 µg/L	>126 µg/L	14.5 µg/L	>79.3 µg/L	1.68 µg/L
FOCUS Step 1	1.941	87	34	64	7	40	0.87
FOCUS Step 2							
North Europe	0.345	492		365	42		4.87
South Europe	0.546	311		230	26		3.08
FOCUS Step 3							
Single							

¹² SANCO/3268/2001 rev. 4 Final (17 October 2002)

¹³ EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues), 2013. Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. EFSA Journal 2013;11(7):3290, 186 pp. doi:10.2903/j.efsa.2013.3290. Available online: www.efsa.europa.eu/efsajournal

application						
D3, ditch	0.195	871	646	74		8.62
D4, pond	0.008	21250	15750	1812		210
D4, stream	0.170	1000	741	85		9.88
D5, pond	0.008	21250	15750	1812		210
D5, stream	0.180	944	700	80		9.33
D6, ditch	0.193	880	652	75		8.70
R1, pond	0.011	15454	11454	1318		152
R1, stream	0.136	1250	926	106		12
R2, stream	0.182	934	692	79		9.23
R3, stream	0.192	885	656	75		8.75
R4, stream	0.134	1268	940	108		12
Multiple application s						
D3, ditch	0.170	1000	741	85		9.88
D4, pond	0.010	17000	12600	1450		168
D4, stream	0.148	1148	851	97		11.35
D5, pond	0.010	17000	12600	1450		168
D5, stream	0.165	1030	763	87		10.18
D6, ditch	0.171	994	736	84		9.82
R1, pond	0.014	12142	9000	1035		120
R1, stream	0.117	1452	1076	123		14.36
R2, stream	0.157	1082	802	92		10.70
R3, stream	0.165	1030	763	87		10.18
R4, stream	0.159	1069	792	91		10.57
Trigger		100	10	100	10	10

Table 43 FOCUS_{sw} step 4 - TERs indoxacarb – Maize at 37.5 g a.s./ha [2 applications]

Organisms *Chironomus
riparius*:

Toxicity endpoint: 1.68 µg/L

Mitigation options	[x] m non-spray buffer zone	[x] m vegetated buffer strip	PEC _{sw} (µg/L)	TER	Trigger
FOCUS Step 4					
Single application					
D3, ditch	10	10	0.034	49	10
D4, stream	10	10	0.038	44	10
D5, stream	10	10	0.040	42	10
D6, ditch	10	10	0.034	49	10
R2, stream	10	10	0.041	41	10
R3, stream	10	10	0.043	39	10
Multiple applications					
D3, ditch	10	10	0.028	60	10
D6, ditch	10	10	0.028	60	10

Sediment exposure**Table 44 FOCUS_{sed} step 1-3 - TERs for indoxacarb – Maize at 37.5 g a.s./ha [2 applications]**

Scenario	PEC global max (µg/kg sed)	Sed. dweller prolonged
<i>Chironomus riparius</i>		
NOEC		
2.92 µg/kg sed		
FOCUS Step 1	81.782	0.04
FOCUS Step 2		
North Europe	14.279	0.20
South Europe	27.299	0.11
FOCUS Step 3		
Single application		

D3, ditch	0.104	28
D4, pond	0.025	116
D4, stream	0.014	208
D5, pond	0.024	121
D5, stream	0.012	243
D6, ditch	0.055	53.
R1, pond	0.042	69
R1, stream	0.315	9.27
R2, stream	0.651	4.49
R3, stream	0.253	11.54
R4, stream	0.667	4.38
Multiple applications		
D3, ditch	0.100	29
D4, pond	0.033	88
D4, stream	0.015	194
D5, pond	0.029	100
D5, stream	0.043	67
D6, ditch	0.141	20
R1, pond	0.083	35
R1, stream	0.316	9.24
R2, stream	0.651	4.49
R3, stream	0.715	4.08
R4, stream	0.667	4.38
Trigger		10

Table 45 FOCUS_{sed} step 4 - TERs indoxacarb – Maize at 37.5 g a.s./ha [2 applications]

Organisms <i>Chironomus riparius</i> :					
Toxicity endpoint: 2.92 µg/kg sed					
Mitigation options	[x] m non-spray buffer zone	[x] m vegetated buffer strip	PEC _{sed}	TER	Trigger

						(µg/kg sed)
FOCUS Step 4						
Single application						
R1, stream	10	10	0.060	48	10	
R2, stream	10	10	0.103	28	10	
R3, stream	10	10	0.047	62	10	
Multiple applications						
R1, stream	10	10	0.060	48	10	
R2, stream	10	10	0.103	28	10	
R3, stream	10	10	0.127	22	10	
R4, stream	10	10	0.147	19	10	

TER for metabolites (use on maize)

IN-JT333

Table 46 FOCUS_{sw} step 1-2 - TERs for IN-JT333 – Maize at 37.5 g a.s./ha [2 applications]

Scenario	PEC global max (µg L)	fish acute	fish chronic	Aquatic invertebrates	Algae
		<i>Oncorhynchus mykiss</i>	<i>Pimephales promelas</i>	<i>Daphnia magna</i>	<i>Pseudokirchneriella subcapitata</i>
		LC ₅₀	NOEC	EC ₅₀	EC ₅₀
		10.5	2.42	>29	>7.5
FOCUS Step 1	0.330	31	7.33	87	22
FOCUS Step 2					
North Europe	0.079	132	30	367	94
South Europe	0.079	132	30	367	94
Trigger		100	10	100	10

Note: IN-KN125 is acutely more toxic for fish than IN-JT333. The toxicity of isomer IN-KN125 was used for the acute TER for fish as this isomer represents the major form of metabolite IN-JT333 (mixture of IN-KN125 and IN-KN124).

Table 47 FOCUS_{sed} step 1-3 - TERs for IN-JT333 – Maize at 37.5 g a.s./ha [2 applications]

Scenario	PEC global max (µg /kg	Sed. dweller prolonged
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sed)		
<i>Chironomus riparius</i>		
NOEC		
96 µg/kg sed		
FOCUS Step 1	30.535	3.14
FOCUS Step 2		
North Europe	2.633	36
South Europe	4.428	21
Trigger		10

IN-JU873**Table 48 FOCUS_{sw} step 1-2 - TERs for IN-JU873 – Maize at 37.5 g a.s./ha [2 applications]**

Scenario	PEC global max (µg L)	fish acute	Aquatic invertebrates	Algae
		<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Pseudokirchneriella subcapitata</i>
		LC ₅₀	EC ₅₀	EC ₅₀
		441	379	>265
FOCUS Step 1	0.151	2920	2509	1754
FOCUS Step 2				
North Europe	0.011	40090	34454	24090
South Europe	0.023	19173	16478	11521
Trigger		100	100	10

IN-KG433**Table 49 FOCUS_{sw} step 1-2 - TERs for IN-KG433 – Maize at 37.5 g a.s./ha [2 applications]**

Scenario	PEC global max (µg L)	fish acute	Aquatic invertebrates	Algae
		<i>Oncorhynchus mykiss</i>	<i>Mysidopsis bahia</i>	<i>Pseudokirchneriella subcapitata</i>
		LC ₅₀	EC ₅₀	EC ₅₀
		>220	1.45*	7.92*
FOCUS Step 1	6.889	31	0.21	1.15
FOCUS Step 2				
North Europe	0.219	1004	6.62	36
South Europe	0.402	547	3.61	19
Trigger		100	100	10

* As no reliable value is available for aquatic invertebrates and algae, the toxicity of the parent compound divided by 10 was used for TER calculations.

Note: The test item IN-KG433 was not found (<LOD) at 0 and 48 hours in the study Dupont-36478 (acute toxicity on *Mysidopsis bahia*). This study cannot be considered reliable and a new study is required. Applicant indicated that a new study has been scheduled.

Based on FOCUS PEC at Step 2, the TER values remain below the trigger. No PECsw values are available at Step 3. The acute risk for aquatic invertebrates is not finalized.

Table 50 FOCUS_{sed} step 1-2 - TERs for IN-KG433 – Maize at 37.5 g a.s./ha [2 applications]

Scenario	PEC global max (µg /kg sed)	Sed. dweller prolonged
		<i>Chironomus riparius</i>
		NOEC
		170 µg/kg sed
FOCUS Step 1	21.568	7.88
FOCUS Step 2		
North Europe	0.675	251
South Europe	1.250	136

Trigger	10
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IN-KT413**Table 51 FOCUS_{sw} step 1-2 - TERs for IN-KT413 – Maize at 37.5 g a.s./ha [2 applications]**

Scenario	PEC global max (µg L)	fish acute	Aquatic invertebrates	Aquatic invertebrates prolonged	Algae	Sed. dweller prolonged
		<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Daphnia magna</i>	<i>Pseudokirchneriella subcapitata</i>	<i>Chironomus riparius</i>
		LC ₅₀	EC ₅₀	NOEC	EC ₅₀	NOEC
		>1060	967	3900	105000	24
FOCUS Step 1	3.679	288	262	1060	28540	6.52
FOCUS Step 2						
North Europe	0.396	2676	2441	9848	265151	60
South Europe	0.396	2676	2441	9848	265151	60
Trigger		100	100	10	10	10

Table 52 FOCUS_{sed} step 1-2 - TERs for IN-KT413 – Maize at 37.5 g a.s./ha [2 applications]

Scenario	PEC global max (µg /kg sed)	Sed. dweller prolonged
		<i>Chironomus riparius</i>
		NOEC
		7500 µg/kg sed
FOCUS Step 1	11.707	640
FOCUS Step 2		
North Europe	1.006	7455
South Europe	1.107	6775
Trigger		10

IN-MK638**Table 53 FOCUS_{sw} step 1-2 - TERs for IN-MK638 – Maize at 37.5 g a.s./ha [2 applications]**

Scenario	PEC global max (µg L)	fish acute	Aquatic invertebrates	Algae
		<i>Oncorhynchus mykiss</i>	<i>Mysidopsis bahia</i>	<i>Pseudokirchneriella subcapitata</i>
		LC ₅₀	EC ₅₀	EC ₅₀
		28000	41100	37200
FOCUS Step 1	2.463	11368	16686	15103
FOCUS Step 2				
North Europe	0.166	168674	247590	224096
South Europe	0.272	102941	151102	136764
Trigger		100	100	10

IN-MK643**Table 54 FOCUS_{sw} step 1-2 - TERs for IN-MK643 – Maize at 37.5 g a.s./ha [2 applications]**

Scenario	PEC global max (µg L)	fish acute	Aquatic invertebrates	Algae
		<i>Oncorhynchus mykiss</i>	<i>Mysidopsis bahia</i>	<i>Pseudokirchneriella subcapitata</i>
		LC ₅₀	EC ₅₀	EC ₅₀
		6990	16400	59700
FOCUS Step 1	0.912	7664	17982	65460
FOCUS Step 2				
North Europe	0.086	81279	190697	694186
South Europe	0.173	40404	94797	345086

Trigger	100	100	10
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IN-KB687**Table 55 FOCUS_{sw} step 1-2 - TERs for IN-KB687 – Maize at 37.5 g a.s./ha [2 applications]**

Scenario	PEC global max (µg L)	fish acute	Aquatic invertebrates	Algae
		<i>Oncorhynchus mykiss</i>	<i>Mysidopsis bahia</i>	<i>Pseudokirchneriella subcapitata</i>
		LC ₅₀	EC ₅₀	EC ₅₀
		11900	7200	>2260
FOCUS Step 1	1.950	6102	3692	1158
FOCUS Step 2				
North Europe	0.071	167605	101408	31830
South Europe	0.071	167605	101408	31830
Trigger		100	100	10

IN-MP819**Table 56 FOCUS_{sw} step 1-2 - TERs for IN-MP819 – Maize at 37.5 g a.s./ha [2 applications]**

Scenario	PEC global max (µg L)	fish acute	Aquatic invertebrates	Algae
		<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Pseudokirchneriella subcapitata</i>
		LC ₅₀	EC ₅₀	EC ₅₀
		>368	60	>358
FOCUS Step 1	0.178	2067	337	2011
FOCUS Step 2				

North Europe	0.083	4433	722	4313
South Europe	0.083	4433	722	4313
Trigger		100	100	10

Table 57 FOCUS_{sed} step 1-2 - TERs for IN-MP819 – Maize at 37.5 g a.s./ha [2 applications]

Scenario	PEC global max (µg /kg sed)	Sed. dweller prolonged
		<i>Chironomus riparius</i>
		NOEC
		86200 µg/kg sed
FOCUS Step 1	3.764	22901
FOCUS Step 2		
North Europe	1.024	84179
South Europe	1.024	84179
Trigger		10

IN-MS775Table 58 FOCUS_{sw} step 1-2 - TERs for IN-MS775 – Maize at 37.5 g a.s./ha [2 applications]

Scenario	PEC global max (µg L)	fish acute	Aquatic invertebrates	Algae
		<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Pseudokirchneriella subcapitata</i>
		LC ₅₀	EC ₅₀	EC ₅₀
		>3.96	>5.67	>52
FOCUS Step 1	0.079	50	71	658
FOCUS Step 2				

North Europe	0.040	>99	141	1300
South Europe	0.040	>99	141	1300
Trigger		100	100	10

The TER_a for IN-MS775 is slightly below the trigger value. However, the respective 96-hour LC₅₀ value is a greater than value, which can be attributed to the solubility limit of the metabolite in the study, indicating a higher actual value. Therefore, RMS assumes a sufficient margin of safety for this metabolite.

Table 59 FOCUS_{sed} step 1-2 - TERs for IN-MS775 – Maize at 37.5 g a.s./ha [2 applications]

Scenario	PEC global max (µg /kg sed)	Sed. dweller prolonged
		<i>Chironomus riparius</i>
		NOEC
		2200 µg/kg sed
FOCUS Step 1	0.571	3852
FOCUS Step 2		
North Europe	0.49	4489
South Europe	0.49	4489
Trigger		10

IN-U8E24

Table 60 FOCUS_{sw} step 1-2 - TERs for IN-U8E24 – Maize at 37.5 g a.s./ha [2 applications]

Scenario	PEC global max (µg L)	fish acute	Aquatic invertebrates	Algae
		<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Pseudokirchneriella subcapitata</i>
		LC ₅₀	EC ₅₀	EC ₅₀
		46500	>12000	55200
FOCUS Step 1	3.546	13113	3384	15567

FOCUS Step 2				
North Europe	0.637	72998	18838	86656
South Europe	1.141	40754	10517	48379
Trigger		100	100	10

IN-UYG24Table 61 FOCUS_{sw} step 1-2 - TERs for IN-UYG24 – Maize at 37.5 g a.s./ha [2 applications]

Scenario	PEC global max (µg L)	fish acute	Aquatic invertebrates	Algae
		<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Pseudokirchneriella subcapitata</i>
		LC ₅₀	EC ₅₀	EC ₅₀
		>115000	>120000	>106000
FOCUS Step 1	0.131	877863	916031	809160
FOCUS Step 2				
North Europe	0.107	1074766	1121495	990654
South Europe	0.107	1074766	1121495	990654
Trigger		100	100	10

IN-ML438Table 62 FOCUS_{sw} step 1-2 - TERs for IN-ML438 – Maize at 37.5 g a.s./ha [2 applications]

Scenario	PEC global max (µg L)	fish acute	Aquatic invertebrates	Algae
		<i>Oncorhynchus mykiss</i>	<i>Mysidopsis bahia</i>	<i>Pseudokirchneriella subcapitata</i>
		LC ₅₀	EC ₅₀	EC ₅₀
		>17*	>12.6*	>7.93*

FOCUS Step 1	0.082	207	154	97
FOCUS Step 2				
North Europe	0.009	1889	1400	881
South Europe	0.012	1417	1050	661
Trigger		100	100	10

*No toxicity data is available for this metabolite. The toxicity of the active substance divided by 10 was used as surrogate.

RMS conclusions on the use on maize:

Considering the toxicity of the active substance on sediment dwelling organisms, a 10 m buffer zone and 10 m Vegetated Filter Strip were considered necessary for the use on maize.

No reliable data on IN-KG433 for acute toxicity on *Mysidopsis bahia* are available. A new study is required. Applicant indicated that a new study has been scheduled. Based on FOCUS PEC at Step 2, the TER values, based on the toxicity of the active substance divided by 10, remain below the trigger. No PEC_{sw} values are available at Step 3. The acute risk for aquatic invertebrates is therefore not finalized for IN-KG433.

For all other metabolites, TER calculations are available for the surface water compartment and show acceptable risks without mitigation measures. As no toxicity data is available for IN-ML438, the toxicity of the active substance divided by 10 was used as surrogate.

TER calculations are also available for the sediment compartment for five metabolites: IN-JT333, IN-KG433, IN-KT413, IN-MP819 and IN-MS775. Acceptable risk via sediment was demonstrated for all five metabolites without mitigation measures.

No TER calculation in sediment is available for IN-JU873, IN-ML438, IN-MK643, IN-MK638, IN-KB687, IN-U8E24, IN-UYG24, IN-MH304 and IN-MF014. Toxicity data on other aquatic organisms are available for IN-JU873, IN-MK643, IN-MK638, IN-KB687, IN-U8E24, IN-UYG24. These metabolites are clearly of low toxicity to other aquatic groups. No TER was considered necessary.

No toxicity data are available for IN-ML438, IN-MH304 and IN-MF014. According to the PEC_{sed} available for Step 2, IN-ML438, IN-MH304 and IN-MF014 are expected at low concentrations in the sediment and the risk via sediment is considered covered by the risk assessment conducted for the active substance.

Use on lettuce:

Table 63 FOCUS_{sw} step 1-3 - TERs for indoxacarb – Lettuce at 37.5 g a.s./ha [4 applications]

Scenario	PEC global max (µg L)	fish acute	fish chronic	Aquatic invertebrate s	Aquatic invertebrate s-prolonged	Algae	Sed. dweller prolonged
		<i>Oncorhynchus mykiss</i>	<i>Pimephales promelas</i>	<i>Mysidopsis bahia</i>	<i>Mysidopsis bahia</i>	<i>Pseudokirchneriella subcapitata</i>	<i>Chironomus riparius</i>

		LC ₅₀	NOEC	EC ₅₀	NOEC	EC ₅₀	EC10
		>170 µg/L	67.5 µg/L	>126 µg/L	14.5 µg/L	>79.3 µg/L	1.68 µg/L
FOCUS Step 1	7.763	21	8.70	16.23	1.87	10.22	0.22
FOCUS Step 2							
North Europe	1.547	109	43	81	9.37		1.09
South Europe	1.547	109	43	81	9.37		1.09
FOCUS Step 3							
Single application							

D3, ditch (1 st)	0.237	531	61	7.09
D3, ditch (2 nd)	0.237	531	61	7.09
D4, pond (1 st)	0.008	15750	1812	210
D4, stream (1 st)	0.189	666	76	8.89
D6, ditch (1 st)	0.232	543	62	7.24
R1, pond (1 st)	0.013	9692	1115	129
R1, pond (2 nd)	0.017	7411	852	98
R1, stream (1 st)	0.156	807	92	10.77
R1, stream (2 nd)	0.157	802	92	10.70
R2, stream (1 st)	0.206	611	70	8.16
R2, stream (2 nd)	0.21	600	69	8.00
R3, stream (1 st)	0.219	575	66	7.67
R3, stream (2 nd)	0.22	572	65	7.64
R4, stream (1 st)	0.156	807	92	10.77
R4, stream (2 nd)	0.155	812	93	10.84
Multiple applications				
D3, ditch (1 st)	0.160	787	90	10.50
D3, ditch (2 nd)	0.160	787	90	10.50
D4, pond (1 st)	0.008	15750	1812	210
D4, stream (1 st)	0.127	992	114	13.23
D6, ditch (1 st)	0.159	792	91	10.57
R1, pond (1 st)	0.062	2032	233	27
R1, pond (2 nd)	0.032	3937	453	52
R1, stream (1 st)	0.165	763	87	10.18
R1, stream (2 nd)	0.207	608	70	8.12
R2, stream (1 st)	0.139	906	104	12.09
R2, stream (2 nd)	0.141	893	102	11.91
R3, stream (1 st)	0.149	845	97	11.28

R3, stream (2 nd)	0.265		475	54		6.34
R4, stream (1 st)	0.370		340	39		4.54
R4, stream (2 nd)	0.250		504	58		6.72
Trigger	100	10	100	10	10	10

Table 64 FOCUS_{sw} step 4 - TERs indoxacarb – Lettuce at 37.5 g a.s./ha [4 applications]

Organisms <i>Chironomus riparius</i> :						
Toxicity endpoint: 1.68 µg/L						
Mitigation options	[x] m non-spray buffer zone	[x] m vegetated buffer strip	PEC _{sw} (µg/L)	TER	Trigger	
FOCUS Step 4*						
Single application						
D3, ditch (1 st)	10	10	Not available	-	10	
D3, ditch (2 nd)	10	10	Not available	-	10	
D4, stream (1 st)	10	10	Not available	-	10	
D6, ditch (1 st)	10	10	Not available	-	10	
R2, stream (1 st)	10	10	0.040	42	10	
R2, stream (2 nd)	10	10	0.041	40	10	
R3, stream (1 st)	10	10	0.088	19	10	
R3, stream (2 nd)	10	10	0.071	23	10	
Multiple applications						
R1, stream (2 nd)	10	10	0.080	21	10	
R3, stream (2 nd)	10	10	0.077	21	10	
R4, stream (1 st)	10	10	0.175	9.60	10	
R4, stream (2 nd)	10	10	0.127	13.23	10	
Single application						
D3, ditch (1 st)	20	20	0.018	93	10	
D3, ditch (2 nd)	20	20	0.018	93	10	
D4, stream (1 st)	20	20	0.019	88	10	
D6, ditch (1 st)	20	20	0.017	98	10	
Multiple applications						
R4, stream (1 st)	20	20	0.092	18	10	

Sediment exposure

Table 65 FOCUS_{sed} step 1-3 - TERs for indoxacarb – Lettuce at 37.5 g a.s./ha [4 applications]

Scenario	PEC global max (µg /kg sediment)	Sed. dweller prolonged
<i>Chironomus riparius</i>		
NOEC		
2.92 µg/kg sed		
FOCUS Step 1	327.128	0.01
FOCUS Step 2		
North Europe	78.497	0.04
South Europe	78.497	0.04
FOCUS Step 3		
Single application		
D3, ditch (1 st)	0.160	18
D3, ditch (2 nd)	0.160	18
D4, pond (1 st)	0.014	208
D4, stream (1 st)	0.127	22
D6, ditch (1 st)	0.159	18
R1, pond (1 st)	0.057	51
R1, pond (2 nd)	0.084	34
R1, stream (1 st)	0.215	13
R1, stream (2 nd)	0.177	16
R2, stream (1 st)	0.139	21
R2, stream (2 nd)	0.141	20
R3, stream (1 st)	0.277	10.54
R3, stream (2 nd)	0.169	17
R4, stream (1 st)	0.385	7.58
R4, stream (2 nd)	0.280	10.43
Multiple applications		
D3, ditch (1 st)	0.139	21

D3, ditch (2 nd)	0.130	22
D4, pond (1 st)	0.050	58
D4, stream (1 st)	0.007	417
D6, ditch (1 st)	0.069	42
R1, pond (1 st)	0.218	13
R1, pond (2 nd)	0.551	5.30
R1, stream (1 st)	3.168	0.92
R1, stream (2 nd)	2.164	1.35
R2, stream (1 st)	2.640	1.11
R2, stream (2 nd)	2.572	1.14
R3, stream (1 st)	1.217	2.40
R3, stream (2 nd)	0.984	2.97
R4, stream (1 st)	0.975	2.99
R4, stream (2 nd)	0.453	6.45
Trigger		10

Table 66 FOCUS_{sed} step 4 - TERs indoxacarb – Lettuce at 37.5 g a.s./ha [4 applications]

Organisms <i>Chironomus riparius</i> :					
Toxicity endpoint: 2.92 µg/kg sed					
Mitigation options	[x] m non-spray buffer zone	[x] m vegetated buffer strip	PECsed (µg/kg sed)	TER	Trigger
FOCUS Step 4*					
Single application					
R4, stream (1 st)	10	10	0.124	23	10
Multiple applications					
R1, pond (2 nd)	10	10	0.229	12	10
R1, stream (1 st)	10	10	0.501	5.83	10
R1, stream (2 nd)	10	10	0.369	7.91	10
R2, stream (1 st)	10	10	0.428	6.82	10
R2, stream (2 nd)	10	10	0.399	7.32	10
R3, stream (1 st)	10	10	0.202	14	10
R3, stream (2 nd)	10	10	0.177	16	10
R4, stream (1 st)	10	10	0.263	11.10	10
R4, stream (2 nd)	10	10	0.117	24	10
Multiple applications					
R1, stream (1 st)	20	20	0.175	16	10
R1, stream (2 nd)	20	20	0.136	21	10
R2, stream (1 st)	20	20	0.152	19	10
R2, stream (2 nd)	20	20	0.137	21	10

TER for metabolites (use on lettuce)

IN-JT333

Table 67 FOCUS_{sw} step 1-3 - TERs for IN-JT333 – Lettuce at 37.5 g a.s./ha [4 applications]

Scenario	PEC global max (µg L)	fish acute	fish chronic	Aquatic invertebrates	Algae
		<i>Oncorhynchus mykiss</i>	<i>Pimephales promelas</i>	<i>Daphnia magna</i>	<i>Pseudokirchneriella subcapitata</i>
		LC ₅₀	NOEC	EC ₅₀	EC ₅₀
		10.5	2.42	>29	>7.5
FOCUS Step 1	0.660	15	3.67	43.94	11.36
FOCUS Step 2					
North Europe	0.079	132	30	367	94
South Europe	0.079	132	30	367	94
Trigger		100	10	100	10

Note: IN-KN125 is acutely more toxic for fish than IN-JT333. The toxicity of isomer IN-KN125 was used for the acute TER for fish as this isomer represents the major form of metabolite IN-JT333 (mixture of IN-KN125 and IN-KN124).

FOCUS_{sed} step 1-3 - TERs for IN-JT333 – Lettuce at 37.5 g a.s./ha [4 applications]

Scenario	PEC global max (µg /kg sed)	Sed. dweller prolonged
<i>Chironomus riparius</i>		
NOEC		
96 µg/kg sed		
FOCUS Step 1	61.069	1.57
FOCUS Step 2		
North Europe	11.481	8.36
South Europe	11.481	8.36
FOCUS Step 3		
Single application		
D3, ditch (1 st)	0.412	233
D3, ditch (2 nd)	0.412	233
D4, pond (1 st)	0.050	1920
D4, stream (1 st)	0.000	96000
D6, ditch (1 st)	0.412	233
R1, pond (1 st)	0.050	1920
R1, pond (2 nd)	0.050	1920
R1, stream (1 st)	0.124	774
R1, stream (2 nd)	0.143	671
R2, stream (1 st)	0.050	1920
R2, stream (2 nd)	0.540	177
R3, stream (1 st)	0.075	1280
R3, stream (2 nd)	0.054	1777
R4, stream (1 st)	0.030	3200
R4, stream (2 nd)	0.022	4363
Multiple applications		
D3, ditch (1 st)	1.112	86

D3, ditch (2 nd)	1.112	86
D4, pond (1 st)	0.125	768
D4, stream (1 st)	0.000	96000
D6, ditch (1 st)	1.112	86
R1, pond (1 st)	0.125	768
R1, pond (2 nd)	0.125	768
R1, stream (1 st)	0.695	138
R1, stream (2 nd)	0.511	187
R2, stream (1 st)	0.182	527
R2, stream (2 nd)	1.523	63
R3, stream (1 st)	0.448	214
R3, stream (2 nd)	0.216	444
R4, stream (1 st)	0.158	607
R4, stream (2 nd)	0.080	1200
Trigger		10

IN-JU873**FOCUS_{sw} step 1-2 - TERs for IN-JU873 – Lettuce at 37.5 g a.s./ha [4 applications]**

Scenario	PEC global max (µg L)	fish acute	Aquatic invertebrates	Algae
		<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Pseudokirchneriella subcapitata</i>
		LC ₅₀	EC ₅₀	EC ₅₀
		441	379	>265
FOCUS Step 1	0.302	1460	1254	877
FOCUS Step 2				
North Europe	0.067	6582	5656	3955
South Europe	0.067	6582	5656	3955
Trigger		100	100	10

IN-KG433**Table 68 FOCUS_{sw} step 1-2 - TERs for IN-KG433 – Lettuce at 37.5 g a.s./ha [4 applications]**

Scenario	PEC global max (µg L)	fish acute	Aquatic invertebrates	Algae
		<i>Oncorhynchus mykiss</i>	<i>Mysidopsis bahia</i>	<i>Pseudokirchneriella subcapitata</i>
		LC ₅₀	EC ₅₀	EC ₅₀
		>220	1.45*	7.92*
FOCUS Step 1	13.778	15	0.11	0.57
FOCUS Step 2				
North Europe	0.820	268	1.77	9.66
South Europe	0.820	268	1.77	9.66
Trigger		100	100	10

* As no reliable value is available for aquatic invertebrates and algae, the toxicity of the parent compound divided by 10 was used for TER calculations.

Note: The test item IN-KG433 was not found (<LOD) at 0 and 48 hours in the study Dupont-36478 (acute toxicity on *Mysidopsis bahia*). This study cannot be considered reliable and a new study is required. Applicant indicated that a new study has been scheduled.

Based on FOCUS PEC at Step 2, the TER values remain below the trigger for aquatic invertebrates and algae. No PEC_{sw} values are available at Step 3. The acute risk for aquatic invertebrates and algae is therefore not finalized.

Table 69 FOCUS_{sed} step 1-2 - TERs for IN-KG433 – Lettuce at 37.5 g a.s./ha [4 applications]

Scenario	PEC global max (µg /kg sed)	Sed. dweller prolonged
		<i>Chironomus riparius</i>
		NOEC
		170 µg/kg sed
FOCUS Step 1	43.136	3.94

FOCUS Step 2		
North Europe	2.557	66
South Europe	2.557	66
Trigger		10

IN-KT413**Table 70 FOCUS_{sw} step 1-2 - TERs for IN-KT413 – Lettuce at 37.5 g a.s./ha [4 applications]**

Scenario	PEC global max (µg L)	fish acute	Aquatic invertebrates	Aquatic invertebrates prolonged	Algae	Sed. dweller prolonged
		<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Daphnia magna</i>	<i>Pseudokirchneriella subcapitata</i>	<i>Chironomus riparius</i>
		LC ₅₀	EC ₅₀	NOEC	EC ₅₀	NOEC
		>1060	967	3900	105000	24
FOCUS Step 1	7.359	144	131	529	14268	3.26
FOCUS Step 2						
North Europe	0.545	1944	1774	7155	192660	44
South Europe	0.545	1944	1774	7155	192660	44
Trigger		100	100	10	10	10

Table 71 FOCUS_{sed} step 1-2 - TERs for IN-KT413 – Lettuce at 37.5 g a.s./ha [4 applications]

Scenario	PEC global max (µg /kg sed)	Sed. dweller prolonged
		<i>Chironomus riparius</i>
		NOEC
		7500 µg/kg sed
FOCUS Step 1	23.414	320

FOCUS Step 2		
North Europe	1.672	4485
South Europe	1.672	4485
Trigger		10

IN-MK638**Table 72 FOCUS_{sw} step 1-2 - TERs for IN-MK638 – Lettuce at 37.5 g a.s./ha [4 applications]**

Scenario	PEC global max (µg L)	fish acute	Aquatic invertebrates	Algae
		<i>Oncorhynchus mykiss</i>	<i>Mysidopsis bahia</i>	<i>Pseudokirchneriella subcapitata</i>
		LC ₅₀	EC ₅₀	EC ₅₀
		28000	41100	37200
FOCUS Step 1	4.927	5682	8341	7550
FOCUS Step 2				
North Europe	0.764	36649	53795	48691
South Europe	0.645	43410	63720	57674
Trigger		100	100	10

IN-MK643**Table 73 FOCUS_{sw} step 1-2 - TERs for IN-MK643 – Lettuce at 37.5 g a.s./ha [4 applications]**

Scenario	PEC global max (µg L)	fish acute	Aquatic invertebrates	Algae
		<i>Oncorhynchus mykiss</i>	<i>Mysidopsis bahia</i>	<i>Pseudokirchneriella subcapitata</i>
		LC ₅₀	EC ₅₀	EC ₅₀

		6990	16400	59700
FOCUS Step 1	1.825	3830	8986	32712
FOCUS Step 2				
North Europe	0.516	13546	31782	115697
South Europe	0.516	13546	31782	115697
Trigger		100	100	10

IN-KB687**Table 74 FOCUS_{sw} step 1-2 - TERs for IN-KB687 – Lettuce at 37.5 g a.s./ha [4 applications]**

Scenario	PEC global max (µg L)	fish acute	Aquatic invertebrates	Algae
		<i>Oncorhynchus mykiss</i>	<i>Mysidopsis bahia</i>	<i>Pseudokirchneriella subcapitata</i>
		LC ₅₀	EC ₅₀	EC ₅₀
		11900	7200	>2260
FOCUS Step 1	3.901	3050	1845	579
FOCUS Step 2				
North Europe	0.103	115533	69902	21941
South Europe	0.103	115533	69902	21941
Trigger		100	100	10

IN-MP819**Table 75 FOCUS_{sw} step 1-2 - TERs for IN-MP819 – Lettuce at 37.5 g a.s./ha [4 applications]**

Scenario	PEC global max (µg L)	fish acute	Aquatic invertebrates	Algae
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		<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Pseudokirchneriella subcapitata</i>
		LC ₅₀	EC ₅₀	EC ₅₀
		>368	60	>358
FOCUS Step 1	0.357	1030	168	1002
FOCUS Step 2				
North Europe	0.083	4433	722	4313
South Europe	0.083	4433	722	4313
Trigger		100	100	10

Table 76 FOCUS_{sed} step 1-2 - TERs for IN-MP819 – Lettuce at 37.5 g a.s./ha [4 applications]

Scenario	PEC global max (µg/kg sed)	Sed. dweller prolonged
		<i>Chironomus riparius</i>
		NOEC
		86200 µg/kg sed
FOCUS Step 1	7.528	11450
FOCUS Step 2		
North Europe	1.564	55115
South Europe	1.564	55115
Trigger		10

IN-MS775Table 77 FOCUS_{sw} step 1-2 - TERs for IN-MS775 – Lettuce at 37.5 g a.s./ha [4 applications]

Scenario	PEC global max (µg/L)	fish acute	Aquatic invertebrates	Algae
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		<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Pseudokirchneriella subcapitata</i>
		LC ₅₀	EC ₅₀	EC ₅₀
		>3.96	>5.67	>52
FOCUS Step 1	0.158	25	35	329
FOCUS Step 2				
North Europe	0.040	>99	141	1300
South Europe	0.040	>99	141	1300
Trigger		100	100	10

The TER_a for IN-MS775 is slightly below the trigger value based on FOCUS Step 2 PEC values. However, the respective 96-hour LC₅₀ value is a greater than value, which can be attributed to the solubility limit of the metabolite in the study, indicating a higher actual value. Therefore, RMS assumes a sufficient margin of safety for this metabolite.

Table 78 FOCUS_{sed} step 1-2 - TERs for IN-MS775 – Lettuce at 37.5 g a.s./ha [4 applications]

Scenario	PEC global max (µg /kg sed)	Sed. dweller prolonged
		<i>Chironomus riparius</i>
		NOEC
		2200 µg/kg sed
FOCUS Step 1	1.142	1926
FOCUS Step 2		
North Europe	0.749	2937
South Europe	0.749	2937
Trigger		10

Table 79 FOCUS_{sw} step 1-2 - TERs for IN-U8E24 – Lettuce at 37.5 g a.s./ha [4 applications]

Scenario	PEC global max (µg L)	fish acute	Aquatic invertebrates	Algae
		<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Pseudokirchneriella subcapitata</i>
		LC ₅₀	EC ₅₀	EC ₅₀
		46500	>12000	55200
FOCUS Step 1	7.09	6559	1693	7786
FOCUS Step 2				
North Europe	2.723	17077	4407	20272
South Europe	2.219	20955	5408	24876
Trigger		100	100	10

IN-UYG24Table 80 FOCUS_{sw} step 1-2 - TERs for IN-UYG24 – Lettuce at 37.5 g a.s./ha [4 applications]

Scenario	PEC global max (µg L)	fish acute	Aquatic invertebrates	Algae
		<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Pseudokirchneriella subcapitata</i>
		LC ₅₀	EC ₅₀	EC ₅₀
		>115000	>120000	>106000
FOCUS Step 1	0.262	438931	458015	404580
FOCUS Step 2				
North Europe	0.16	718750	750000	662500
South Europe	0.16	718750	750000	662500
Trigger		100	100	10

IN-ML438**Table 81 FOCUS_{sw} step 1-2 - TERs for IN-ML438 – Lettuce at 37.5 g a.s./ha [4 applications]**

Scenario	PEC global max (µg L)	fish acute	Aquatic invertebrates	Algae
		<i>Oncorhynchus mykiss</i>	<i>Mysidopsis bahia</i>	<i>Pseudokirchneriella subcapitata</i>
		LC ₅₀	EC ₅₀	EC ₅₀
		>17*	>12.6*	>7.93*
FOCUS Step 1	0.164	104	77	48
FOCUS Step 2				
North Europe	0.035	486	360	227
South Europe	0.035	486	360	227
Trigger		100	100	10

*No toxicity data is available for this metabolite. The toxicity of the active substance divided by 10 was used as surrogate.

RMS conclusions on the use on lettuce:

Considering the toxicity of the active substance on sediment dwelling organisms, a 20 m buffer zone and 20 m Vegetated Filter Strip were considered necessary for the use on lettuce.

No reliable data on IN-KG433 for acute toxicity on *Mysidopsis bahia* are available. A new study is required. Applicant indicated that a new study has been scheduled. Based on FOCUS PEC at Step 2, the TER values, based on the toxicity of the active substance divided by 10, remain below the trigger. No PEC_{sw} values are available at Step 3. The acute risk for aquatic invertebrates is therefore not finalized for IN-KG433.

For all other metabolites, TER calculations are available for the surface water compartment and show acceptable risks without mitigation measures. As no toxicity data are available for IN-ML438, the toxicity of the active substance divided by 10 was used as surrogate.

TER calculations are also available for the sediment compartment for five metabolites: IN-JT333, IN-KG433, IN-KT413, IN-MP819 and IN-MS775. Acceptable risk via sediment was demonstrated for all five metabolites without mitigation measures.

No TER calculation in sediment is available for IN-JU873, IN-ML438, IN-MK643, IN-MK638, IN-KB687, IN-U8E24, IN-UYG24, IN-MH304 and IN-MF014. Toxicity data on other aquatic organisms are available for IN-JU873, IN-MK643, IN-MK638, IN-KB687, IN-U8E24, IN-UYG24. These metabolites are clearly of low toxicity to other aquatic groups. No TER was considered necessary.

No toxicity data are available for IN-ML438, IN-MH304 and IN-MF014. According to the PEC_{sed} available for Step 2, IN-ML438, IN-MH304 and IN-MF014 are expected at low concentrations in the sediment and the risk via sediment is considered covered by the risk assessment conducted for the active substance.

B.9.5. EFFECTS ON ARTHROPODS**B.9.5.1. Effects on bees**

A laboratory oral and contact toxicity test with Indoxacarb 150 g/L EC on honey bees was conducted. A summary of this test is presented below.

Report: Schmitzer, S. (2006); Indoxacarb (DPX-KN128) 150 g/L EC: Acute oral and contact toxicity to the honey bee, *Apis mellifera* L.

DuPont Report No.: DuPont-18924

Guidelines: OECD 213 (1998), OECD 214 (1998) **Deviations:** None

Testing Facility: IBACON, Rossdorf, Germany

Testing Facility Report No.: 29521035

GLP: Yes

Certifying Authority: Hessisches Ministerium für Umwelt, Landwirtschaft und Forsten

Executive summary:

Acute 48-hour oral and contact toxicity tests on honey bees (*Apis mellifera* L.) were conducted with Indoxacarb 150 g/L EC in the laboratory according to OECD Guidelines 213 and 214 (1998). Treatments consisted of four toxic standard treatment rates, a dilution water control, and six concentrations of Indoxacarb 150 g/L EC/bee. Nominal test rates in the contact test were 0.016, 0.031, 0.06, 0.13, 0.25, and 0.50 µg a.s./bee. Nominal dosages in the oral test were 0.03, 0.06, 0.13, 0.25, 0.50, and 1.0 µg a.s./bee. The treatment rates based on measured dietary intake (actual consumption) were 0.033, 0.069, 0.13, 0.27, 0.54, and 1.09 µg a.s./bee. The 48-hour and 96-hour oral LD₅₀ for honey bees based on mortality and mean measured dietary intake were 0.11 and 0.10 µg a.s./bee, respectively (equivalent to 0.69 and 0.63 µg formulated product/bee). The 48-hour contact LD₅₀ for honey bees based on mortality and nominal concentrations was 0.08 µg a.s./bee (equivalent to 0.50 µg Indoxacarb 150 g/L EC/bee).

I. MATERIALS AND METHODS

A. MATERIALS

- | | |
|-----------------------------|--|
| 1. Test material: | Indoxacarb 150 g/L EC |
| Lot/Batch #: | KN128-150 |
| Purity: | 150 g a.s./L |
| CAS#: | None for the formulation |
| | 173584-44-6 for indoxacarb active substance |
| Description: | Liquid |
| Stability of test compound: | Not determined in the test system |
| 2. Control: | Tap water |
| Test vehicle: | Tap water |
| Toxic reference: | Dimethoate a.s. |
| 3. Test organism: | Honey bees |
| Species: | <i>Apis mellifera</i> |
| Age at dosing: | Adult female worker bees |
| Source: | Bee hives located at IBACON, Rossdorf, Germany |
| Diet: | 50% sucrose solution |
| Water: | See diet |
| Test chamber: | Stainless steel, 10 cm × 8.5 cm × 5.5 cm (l × w × h) |
| 4. Environmental conditions | |
| (In-life period) | |
| Temperature: | 25°C |
| Relative humidity: | 53-94% |
| Photoperiod: | Continuous dark |

B. STUDY DESIGN AND METHODS

1. Experimental start/completed
20-June-2006 to 30-June-2006
2. Experimental treatments
The acute 48-hour oral and contact toxicity of Indoxacarb 150 g/L EC was determined in honey bees (*Apis mellifera*). Treatments consisted of four toxic standard treatment rates, a dilution water control, and six nominal concentrations of Indoxacarb 150 g/L EC. Nominal test rates in the contact test were 0.016, 0.031, 0.06, 0.13, 0.25, and 0.50 µg a.s./bee. Nominal dosages in the oral test were 0.03, 0.06, 0.13, 0.25, 0.50, and 1.0 µg a.s./bee. The treatment rates based on measured dietary intake (actual consumption) were 0.033, 0.069, 0.13, 0.27, 0.54, and 1.09 µg a.s./bee. Five replicates per treatment and 10 honey bees per replicate (total 50 bees per treatment) were used for the test item concentration, control, and toxic reference. Dimethoate was the toxic standard used in these tests. In the oral test, bees were offered the test solutions in 50% aqueous sugar solution. In the contact test bees were dosed with Indoxacarb 150 g/L EC by topical application with a 1 µL drop to the dorsal thorax of each bee.
3. Observations
Assessments for mortalities and sublethal effects were carried out after 4 hours (first day); 24 and 48 hours (contact and oral test) and additionally 72 and 96 hours in the oral test after treatment. The rates producing the oral and contact LD₅₀ (rate resulting in 50% inhibitory response) were determined.
4. Statistics
The contact and oral LD₅₀ of the test item and the toxic standard were estimated with the moving average method.

II. RESULTS AND DISCUSSION

A. FINDINGS

Control mortality in the oral and contact test after 48 hours was 6.7 and 0%, respectively. The oral and contact toxicity of the toxic reference standard, dimethoate, to honey bees in these tests fell within the accepted range, indicating the validity of these tests.

Transient sublethal effects were observed in the two highest doses in the contact toxicity tests. Dose-dependent sublethal effects were observed throughout the oral test.

Actual test item intake in the oral tests and mortality results for the oral and contact tests at 4, 24, 72, and 96 hours (oral test) and 4, 24, and 48 hours (contact test) are reported in the following tables.

Table 82
Acute oral toxicity of Indoxacarb 150 g/L EC to honey bees

Nominal concentration (µg a.s./bee) ^a	Measured dietary intake (µg a.s./bee) ^b	Mean cumulative mortality (%) ^c				
		4 h	24 h	48 h	72 h	96 h
0	N/A	0	0	0	3.3	6.7
0.03	0.033	0	0	0	0.0	3.3
0.06	0.069	0	6.7	16.7	20.0	26.7
0.13	0.13	0	43.3	56.7	60.0	63.3
0.25	0.27	0	86.7	100	100.0	100.0
0.50	0.54	0	86.7	96.7	100.0	100.0
1.00	1.09	0	36.7	80.0	93.3	96.7

^a Treatments are specified as intended uptake, in mean µg a.s./bee per treatment rate.

^b Test item intake is specified as actual uptake, in mean µg a.s./bee per treatment rate.

^c Test mortality for treatments is corrected for control mortality (mortality at 0 µg a.s./bee).

Table 83
Acute contact toxicity of Indoxacarb 150 g/L EC to honey bees

Nominal concentration (µg a.s./bee) ^a	Mean cumulative mortality (%)		
	4 hours	24 hours	48 hours
0.0	0	0	0
0.016	0	0	0
0.031	0	0	3.3
0.06	0	0	10.0
0.13	0	70.0	100.0
0.25	0	100.0	100.0
0.50	0	100.0	100.0

^a Treatments are specified as mean µg a.s./bee per treatment rate, applied dorsally in 1 µL droplets

III. CONCLUSIONS

The 48- and 96-hour oral LD₅₀ for honey bees based on mortality and measured dietary intake were 0.11 and 0.10 µg a.s./bee (equivalent to 0.69 and 0.63 µg Indoxacarb 150 g/L EC/bee), respectively.

The 48-hour contact LD₅₀ for honey bees based on mortality and nominal concentrations was 0.08 µg a.s./bee (equivalent to 0.50 µg Indoxacarb 150 g/L EC).

(Schmitzer, S., 2006)

RMS comment

Study submitted to the EU for the first time in this submission.

This study was conducted in compliance with the current guideline. The 48- and 96-hour oral LD₅₀ for honey bees were 0.11 and 0.10 µg a.s./bee (equivalent to 0.69 and 0.63 µg Indoxacarb 150 g/L EC/bee), respectively. The 48-hour contact LD₅₀ for honey bees was 0.08 µg a.s./bee (equivalent to 0.50 µg Indoxacarb 150 g/L EC). This study is acceptable.

Report: Haupt, S., (2014); Indoxacarb (DPX-KN128) 150 g/L EC: Acute oral and contact toxicity to the bumblebee, *Bombus terrestris* L. (Hymenoptera)

DuPont Report No.: DuPont-38351

Guidelines: OECD 213 (1998), OECD 214 (1998) with modifications and adaptations **Deviations:** None

Testing Facility: IBACON, Rossdorf, Germany

Testing Facility Report No.: 83785105

GLP: Yes

Certifying Authority: Hessisches Ministerium für Umwelt, Energie, Landwirtschaft und Verbraucherschutz (Wiesbaden, Germany).

Executive summary:

The aim of this study was to determine the acute contact and oral toxicity of Indoxacarb 150 g/L EC to the bumblebee (*Bombus terrestris* L.) in a laboratory study. A contact test with 4.0, 2.0, 1.0, 0.5 and 0.25 µg indoxacarb/bee and an oral test with 0.8, 0.4, 0.2, 0.1 and 0.05 µg a.s./bee were conducted according to van der Steen (2001), and OECD 213/214 (1998), with modifications and adaptations, and current recommendations of the non-Apis ring test group (2014).

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Indoxacarb 150 g/L EC
 Lot/Batch #: DPX-KN128-311
 Purity: 150 g a.s./L
 Description: Liquid
 CAS#: None for the formulation, 173584-44-6 for the active substance)
 Stability of test compound: 98.2% of the indoxacarb remains in the delivery vehicle after one hour under agitation
 Test vehicle: Oral test: 50% w/v sucrose solution (500 g sucrose/L tap water);
 Contact test: tap water with 0.5% Tween80*
2. Control Vehicle without test item
- Reference item: Perfekthion (BAS 152 11 I)
3. Test organism: Worker bumblebees (Insecta, Hymenoptera)
 Species: Adult *Bombus terrestris* L.
 Stage and sex: Female worker bees
 Source: Bumblebee colonies, healthy and queen-right, obtained from a commercial bumblebee breeding company (Biobest Belgium N.V., Ilse Velden 18, 2260 Westerlo, Belgium) in a plastic box.
 Acclimatisation: Contact Test: 24 hours 15 minutes
 Oral Test: 26 hours 50 minutes
4. Test Units
 Type and size: Cylindrical, latticed plastic cages with a length of approximately 7 cm and a diameter of 2.2 cm at the large and 1.7 cm at the small opening.
 The bees were kept in the above mentioned test units. The contact application was conducted outside of the test unit. The test units were laid on a plate, the small opening was closed by a rubber plug holding a syringe which contained the feeding solution. The large opening was closed by a lid.
 No. of Individuals: 1 per test unit
 Replicates: 30 per treatment group/control
5. Environmental conditions
 Temperature: Acclimatisation: 23–27°C
 Exposure: 23–27°C
 Relative humidity: Acclimatisation: 55–64%
 Exposure: 54–64%

B. STUDY DESIGN AND METHODS

1. Application information

Application in the Oral Test:

The test item and the reference item were dissolved in 50% w/v sucrose solution which was used as carrier (food) in the oral test. For the untreated control pure 50% w/v sucrose solution was used.

The treated food was offered in syringes, which were weighed before and after introduction into the cages. After a maximum of 2 hours 30 minutes, uptake of the test item treated food was complete and the syringes containing the treated food were removed, weighed and replaced by ones containing fresh, untreated food.

The mean target dose levels (e.g. 0.8 µg a.s./bee) would have been obtained if exactly 40 mg/bee of the treated food were ingested. In practice, uptake of the treated sugar solutions differed slightly from 40 mg/bee and results are given based on the measured consumption.

Application in the contact test:

One single 5 µL droplet of Indoxacarb 150 g/L EC in an appropriate carrier (tap water with 0.5% Tween®80) was placed on the dorsal bee thorax using a pipette.

For the control one 5 µL droplet of tap water containing 0.5% Tween®80 was used. The reference item was also applied in 5 µL tap water (dimethoate made up in tap water containing 0.5% Tween®80). The Tween®80 was used to improve the adhesion of the droplet on the bee body. Tween80 is non-toxic to bumblebees.

2. Statistic

Results, obtained from the bees treated with the test item and the reference item, were compared to those obtained from the control in both the contact and oral tests.

The contact and oral LD₅₀ values of the test item were estimated with Logit Analysis using linear maximum likelihood regression.

The contact and oral LD₅₀ values of the reference item were estimated with Probit Analysis (according to Finney 1971).

If necessary, the LD₅₀ calculation was carried out taking into account the mortality data corrected by control mortality using Abbott's formula (1925).

The software used to perform the statistical analysis was ToxRat Professional, Version 2.10.05, ToxRat Solutions GmbH

II. RESULTS AND DISCUSSION

All study validity criteria were achieved.

In the contact test, 30 worker bees per treatment group were exposed to doses of 4.0, 2.0, 1.0, 0.5 and 0.25 µg a.s./bee by dorsal application of a 5 µL droplet (test item in tap water containing 0.5% Tween®80) on the bumblebee thorax. At test end (96 hours after application) there was 100% mortality in the two highest dosing groups (4.0 and 2.0 µg a.s./bee) and 96.7% in the 1.0 µg a.s./bee group. 76.7 and 36.7% mortality occurred at 0.5 µg a.s./bee and 0.25 µg a.s./bee, respectively and 3.3% mortality occurred in the control group (tap water containing 0.5% Tween®80). From 24 hours onwards behavioural abnormalities (e.g. bees were affected (bees still upright and attempting to walk but showing signs of reduced coordination) or moribund) in a few of the surviving bumblebees were observed among the test item dose groups. These behavioural abnormalities were increasing with dose levels and decreasing with time. In the lowest dosing group (0.25 µg a.s./bee) behavioural abnormalities only occurred during the last assessment (96 hours).

In the oral test the target dose levels of 0.8, 0.4, 0.2, 0.1 and 0.05 µg a.s./bee would have been achieved if exactly 40 mg treated feeding solution were consumed per bumblebee. Actually, the uptake differed slightly and corresponded to 0.812, 0.404, 0.214, 0.114 and 0.0548 µg a.s./bee. At test end (96 hours after application) there was 100% mortality in the 0.812 µg a.s./bee dosing group, 80% mortality occurred at a dose level of 0.404 µg a.s./bee, and 96.7% in the 0.214 µg a.s./bee treatment group had died. At 0.114 µg a.s./bee, 66.7% mortality occurred, 3.3% mortality was observed at 0.0548 µg a.s./bee. There was no mortality in the control group (50% w/v sucrose solution). Behavioural abnormalities (e.g. bees were affected or moribund) were observed in the 0.812, 0.404, 0.214 and 0.114 µg a.s./bee dose levels during the 24- and 48-hour assessments. During the 72- and 96-hour assessment these behavioural impairments were exclusively observed in the 0.214 and 0.114 µg a.s./bee dosing groups.

No behavioural abnormalities occurred in the 0.0548 µg a.s./bee dose level.

Mortality of the bumblebees treated with reference item (dimethoate, 400 g/L EC) was 93.3% in the contact test (8 µg a.s./bee) and 100% in the oral test (4.09 µg a.s./bee) at test end (96 hours after application), respectively. The results are summarized in the Table 84 that follows.

Table 84
Toxicity of Indoxacarb 150 g/L EC to bumblebees (*Bombus terrestris* L.) in an acute contact and oral toxicity test

Test item	Indoxacarb 150 g/L EC	
Test object	<i>Bombus terrestris</i> L.	
Exposure	contact (solution in water + 0.5% Tween®80)	oral (50% w/v sucrose solution)
LD ₅₀ [µg a.s./bee]	0.32 (96 h)	0.11 (96 h)

III. CONCLUSIONS

The effects of Indoxacarb 150 g/L EC on the bumblebee (*Bombus terrestris* L.) were assessed in an acute contact and oral toxicity test, conducted in the laboratory. The contact LD₅₀ (96 h) based on measured concentration was 0.32 µg a.s./bee. The oral LD₅₀ (96 h) based on measured concentration was 0.11 µg a.s./bee.

(Haupt, S., 2014)

RMS comment

Study submitted to the EU for the first time in this submission.

Tables of results were added by RMS for clarity:

Mortality and behavioural abnormalities of the bumblebees in the contact toxicity test

Treatment group	After 4 hours		After 24 hours		After 48 hours		After 72 hours		After 96 hours	
	Mortality Mean %	Beh. Abnormal. Mean %	Mortality Mean %	Beh. Abnormal. Mean %	Mortality Mean %	Beh. Abnormal. Mean %	Mortality Mean %	Beh. Abnormal. Mean %	Mortality Mean %	Beh. Abnormal. Mean %
4.0 µg/bee	0.0	0.0	16.7	80.0	96.7	3.3	96.7	3.3	100.0	0.0
2.0 µg/bee	0.0	0.0	0.0	63.3	83.3	16.7	100.0	0.0	100.0	0.0
1.0 µg/bee	0.0	0.0	0.0	46.7	63.3	30.0	93.3	6.7	96.7	0.0
0.5 µg/bee	0.0	0.0	0.0	0.0	10.0	26.7	46.7	33.3	76.7	20.0
0.25 µg/bee	0.0	0.0	0.0	0.0	10.0	0.0	26.7	0.0	36.7	30.0
control	0.0	0.0	3.3	0.0	3.3	0.0	3.3	0.0	3.3	0.0
Ref: 8 µg dimethoate/bee	23.3	76.7	76.7	16.7	86.7	0.0	90.0	0.0	93.3	0.0

Mortality and behavioural abnormalities of the bumblebees in the oral toxicity test

Treatment group	After 4 hours		After 24 hours		After 48 hours		After 72 hours		After 96 hours	
	Mortality Mean %	Beh. Abnormal. Mean %	Mortality Mean %	Beh. Abnormal. Mean %	Mortality Mean %	Beh. Abnormal. Mean %	Mortality Mean %	Beh. Abnormal. Mean %	Mortality Mean %	Beh. Abnormal. Mean %

0.812 µg/bee	3.3	0.0	90.0	10.0	96.7	3.3	100.0	0.0	100.0	0.0
0.404 µg/bee	0.0	0.0	16.7	50.0	73.3	3.3	73.3	0.0	80.0	0.0
0.214µg/bee	0.0	0.0	0.0	10.0	53.3	36.7	90.0	6.7	96.7	3.3
0.114µg/bee	0.0	0.0	0.0	3.3	10.0	50.0	36.7	43.3	66.7	20.0
0.0548 µg/bee	3.3	0.0	3.3	0.0	3.3	0.0	3.3	0.0	3.3	0.0
control	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ref: 4 µg dimethoate/bee	36.7	63.3	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0

There is currently no guideline to test the acute toxicity on bumble. The test was based on guidelines available for bees and adapted. It is valid according to validity criteria of OECD 213 (1998), OECD 214 (1998). The contact LD₅₀ (96 h) based on measured concentration was 0.32 µg a.s./bumble bee. The oral LD₅₀ (96 h) based on measured concentration was 0.11 µg a.s./bumble bee. This study is acceptable.

Report: Kling, A. (2014); Indoxacarb 150 g/L EC: Assessment of chronic effects to the honeybee, *Apis mellifera* L., in a 10 days continuous laboratory feeding test

DuPont Report No.: DuPont-36492

Guidelines: None cited **Deviations:** None

Testing Facility: Eurofins Agrosience Services EcoChem GmbH, Niefern-Öschelbronn, Germany

Testing Facility Report No.: S13-01457

GLP: Yes

Certifying Authority: Landesanstalt Fur Umwelt, Messungen Und Naturschutz Baden-Wurttemberg

Executive summary:

Chronic effects of the test item Indoxacarb 150 g/L EC on the honey bee, *Apis mellifera* L., were assessed in a 10-day laboratory feeding test. Honey bees were fed *ad libitum* with a 50% (w/v) aqueous sucrose feeding solution containing Indoxacarb 150 g/L EC at the concentration levels of 188, 375, 750, 1500 and 3000 µg a.s./kg.

The control group was exposed for the same period of time under identical exposure conditions to untreated 50% (w/v) aqueous sucrose feeding solution. Assessments of mortality, sub-lethal effects or behavioural differences were carried out daily during the 10-days test period. Furthermore, the daily food uptake was determined.

In the control group there was no mortality at the final assessment after 10 days.

In the test item group mortality of 0.0, 7.5, 37.5, 82.5 and 100.0% was observed at the test item concentration levels of 188, 375, 750, 1500 and 3000 µg a.s./kg at the end of the test. The concentration level of 188 µg a.s./kg was determined to be the NOEC (No Observed Effect Concentration) based on mortality. The mortality at all other test item levels was statistically significantly higher compared to the control group. The concentration level of 375 µg a.s./kg was therefore determined to be the LOEC (Lowest Observed Effect Concentration).

There were no remarkable sub-lethal effects recorded at the lowest treatment level of 188 µg a.s./kg. In the treatment group of 375 µg a.s./kg, few affected bees were observed on all assessments from assessment E6 (assessment Day 6) on. A larger number of affected or apathetic bees were observed on most assessments at the concentration level of 750 µg a.s./kg, tending to increase with time of exposure. Several affected bees were

observed at the concentration level of 1500 µg a.s./kg, also increasing with increasing time of exposure. At the highest treatment level many bees were recorded as affected, until all of them had died at assessment E4.

The daily mean food consumption of aqueous sucrose feeding solution in the test item groups were not statistically significantly lower compared to the control group (day-by-day comparison) in all test item groups throughout the entire test period. These observations do not indicate a repellent effect of the test item at any tested concentration level.

Also the overall daily mean food consumption (*i.e.* the average value per replicate over 10 days) was not statistically significantly lower in any test item treatment group compared to the control group.

The 10-day LC₅₀ was determined to be 884 µg a.s./kg (with the 95% confidence limits of 765 to 1049 µg a.s./kg).

The 10-day LD₅₀ based on the test item consumption per bee per day was determined to be 39.9 ng a.s./bee/day (with the 95% confidence limits of 33.6 to 48.2 ng a.s./bee/day).

I. MATERIALS AND METHODS

A. MATERIALS

- | | |
|---|---|
| 1. Test material: | Indoxacarb 150 g/L EC |
| Lot/Batch#: | KN128-311 |
| Purity: | 150 g a.s./L |
| Description: | liquid/light amber |
| CAS #: | None for the formulation
173584-44-6 for the active substance (indoxacarb) |
| Stability in solution: | Sufficient for test purpose |
| 2. Control: | 50% (w/v) aqueous sucrose feeding solution |
| Test vehicle | Tap water |
| 3. Test organism: | Honey bees |
| Species: | <i>Apis mellifera</i> L. |
| Age at dosing: | Young adult worker bees (newly hatched; 1 to 4 days old) |
| Source: | Beekeeper Mr. Wolters, Im Bannen 38-54,
56727 Mayen, Germany |
| Test chamber: | Stainless steel cages, base: 8 x 4 cm, height: 6 cm |
| 4. Environmental conditions (In-life phase) | |
| Temperature: | 31.5–33.2°C |
| Relative humidity: | 53.4–70.1% |
| Photoperiod: | Continuous dark, except during the assessments |

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
27-August-2013 to 06-September-2013
2. Experimental treatments
One control and five test item concentrations of 188, 375, 750, 1500 and 3000 µg a.s./kg were tested. Four replicates per concentration level and 10 replicates in the control group each with 10 honey bees per replicate were used. The feeding solutions were offered to the bees continuously and *ad libitum* over a test period of 10 days.
3. Observations
Assessments of mortality, sub-lethal effects or behavioural differences were carried out daily during the 10-days test period. Furthermore, the daily food uptake was determined.

4. Statistics

The LC₅₀ and LD₅₀ values with 95% confidence intervals of the test item group were calculated by means of a probit analysis using the statistic, SAS[®] Proprietary Software 9.3, (Ed. 2002-2010).

Fisher's Exact Test (Bonferroni-Holms corrected, right-sided, $\alpha \leq 0.05$) was used to evaluate whether there are significant differences between the mortality data of the test item treatment group and the control group and to determine the NOEC value.

For the statistical comparison of the food consumption, non-rounded values were taken. Data were analysed for normality (Shapiro Wilks test) and homoscedasticity (Bartlett test when Shapiro Wilks test $\alpha > 0.2$, Levene test when Shapiro Wilks test $\alpha \leq 0.2$). Data of food consumption were statistically analysed by using the Welch t-Test (Bonferroni-Holms corrected, left sided; $\alpha \leq 0.05$).

II. RESULTS AND DISCUSSION

A. FINDINGS

In the control group the mortality was 0.0% at the final assessment after 10 days. Consequently, validity criterion for the test was met and the test was deemed valid.

In the test item group mortality of 0.0, 7.5, 37.5, 82.5 and 100.0% was observed at the test item concentration levels of 188, 375, 750, 1500 and 3000 µg a.s./kg at the end of the test. The concentration level of 188 µg a.s./kg was determined to be the NOEC based on mortality. The concentration level of 375 µg a.s./kg was determined to be the LOEC.

Actual results of mortality, corrected mortality and food consumption, as well as a summary of the calculated values are given in the table below.

Table 85
Summary of chronic effects of Indoxacarb 150 g/L EC after continuous feeding on honey bees

Treatment	Control	Indoxacarb 150 g/L EC [µg a.s./kg]				
		188	375	750	1500	3000
Cumulative mortality [%]	0.0	0.0	7.5 ^d	37.5 ^d	82.5 ^d	100.0 ^d
Overall mean daily consumption of aqueous sucrose feeding solution per replicate [mg/bee] ^a	44.4	41.3	41.6	43.9	50.1	53.0
Mean intake accumulated over test days [ng a.s./bee]	-	78.4	157	334	744	642
Mean intake per bee per day [ng a.s./bee/day]	-	7.84	15.7	33.4	74.4	64.2
LC ₅₀ (95% confidence limits)	884 µg a.s./kg (765 to 1049 µg a.s./kg)					
LD ₅₀ (95% confidence limits)	39.9 ng a.s./bee/day (33.6 to 48.2 ng a.s./bee/day)					
NOEC ^b	188 µg a.s./kg (equivalent to 7.84 ng a.s./bee/day)					
LOEC ^c	375 µg a.s./kg (equivalent to 15.7 ng a.s./bee/day)					

^a The mean values per replicate over the test period (non-rounded values) were used as basis for the calculation of the overall mean daily consumption of the aqueous sucrose feeding solution per treatment over the test period

^b Determined to be the NOEC based on mortality (not statistically significantly different compared to the control; Fischer's Exact Test (Bonferroni-Holms corrected; right sided; $\alpha \leq 0.05$))

^c Determined to be the LOEC based on mortality (statistically significantly different compared to the control; Fischer's Exact Test (Bonferroni-Holms corrected; right sided; $\alpha \leq 0.05$))

^d Statistically significantly different compared to the control; Fischer's Exact Test (Bonferroni-Holms corrected; one side; $\alpha = 0.05$)

III. CONCLUSIONS

The effects of Indoxacarb 150 g/L EC was assessed in a 10-day oral laboratory toxicity test.

The 10-day LC_{50} was determined to be 884 $\mu\text{g a.s./kg}$ (with the 95% confidence limits of 765 to 1049 $\mu\text{g a.s./kg}$).

The 10-day LD_{50} – based on the test item consumption per bee per day – was determined to be 39.9 ng a.s./bee/day (with the 95% confidence limits of 33.6 to 48.2 ng a.s./bee/day).

(Kling A., 2014)

RMS comment

Study submitted to the EU for the first time in this submission.

RMS notes that no toxic reference was used.

This study was completed before the honey bee chronic adult ring test was officially started, but follows Kling, A. & Schmitzer, S. (2015): Proposal for a new OECD guideline for the testing of chemicals on adult honey bees (*Apis mellifera* L.) in a 10 day chronic feeding test in the laboratory and results of the recent ring test 2014. Hazards of pesticides to bees - 12th International Symposium of the ICP-PR Bee Protection Group, Ghent (Belgium), 15-17 September 2014. Julius-Kühn-Archiv, 450, pp. 69-74. Mean mortality in control was $\leq 15\%$ at the end of the test fulfilling the validity criteria. The 10-day LC_{50} was determined to be 884 $\mu\text{g a.s./kg}$. The 10-day LD_{50} was determined to be 39.9 ng a.s./bee/day.

Report: Kleinhenz, M. (2014); Indoxacarb (DPX-KN128) 150 g/L EC: A feeding study to evaluate effects on the brood of honey bees (*Apis mellifera*; Hymenoptera, apidae) in Germany 2013

DuPont Report No.: DuPont-37488

Guidelines: OEPP/EPPO Bulletin No. 22 (OOMEN et al., 1992), OECD 75 (2007) **Deviations:** None

Testing Facility: Eurofins Agrosience Services EcoChem GmbH, Niefern-Öschelbronn, Germany

Testing Facility Report No.: S13-00882

GLP: Yes

Certifying Authority: Landesanstalt Für Umwelt, Messungen Und Naturschutz Baden-Württemberg

Executive summary:

The effects of the test item Indoxacarb 150 g/L EC were tested on the honey bee (*Apis mellifera* L.) under field conditions in Germany, following Oomen *et al.* (1992) and OECD guidance document No. 75, (2007) with partly integration of the recommendations by EFSA (2012). This study was conducted in Niefern-Öschelbronn in Southern Germany (region: Baden-Württemberg) from May to July 2013 and included a total of three treatment groups:

Indoxacarb 150 g/L EC treatment group T with the daily application of a feeding solution (200 mL 50% sucrose solution per hive per day) at a concentration of 100 μg indoxacarb/kg over a period of 9 days.

Reference item treatment group R with the daily application of a feeding solution (200 mL 50% sucrose solution per hive per day) at rate of 0.167 g Insegar 25WG (a.s. fenoxycarb)/hive/day over a period of 9 days.

Control group C with the daily application of a pure 200 mL 50% sucrose solution per hive per day over a period of 9 days.

Feeding was done over a period of 9 days from 11 June 2013 (0DAF; DAF = days after first feeding) to 19 June 2013 (8DAF). The first application of the feeding solution was performed after the first photographic assessment of the brood combs (BFD0; BFD = brood area fixing day) on the same day. Feeding was done once per day to allow the bees to take up the feeding solution in the meantime. To avoid contamination, feeding was first done in hives of the control group C, followed by those from test item treatment group T and then from treatment group R. Fresh feeding solution was prepared each day shortly before feeding.

The effects of the test item treatments were examined on small colonies placed in an area with no flowering main crops. The influence of the 9 test item feedings of Indoxacarb 150 g/L EC was evaluated by comparing the results in the test item treatment to the data in the control treatment as well as in the reference item treatment regarding the following observations:

Mortality: number of dead bees in the dead bee traps in front of the hives,

Condition of the colonies and amount of brood,

Detailed observation of the brood development in >600 selected cells (>200 cells containing eggs, >200 cells containing young larvae, and >200 cells containing old larvae at the first assessment (BFD0), shortly before the first feeding),

Behaviour of the honey bees in front of the hives.

I. MATERIAL AND METHODS

A. MATERIALS:

- | | |
|--|--|
| 1. Test material: | Indoxacarb 150 g/L EC |
| Lot/Batch#: | DPX-KN128-311 |
| Purity: | 150 g a.s./L |
| Description: | Liquid |
| CAS#: | None for the formulation
173584-44-6 for the active substance |
| Stability of test compound: | 98.2% of the indoxacarb remains in the delivery vehicle after one hour under agitation |
| Reference item: | Insegar 25WG |
| Batch: | SMO2K433 |
| Content of a.s., nominal: | 25.0% (w/w) |
| Description: | Insegar 25 WG (fenoxycarb) |
| CAS#: | 72490-01-8 |
| Stability in solution: | Sufficient for test purpose (at least 1 hour) |
| 2. Vehicle and/or control: | 50% sucrose solution |
| 3. Test organism | |
| Species: | <i>Apis mellifera</i> L. |
| Age at dosing: | Direct exposure of adult honey bees through feeding, indirect exposure of all stages of development |
| Source: | Eurofins Agrosience Services EcoChem GmbH |
| Diet: | Honey bees are freely flying, feeding of the test item, reference item and control as a 50% sucrose solution |
| 4. Environmental conditions during the exposure period (07 June – 09 July 2013): | |
| Temperature (min/max): | 7.7–34.5°C |
| Relative Humidity: | 32.0–100.0% |
| Photoperiod (exposure): | natural light conditions |

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed

27-May-2013 to 09-July-2013

2. Experimental treatments

Indoxacarb 150 g/L EC treatment group T with the daily application of a feeding solution (200 mL 50% sucrose solution per hive per day) at a concentration of 100 µg indoxacarb/kg over a period of 9 days.

Reference item treatment group R with the daily application of a feeding solution (200 mL 50% sucrose solution per hive per day) at rate of 0.167 g Insegar 25WG (a.s. fenoxycarb)/hive/day over a period of 9 days.

Control group C with the daily application of a pure 200 mL 50% sucrose solution per hive and day over a period of 9 days.

3. Observations

The influence of the 9 test item feedings of Indoxacarb 150 g/L EC was evaluated by comparing the results in the test item treatment to the data in the control treatment as well as in the reference item treatment regarding the following observations:

Mortality: number of dead bees in the dead bee traps in front of the hives,

Condition of the colonies and amount of brood,

Detailed observation of the brood development in >600 selected cells (>200 eggs, >200 young larvae, and >200 old larvae),

Behaviour of the honey bees in front of the hives.

II. RESULTS AND DISCUSSION

A. MORTALITY

Mortality after installation of the colonies until the first feeding of the sucrose test solution (4DBF to 0DBF; DBF = days before first application of the feeding solution) was comparable in all three treatment groups: mean daily mortality was in the range from 10.8 to 23.3 dead honey bees/day in the control, from 14.0 to 26.3 dead honey bees/day in T and from 8.8 to 21.8 dead honey bees/day in R. Mean daily mortality over the whole period (4DBF to 0DBF) was 17.2 dead honey bees/day in the control, 19.4 dead honey bees/day in T and 13.8 dead honey bees in R. There were no significant differences between the three treatment groups (Tukey-test; $p \leq 0.05$) in the period before the first application of the feeding solution.

During the period of daily feeding of the sucrose test solution (0DAF to 8DAF, corresponding to subsequent mortality assessments on 1DAF to 9DAF; DAF = days after first application of the feeding solution) the daily mortality values were in the range from 7.8 to 42.8 dead honey bees/day in the control, 9.3 to 41.8 dead honey bees/day in T and 12.8 to 41.8 dead honey bees/day in R. The mean daily mortality during this period was 20.5 dead honey bees/day in the control, 20.0 dead honey bees/day in T and 24.9 dead honey bees/day in R. There was no statistically significant difference between the treatment groups T or R compared to the control (pooled t-Test or Satterthwaite t-Test, $p \leq 0.05$).

During further observation of the colonies after the end of the feeding period (10DAF to 28DAF) daily mortality was in the range from 7.5 to 39.3 dead honey bees/day in the control, from 6.5 to 40.8 dead honey bees/day in T and from 21.3 to 603.0 dead honey bees/day in R. Mortality in the test item treatment group T was not significantly different from the control on any of these days. Mortality in the treatment group R was significantly different from the control on most days of the period from 11DAF to 26DAF (pooled

t-Test or Satterthwaite t-Test, $p \leq 0.05$) with few exceptions (12DAF, 19DAF and 23DAF). Increased mortality in R during this period resulted mainly from large numbers of pupae that died and were removed from the combs, an effect that could be expected from this reference item.

The mean daily mortality over the entire period after the applications of the feeding solution (10DAF to 28DAF) was 20.7 dead honey bees/day in the control, 22.6 dead honey bees/day in T (not significantly different from the control; pooled t-test or Satterthwaite t-test, $p \leq 0.05$) and 160.6 dead honey bees/day in R (significantly different from the control; pooled t-test or Satterthwaite t-test, $p \leq 0.05$).

During the period from installation of the hive to the first feeding (4DBF to 0 DBF), the mean total number of dead pupae, dead young bees, malformed bees and pupae was similar in the three treatments: there were 20.8 dead bees in the control, C, 15.5 dead bees in T and 10.0 dead bees in the reference item treatment group R.

Table 86
Mean mortality (mean number of dead honey bees, larvae and pupae) per day and treatment group

Date	Timing	C	T Indoxacarb 150 g/L EC	R (Insegar/ Fenoxycarb)
07 Jun 2013	4DBF	23.3	26.3	21.8
08 Jun 2013	3DBF	19.5	21.0	15.0
09 Jun 2013	2DBF	17.3	14.0	9.8
10 Jun 2013	1DBF	15.3	16.8	13.8
11 Jun 2013 ^a	0DBF	10.8	18.8	8.8
Mean (pre-application period, 4DBF to 0DBF)		17.2	19.4	13.8
STD		13.6	11.4	5.6
12 Jun 2013	1DAF ^b	42.8	41.8	41.8
13 Jun 2013	2DAF	23.3	18.0	26.5
14 Jun 2013	3DAF	15.0	11.0	15.3
15 Jun 2013	4DAF	25.5	23.0	31.8
16 Jun 2013	5DAF ^b	24.5	15.3	31.0
17 Jun 2013	6DAF	23.0	18.8	26.8
18 Jun 2013	7DAF	10.0	25.0	12.8
19 Jun 2013 ^c	8DAF ^c	7.8	17.5	16.3
20 Jun 2013	9DAF	12.3	9.3	21.5
Mean (application period, 1DAF to 9DAF)		20.5	20.0	24.9
STD		14.6	15.1	12.5
21 Jun 2013	10DAF	9.0	11.0	21.3
22 Jun 2013	11DAF ^b	19.0	18.5	58.5*
23 Jun 2013	12DAF	15.3	28.8	90.8
24 Jun 2013	13DAF	7.5	12.8	81.8*
25 Jun 2013	14DAF	11.8	6.5	82.0*
26 Jun 2013	15DAF	12.5	14.5	61.3*
27 Jun 2013	16DAF ^b	36.5	33.3	229.3*
28 Jun 2013	17DAF	20.3	21.5	125.8*
29 Jun 2013	18DAF	32.5	32.0	324.0*
30 Jun 2013	19DAF	30.0	22.5	123.3
01 Jul 2013	20DAF	39.3	39.0	502.3*
02 Jul 2013	21DAF	17.8	24.8	603.0*
03 Jul 2013	22DAF	15.5	22.0	277.5*
04 Jul 2013	23DAF	26.3	22.5	109.0
05 Jul 2013	24DAF ^b	30.5	40.8	136.0*
06 Jul 2013	25DAF	14.8	25.0	77.8*
07 Jul 2013	26DAF	9.8	21.0	59.3*
08 Jul 2013	27DAF	22.5	16.5	53.5
09 Jul 2013	28DAF	22.3	17.0	35.8
Mean (post-application, 10DAF to 28DAF)		20.7	22.6	160.6*
STD		15.5	6.3	111.5

DBF = Days before first application of feeding solution

DAF = Days after first application of feeding solution (0DAF = first day of feeding)

STD = Standard deviation

^a Feeding started in the evening of 11 Jun 2013, after the mortality assessment on that day (= 0DAF)

^b Potential higher mortality due to a colony assessment that was carried out in all treatments the day before

^c Last feeding was done on 18 Jun 2013, after the mortality assessment on that day

* Significantly different from the control (pooled t-Test or Satterthwaite t-Test, $p \leq 0.05$)

During the whole feeding period of the sucrose test solutions (1DAF to 9DAF) the mean total number of dead pupae, dead young bees and dead malformed pupae and bees was similar in all three treatments (12.0 dead bees in the control, 3.8 dead bees in T and 10.0 dead bees in R).

During the whole period after start of feeding (1DAF to 28DAF) there were 25.5 dead bees in the control and 22.8 dead bees in T. In R there were 2648.5 dead bees and pupae during this period. This increase pupal mortality in R and the presence of dead bees and pupae with sickle shadowed eyes are typical for this kind of reference item, confirming that the study design was suitable to ensure exposure of the honey bees and their brood to the sucrose test solution.

Overall, there was no impact of the Indoxacarb 150 g/L EC treatments on honey bee adult mortality, pupal mortality or malformations, while there was a clear impact on pupal mortality and occurrence of malformations in the reference item treatment R.

B. BEHAVIOUR OF THE HONEY BEES

No unusual behaviour was recorded in all treatment groups before the first feeding of the sucrose test solution (4DBF to 0DBF).

On the following days (starting on 1DAF) few bees in the control occasionally displayed other than normal behaviour. There were mainly inactive (motionless) bees, in few cases there were bees with locomotion problems, two cases of cramping and 1 trembling bee. This may be due to *e.g.* guard bees stinging foreign bees that occasionally enter the hive through the entrance or try to scavenge the open and easily accessible hives when the hive covers are removed during the colony assessments and photo sessions that are part of this study.

Similar observations were made in the test item treatment T. There were mainly inactive bees, and in few cases also bees with locomotion problems, cramping or trembling bees were noted. Intensive self-grooming was observed twice and aggressiveness to the observer occurred in one hive on one day (3DAF). Since the number of bees showing these symptoms was small (in most cases only 1-2 bees in 1 or 2 of the four hives) and similar observations were made in the control, these symptoms are considered of no biological relevance.

Similar observations as in the control and T, though in slightly more bees, were seen in the reference item treatment R.

Overall, there was no negative impact of the Indoxacarb 150 g/L EC treatments on honey bee behaviour having any biological relevance.

C. BROOD DEVELOPMENT AND COLONY CONDITION

Colony Size:

At the first assessment (0DBF) before the applications of the feeding solutions started, all colonies were in a good and healthy condition with brood of all stages and food (nectar and pollen) present in all hives except hives Cb, Tb, Tc, and Rc where no significant amounts of pollen were found.

At the first assessment (0DBF) the mean colony sizes were similar in all three treatments. There were 13547 honey bees/hive in C, 12891 honey bees/hive in T and 13453 honey bees/hive in R.

During the study, the development of the colony sizes in C and T were similar and no negative influence of the test item could be discerned. In the control there was a slight growth of the colonies (15422 honey bees/hive at the last assessment on 28DAF) whereas in T the colony size remained rather stable (13000 honey bees/hive on 28DAF) with only slight fluctuations observed during the previous assessments. However, these slight differences are not considered to be due to the treatment and they are not considered to be of any biological relevance.

In the reference item treatment R, the colony sizes remained on a rather stable level with only a slight decline until 15DAF (12000 honey bees/hive) but then clearly decreased to only 7485 honey bees/hive on 28DAF, mainly due to the high rate of failing brood, which implies a low hatch rate of new worker bees. Additionally, after swarming of hive Ra on 2DAF there were only small numbers of eggs present at the next assessment (4DAF) and there was no young brood (eggs, larvae) on 10DAF and later, indicating that the new queen was not mated successfully, or she had died. However, the small mean colony size in R was not due to swarming and subsequent queen failure in Ra, since this hive had been strong at all assessment dates.

Overall, there was no negative effect of the Indoxacarb150 g/L EC treatment on the size of the colonies (mean number of honey bees per hive) while there was a clear impact in the reference item treatment R.

D. BROOD DEVELOPMENT

Brood of all stages (eggs, larvae, capped brood) was present in all colonies at all assessments during the study in the control group C and in the test item treatment group T. The mean total amount of brood cells was in the range from 24600 to 30600 brood cells/hive in the control and from 26500 to 32100 brood cells/hive in T. There was no negative impact of the treatment on the amount of brood.

In the reference item treatment group R, the amount of brood cells decreased from 28400 brood cells/hive on 0DBF to 9050 and 9700 brood cells/hive on 23DAF and 28DAF. This decline was mainly due to the removal of brood and a small number of larvae in Rb, an intermittent lack of larvae in Rc on 15DAF, a lack of certain brood stages in Rd from 15DAF to 23DAF and the end of brood rearing after swarming in hive Ra. Thus, a clear effect of the reference item was observed, confirming that the study design was suitable to ensure exposure of the honey bees and their brood to the fed substances.

Overall, there was no effect of the Indoxacarb 150 g/L EC treatments on the amount of honey bee brood while there was a clear impact in the reference item treatment R.

E. DETAILED ASSESSMENT OF BROOD CELL CONTENTS

Development of Honey Bee Eggs in Individual Cells:

According to the development time of a worker honey bee from egg to adult bee which normally averages approx. 21 days, it can be assumed that all honey bees from those cells that were initially (BFD0) marked as cells containing eggs hatch until the assessment date BFD+23. Thus, the study period covered one complete development cycle of the observed brood cells.

In the control C and in the test item treatment group T, successful development was observed in the majority of the marked cells that contained eggs at the assessment at BFD0, before application of the feeding solution started. Brood indices and compensation indices were rising throughout the entire assessment period except for the assessment on BFD+15 where the brood indices and compensation indices remained on almost the same level in comparison to the previous assessment on BFD+10. This is not unusual because a normal developing bee is expected to be in the same stage (pupa) at both assessment dates, and therefore no increase of the indices is expected.

The brood index values of the test item treatment were similar and comparable to the control. At the last assessment (BFD+23), the mean brood indices were 4.25 in the control and 4.24 in T. The slight differences of the test item treatment to the control were not statistically significant at any assessment (pooled t-test or Satterthwaite t-test, $p \leq 0.05$).

In the reference item treatment group R, the brood indices were lower than the corresponding control values at all assessments (significant on BFD+4, BFD+15 and BFD+23; pooled t-test or Satterthwaite t-test, $p \leq 0.05$), with a mean brood index of 1.98 at the assessment on BFD+23, indicating a high proportion of failing brood.

Also the compensation indices in the test item treatment were similar and comparable to the control. At the last assessment (BFD+23) the mean compensation indices were 4.57 for the control and 4.50 for the

treatment group T. There was no statistically significant difference between the control and T (pooled t-test or Satterthwaite t-test, $p \leq 0.05$) at any of the assessments. In the reference item treatment group R, the compensation indices were low throughout the observation period, resulting in a mean of 2.14 at the last assessment (BFD+23). Differences between the reference item treatment group and the control were statistically significant at BFD+4, BFD+15 and BFD+23 (pooled t-test or Satterthwaite t-test, $p \leq 0.05$).

Table 87
Brood/compensation indices at +4, +10, +15 and +23 days after brood area fixing day (BFD0) for marked cells
(mean values of 4 replicates per treatment)

Treatment Group	BFD0	BFD+4	BFD+10	BFD+15	BFD+23
Marked cells containing eggs (index value = 1) at the first assessment (BFD0):					
Mean C	1.00/1.00	2.45/2.46	3.69/3.75	3.64/3.76	4.25/4.57
Mean T	1.00/1.00	2.29/2.30	3.48/3.61	3.42/3.62	4.24/4.50
Mean R	1.00/1.00	1.84*/1.90*	2.30/2.41	1.64*/1.72*	1.98*/2.14*
Marked cells containing young larvae (index value = 2) at the first assessment (BFD0):					
Mean C	2.00/2.00	3.40/3.40	3.75/3.78	3.88/3.94	4.68/4.35
Mean T	2.00/2.00	3.10/3.16	3.51/3.70	3.62/3.84	4.39/4.32
Mean R	2.00/2.00	3.08/3.14	2.76*/2.78*	3.19/3.23	3.43/1.55*
Marked cells containing old larvae (index value = 3) at the first assessment (BFD0):					
Mean C	3.00/3.00	3.93/3.94	3.86/3.89	4.73/4.86	n.a.
Mean T	3.00/3.00	3.81/3.86	3.76/3.86	4.69/4.84	n.a.
Mean R	3.00/3.00	3.80/3.82	3.09*/3.10*	3.54*/3.81*	n.a.

BFD0= Brood area fixing day

* significantly different from the control (pooled t-test or Satterthwaite t-test, $p \leq 0.05$)

n.a.= not applicable (development cycle of old larvae completed before BFD+23)

The termination rates for the treatment group T were similar and comparable to the control at all assessment dates, indicating a high rate of successful hatching of the developed brood. At the assessment on BFD+23 (end of the natural development cycle) the mean termination rates were 15.03 in the control, 15.16 in the treatment group T and 60.37 in the reference item group R. There were no statistically significant differences between the control and the treatment group T at any assessment date. In the reference item treatment R, there were statistically significant differences compared to the control at BFD+15 and BFD+23 (Satterthwaite t-test, $p \leq 0.05$).

The low brood indices and low compensation indices as well as the high termination rates in the reference item treatment R compared to the control could be expected from this kind of reference item as it is known to have a negative effect on the development of the honey bee brood, confirming that the study design was appropriate to ensure feeding exposure of the honey bees and their brood to the test solution.

Overall, there was no effect of the Indoxacarb 150 g/L EC treatments on the brood index, compensation index and termination rate of the eggs while there was a clear impact in the reference item treatment R.

Table 88
Termination rates at +4, +10, +15 and +23 days after brood area fixing day (BFD0) for marked cells
(mean values of 4 replicates per treatment)

Treatment group	BFD+4	BFD+10	BFD+15	BFD+23
Marked cells containing eggs at the first assessment (BFD0):				
Mean C	3.84	7.91	9.04	15.03
Mean T	4.49	12.77	14.43	15.16
Mean R	20.12	42.44	59.00*	60.37*
Marked cells containing young larvae at the first assessment (BFD0):				
Mean C	2.18	6.34	6.34	6.42
Mean T	8.97	12.27	12.27	12.27
Mean R	13.49	31.10*	31.10*	31.53*
Marked cells containing old larvae at the first assessment (BFD0):				
Mean C	1.72	3.48	5.50	n.a.
Mean T	4.75	6.09	6.22	n.a.
Mean R	4.94	22.77*	29.26*	n.a.

BFD0 = Brood area fixing day

* Significantly different from the control (pooled t-Test or Satterthwaite t-Test, $p \leq 0.05$)

n.a.= not applicable (development cycle of old larvae completed before BFD+23)

F. DEVELOPMENT OF YOUNG HONEY BEE LARVAE IN INDIVIDUAL CELLS

According to the development time of a worker honey bee from young larvae to adult bee which normally averages approximately 15-17 days, it can be assumed that all honey bees from those cells that were initially (BFD0) marked as cells containing young larvae hatch until the assessment date BFD+23. Thus, the study period covered one complete development cycle of the observed brood cells.

The brood index values during further development of young larvae were similar in the test item treatment T and in the control, showing rising brood indices over the whole assessment period and indicating successful development in the majority of the observed cells. At the last assessment (BFD+23), the mean brood indices were 4.68 in the control and 4.39 in T. The slight differences of the test item treatment compared to the control were not statistically significant at any assessment date (pooled t-test or Satterthwaite t-test, $p \leq 0.05$). In the reference item treatment group R, the mean brood index was lower than the values in the control at all assessment dates (significantly different only on BFD+10; one-sided Satterthwaite t-test, $p \leq 0.05$) and at the assessment on BFD+23 it was 3.43, indicating a higher proportion of failing brood at that assessment date for R, resulting in a lower mean brood index at the later assessment dates for the reference item group R compared to the control and the treatment group T.

Also the compensation indices in the test item treatment T were similar and comparable to the control throughout the observation period. At the assessment on BFD+23 the mean compensation indices were 4.35 for the control and 4.32 for the treatment group T. There were no statistically significant differences between the control and T at any assessment date (one-sided pooled t-test or Satterthwaite t-test, $p \leq 0.05$).

In the reference item treatment group R, the compensation indices were lower than the corresponding values in the control (significantly different at BFD+10 and BFD+23; one-sided pooled t-test or Satterthwaite t-test, $p \leq 0.05$), reaching a level as low as 1.55 at BFD+23.

The termination rates in the test item treatment T were slightly higher than the values in the control at all assessment dates, but they were still on an acceptable level and none of these differences were statistically significant (one-sided pooled t-test or Satterthwaite t-test, $p \leq 0.05$). The mean termination rates at BFD+23

were 6.42 for the control, 12.27 for T and 31.53 for R. On BFD+10, BFD+15 and BFD+23, the termination rates in the reference item group R were significantly higher than those in the control (one-sided pooled t-test or Satterthwaite t-test, $p \leq 0.05$).

Overall, there was no significant effect of the Indoxacarb 150 g/L EC treatments on the brood index, compensation index and termination rate of young larvae while there was a clear impact in the reference item treatment R.

G. DEVELOPMENT OF OLD HONEY BEE LARVAE IN INDIVIDUAL CELLS

According to the development time of a worker honey bee from old larvae to adult bee which normally averages approximately 12-14 days, it can be assumed that all honey bees from those cells that were initially (BFD0) marked as cells containing old larvae hatched until the assessment date BFD+15. Thus, the study period covered one complete developmental cycle of the observed brood cells, and no evaluation of the assessment on BFD+23 was done for the cells initially containing old larvae.

The development of the brood index, compensation index and termination rate was very similar in the test item treatment T and in the control, showing that the test item treatment had no effect on the development of the old larvae. The brood and compensation indices on BFD+15 were 4.69 and 4.84 in T compared to 4.73 and 4.86 in the control. The termination rate at the end of the development cycle of old larvae (BFD+15) was 6.22 in T compared to 5.50 in the control. None of these differences was statistically significant.

In the reference item treatment R, the brood and compensation indices were significantly lower and the termination rates were significantly higher than in the control on BFD+10 and BFD+15. At the end of the development cycle of old larvae (BFD+15), the termination rate in R was 29.26 compared to 5.50 in the control.

Overall, there was no effect of the Indoxacarb 150 g/L EC treatments on the brood index, compensation index and termination rate of old larvae while there was a clear impact in the reference item treatment R.

III. CONCLUSIONS

Daily feeding of Indoxacarb 150 g/L EC in a 50% (w/w) sucrose solution (200 mL per day) at a concentration of 100 µg indoxacarb/kg over a period of 9 days had no effects on honey bee mortality, pupal mortality, malformations, behaviour, colony size, amount of brood, amount of food and colony condition.

There was no effect of the test item treatments on the brood indices, compensation indices and termination rates of the individual brood cells initially marked as eggs, young larvae or old larvae.

In the reference item treatment R, there were clear impacts on honey bee mortality, pupal mortality, malformations, colony size, amount of brood, and on the brood indices, compensation indices and termination rates of individually marked brood cells.

(Kleinhenz, M., 2014)

RMS comment

Study submitted to the EU for the first time in this submission.

This study is considered valid. The mean brood termination rate for eggs, young larvae and old larvae in the control group was $\leq 50\%$ at the end of the study (as required by the study plan).

No analytical confirmation of the level to which the bees were exposed is available.

There were four bee colonies per treatment group.

RMS agrees with the conclusions above. RMS however notes that, for eggs, young larvae and with a lesser extend old larvae, the termination rates on almost all assessments from BFD+5 to BFD+21 are higher than in control (even if not significant according to the study report). Even if these values are low and not

statistically different from the control, it is not known if this higher termination rates are treatment related or not.

Report: Klank, C. (2014); Indoxacarb (DPX-KN128) 150 g/L EC: Honey bee (*Apis mellifera* L.) larval toxicity test (single feeding exposure)

DuPont Report No.: DuPont-34817

Guidelines: OECD 237 (2013) **Deviations:** None

Testing Facility: Eurofins Agrosience Services EcoChem GmbH, Niefern-Öschelbronn, Germany

Testing Facility Report No.: S14-00332

GLP: Yes

Certifying Authority: Landesanstalt Für Umwelt, Messungen Und Naturschutz Baden-Württemberg

Executive summary:

Effects of the test substance Indoxacarb 150 g/L EC on the honey bee, *Apis mellifera* L., were assessed in a 7-day laboratory test. Synchronised honey bee larvae (first instar, L1) were transferred into well plates where they were fed a standardized amount of artificial diet. On day 4 (D4) of the test, five different doses (0.37, 1.11, 3.33, 10 and 30 µg a.s./larva) of the test substance were applied to the larvae with the diet. The control group was exposed for the same period of time under identical exposure conditions to untreated artificial diet. Assessments of mortality were carried out on D5, D6, and D7 (24 h, 48 h, and 72 h after application of treated and untreated food, respectively). The dose which caused 10, 20, and 50% mortality (LD₁₀, LD₂₀, and LD₅₀) and the NOED (No Observed Effect Dose) 72 h after application of the test substance to larvae of *Apis mellifera* L. were determined.

The analytical dose verification showed recovery rates of indoxacarb in all test substance solutions used to prepare treated diet C between 107 and 113%. Thus, the concentration of test substance solution for T1-T5 was confirmed.

No mortality occurred in the control group on all assessment days over the whole test duration.

The 72-h LD₁₀ was calculated as 2.38 µg a.s./larva (Probit analysis using linear max. likelihood regression; lower 95 % confidence limit of 1.27 µg a.s./larva; upper 95% confidence limit of 3.59 µg a.s./larva).

The 72-h LD₂₀ was calculated as 4.62 µg a.s./larva (Probit analysis using linear max. likelihood regression; lower 95 % confidence limit of 2.97 µg a.s./larva; upper 95% confidence limit of 6.50 µg a.s./larva).

The 72-h LD₅₀ was calculated as 16.42 µg a.s./larva (Probit analysis using linear max. likelihood regression; lower 95 % confidence limit of 11.60 µg a.s./larva; upper 95% confidence limit of 26.24 µg a.s./larva).

The 72 h-NOED was determined as 1.11 µg a.s./larva.

In the reference item treatment group (8.8 µg dimethoate/larva) the adjusted mortality was 83.3% across all replicates at the final evaluation on D7.

The study was considered valid since all validity criteria were met.

I. MATERIALS AND METHODS

A. MATERIALS

- | | |
|---|--|
| 1. Test material: | Indoxacarb 150 g/L EC |
| Lot/Batch#: | KN128-434 |
| Purity: | 150 g a.s./L |
| Description: | Liquid |
| CAS#: | None for the formulation
173584-44-6 for the active substance |
| Stability in Solution: | Sufficient for test purpose (at least 1 hour) |
| 2. Control: | Diet C containing autoclaved, deionized water as solvent |
| Test vehicle | Diet C (50% weight of fresh royal jelly + 50% weight of an aqueous solution containing 4% weight of yeast extract, 18% weight of glucose and 18% weight of fructose) containing Indoxacarb 150 g/L EC diluted with autoclaved, deionized water |
| 3. Test organism: | Honey bee larvae |
| Species: | <i>Apis mellifera</i> L. |
| Age at grafting: | First instar larvae (L1) |
| Source: | Eurofins Agrosience Services, EcoChem GmbH,
Eutinger Strasse 24, D-75223 Niefern-Öschelbronn, Germany |
| Place of Test: | Eurofins Agrosience Services EcoChem Field Station
Neulingen-Göbrichen, Nordweg 10, 75245 Neulingen-Göbrichen, Germany |
| Test chamber: | Crystal polystyrene grafting cells (NICOTPLAST) diameter of 9 mm; cells placed into a well of a 48-well cell culture plate; plates placed into desiccator; desiccator placed into an incubator with forced air circulation |
| 4. Environmental conditions (In-life phase) | |
| Temperature | 32.9 to 34.3°C |
| Relative Humidity | 54.4* to 100.0% |
| | *Only short term deviations <2h |
| Exposure to light | Constant darkness except during feeding and assessment. |

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
11-July-2014 to 13-August-2014
2. Experimental treatments
One control and five test substance concentrations of 0.37, 1.11, 3.33, 10 and 30 µg a.s./larva were tested. In total, 42 larvae per treatment group from three different colonies (each colony representing a replicate), each containing 14 test organisms, were used. On day 4 (D4) of the test, the diet C containing the application solutions was applied to the larvae.
3. Observations
Assessments of mortality were carried out after 24 h, 48 h and 72 h (D5, D6 and D7) after feeding with treated diet.
4. Statistics
Fisher's Exact Test with Bonferroni Correction (one-sided greater, $\alpha = 0.05$) was used to evaluate whether there were significant differences between the mortality data of the test item group and the control group and to determine the NOED value. For calculation of the LD₁₀, LD₂₀, LD₅₀ with 95%

confidence limits the Probit analysis using linear maximum likelihood regression was used. Fisher's Exact Test (one-sided greater, $\alpha = 0.05$) was used to evaluate whether there were significant differences between the mortality data of the reference item group and the control group.

II. RESULTS AND DISCUSSION

A. FINDINGS

Table 89
The effects on mortality of honey bee larvae 72 hours after exposure to treated and untreated food in the laboratory

Treatment group	Dose Level ($\mu\text{g a.s./larva}$)	Mortality (%) on D7 (72h after feeding)	Adjusted Mortality (%) on D7 (72h after feeding)
Control	0	0.0	-
Indoxacarb 150 g/L EC	0.37	0.0	0.0
	1.11	2.4	2.4
	3.33	21.4 ^a	21.4
	10	31.0 ^a	31.0
	30	66.7 ^a	66.7
Reference item (dimethoate)	8.8	83.3 ^b	83.3
Endpoints ($\mu\text{g a.s./larva}$)			
72-hour NOED		1.11	
72-hour LD ₁₀ (95% confidence limits)		2.38 (1.27–3.59)	
72-hour LD ₂₀ (95% confidence limits)		4.62 (2.97–6.50)	
72-hour LD ₅₀ (95% confidence limits)		16.42 (11.60–26.24)	

^a Significantly increased compared to control (Fisher's Exact Test with Bonferroni Correction, one-sided greater, $\alpha = 0.05$)

^b Significantly increased compared to control (Fisher's Exact Test, one-sided greater, $\alpha = 0.05$)

III. CONCLUSIONS

According to the results of the test, the 72 h-LD₁₀ was calculated as 2.38 $\mu\text{g a.s./larva}$ (95 % confidence limits: 1.27 μg –3.59 $\mu\text{g a.s./larva}$).

The 72 h-LD₂₀ was calculated as 4.62 $\mu\text{g a.s./larva}$ (95% confidence limits: 2.97 μg –6.50 $\mu\text{g a.s./larva}$).

The 72 h-LD₅₀ was calculated as 16.42 $\mu\text{g a.s./larva}$ (95% confidence limits: 11.60 μg –26.24 $\mu\text{g a.s./larva}$).

The 72 h-NOED was determined as 1.11 $\mu\text{g a.s./larva}$.

The study was considered valid since all validity criteria were met.

(Klank C., 2014)

RMS comment

Study submitted to the EU for the first time in this submission.

This study was conducted in compliance with the current guideline. This study is considered valid. RMS agrees with the conclusions above.

Report: Giffard, M. H. (2006); Evaluation des effets sur l'abeille domestique d'une application de Steward et de DPX-KN128 150 EC (indoxacarbe) sur culture de phacélie. (Evaluation of the effects on domestic bees from one application of Steward and of DPX-KN128 150 EC (indoxacarb) on a *Phacelia* crop)

DuPont Report No.: Etude No. 92-2006

Guidelines: CEB Guideline No. 230 (November 2003) **Deviations:** None

Testing Facility: Testapi à Sarré, Gennes, France

Testing Facility Report No.: 92-2006

GLP: Yes

Certifying Authority: Groupe Interministeriel des Produits Chimiques (GIPC) (Paris, France)

Executive summary:

A semi-field toxicity study was conducted on foraging bees (*Apis mellifera* L.) in *Phacelia* treated with DPX-MP062 30WG and Indoxacarb 150 g/L EC in Gennes, in western France under insect-proof tunnels (August 2006). The *Phacelia* crop was treated during bee foraging times and in the evening when bees were not present. The study consisted of six treatment groups: a water control group, a toxic reference (Dimezyl 40 EC, 400 g a.s./ha), DPX-MP062 30WG (50 g a.s./ha) applied during bee forage times, DPX-MP062 30WG (50 g a.s./ha) applied outside of bee forage times, Indoxacarb 150 g/L EC (50 g a.s./ha) during bee forage times, and Indoxacarb 150 g/L EC (50 g a.s./ha) outside of bee forage times. One tent enclosing 140 m² was set up in each treatment and control area, and active honeybee colonies derived from the same hive were established in the enclosures prior to application. Results of this study indicated that Steward and DPX-KN128 150 g/L EC applied at night, out of the presence of foraging bees, appears to be safe in regard to bee mortality.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test materials: DPX-MP062 30WG
Indoxacarb 150 g/L EC
Lot/Batch #: MP062-442
E2000598-69 (for Indoxacarb 150 g/L EC)
Purity: DPX-MP062 30WG: 300 g a.s./kg (30.6% by analysis)
Indoxacarb 150 g/L EC: 150 g/L (151.3 g a.s./L by analysis)
CAS#: 173584-44-6 for indoxacarb active substance
None for the formulation
Description: Brown granules (DPX-MP062 30WG)
Clear amber liquid (Indoxacarb 150 g/L EC)
Stability of test compound: 99.9% and 98.8% of the indoxacarb remains in the delivery vehicle after one hour under agitation for DPX-MP062 30WG and Indoxacarb 150 g/L EC, respectively
2. Vehicle and/or positive control Water
- Toxic reference Diméthyl 40 EC (dimethoate a.s.)
3. Test organism European honeybee
Species: *Apis mellifera* L.
Source: The source of the bees was not provided
Age at dosing: Direct exposure of foraging adults; indirect exposure of all stages of development
Crop: Blue tansy (*Phacelia tanacetifolia* var. Balo)
Water: A watering trough was provided in the field
Tunnel tents (exposure): Tunnel tent: tents (7.0 m × 20.0 m and a height of 3.0 m) placed over plots of *Phacelia* were made of galvanised steel and covered with an insect proof net; 1 replicate tunnel tent per treatment
Crop area: 4 crop plots per tent; crop plots furnished *Phacelia tanacetifolia* in full bloom to bees for forage
Plastic sheet: The remaining surface was covered with plastic sheets for the assessment of dead bees in the tunnels.
4. Environmental conditions
Temperature: 23°C during foraging times; 18°C during non-foraging times
Photoperiod (exposure): natural light conditions

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
07-August-2006 to 20-August-2006

2. Experimental treatments

A semi-toxicity test on honeybees (*Apis mellifera* L.) in blue tansy, *Phacelia tanacetifolia* (var. Balo) was conducted with DPX-MP062 30WG and Indoxacarb 150 g/L EC in Gennes, in western France. The study consisted of six treatments: the water-treated control, the toxic reference treatment, Diméthyl 40 EC applied at 1 L product/ha (equivalent to 400 g dimethoate/ha), DPX-MP062 30WG applied during normal foraging times at 0.167 kg/ha (equivalent to 50.0 g a.s./ha), DPX-MP062 30WG applied outside foraging times at 0.167 kg/ha (equivalent to 50.0 g a.s./ha), Indoxacarb 150 g/L EC applied during normal foraging times at 0.333 L product/ha (50 g a.s./ha), and Indoxacarb 150 g/L EC applied outside foraging times at 0.333 L product/ha (50 g a.s./ha). All applications were carried out with a volume ranging between 100 and 300 L/ha. The effects of the both the DPX-MP062 30WG and Indoxacarb 150 g/L EC treatments were examined on small honey bee colonies in tunnel tents placed over plots of *Phacelia tanacetifolia*. One replicate was used per treatment (small healthy colonies with 15000 to 20000 individuals).

The applications were done after the mortality of the bees had stabilized, which was seven days after the introduction of the colonies to the tents. The treatments in the tents which were done outside of foraging were performed in the evening after the bees had finished foraging and had returned to their colonies. All plots were treated in as short a time as possible in order to limit any changes to foraging done by the bees.

The mortality counts were done in each tent in the mornings and in the evenings by counting bee corpses found on plastic films placed on the ground under each tent. The counts were done each day for six days prior to and after the applications. Entry into each tent was tightly controlled, with special attention paid to times just after application in order to avoid placing additional stress on the bees.

3. Observations

Mortality:	The numbers of dead honey bees within the tunnels were assessed daily on 6 plastic sheets (lanes) starting 6 days before the applications to 6 days after the applications.
Foraging activity	All forager honey bees were counted daily on the 4 crop plots of each tunnel.
Colony assessment:	Two apiarist visits were conducted: At the beginning and at the end of the experimental phase the bee colonies were inspected in order to assess the presence of a healthy bee population, including the number of adults, eggs, and larva, the number of combs with brood and honey, and the construction of new combs.

4. Statistics

The data did not warrant statistical analysis; only one replicate of each treatment and control was used.

II. RESULTS AND DISCUSSION

A. FINDINGS

1. Effects on Honey Bee Mortality

In the course of the six days prior to application, the bee mortalities remained homogenous for all colonies in the test site. On the morning of the application, the mortality count was less than 180 individuals for each tunnel. The control mortality was quite stable over the entire study period after application, validating the test. The toxic reference (400 g dimethoate/ha) induced a high peak of mortality the day after spray application proving the sensitivity of the test system. The impact of the toxic reference treatment on mortality was very high. The counts of dead honey bees performed on the evening at Day 0aa (after application) and the following morning (Day +1) summed up to the total of 1182 individuals.

When DPX-MP062 30WG was applied under the same conditions as the negative and positive controls during foraging hours, an elevated number of bee mortalities was noted on Day 0 through Day 2. The level of bee mortalities was comparable to that seen in the negative control group for Day 3 through Day 6 of the study. The plots treated outside of bee forage times did not see an overall significant increase in bee mortality throughout the remainder of the study.

Application of Indoxacarb 150 g/L EC during bee foraging hours resulted in a strong elevation of bee mortality on Day 1 after application, continuing into Day 2, and then was similar to the mortalities seen with the negative control on Day 3 through the remainder of the study. When Indoxacarb 150 g/L EC was applied outside of foraging times, there was little to no increase in bee mortality throughout the study after application.

The toxicity of the dimethoate was greater than the toxicity exhibited by either DPX-MP062 30WG or Indoxacarb 150 g/L EC and lasted longer; heavy bee mortality was seen for Day 1 and Day 2 after

treatment before the numbers began to be reduced. In comparison, DPX-MP062 30WG and Indoxacarb 150 g/L EC bee toxicity levels became comparable to the negative control by Day 2 and remained steady throughout the remainder of the study. The main toxic effect of DPX-MP062 30WG and Indoxacarb 150 g/L EC was observed when applied during foraging hours; application of DPX-MP062 30WG and Indoxacarb 150 g/L EC in the evening after the bees were absent from the crop resulted in mortality levels that were near the mortality seen with the negative control.

Table 90
Total number of dead honey bees/tunnel

Date	Day	Treatment groups					
		DPX-MP062 30WG, during forage (50 g a.s./ha)	DPX-MP062 30WG, outside forage (50 g a.s./ha)	Indoxacarb 150 g/L EC, during forage (50 g a.s./ha)	Indoxacarb 150 g/L EC, outside forage (50 g a.s./ha)	Diméthyl 40 EC	Control (water)
8 August 2006	-6	238	136	140	223	137	174
9 August 2006	-5	76	70	102	121	61	84
10 August 2006	-4	91	57	105	80	109	116
11 August 2006	-3	132	54	147	150	160	133
12 August 2006	-2	83	35	73	114	62	75
13 August 2006	-1	73	53	104	102	87	146
14 August 2006	0ba	128	51	177	94	79	113
14 August 2006	0aa	137	-- ^a	207	-- ^a	284	110
15 August 2006	+1	208	95	585	163	898	65
16 August 2006	+2	581	138	588	189	1259	158
17 August 2006	+3	99	134	65	127	515	69
18 August 2006	+4	120	121	159	180	350	94
19 August 2006	+5	122	94	201	180	288	77
20 August 2006	+6	221	134	229	148	249	173

Day 0ba = Day of treatment before application

Day 0aa = Day 0 after application of test item

^a Day 0aa treatments of DPX-MP062 30WG and Indoxacarb 150 g/L EC were done in the evening; mortality counts were done the following morning.

2. Effects on Foraging Activity

The numbers of foraging bees were similar for all of the introduced colonies beneath the tents. The level of activity varied between 0 and 12 bees per square meter during the acclimation period. Determination of the number of foraging bees were performed twice daily before and after treatment in all plots. After treatment, the levels of foraging bees in the plots treated with DPX-MP062 30WG and Indoxacarb 150 g/L EC were near the number of foraging bees seen in the negative control. Almost all bee foraging ceased after the plot was treated with dimethoate, partly due to the high mortality rate of bees overall.

Prior to treatment, there were no foraging bees present in the two tents that were to be treated with DPX-MP062 30WG and with Indoxacarb 150 g/L EC, and so the numbers of foraging bees were not calculated for these two treatment groups. Treatment with dimethoate created a massive difference between the number of bees foraging after treatment compared to those foraging after treatment with the negative control substance. These indices do confirm a slight level of elevated toxicity to foraging

bees for DPX-MP062 30WG during normal foraging times. The level of toxicity is slightly more elevated for Indoxacarb 150 g/L EC when applied while the bees are foraging.

Table 91
Foraging activity (mean number of honeybees/m²)

Date	Day	Treatment groups					
		DPX-MP062 30WG, during forage	DPX-MP062 30WG, outside forage	Indoxacarb 150 g/L EC, during forage	Indoxacarb 150 g/L EC, outside forage	Dimézy 40 EC	Control, water
10 August 2006	-4	6	5	9	2	3	4
11 August 2006	-3	5	2	5	2	0	1
12 August 2006	-2	6	4	8	3	2	4
13 August 2006	-1	4	4	7	1	2	1
14 August 2006	0 ba	8	7	12	5	4	6
14 August 2006	0 aa	8	7	13	5	1	6
15 August 2006	+1	7.3	6.5	9.4	5.9	0.0	6.8
16 August 2006	+2	3.6	4.4	3.9	3.9	0.0	2.5
17 August 2006	+3	4.6	4.9	4.3	3.8	0.0	5.5
Index of relative toxicity							
		DPX-MP062 30WG, during forage	DPX-MP062 30WG, outside forage	Indoxacarb 150 g/L EC, during forage	Indoxacarb 150 g/L EC, outside forage	Dimézy 40 EC	Control, water
Toxicity, Day 1		1.7	1.2	2.9	1.1	9.7	1.0
Toxicity, Day 2		3.2	1.9	2.4	1.4	11.4	1.0
Index of foraging bee mortality							
		DPX-MP062 30WG, during forage	DPX-MP062 30WG, outside forage	Indoxacarb 150 g/L EC, during forage	Indoxacarb 150 g/L EC, outside forage	Dimézy 40 EC	Control, water
Simple calculation		0.4	--	0.7	--	2.9	0.1
Relative to control		3.1	--	5.8	--	23.9	1.0

Day 0ba = day of treatment before application

Day 0aa = in the evening after application of test item

3. Effects on Honey Bee Brood Development:

Some changes were detected between the two colony assessments at the beginning and end of the experimental phase. Some changes in the health of the hives were noted between the two visits, especially in the number of larva which had developed within the hives. However, due to the short period of time between the two visits (approximately two weeks), and given the stresses induced by being trapped beneath the tents, the smaller number of bee larvae produced was not considered to be significant and therefore not considered to be the result of any one factor during the course of the study.

III. CONCLUSIONS

For the test items DPX-MP062 30WG (0.167 kg a.s./ha) and Indoxacarb 150 g/L EC (0.333 L/ha), applications made outside of normal forage times for honeybees appear to be perfectly innocuous in regard to honeybee toxicity. Treatment with these reagents during forage time shows slight toxicity in regard to honeybees.

(Giffard, M. H., 2006)

RMS comment

Study submitted to the EU for the first time in this submission.

This study is considered acceptable. This study was conducted according to the French guideline CEB 230 and is in compliance with the EPPO recommendations but with only one replicate for each treatment. The study report is written in french and the study summary above was translated by the applicant.

The effects of Indoxacarb 150 g/L EC and another formulation, Steward (WG formulation), were tested when applied during and after bee flight (One tent per treatment group).

RMS notes that the quantity of brood and the population decreased but no raw data is available in the study report (no comparison can be made between control and the test items). Due to the short duration of the study, no relevant observations can be made on the bee brood.

RMS notes that the mortality increased in the tunnels treated during foraging activity for both formulations. This increase lasted until DAA+2 and returned to a normal level afterwards. The peak of mortality seems higher in the tunnel treated with Indoxacarb 150 g/L EC. This resulted in higher cumulative mortality in this tunnel. It is not known if the difference in mortality values might reflect a difference of toxicity of the different types of formulations (EC and WG) when the bees are exposed to fresh residues.

In the tunnels treated in the evening after foraging activity, no increase of mortality was observed for both formulations.

No data on bee behaviour is available.

No effect on foraging activity due to treatment was found in the tunnels treated with both test items. A higher decrease is observed in the tunnel treated with Indoxacarb 150 g/L EC during foraging but the number of foraging bees was higher in this tunnel during the pre-application period and the trend for decrease was observed in all tunnels including control. Thus the decrease in foraging activity is not considered treatment related.

Report: Gonsior, G. (2006); Indoxacarb (DPX-KN128) 150 g/L EC: A semi-field study to evaluate effects on the honey bee (*Apis mellifera carnica*; Hymenoptera, Apidae) in Phacelia in Germany 2006

DuPont Report No.: DuPont-19449

Guidelines: OEPP/EPPO 170 (2001) **Deviations:** None

Testing Facility: GAB Biotechnologie, GmbH, Niefern-Oeschelbronn, Germany

Testing Facility Report No.: 20061269/G1-BZEU

GLP: Yes

Certifying Authority: Ministerium für Umwelt und Verkehr (Baden-Württemberg, Germany)

Executive summary:

The effect of Indoxacarb 150 g/L EC was tested on the honey bee (*Apis mellifera carnica* L.) under semi-field conditions following the OEPP/EPPO Guideline No. 170 (3) (2001).

This study was conducted near Niefern in southern Germany (region: Baden-Württemberg) in July/August 2006 and included three treatment groups (each with three tunnel tents). In all treatment groups the application was performed during bee flight. The application rate of the test item was 370 mL Indoxacarb 150 g/L EC per ha (equivalent to 55.5 g a.s./ha, nominal). A second group was treated with tap water and served as the control. As reference item, "Perfekthion" (dimethoate) was applied at a rate of 650 g product/ha (equivalent to 243.23 g dimethoate a.s./ha, nominal) in the third group. The test item applications were carried out with a spray volume of 500 L/ha, whereas the control and the reference item treatments were carried out with a spray volume of 400 L/ha.

The effects of the test item treatment were examined on small honey bee colonies in tunnel tents (5.0 m × 10.0 m and a height of 3.5 m) placed over plots of *Phacelia tanacetifolia*. The semi-field test comprised 3 replicates for each treatment group.

The effects of Indoxacarb 150 g/L EC were evaluated by comparing the results in the test item treatment to the data in the control treatment as well as in the reference item treatment regarding the following observations:

- Number of dead honey bees at the edge of the treated area (linen sheets) and in the dead honey bee traps in front of the hives.
- Foraging activity on the crop (number of forager honey bees/m²).
- Condition of the colonies and development of the honey bee brood.
- Behaviour of the honey bees in the crop area and around the hives.

It was concluded, that Indoxacarb 150 g/L EC had no harmful effects on honey bee mortality, flight intensity, brood development, and behaviour, when applied at 370 mL Indoxacarb 150 g/L EC/ha (equivalent to 55.5 g a.s./ha) to *Phacelia tanacetifolia* during bee flight.

I. MATERIALS AND METHODS

A. MATERIALS

- | | | |
|----|----------------------------------|---|
| 1. | Test material: | Indoxacarb 150 g/L EC |
| | Lot/Batch #: | DPX-KN128-150 |
| | Purity: | 150 g a.s./L |
| | CAS#: | None for the formulation
173584-44-6 for indoxacarb active substance |
| | Description: | Liquid/Amber |
| | Stability of test compound: | 98.8% of the indoxacarb remains in the delivery vehicle after one hour under agitation |
| 2. | Vehicle and/or positive control: | Tap water |
| 3. | Test organism | |
| | Species: | <i>Apis mellifera</i> L. |
| | Age at dosing: | Direct exposure of adults; indirect exposure of all stages of development |
| | Source: | Beekeeper, Berthold Nengel (Dahlheim, Germany). |
| | Diet: | Nectar and pollen of flowering <i>Phacelia tanacetifolia</i> |
| | Tunnel tents (exposure): | Within the test field tents (5.0 m × 10.0 m and a height of 3.5 m) were installed before moving the hives to the experimental field. The tent frames were covered with light plastic gauze. Before start of the test, paths were created in each tunnel by removal of the plants and smoothing the ground for walking inside the tunnel. The crop area per tent was approx. 40 m ² . |
| | Test age: | Direct exposure of adults; indirect exposure of all stages of development |
| 4. | Environmental conditions | |
| | Temperature: | 10.9 to 31.5°C |
| | Relative humidity: | 67.6–95.2% (daily means) |
| | Photoperiod (exposure): | Not reported |

B. STUDY DESIGN AND METHODS

1. Experimental start/completion
27-July-2006 to 22-August-2006
2. Experimental treatments
This study was conducted near Niefern in southern Germany (region: Baden-Württemberg) in July/August 2006 and included three treatment groups (each with three tunnel tents). In all treatment groups the application was performed during bee flight. The application rate of the test item was 370 mL Indoxacarb 150 g/L EC per ha (equivalent to 55.5 g a.s./ha). A second group was treated with tap water and served as the control. As reference item, "Perfekthion" (dimethoate) was applied at a rate of 650 g product/ha (equivalent to 243.23 g dimethoate/ha) in the third group. The test item applications were carried out with a spray volume of 500 L/ha, whereas the control and the reference item treatments were carried out with a spray volume of 400 L/ha.
3. Observations
The effects of Indoxacarb 150 g/L EC were evaluated by comparing the results in the test item treatment to the data in the control treatment as well as in the reference item treatment regarding the following observations:
 - Number of dead honey bees at the edge of the treated area (linen sheets) and in the dead honey bee traps in front of the hives.

- Foraging activity on the crop (number of forager honey bees/m²).
- Condition of the colonies and development of the honey bee brood.
- Behaviour of the honey bees in the crop area and around the hives.

II. RESULTS AND DISCUSSION

A. FINDINGS

1. Effects on honey bee mortality

During the pre-application period (from 3 days before application (DAA -3) until the day of application before treatment, (DAA 0ba) a mean number of 4.6 dead bees/day was calculated in the Indoxacarb 150 g/L EC treatment group (T) compared to 8.9 dead bees/day in the control group (C) and 10.5 dead bees/day the reference item treatment group (R) respectively.

On the day of treatment before application (DAA 0ba) the mean number of 2.7 dead bees was observed in the Indoxacarb 150 g/L EC group compared to 4.7 dead bees in the control and 6.7 dead bees in the reference item treatment group.

On the day of treatment after application (DAA 0aa) the mean number of dead bees in the Indoxacarb 150 g/L EC group increased to 23.0 dead bees. In the control treatment, a mean number of 15.3 dead bees were calculated while in the reference item treatment group the mean number of dead bees increased to 256.3.

The mean post-application mortality (DAA 0aa to +7) was determined to be 21.9 dead bees/day in the Indoxacarb 150 g/L EC treatment group compared to 12.5 dead bees/day in the control group and 76.5 dead bees/day in the reference item treatment group. There were no statistically significant differences between the post-application mortality of the Indoxacarb 150 g/L EC treatment group and the control group. The post application mortality of the reference item group was significantly different compared to the control group as well as to the Indoxacarb 150 g/L EC treatment group (t-Test; $p < 0.05$).

Table 92
Mean number of dead honey bees/tent (linen sheets plus dead bee trap)

Date	DAA	Treatment groups		
		Indoxacarb 150 g/L EC (T)	Reference item (R)	Control (C)
30 July 06	-3	5.0	11.7	6.0
31 July 06	-2	6.3	17.7	15.7
01 August 06	-1	4.3	6.0	9.3
02 August 06	0ba	2.7	6.7	4.7
Mean number of dead honey bees (DAA -3 to 0ba)		4.6	10.5	8.9
02 August 06	0aa	23.0	256.3	15.3
03 August 06	+1	31.7	49.0	5.7
04 August 06	+2	22.3	121.0	6.3
05 August 06	+3	25.3	67.0	13.7
06 August 06	+4	17.7	31.7	12.0
07 August 06	+5	11.3	18.0	8.0
08 August 06	+6	30.3	42.7	26.3
09 August 06	+7	13.3	26.3	12.3
Mean number of dead honey bees (DAA 0aa to +7)		21.9	76.5*	12.5

DAA = Days after application

0ba = Mortality on the day of treatment before application

0aa = Mortality assessed on the day of application after treatment

* = Post-application period significantly different to control and test item treatment, t-test, $p < 0.05$

2. Effects on honey bee flight intensity

The daily mean flight intensity during the pre-application period was 9.7 honey bees/m² in the Indoxacarb 150 g/L EC treatment group (T), 9.3 honey bees/m² in the reference item treatment group (R), and 9.0 honey bees/m² in the control treatment group (C).

Shortly before application a mean of 7.7 honey bees/m² was observed in the Indoxacarb 150 g/L EC treatment group. After the application, flight intensity slightly increased to a mean of 8.6 bees/m². In the reference item treatment group, a mean flight intensity of 7.7 honey bees/m² was calculated shortly before the application which decreased to a mean of 1.8 honey bees/m², based on the values of the assessments on the day of treatment after the application. In the control treatment group the mean flight intensity before and after the application was similar (9.3 and 8.9 honey bees/m²).

From DAA +1 up to +7 there was no difference in the flight intensity of the control (mean: 7.8 honey bees/m²) and the Indoxacarb 150 g/L EC treatment group (mean: 7.9 honey bees/m²). The flight intensity from DAA +1 to DAA +7 was very low (mean: 0.2 honey bees/m²) in the reference item treatment group.

Table 93
Flight intensity (mean number of honey bees/m²)

Date	DAA	Treatment groups		
		Indoxacarb 150 g/L EC (T)	Reference item (R)	Control (C)
30 July 06	-3	13.0	10.7	9.3
31 July 06	-2	10.7	11.3	11.0
01 August 06	-1	7.3	7.3	6.3
02 August 06	0ba	7.7	7.7	9.3
Mean number of honey bees/m² (DAA -3 to 0ba)		9.7	9.3	9.0
02 August 06	0aa	8.6	1.8	8.9
03 August 06	+1	8.6	0.0	7.8
04 August 06	+2	12.1	0.0	11.9
05 August 06	+3	9.3	0.0	7.3
06 August 06	+4	4.7	0.0	1.3
07 August 06	+5	7.3	0.0	12.7
08 August 06	+6	4.7	0.0	2.3
09 August 06	+7	8.0	0.0	10.3
Mean number of honey bees/m² (DAA 0aa to +7)		7.9	0.2	7.8

DAA = Days after application

0ba = Flight intensity on the day of treatment before application

0aa = Flight intensity assessed on the day of application after treatment

3. Effects on honey bee brood development

The strength of the colonies (number of bee ways between combs filled with honey bees) in the Indoxacarb 150 g/L EC colonies increased from 3.0 to 3.5 (Replicate 1), 3.0 to 3.0 (Replicate 2), and 3.0 to 4.0 (Replicate 3) over the three brood assessments carried out (one assessment on the day before placing the colonies in the tunnel tents and two assessments 1 and 3 weeks after treatment).

At all three brood assessments, all brood stages (egg, larval, and pupal stages) were observed in the colonies of all three treatment groups. The changes recorded in the nine hives of the three treatment groups are regarded as normal and cannot be linked to the treatments.

4 Behaviour of the honey bees

The colonies in the control and Indoxacarb 150 g/L EC treatment group showed no abnormal behaviour in the pre-application or post-application periods. In the tents of the reference item treatment group abnormal honey bee behaviour (grooming; motionless bees) was noticed on the day of application after the treatment.

III. CONCLUSIONS

Indoxacarb 150 g/L EC when applied at 370 mL/ha (equivalent to 55.5 g a.s./ha) to *Phacelia tanacetifolia* during bee flight had no harmful effects on honey bee mortality, flight intensity, brood development, and behaviour.

(Gonsior, G., 2006)

RMS comment

Study submitted to the EU for the first time in this submission.

This study is considered acceptable.

RMS notes the mortality was higher in one replicate (one of the three tunnels treated with Indoxacarb 150 g/L EC). This explains the higher mortality observed after treatment on the basis of mean mortality values. According to the study author, this difference between replicates is possibly due to biological differences between hives. Excluding this colony the post-application mortality would be in the same range as the control. However, RMS considers that transient effects on mortality cannot be excluded as they appear precisely after the day of application.

Indoxacarb 150 g/L EC when applied at 370 mL/ha (equivalent to 55.5 g a.s./ha) to *Phacelia tanacetifolia* during bee flight had no harmful effects (2 tunnels) or transient effects (1 tunnel) on honey bee mortality, and no effect on flight intensity, brood development, and behaviour.

Report: Gonsior, G. (2007a); Indoxacarb (DPX-KN128) 150 g/L EC: A semi-field study to evaluate effects on the honey bee (*Apis mellifera carnica*; Hymenoptera, Apidae) in *Phacelia tanacetifolia* in France 2007

DuPont Report No.: DuPont-19450

Guidelines: OEPP/EPPO 170 (2001) **Deviations:** None

Testing Facility: GAB Biotechnologie, GmbH, Niefern-Oeschelbronn, Germany

Testing Facility Report No.: 20071084/F1-BZEU

GLP: Yes

Certifying Authority: Ministerium für Umwelt und Verkehr (Baden-Württemberg, Germany)

Executive summary:

The effect of Indoxacarb 150 g/L EC was tested on the honey bee (*Apis mellifera carnica* L.) under semi-field conditions following the OEPP/EPPO Guideline No. 170 (3) (2001).

The application rate was 371 mL Indoxacarb 150 g/L EC per ha (equivalent to 55.6 g a.s./ha, nominal). A second group was treated with tap water and served as the control. As reference item, "Perfekthion" (dimethoate) was applied at a rate of 1 L product/ha (equivalent to 400 g dimethoate a.s./ha, nominal) in the third group. All applications were carried out with a spray volume of 500 L/ha.

The effects of the test item treatment were examined on small honey bee colonies in tunnel tents (5.0 m × 10.0 m and a height of 3.5 m) placed over plots of *Phacelia tanacetifolia*. The semi-field test comprised 3 replicates for each treatment group.

The effects of Indoxacarb 150 g/L EC were evaluated by comparing the results in the test item treatment to the data in the control treatment as well as in the reference item treatment regarding the following observations:

- Number of dead honey bees at the edge of the treated area (linen sheets) and in the dead honey bee traps in front of the hives.
- Foraging activity on the crop (number of forager honey bees/m²).
- Condition of the colonies and development of the honey bee brood.
- Behaviour of the honey bees in the crop area and around the hives.

It was concluded that Indoxacarb 150 g/L EC, when applied at 371 mL Indoxacarb 150 g/L EC/ha (equivalent to 55.6 g a.s./ha) to *Phacelia tanacetifolia* during bee flight, had no effects on honey flight intensity and brood development. Concerning mortality, an increase could be noticed on the day of application after application.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Indoxacarb 150 g/L EC
 Lot/Batch #: DPX-KN128-206
 Purity: 150 g a.s./L (nominal)
 CAS#: None for the formulation
 173584-44-6 for indoxacarb active substance
 Description: Liquid/amber
 Stability of test compound: 99.7% of indoxacarb remains in the delivery vehicle after one hour under agitation
2. Vehicle and/or positive control: Tap water
3. Test organism
 Species: *Apis mellifera* L.
 Age at dosing: Direct exposure of adults; indirect exposure of all stages of development
 Source: Beekeeper, Berthold Nengel (Dahlheim, Germany).
 Diet: Nectar and pollen of flowering *Phacelia tanacetifolia*
 Tunnel tents (exposure): Within the test field tents (5.0 m × 10.0 m and a height of 3.5 m) were installed before moving the hives to the experimental field. The tent frames were covered with light plastic gauze. Before start of the test, paths were created in each tunnel by removal of the plants and smoothing the ground for walking inside the tunnel. The crop area per tent was approx. 40 m².
 Test age: Direct exposure of adults; indirect exposure of all stages of development
4. Environmental conditions
 Temperature: 7.7 to 34.0°C
 Relative humidity: 31.0–99.0% (daily means)
 Photoperiod (exposure): Not reported
 Precipitation: 0.0–11.0 mm

B. STUDY DESIGN AND METHODS

1. Experimental start/completion
 07-July-2007 to 03-August-2007

2. Experimental treatments

This study was conducted in Drusenheim, France in July/August 2007 and included three treatment groups (each with three tunnel tents). In all treatment groups, the application was performed during honey bee flight. The application rate was 371 mL Indoxacarb 150 g/L EC per ha (equivalent to 55.6 g a.s./ha, nominal). A second group was treated with tap water and served as the control. As reference item, "Perfekthion" (dimethoate) was applied at a rate of 1 L product/ha (equivalent to 400 g dimethoate a.s./ha, nominal) in the third group. All applications were carried out with a spray volume of 500 L/ha.

3. Observations

The effects of Indoxacarb 150 g/L EC were evaluated by comparing the results in the test item treatment to the data in the control treatment as well as in the reference item treatment regarding the following observations:

- Number of dead honey bees at the edge of the treated area (linen sheets) and in the dead honey bee traps in front of the hives.
- Foraging activity on the crop (number of forager honey bees/m²).
- Condition of the colonies and development of the honey bee brood.
- Behaviour of the honey bees in the crop area and around the hives.

II. RESULTS AND DISCUSSION

A. FINDINGS

1. Effects on honey bee mortality

During the pre-application period (from 3 days before application (DAA -3) until the day of application before treatment, (DAA 0ba)), a mean number of 32.8 dead honey bees/day was calculated in the Indoxacarb 150 g/L EC treatment group (T) compared to 21.1 dead honey bees/day in the control group (C) and 23.7 dead honey bees/day in the reference item treatment group (R) respectively.

On the day of treatment before application (DAA 0ba), the mean number of 37.3 dead honey bees was observed in the Indoxacarb 150 g/L EC group compared to 29.3 dead honey bees in the control and 31.3 dead honey bees in the reference item treatment group.

On the day of treatment after application (DAA 0aa), the mean number of dead honey bees in the Indoxacarb 150 g/L EC group increased to 118.3 dead honey bees. In the control treatment a mean number of 13.3 dead honey bees were calculated while in the reference item treatment group the mean number of dead honey bees increased to 341.7.

The mean post-application mortality (DAA 0aa to +7) was determined to be 36.8.0 dead honey bees/day in the Indoxacarb 150 g/L EC treatment group compared to 25.2 dead honey bees/day in the control group and 103.6 dead honey bees/day in the reference item treatment group. There were statistically significant differences between post-application mortality values of the Indoxacarb 150 g/L EC treatment group and the control group on DAA 0aa and +1. Concerning post-application mortality values of the reference item group, there were significant differences compared to the control group on DAA 0aa, +1, +2, and +3 as also concerning the mean post application mortality (t-Test; $p < 0.05$).

Table 94
Mean number of dead honey bees/tent (linen sheets plus dead bee trap)

Date	DAA	Treatment groups		
		Indoxacarb 150 g/L EC (T)	Reference item (R)	Control (C)
11 July 07	-3	49.0	35.0	24.0
12 July 07	-2	25.7	16.3	17.0
13 July 07	-1	19.3	12.0	14.0
14 July 07	0ba	37.3	31.3	29.3
Mean number of dead honey bees (DAA -3 to 0ba)		32.8	23.7	21.1
14 July 07	0aa	118.3*	341.7*	13.3
15 July 07	+1	37.3*	111.0*	12.0
16 July 07	+2	26.7	123.0*	14.7
17 July 07	+3	17.3	56.0*	18.0
18 July 07	+4	24.3	43.3	35.7
19 July 07	+5	28.3	67.7	43.0
20 July 07	+6	18.7	52.3	32.7
21 July 07	+7	23.3	33.7	32.0
Mean number of dead honey bees (DAA 0aa to +7)		36.8	103.6*	25.2

DAA Days after application

0ba Mortality on the day of treatment before application

0aa Mortality assessed on the day of application after treatment

* Post-application period significantly different to control and test item treatment, t-Test, $p < 0.05$

2. Effects on honey bee flight intensity

The daily mean flight intensity during the pre-application period was 14.4 honey bees/m² in the Indoxacarb 150 g/L EC treatment group (T), 15.1 honey bees/m² in the reference item treatment group (R), and 14.0 honey bees/m² in the control treatment group (C).

Shortly before application a mean of 16.0 honey bees/m² was observed in the Indoxacarb 150 g/L EC treatment group. After the application, flight intensity stayed on the same level with a mean of 15.0 honey bees/m². In the reference item treatment group a mean flight intensity of 19.3 honey bees/m² was calculated shortly before the application which decreased to a mean of 3.4 honey bees/m², based on the values from the assessments on the day of treatment after the application. In the control treatment group the mean flight intensity before treatment was determined to be 16.7 honey bees/m². After the application flight intensity slightly increased to a mean of 19.8 honey bees/m² on that day.

From DAA +0 up to +7, the mean flight intensity of the control was 15.8 honey bees/m², slightly above the mean flight intensity of the test item treatment (10.4 honey bees/m²). For the reference item group a mean flight intensity of only 0.7 honey bees/m² was determined.

On DAA +5, the flight activity throughout the trial was reduced due to rainy and cool weather conditions.

Table 95
Flight intensity (mean number of honey bees/m²)

Date	DAA	Treatment groups		
		Indoxacarb 150 g/L EC (T)	Reference item (R)	Control (C)
11 July 07	-3	13.3	14.3	11.3
12 July 07	-2	12.3	9.3	7.0
13 July 07	-1	16.0	17.3	21.0
14 July 07	0ba	16.0	19.3	16.7
Mean number of honey bees/m² (DAA -3 to 0ba)		14.4	15.1	14.0
14 July 07	0aa	15.0	3.4	19.8
15 July 07	+1	14.8	0.2	23.9
16 July 07	+2	14.4	0.0	24.2
17 July 07	+3	15.3	0.0	14.0
18 July 07	+4	10.0	0.0	17.0
19 July 07	+5	0.0	0.0	0.7
20 July 07	+6	9.3	1.7	16.3
21 July 07	+7	4.3	0.0	10.3
Mean number of honey bees/m² (DAA0aa to +7)		10.4	0.7	15.8

DAA Days after application

0ba Flight intensity on the day of treatment before application

0aa Flight intensity assessed on the day of application after treatment

3. Effects on honey bee brood development

The strength of the colonies (number of bee ways between combs filled with honey bees) in the Indoxacarb 150 g/L EC colonies increased from 3.0 to 4.0 (Replicate 1 and 3) and 3.0 to 4.5 (Replicate 2) over the three brood assessments carried out (first assessment two days before placing the colonies in the tunnel tents, second and third assessment 9 and 20 days after treatment, respectively). Within the same time the strength of the colonies of the control treatment increased from 3.0 to 4.0 in all three replicates. In the reference item treatment, the colonies of the three replicates decreased in strength from 3.0 to 2.5 (Replicate 1 and 2) or slightly increased from 3.0 to 3.5 (Replicate 2).

At all three brood assessments, all brood stages (egg, larval, and pupal stages) were observed in the colonies of all three treatment groups. The changes recorded in the nine hives of the three treatment groups are regarded as normal and cannot be linked to the treatments.

4. Behaviour of the honey bees

The colonies in the control treatment group showed no abnormal behaviour in the pre-application or post-application period. In the tents of the test item treatment group only once abnormal behaviour was observed, on the day of application, two hours after treatment (normal behaviour of most of the honey bees, and some cramping honey bees on the linen and on the net, some honey bees spinning around themselves). In the reference item treatment group, abnormal honey bee behaviour was observed about 45 minutes after the application (cramping honey bees on the linen and in the dead bee traps).

III. CONCLUSIONS

Indoxacarb 150 g/L EC when applied at 371 mL Indoxacarb 150 g/L EC/ha (equivalent to 55.6 g a.s./ha) to *Phacelia tanacetifolia* during bee flight had no effects on honey flight intensity and brood development. Concerning mortality, an increase could be noticed on the day of application after application.

(Gonsior, G., 2007a)

RMS comment

Study submitted to the EU for the first time in this submission.

This study is considered acceptable.

RMS notes that flight intensity in tunnels treated with Indoxacarb 150 g/L EC is lower than in control after the application but is at the same level as before the application. RMS also notes abnormal behaviour on the day of application. Indoxacarb 150 g/L EC when applied at 371 mL Indoxacarb 150 g/L EC/ha (equivalent to 55.6 g a.s./ha) to *Phacelia tanacetifolia* during bee flight had no effects on honey flight intensity and brood development. Concerning mortality, an increase occurred on the day of application and the day after application.

Report: Gonsior, G. (2007b); Indoxacarb (DPX-KN128) 150 g/L EC: A semi-field study to evaluate effects on the honey bee (*Apis mellifera carnica*; Hymenoptera, Apidae) in *Phacelia tanacetifolia* in Germany 2007

DuPont Report No.: DuPont-19451

Guidelines: OEPP/EPPO 170 (2001) **Deviations:** None

Testing Facility: GAB Biotechnologie, GmbH, Niefern-Oeschelbronn, Germany

Testing Facility Report No.: 20071084/G1-BZEU

GLP: Yes

Certifying Authority: Ministerium für Umwelt und Verkehr (Baden-Württemberg, Germany)

Executive summary:

This study was conducted in Niefern, Germany in July/August 2007 and included three treatment groups (each with three tunnel tents). In all treatment groups the application was performed during bee flight. The application rate was 371 mL Indoxacarb 150 g/L EC/ha nominal (359 mL Indoxacarb 150 g/L EC/ha, equivalent to 55.6 g a.s./ha, analysed). A second group was treated with tap water and served as the control. As reference item, "Perfekthion" (dimethoate) was applied at a rate of 1 L product/ha (equivalent to 400 g dimethoate/ha, nominal) in the third group. All applications were carried out with a spray volume of 500 L/ha.

The effects of the test item treatment were examined on small honey bee colonies in tunnel tents (6.0 m × 10.5 m and a height of 2.3 m) placed over plots of *Phacelia tanacetifolia*. The semi-field test comprised 3 replicates for each treatment group.

The effects of Indoxacarb 150 g/L EC were evaluated by comparing the results in the test item treatment to the data in the control treatment as well as in the reference item treatment regarding the following observations:

- Number of dead honey bees at the edge of the treated area (linen sheets) and in the dead honey bee traps in front of the hives.
- Foraging activity on the crop (number of forager honey bees/m²).

- Condition of the colonies and development of the honey bee brood.
- Behaviour of the honey bees in the crop area and around the hives.

Indoxacarb 150 g/L EC, when applied at 371 mL Indoxacarb 150 g/L EC/ ha nominal (359 mL Indoxacarb 150 g/L EC/ha equivalent to 55.6 g a.s./ha, analysed) to *Phacelia tanacetifolia* during bee flight, had no effects on honey flight intensity and brood development. Concerning mortality, an increase could be noticed on the day of application, after application, and on the following day.

I. MATERIALS AND METHODS

A. MATERIALS

- Test material: Indoxacarb 150 g/L EC
 Lot/Batch #: DPX-KN128-206
 Purity: 150 g a.s./L (nominal)
 CAS#: None for the formulation
 173584-44-6 for indoxacarb active substance
 Description: Liquid/Amber
 Stability of test compound: 98.8% of the indoxacarb remains in the delivery vehicle after one hour under agitation
- Vehicle and/or positive control: Tap water
- Test organism
 Species: *Apis mellifera* L.
 Age at dosing: Direct exposure of adults; indirect exposure of all stages of development
 Source: Beekeeper, Berthold Nengel (Dahlheim, Germany).
 Diet: Nectar and pollen of flowering *Phacelia tanacetifolia*
 Tunnel tents (exposure): Within the test field tents (6.0 m × 10.5 m and a height of 2.3 m) were installed before moving the hives to the experimental field. The tent frames were covered with light plastic gauze. Before start of the test, paths were created in each tunnel by removal of the plants and smoothing the ground for walking inside the tunnel. The crop area per tent was approx. 40 m².
 Test age: Direct exposure of adults; indirect exposure of all stages of development
- Environmental conditions
 Temperature: 7.1 to 31.1°C
 Relative humidity: 61.9–100.0% (daily means)
 Photoperiod (exposure): Not reported

B. STUDY DESIGN AND METHODS

- Experimental start/completion
 30-July-2007 to 23-August-2007

- Experimental treatments

This study included three treatment groups (each with three tunnel tents). In all treatment groups the application was performed during honey bee flight. The application rate was 371 mL Indoxacarb 150 g/L EC per ha nominal (359 mL Indoxacarb 150 g/L EC per ha equivalent to 55.6 g a.s./ha, analysed). A second group was treated with tap water and served as control. As reference item "Perfekthion" (dimethoate) was applied at a rate of 1 L product/ha (equivalent to 400 g dimethoate a.s./ha, nominal) in the third group. All applications were carried out with a spray volume of 500 L/ha.

3. Observations

The effects of Indoxacarb 150 g/L EC were evaluated by comparing the results in the test item treatment to the data in the control treatment as well as in the reference item treatment regarding the following observations:

- Number of dead honey bees at the edge of the treated area (linen sheets) and in the dead honey bee traps in front of the hives.
- Foraging activity on the crop (number of forager honey bees/m²).
- Condition of the colonies and development of the honey bee brood.
- Behaviour of the honey bees in the crop area and around the hives.

II. RESULTS AND DISCUSSION

A. FINDINGS

1. Effects on honey bee mortality

During the pre-application period (from 4 days before application (DAA -4) until the day of application before treatment, (DAA 0ba)) a mean number of 13.0 dead honey bees/day was calculated in the Indoxacarb 150 g/L EC treatment group (T) compared to 15.7 dead honey bees/day in the control group (C) and 11.1 dead honey bees/day in the reference item treatment group (R) respectively.

On the day of treatment before application (DAA 0ba), the mean number of 18.0 dead honey bees was observed in the Indoxacarb 150 g/L EC group compared to 19.3 dead honey bees in the control and 18.0 dead honey bees in the reference item treatment group.

On the day of treatment after application (DAA 0aa), the mean number of dead honey bees in the Indoxacarb 150 g/L EC group increased to 159.0 dead honey bees. In the control treatment, a mean number of 11.3 dead honey bees were calculated while in the reference item treatment group, the mean number of dead honey bees increased to 512.0.

The mean post-application mortality (DAA 0aa to +7) was determined to be 42.3 dead honey bees/day in the Indoxacarb 150 g/L EC treatment group compared to 18.4 dead honey bees/day in the control group and 125.6 dead honey bees/day in the reference item treatment group. There were statistically significant differences between post-application mortality values of the Indoxacarb 150 g/L EC treatment group and the control group on DAA 0aa, +1 concerning the mean post-application mortality. Regarding post-application mortality values of the reference item group, there were significant differences compared to the control group on DAA 0aa, +1, +2, and +3 as to the mean post-application mortality (t-Test; $p < 0.05$).

Table 96
Mean number of dead honey bees/tent (linen sheets plus dead bee trap)

Date	DAA	Treatment groups		
		Indoxacarb 150 g/L EC (T)	Reference item (R)	Control (C)
31 July 07	-4	2.7	1.7	2.3
01 August 07	-3	20.3	14.3	27.7
02 August 07	-2	11.0	11.0	11.0
03 August 07	-1	13.0	10.3	14.7
04 August 07	0ba	18.0	18.0	19.3
Mean number of dead honey bees (DAA -4 to 0ba)		13.0	11.1	15.7
04 August 07	0aa	159.0*	512.0*	11.3
05 August 07	+1	56.0*	111.7*	7.0
07 August 07	+2	17.3	219.3*	11.7
07 August 07	+3	12.7	75.7*	10.3
08 August 07	+4	18.3	23.3	32.0
09 August 07	+5	29.0	18.3	28.3
10 August 07	+6	20.3	22.3	22.0
11 August 07	+7	26.0	22.0	24.7
Mean number of dead honey bees (DAA 0aa to +7)		42.3*	125.6*	18.4

DAA Days after application

0ba Mortality on the day of treatment before application

0aa Mortality assessed on the day of application after treatment

* Post-application period significantly different to control, t-test, $p < 0.05$

2. Effects on honey bee flight intensity

The daily mean flight intensity during the pre-application period was 15.1 honey bees/m² in the Indoxacarb 150 g/L EC treatment group (T), 14.0 honey bees/m² in the control treatment group (C), and 13.8 honey bees/m² in the reference item treatment group (R).

Shortly before application, a mean of 24.7 honey bees/m² was observed in the Indoxacarb 150 g/L EC treatment group. After the application, flight intensity slightly decreased to a mean of 16.3 honey bees/m². In the control treatment group, the mean flight intensity shortly before the application was 21.0 honey bees/m². After the application, the flight intensity was calculated with 26.7 honey bees/m². In the reference item treatment group, a mean flight intensity of 26.7 honey bees/m² was calculated shortly before the application which decreased to a mean of 5.8 honey bees/m², based on the values of the assessments on the day of treatment after the application.

From DAA +1 to +7, the flight intensity in the control group and in the Indoxacarb 150 g/L EC treatment group stayed on a comparable level and resulted in a mean flight intensity of 9.0 honey bees/m² in the control group and 7.2 honey bees/m² in the test item treatment group (DAA 0aa to +7). The flight intensity from DAA 0aa to DAA +7 was very low in the reference item treatment group (mean: 0.7 honey bees/m²). On DAA +3 to +7, the weather conditions were very bad with continuous rain, thus flight intensity recorded on these days was very low or zero.

Table 97
Flight intensity (mean number of honey bees/m²)

Date	DAA	Treatment groups		
		Indoxacarb 150 g/L EC (T)	Reference item (R)	Control (C)
31 July 07	-4	11.3	11.3	15.0
01 August 07	-3	12.3	10.3	8.7
02 August 07	-2	14.3	12.3	13.0
03 August 07	-1	12.7	8.3	12.3
04 August 07	0ba	24.7	26.7	21.0
Mean number of honey bees/m² (DAA -4 to 0ba)		15.1	13.8	14.0
04 August 07	0aa	16.3	5.8	26.7
05 August 07	+1	17.8	0.0	19.9
07 August 07	+2	17.8	0.0	22.2
07 August 07	+3	0.0	0.0	0.0
08 August 07	+4	0.0	0.0	0.0
09 August 07	+5	0.0	0.0	0.0
10 August 07	+6	0.0	0.0	0.0
11 August 07	+7	5.7	0.0	3.3
Mean number of honey bees/m² (DAA0aa to +7)		7.2	0.7	9.0

DAA Days after application

0ba Flight intensity on the day of treatment before application

0aa Flight intensity assessed on the day of application after treatment

3. Effects on honey bee brood development

The strength of the colonies (number of bee ways between combs filled with honey bees) in the colonies of the Indoxacarb 150 g/L EC treatment group decreased from 3.5 to 2.5 (Replicate 1 and 3) and from 3.5 to 2.0 (Replicate 2) over the three brood assessments carried out (one assessment on the day before placing the colonies in the tunnel tents and two assessments 1 and 3 weeks after treatment). The strength of the colonies in the colonies of the control treatment group decreased from 3.5 to 2.5 (Replicate 1 and 2) and 3.5 to 3.0 (Replicate 3) in the corresponding time. In the reference item treatment group the strength of the colonies decreased from 4.0 to 2.5 (Replicate 1) and from 3.5 to 2.0 (Replicate 2 and 3).

At all three brood assessments, all brood stages (egg, larval, and pupal stages) were observed in the colonies of all three treatment groups except in colony T2, where no pupae were recorded at the third assessment and except in colony R2 where no larval and pupal stage could be observed at the second brood assessment at the end of exposure. The changes recorded in the nine hives of the three treatment groups are regarded as normal and cannot be linked to the treatments.

4. Behavior of the honey bees

The colonies in the control treatment group showed no abnormal behaviour in the pre-application or post-application period. The behaviour of the bees of the Indoxacarb 150 g/L EC treatment group only differed on the day of application 2-6 hours after the application where bees were nervous and clustering at the hive entrance. In the reference item treatment group, abnormal honey bee behaviour (cramping bees in the trap, on the linen and on the net, clustering at the hive entrance) was noticed on the day of application after the treatment.

III. CONCLUSIONS

Indoxacarb 150 g/L EC, when applied at 371 mL Indoxacarb 150 g/L EC/ha nominal (359 mL Indoxacarb 150 g/L EC/ha equivalent to 55.6 g a.s./ha, analysed) to *Phacelia tanacetifolia* during bee flight, had no effects on honey flight intensity and brood development. Concerning mortality, an increase could be noticed on the day of application after application and on the following day.

(Gonsior, G., 2007b)

RMS comment

Study submitted to the EU for the first time in this submission.

This study is considered acceptable.

RMS notes that no pupae were seen at third assessment (19 DAA) in one replicate with Indoxacarb 150 g/L EC. Other development stages were observed and this absence of pupae is not considered treatment related. Effects on flight intensity and behaviour were seen on the day of application. RMS also notes that flight intensity was null between DAA3 and DAA6 due to bad weather. Indoxacarb 150 g/L EC, when applied at 371 mL Indoxacarb 150 g/L EC/ha nominal (359 mL Indoxacarb 150 g/L EC/ha equivalent to 55.6 g a.s./ha, analysed) to *Phacelia tanacetifolia* during bee flight, had no effects on brood development. Concerning mortality, an increase could be noticed on the day of application after application and on the following day.

Report: Giffard, H. (2008); DPX-KN128 150EC [150 g a.s./L (w/v)]: A semi-field study to evaluate effects on the honey bee (*Apis mellifera mellifera*; Hymenoptera, Apidae) on *Phacelia* in France 2007

DuPont Report No.: DuPont-19453

Guidelines: CEB 230 (November 2003) **Deviations:** None

Testing Facility: Testapi, Gennes, France

Testing Facility Report No.: 115-2007

GLP: Yes

Certifying Authority: Groupe Interministeriel des Produits Chimiques (GIPC) (Paris, France)

Executive summary:

A semi-field toxicity study on honey bees (*Apis mellifera mellifera* L.) in *Phacelia tanacetifolia* (var. Balo) was conducted with Indoxacarb 150 g/L EC in Gennes, in south-western France under CEB 230 (November 2003). The study consisted of four treatment groups: the water-treated control applied during bee flight, Indoxacarb 150 g/L EC at 0.333 L formulated product/ha (50 g a.s./ha) applied after bee flight, Indoxacarb 150 g/L EC at 0.333 L formulated product/ha (50 g a.s./ha) applied during bee flight, and toxic standard, 1 L Diméthyl 40EC (400 g diméthoate a.s./ha) applied during bee flight. All treatments were applied at 200 L/ha spray volume. One tent enclosing 140 m² was set up in each treatment and control area, and active honey bee colonies were established in the enclosures prior to application. A *Phacelia* crop area of 64 m² was available for foraging. Results of this study indicated that Indoxacarb 150 g/L EC applied during or after bee flight at a nominal rate of 0.333 L formulated product/ha (50 g a.s./ha) to *Phacelia* did not have a harmful effect on honey bees.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Indoxacarb 150 g/L EC
Lot/Batch #: KN128-206
Purity: 150 g a.s./L
CAS#: None for the formulation
173584-44-6 for indoxacarb active substance
Description: Deep brown liquid
Stability of test compound: 100% of the indoxacarb remains in the delivery vehicle after one hour under agitation
2. Vehicle and/or positive control: Tap water
Toxic reference Diméthyl 40EC (dimethoate a.s.)
3. Test organism
Species: *Apis mellifera* L.
Age at dosing: Direct exposure of adults; indirect exposure of all stages of development
Source: Supplied by a professional beekeeper, Meli-Bocage, in St. Michel
Crop: *Phacelia tanacetifolia* (var. Balo)
Water: Water was supplied in a container filled with 0.3 L water
Tunnel tents (exposure): Tunnel tent: tents (7.0 m × 20.0 m and a height of 3.5 m) placed over plots of *Phacelia tanacetifolia* were made of iron and covered with an insect proof net; 1 replicate tunnel tent per treatment.
Crop area: 4 crop plots per tent; crop plots (2 m × 8 m) furnished 64 m² to bees to forage on *Phacelia tanacetifolia*.
Plastic sheet: The remaining surface was covered with plastic sheets for the assessment of dead bees in the tunnels.
4. Environmental conditions
Temperature: 8 to 28°C
Relative humidity: Not reported
Photoperiod (exposure): natural light conditions

B. STUDY DESIGN AND METHODS

1. Experimental start/completion
28-April-2007 to 10-May-2007
2. Experimental treatments
A semi-field toxicity test on honey bees (*Apis mellifera mellifera* L.) in *Phacelia tanacetifolia* (var. Balo) was conducted with Indoxacarb 150 g/L EC in Gennes, in south-western France. The study consisted of four treatments: the water-treated control, Indoxacarb 150 g/L EC applied during bee flight at 0.333 L formulated product/ha (equivalent to 50 g a.s./ha), Indoxacarb 150 g/L EC applied after bee flight at 0.333 L formulated product/ha (equivalent to 50 g a.s./ha), and the toxic reference treatment, Diméthyl 40EC applied at 1 L product/ha (equivalent to 400 g dimethoate active substance/ha). All applications were carried out with a volume of 200 L/ha. Toxic reference and control tunnels were common with Testapi study 114-2007 conducted in the same conditions in the same time. The effects of the Indoxacarb 150 g/L EC treatment were examined on small honey bee colonies in tunnel tents placed over plots of *Phacelia tanacetifolia*. One replicate was used per treatment (small healthy colonies with 6 combs).
3. Observations
Mortality: The numbers of dead honey bees within the tunnel were assessed daily on 6 plastic sheets (lanes) starting 3 days before application to 5 days after application.

Foraging activity: All forager honey bees were counted daily on the 4 crop plots of each tunnel.

Colony assessment: Two apiarist visits were conducted. At the beginning and at the end of the experimental phase the bee colonies were inspected in order to assess the presence of a healthy queen and eggs, and the number of combs with brood and honey.

Behavioural observations: Any abnormal behaviour was recorded

4. Statistics

The data did not warrant statistical analysis since only one replicate of each treatment and control was used.

II. RESULTS AND DISCUSSION

A. FINDINGS

Effects on honey bee mortality:

The daily honey bee mortalities were quite homogeneous and decreased from Day -4 to Day -1. The control mortality was very stable over the entire study period after spray application with about 100 to 200 dead honey bees per day validating the test. The toxic reference (400 g dimethoate/ha) induced a high peak of mortality the day after spray application proving the sensitivity of the test system. The impact of the toxic reference treatment on the mortality was very high. The counts of dead honey bee performed the evening at Day 0aa (after application) and the following morning (Day +1) summed up to the total of 2164 individuals. Also at Day +2 a strong effect (546 dead honey bees) was determined in the toxic reference treatment.

When Indoxacarb 150 g/L EC was applied at 0.333 L formulated product/ha during bee flight a slight increase in mortality was recorded on the following day after spray application (Day +1) from 174 to 690 dead honey bees. This effect is nevertheless limited on time as the mortality level recorded on D+2 was similar to the day before application (263 dead honey bees).

When Indoxacarb 150 g/L EC was applied at 0.333 L formulated product/ha after daily bee flight, similar numbers of dead honey bees were found as in the control tunnel. Over the entire observation period the numbers of dead honey bees in control and the Indoxacarb 150 g/L EC treatment applied out of bee flight showed the same pattern. No impact on mortality followed the spray application of Indoxacarb 150 g/L EC, and the mortality data were stable over the whole trial period.

Over the whole post-treatment period, Indoxacarb 150 g/L EC application after daily bee flight induced no peak of mortality whereas it induced a slight increase for one day when applied during bee flight.

The calculated toxicity index for the day of application until Day +1 after application of 14.2 confirms the high toxicity of toxic reference, Diméthyl 40EC, relative to the control (toxicity index = 1). In contrast the calculated toxicity indices for both Indoxacarb 150 g/L EC treatments (application during bee flight and application after bee flight) were 3.9 and 0.9, respectively. Effects of the spray application after bee flight were close to the water control demonstrating the similarity of effects observed in the Indoxacarb 150 g/L EC treatment and the water control.

Table 98
Honey bee mortality

Date	Day	Treatment groups (number of dead bees/tunnel)			
		Indoxacarb 150 g/L EC treatment after bee-flight	Indoxacarb 150 g/L EC treatment during bee-flight	Toxic standard, Dimézyt 40EC	Control
30 April 2007	-4	176	373	200	354
1 May 2007	-3	63	148	111	86
2 May 2007	-2	43	130	92	125
3 May 2007	-1	45	149	87	95
4 May 2007	0ba	50	174	151	122
4 May 2007	0aa	NA	142	777	61
5 May 2007	+1	51	548	1387	62
6 May 2007	+2	35	263	546	122
7 May 2007	+3	33	108	378	168
8 May 2007	+4	62	47	131	65
9 May 2007	+5	48	60	150	88
10 May 2007	+6	84	81	237	244
Toxicity index $i_{tox} (Day\ 0ba - Day\ 1aa)$		0.9	3.9	14.2	1.0

Day = days relative to application

NA: not applicable

Day 0ba = Day of treatment before application

Day 1aa = Day 1 after application of test item

$i_{tox}(0\ ba-1aa)$ = toxicity index (Day 0ba – Day 1aa): Evolution quotient of the daily mean mortality after/before treatment in the treated tunnel.

$$i_{tox} = \frac{Qm}{Qt} = \frac{Mt}{Ma} \times \frac{Ta}{Tt} = \frac{Mt \times Ta}{Ma \times Tt}$$

Qm = Mortality evolution after/before treatment in the test item tunnel

Qt = Mortality evolution after/before treatment in the water control tunnel

Mt = daily mortality in the test item tunnel after treatment

Ma = daily mortality in the test item tunnel before treatment

Tt = daily mortality in the water control tunnel after treatment

Ta = daily mortality in the water control tunnel before treatment

Effects on honey bee foraging activity:

The daily mean honey bee foraging activity during the pre-application period varied between 6 to 20 honey bees/m² in the 4 different tents. The honey foraging activity was above the required level of >5 bees/m² in all treatments.

During the day of spray application (Day 0ba to Day 0aa) the honey bee foraging activity stayed at about the same level in the control and Indoxacarb 150 g/L EC tunnel to be treated out of bee flight. This activity level slightly decreased in Indoxacarb 150 g/L EC tunnel treated during bee flight, from 14.7 to 9.9 bees/m². On the contrary, in the reference tunnel, the foraging activity dropped close to zero in the afternoon after spray application and almost no honey bees were foraging any longer.

In Indoxacarb 150 g/L EC tunnels treated during bee flight, the foraging activity remained stable during the whole post-treatment period of the trial, with 8 to 12 foraging honey bees per m². The number of forager bees in this treatment was close to the control group from Day +1 to Day +3. In both Indoxacarb 150 g/L EC tunnels treated during and after bee flight, the foraging activity increased during Day +1 in a similar way as the control tunnel. On Day +3 the foraging activity decreased slightly in the control tunnel, while it increased in both Indoxacarb 150 g/L EC tunnels treated during and after bee flight. On the

contrary in the reference tunnel the foraging activity stayed zero until the end of the experimental recording phase (Day +1 to Day +3). The calculated forager mortality indices for the day of application (before application) until Day +1 after application for the water control according the two ways of calculation (option 1 or option 2) were 0 and 1, respectively. The calculated forager mortality indices for the Indoxacarb 150 g/L EC treatment that was sprayed during bee flight were 0.6 and 256.8, respectively, demonstrating the limited effects observed in the Indoxacarb 150 g/L EC treatment compared with the water control. In contrast the forager mortality indices for the toxic reference, Diméthyl 40EC, were 2.6 and 1206.8, respectively. These calculated values demonstrate the difference between the control and the toxic reference, dimethoate, and the similarity of effects between Indoxacarb 150 g/L EC and control.

Table 99
Foraging activity

Date	Day	Treatment Groups (mean number of honey bees/m ²)			
		Indoxacarb 150 g/L EC treatment after bee-flight	Indoxacarb 150 g/L EC treatment during bee-flight	Toxic standard, Diméthyl 40EC	Control
30 April 2007	-4	6.7	7.9	7.2	6.2
1 May 2007	-3	7.4	8.6	10.9	7.4
2 May 2007	-2	10.0	14.2	15.3	12.0
3 May 2007	-1	12.6	19.8	16.7	12.0
4 May 2007	0ba	9.2	14.7	12.2	7.3
4 May 2007	0aa	9.0	9.9	0.7	8.5
5 May 2007	+1	11.2	11.7	0.1	10.1
6 May 2007	+2	7.7	8.4	0.0	9.7
7 May 2007	+3	9.6	11.9	0.0	7.9

Table 100
Forager mortality index

Forager Mortality Index (I)	Treatment groups (mean number of honey bees/m ²)			
	Indoxacarb 150 g/L EC treatment after bee-flight	Indoxacarb 150 g/L EC treatment during bee-flight	Toxic standard, Diméthyl 40EC	Control
i _F option 1 (Day 0ba – Day 1aa)	NA	0.6	2.6	0.0
i _F option 2 (Day 0ba – Day 1aa)	NA	256.9	1206.8	1.0

Day 0ba = day of treatment before application

Day 0aa = in the evening after application of test item

NA: not applicable

$$iF_{\text{option 1}} = \frac{Mt - Ma}{Nm} \times \frac{Nt}{Tt - Ta}$$

$$iF_{\text{option 2}} = \frac{Mt - Ma}{Nm}$$

Mt = Number of dead bees in the test item tunnel after treatment

Ma = Number of dead bees in the test item tunnel before treatment

Nm = number of foraging bees in the test item tunnel before treatment

Nt = number of foraging bees in the water control tunnel before treatment

Effects on Honey Bee Brood Development:

Only minor changes were detected between the two colony assessments at the beginning and end of the experimental phase. Due to the attractiveness of the *Phacelia* crop, honey bees foraged actively and so the colonies had about the same level of food frame storage at the end of the test compared to the first assessment at the beginning of the study. The proportion of different brood stages remained stable in all colonies.

The two colony assessments before and after application did not show any significant changes due to the test item, Indoxacarb 150 g/L EC, applied during or after bee flight relative to the control. The adult honey bee population slightly decreased in all four colonies, due to the limited foraging resources under the insect-proof tunnels.

Honey Bee Behaviour:

No symptoms of poisoning or abnormal behaviour were recorded during the whole trial period in either of the Indoxacarb 150 g/L EC treatment groups, relative to the water control.

III. CONCLUSIONS

It was concluded that, in these experimental conditions, Indoxacarb 150 g/L EC applied during and after bee flight at 0.333 L formulated product/ha (equivalent to 50 g a.s./ha) did not have a harmful effect on honey bees.

(Giffard, H., 2008)

RMS comment

Study submitted to the EU for the first time in this submission.

This study was conducted according to the French guideline CEB 230 and is in compliance with the EPPO recommendations but with only one replicate for each treatment. This study is considered acceptable.

The effects of Indoxacarb 150 g/L EC were tested when applied during and after bee flight.

RMS notes that the mortality in the tunnel treated during foraging activity was increased from the day of application to DAA+2. Foraging also appears lower on the day of application after the treatment (when the product is applied during the foraging activity).

No effects were observed when Indoxacarb 150 g/L EC is applied after the bee flight (in evening).

No raw data was available for bee brood development. No effect due to treatment was observed on bee brood but the evaluation period is too short to be conclusive.

Report: Gonsior, G. (2008a); Indoxacarb (DPX-KN128) 150 g/L EC: A semi-field study to evaluate effects on the honey bee (*Apis mellifera carnica*; Hymenoptera, Apidae) in *Phacelia tanacetifolia* in Alsace, France 2007

DuPont Report No.: DuPont-21945

Guidelines: CEB 230 (November 2003), EPPO 170 **Deviations:** None

Testing Facility: eurofins-GAB GmbH, Niefern-Oeschelbronn, Germany

Testing Facility Report No.: 20071084/F3-BZEU

GLP: Yes

Certifying Authority: Ministerium für Umwelt und Verkehr (Baden-Württemberg, Germany)

Executive summary:

The effects of the test item Indoxacarb 150 g/L EC were tested on the honey bee (*Apis mellifera carnica* L.) under semi-field conditions.

This study was conducted in Drusenheim in northern France (region: Alsace) in July 2007 and included four treatment groups (one tunnel tent each). Indoxacarb 150 g/L EC was applied in the evening after bee-flight and on the following day during bee-flight in separated tunnel tents. The effects of the test item treatment were examined on honey bee colonies in tunnel tents (5.0 m × 20.0 m and a height of 3.5 m) placed over plots of *Phacelia tanacetifolia*. The semi-field test comprised 1 replicate tunnel tent in each of the treatments (test item treatment after and during bee-flight, reference item and control).

The influence of Indoxacarb 150 g/L EC was evaluated by comparing the results in the test item treatments K1 (applied after bee-flight) and K2 (applied during bee-flight) to the data in the control treatment as well as in the reference item treatment regarding the following observations:

- Number of dead honey bees at the edge of the treated area (linen sheets) and in the dead honey bee traps in front of the hives.
- Foraging activity on the crop (number of forager honey bees/m² wheat).
- Condition of the colonies and development of the honey bee brood.
- Behaviour of the honey bees in the crop area and around the hives.

It can be concluded that Indoxacarb 150 g/L EC applied at 371 mL formulated product/ha (equivalent to 55.6 g a.s./ha) to *Phacelia tanacetifolia* after and during bee flight has no effect on honey bee flight intensity, behaviour and brood development. The number of dead honey bees increased after application of Indoxacarb 150 g/L EC during bee-flight relative to the control treatment on the day of application (DAA 0aa). When Indoxacarb 150 g/L EC was applied after bee-flight the number of dead honey bees increased on the following day (DAA 0).

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Indoxacarb 150 g/L EC
Lot/Batch #: KN128-206
Purity: 150 g a.s./L
Description: Dark brown liquid
CAS#: None for the formulation
173584-44-6 for indoxacarb active substance
Stability of test compound: 99.7% of the indoxacarb remains in the delivery vehicle after one hour under agitation
2. Vehicle and/or positive control: Tap water
3. Test organism
Species: *Apis mellifera* L.
Age at dosing: Direct exposure of adults; indirect exposure of all stages of development
Source: Beekeeper, Berthold Nengel (Dahlheim, Germany).
Diet: Nectar and pollen of flowering *Phacelia tanacetifolia*
Tunnel tents (exposure): Within the test field tents (5.0 m × 20.0 m and a height of 3.5 m) were installed soon before moving the hives to the experimental field. The tent frames were covered with light plastic gauze. Before start of the test, paths were created in each tunnel by removal of the plants and smoothing the ground. The crop area per tent was approx. 68 m². Subsequently, the paths (approx. 32 m²) were covered with linen sheets for the assessment of dead bees in the crop area.
Test age: Direct exposure of adults; indirect exposure of all stages of development
4. Environmental conditions
Temperature: 9.8 to 34.0°C
Relative humidity: 31 to 99%
Photoperiod (exposure): natural light conditions

B. STUDY DESIGN AND METHODS

1. Experimental start/completion
07-July-2007 to 20-July-2007
2. Experimental treatments
The application rate was 371 mL formulated product/ha (equivalent to 55.6 g a.s./ha). A third group (tunnel) treated with tap water served as the control. As reference item, "Perfekthion" (dimethoate) was applied at a rate of 1 L product/ha. The applications in the control and reference item treatment were carried out during daily bee-flight. All applications were carried out with a rate of 300 L water/ha.

The effects of the test item treatment were examined on small honey bee colonies in tunnel tents (5.0 m × 20.0 m and a height of 3.5 m) placed over plots of *Phacelia tanacetifolia*. The semi-field test comprised 1 replicate tunnel tent in each of the treatments (test item treatment after and during bee-flight, reference item and control).
3. Observations
The influence of Indoxacarb 150 g/L EC was evaluated by comparing the results in the test item treatments K1 (applied after bee-flight) and K2 (applied during bee-flight) to the data in the control treatment as well as in the reference item treatment regarding the following observations:

- Number of dead honey bees at the edge of the treated area (linen sheets) and in the dead honey bee traps in front of the hives.
- Foraging activity on the crop (number of forager honey bees/m² *P. tanacetifolia*).
- Condition of the colonies and development of the honey bee brood.
- Behaviour of the honey bees in the crop area and around the hives.

II. RESULTS AND DISCUSSION

A. FINDINGS

1. Effects on Honey Bee Mortality

During the pre-application period (DAA-4 to DAA-1ba/0ba) a mean number of 174.5 dead honey bees/day was calculated in the Indoxacarb 150 g/L EC treatment group applied after bee-flight (K1) and 40.5 dead honey bees/day in the Indoxacarb 150 g/L EC treatment group applied during bee-flight (K2). During the same time period the control group showed a daily mean mortality of 31.6 dead honey bees/day and the reference item treatment group 64.0 dead honey bees/day.

On the day of application during bee-flight (DAA0aa) a total mortality of 310 dead honey bees/day was observed in the Indoxacarb 150 g/L EC group with application after bee-flight (K1). During the same time period the total mortality of the treatment group where the test item (Indoxacarb 150 g/L EC) was applied during bee-flight (K2) was recorded with 315 dead honey bees. In the control treatment 30 dead bees were counted within the same time. The reference item was with 1086 dead honey bees clearly above the level of both test item treatments.

On the following assessment days (DAA+1 to +5) the daily total mortality was in the range of 62 to 172 dead honey bees/day in the test item treatment with application after bee-flight (K1) and of 15 to 36 dead honey bees/day in the treatment group where Indoxacarb 150 g/L EC was applied during bee-flight (K2). Whereas the daily total number of dead honey bees in control group varied between 6 and 32 dead honey bees/day and between 151 and 466 dead honey bees/day in the reference item treatment. However, the highest value of the daily total mortality in the test item treatment group with application after bee-flight (K1) was observed on the day after application, the first day of exposure to the test item, DAA 0 with 310 dead honey bees/day, followed by DAA +2 with 172 dead honey bees/day. In the test item treatment with application during bees flight the highest value was recorded on the day of application (DAA0) where 315 dead honey bees/day were counted. In the reference item treatment the highest number of dead honey bees also was recorded on the day of application (DAA 0) with 1086 dead honey bees/day followed by the value of DAA +2 with 466 dead honey bees/day.

The mean post-application mortality was determined to be 141.8 dead honey bees/day in the treatment group where test item was applied after bee-flight (K1), 72.3 dead honey bees/day with Indoxacarb 150 g/L EC application during bee-flight (K2), 21.3 dead honey bees/day in the control and 372.3 dead honey bees/day in the reference item treatment.

Table 101
Honey bee mortality

Date	DAA	Treatment groups (total number of dead honey bees/tent)			
		Indoxacarb 150 g/L EC; Treatment after bee-flight (K1)	Indoxacarb 150 g/L EC; Treatment during bee-flight (K2)	Reference item	Control
10 July 07	-4	133	18	30	12
11 July 07	-3	188	59	76	34
12 July 07	-2	121	38	74	28
13 July 07	-1	181	11	55	11
13/14 July 07	-1ba/0ba	75	47	85	73
14 July 07	0aa	310	315	1086	30
15 July 07	+1	62	28	151	6
16 July 07	+2	172	36	466	13
17 July 07	+3	99	24	158	15
18 July 07	+4	95	16	161	32
19 July 07	+5	113	15	212	32
Mean number of dead honey bees (DAA-4 to DAA-1ba/0ba)		174.5	40.5	64.0	31.6
Mean number of dead honey bees (DAA0aa to +5)		141.8	72.3	372.3	21.3
Toxicity index $i_{tox(0aa\ to\ +1)}$		10.1	14.8	29.5	--
Toxicity index $i_{tox(0aa\ to\ +5)}$		6.5	5.3	15.0	--

DAA Days after application during bee-flight

ba Mortality before application

aa Mortality after application

The toxicity index for day of application during bee-flight ($i_{tox(0aa\ to\ +1)}$) was calculated to 10.1 for the Indoxacarb 150 g/L EC applied after bee-flight (K1) and 14.8 for the treatment group with application during bee-flight (K2). In the reference item a toxicity index of 29.5 was calculated. The calculation of the toxicity index for day of application till day five after application during bee flight ($i_{tox(0aa\ to\ +5)}$) showed a value for Indoxacarb 150 g/L EC applied after bee-flight (K1) of $i_{tox(0aa\ to\ +5)} = 6.5$ and for the test item application during bee-flight (K2) of $i_{tox(0aa\ to\ +5)} = 5.3$. For the reference item the $i_{tox(0aa\ to\ +5)}$ value was 15.0.

2. Effects on Honey Bee Flight Intensity

The daily mean flight intensity during the pre-application period was 12.7 honey bees/m² in the Indoxacarb 150 g/L EC treatment with application after bee-flight (K1), 12.2 honey bees/m² in the test item treatment with application during bee-flight (K2), 12.5 honey bees/m² in the reference item treatment and 10.0 honey bees/m² in the control treatment.

Shortly before application during bee-flight a mean of 14.8 honey bees/m² was observed in the Indoxacarb 150 g/L EC treatment K2, compared to a mean of 15.2 honey bees/m² across the assessments on that day after the application. In Indoxacarb 150 g/L EC treatment K1 with application after bee-flight on the evening before, mean flight intensity on DAA 0 was recorded with 17.2 honey bees/m². The mean flight intensity on the day of application during bee-flight before the application was calculated as 14.3 honey bees/m² in the control, compared to the mean of 16.2 honey bees/m² based on the values of all assessments on that day after treatment. In the reference item treatment a mean of 20.3 honey bees/m² was observed shortly before the start of application. After the application the flight intensity in the reference item treatment decreased over the time of assessments and resulted in a mean of 4.1 honey bees/m².

From DAA+1 to +5 the flight intensity in the Indoxacarb 150 g/L EC treatment K1 ranged between 18.1 and 1.3 (DAA +5) honey bees/m² and in treatment K2 between 10.6 and 1.5 (DAA +5) honey bees/m². In the control the highest value was recorded with 20.1 and the lowest with 3.8 honey bees/m² (DAA +5). In the reference item treatment from DAA+1 till DAA+5, low or no flight intensity was observed. On DAA+5 the weather conditions were cloudy and rainy, thus the flight intensity on that day was low throughout all treatments.

The daily mean post-application level of flight intensity (DAA 0aa to +5) was 11.7 honey bees/m² in the Indoxacarb 150 g/L EC treatment with application after bee-flight (K1), 8.6 honey bees/m² in the Indoxacarb 150 g/L EC treatment with application during bee-flight (K2), 13.2 honey bees/m² in the control treatment and 0.7 honey bees/m² in the reference item treatment.

Table 102
Flight intensity

Date	DAA	Treatment groups (mean number of honey bees/m ²)			
		Indoxacarb 150 g/L EC; Treatment after bee-flight (K1)	Indoxacarb 150 g/L EC; Treatment during bee-flight (K2)	Reference item	Control
10 July 07	-4	13.3	8.5	15.3	7.0
11 July 07	-3	18.8	17.5	17.5	18.0
12 July 07	-2	7.3	13.5	4.0	6.0
13 July 07	-1	11.5	6.5	5.5	4.5
13/14 July 07	-1ba/0ba	0.0 ^a	14.8	20.3	14.3
14 July 07	0aa	17.2	15.2	4.1	16.2
15 July 07	+1	18.1	8.2	0.0	17.6
16 July 07	+2	15.1	10.6	0.1	20.1
17 July 07	+3	9.4	7.3	0.0	12.0
18 July 07	+4	9.0	6.4	0.0	9.5
19 July 07	+5	1.3	1.5	0.0	3.8
mean number of honey bees/m ² (DAA-4 to DAA-1ba/0ba)		12.7	12.2	12.5	10.0
mean number of honey bees/m ² (DAA0aa to +5)		11.7	8.6	0.7	13.2
i_{for1} (0aa to +1)		-9.9	-7.7	-21.9	1.0
i_{for2} (0aa to +1)		25.8	20.0	56.7	-2.6
i_{for1} (0aa to +5)		-1.6	-0.5	-3.9	1.0
i_{for2} (0aa to +5)		5.8	1.7	14.2	-3.6

DAA Days after application during bee-flight

ba Flight intensity before application

aa Flight intensity after application

^a Assessment performed after bee-flight and excluded from further evaluation

The forager mortality index for day of application ($i_{for1(0aa\ to\ +1)}$) was calculated with -9.9 for the Indoxacarb 150 g/L EC applied after bee-flight (K1), with -7.7 for the Indoxacarb 150 g/L EC applied during bee-flight (K2) and -21.9 for the reference item group. The forager mortality index i_{for2} for day of application ($i_{for2(0aa\ to\ +1)}$) was calculated with 25.8 for the Indoxacarb 150 g/L EC applied after bee-flight (K1), with 20.0 for the Indoxacarb 150 g/L EC applied during bee-flight (K2) and 56.7 for the reference item group.

Regarding the forager mortality index beginning from day of application of the Indoxacarb 150 g/L EC group (K2) until Day +5 (DAA0aa to DAA+5: $i_{for1(0aa\ to\ +5)}$) a value for Indoxacarb

150 g/L EC applied after bee-flight (K1) with $i_{\text{for1}(0\text{aa to } +5)} = -1.6$ and for the test item application during bee-flight (K2) with $i_{\text{for1}(0\text{aa to } +5)} = -0.5$ was calculated. For the reference item the $i_{\text{for1}(0\text{aa to } +5)}$ value was -3.9.

Regarding the forager mortality index i_{for2} beginning from day of application of the test item group (K2) until Day +5 (DAA0aa to DAA+5: $i_{\text{for2}(0\text{aa to } +5)}$) a value for Indoxacarb 150 g/L EC applied after bee-flight (K1) with $i_{\text{for2}(0\text{aa to } +5)} = 5.8$ and for the test item application during bee-flight (K2) with $i_{\text{for2}(0\text{aa to } +5)} = 1.7$ was calculated. For the reference item the $i_{\text{for2}(0\text{aa to } +5)}$ value was calculated with 14.2.

Honey bee brood

The strength of the colonies (no. of bee ways between combs filled with honey bees) in both colonies of Indoxacarb 150 g/L EC treatments and in the colonies of the control and the reference item treatment decreased from the brood assessment carried out before placing the colonies in the tunnel tents to the assessment after end of exposure of the honeybees (K1: 8.0 to 6.0; K2: 7.0 to 6.0; C: 7.0 to 6.0 and R even from 9.0 to 6.0).

At the brood assessments carried out before application all brood stages (egg stage, larval and pupal stage) in the colonies of all treatment groups were available and the colonies contained 6-8 combs covered with brood. After the end of exposure in all treatment groups the number of combs covered with brood ranged from 4-6. Only in the reference item group no larvae were recorded at the second brood assessment.

In the assessment carried out after end of exposure of the bees in the tunnel tents all colonies of the treatment groups K1, K2 and the control had sufficient pollen and nectar resources.

3. Effects on Honey Bee Behavior

Within the pre-application period all colonies of all treatment groups showed (DAA -4 to DAA +5) normal behaviour of the bees. After the application during bee-flight in test item treatment K2, very few honey bees (5) were observed on the linen, being unable to fly away (2-6 hours after application). In comparison the bees of the reference item treatment showed abnormal behaviour from shortly after the application up to the first assessment on DAA +1 (hanging bees on the flowers, cramping bees, clustering at the bee hive entrance). The bees of the Indoxacarb 150 g/L EC treatment group with application after bee-flight as well as the bees of the control treatment showed normal behaviour throughout the five days of the post-application period.

III. CONCLUSIONS

It can be concluded that Indoxacarb 150 g/L EC applied at 371 mL formulated product/ha (equivalent to 55.6 g a.s./ha) to *Phacelia tanacetifolia* after and during bee-flight has no effect on honey bee flight intensity, behaviour and brood development. The number of dead honey bees increased after application of Indoxacarb 150 g/L EC during bee-flight relative to the control treatment on the day of application (DAA 0aa). When Indoxacarb 150 g/L EC was applied after bee-flight the number of dead honey bees increased on the following day (DAA 0).

(Gonsior, G., 2008a)

RMS comment

Study submitted to the EU for the first time in this submission.

This study is considered acceptable.

The effects of Indoxacarb 150 g/L EC were tested when applied during and after bee flight (One tent per treatment group).

RMS notes that the mortality in the tunnel treated after the foraging activity was higher before the application than in other tunnels. The mortality in control increased just before the application. RMS doubts the reliability on index toxicity values.

Indoxacarb 150 g/L EC applied at 371 mL formulated product/ha (equivalent to 55.6 g a.s./ha) to *Phacelia tanacetifolia* after bee-flight has no effect on honey bee flight intensity and behaviour.

Foraging decreased during 4 days after the treatment when the product was applied during the foraging activity. Only slight effect on behaviour when Indoxacarb 150 g/L EC is applied during bee flight.

When Indoxacarb 150 g/L EC was applied after bee-flight the number of dead honey bees increased on the following day (DAA 0). However RMS notes that mortality was higher before application than during the days after the application. Besides, mortality in this tunnel was also much higher than in the control tunnel. This tunnel is not considered reliable by RMS.

The number of dead honey bees increased after application of Indoxacarb 150 g/L EC during bee-flight relative to the control treatment on the day of application (DAA 0aa). No effect due to treatment was observed on bee brood but the evaluation period is too short to be conclusive.

Report: Berg, C. (2013); Indoxacarb (DPX-KN128) 150 g/L EC: A semi-field study to evaluate effects on the honey bee (*Apis mellifera*; Hymenoptera, Apidae) in maize (*Zea mays*) in Germany 2013

DuPont Report No.: DuPont-37487

Guidelines: EPPO 170 **Deviations:** None

Testing Facility: Eurofins Agrosience Services GmbH, Niefern-Oeschelbronn, Germany

Testing Facility Report No.: S13-00879

GLP: Yes

Certifying Authority: Landesanstalt Fur Umwelt, Messungen Und Naturschutz Baden-Wurtemberg

Executive summary:

The effects of the test item Indoxacarb 150 g/L EC were tested on the honey bee (*Apis mellifera* L.) under semi-field conditions following the OEPP/EPPO guideline 170 (4), 2010. The field phase of this study was conducted in Pforzheim in southern Germany (region: Baden-Württemberg) in *Zea mays* in September to October 2013 and included a total of four treatment groups:

- Indoxacarb 150 g/L EC treatment group T1 with one application of the test item in each replicate (tunnel tent). The application was performed during flowering of *Zea mays* (BBCH 63-65) on 05 September 2013, 3 days after set-up of the bee hives in the tunnels in the evening, after bee-flight. The application was carried out at a target rate of 37.5 g indoxacarb/ha.
- Indoxacarb 150 g/L EC treatment group T2 with one application of the test item in each replicate (tunnel tent). The application was performed during flowering of *Zea mays* (BBCH 63-65) on 06 September 2013, 4 days after set-up of the bee hives in the tunnels and during daily honey bee flight. The application was carried out at a target rate of 37.5 g a.s./ha.
- Reference item treatment group R with one application of BAS 152 11 I (a.s. dimethoate) at a target rate of 1000 mL product/ha (equivalent to 400 g dimethoate./ha nominal) during flowering of *Zea mays* and daily honey bee flight, on the same day as the application in the treatment group T2 and in the control.
- Control group C with one application of tap water during flowering (BBCH 63-65) of *Zea mays* and daily honey bee flight, on the same day as the applications in the treatment group T2 and the reference item treatment group R.

All applications were carried out with a target spray volume of 400 L water per ha.

The effects of the test item treatments were examined on honey bee colonies in tunnel tents (5.0 m × 40.0 m and a height of 3.5 m in the centre) placed over plots of *Zea mays*. The semi-field test comprised 3 replicate tunnel tents in each treatment group.

The influence of Indoxacarb 150 g/L EC was evaluated by comparing the results in the test item treatment group T1 (applied after honey bee flight) and T2 (applied during honey bee flight) to the data in the control treatment as well as in the reference item treatment regarding the following observations:

- Number of dead honey bees within the crop area (on the linen sheets) and in the dead honey bee trap at the entrance of the hives,
- Flight intensity on the crop (number of forager honey bees per 30 plants),
- Counting of returning forager bees with maize pollen at the entrance of the hives,
- Condition of the colonies and development of the brood,
- Behaviour of the honey bees in the crop area and around the hives.

Indoxacarb 150 g/L EC, applied once during flowering of *Zea mays* in the evening after honey bee flight (treatment T1) or during daily honey bee flight (treatment T2) had no effect on honey bee flight activity, brood development and colony condition in both test item treatments T1 and T2.

There was a slight increase in honey bee mortality in the Indoxacarb 150 g/L EC treatment T1 on the day of application and considering the post-application period from 0DAA to 7DAA. There was an increased daily mortality from 0DAA to 3DAA and considering the whole post-application period in the Indoxacarb 150 g/L EC treatment T2.

Additionally, there was a slight effect on behaviour in the treatment group T1 and an effect on behaviour in the treatment group T2.

I. MATERIAL AND METHODS**A. MATERIALS:**

1. Test material:
Lot/Batch#: DPX-KN128-311
Purity: 150 g a.s./L
Description: Liquid
CAS#: 173584-44-6 for the active substance
None for the formulation
Stability of test compound: 98.2% of the indoxacarb remains in the delivery vehicle after one hour under agitation
Reference item: BAS 152 11 I (a.s. dimethoate)
Lot/Batch: 0001017331
Purity: 400 g/L
Description: BAS 152 11 I (dimethoate)
CAS#: 60-51-5
Stability in solution: Sufficient for test purpose (at least 1 hour)
2. Vehicle and/or control: Tap water
3. Test organism
Species: *Apis mellifera* L.
Age at dosing: Direct exposure of adult honey bees; indirect exposure of all stages of development.
Source: Karl-Heinz Baur, Lautenbach 5, 77955 Ettenheim, Germany
Diet: Pollen of flowering *Zea mays* and additional feeding of maltose solution on 5DAA during the exposure period in the tunnels.
Tunnel tents (exposure): Within the test field 12 plots (each 5.0 m × 40.0 m) were marked and labelled before set-up in the tunnel tents. Paths (0.6 m) were made in each plot by removing the plants and smoothing the ground. Before set-up of the bee colonies, tunnel tents (5.0 m × 40.0 m and a height of 3.5 m in the centre) were installed over the marked plots. The tent frames were covered with light plastic gauze. The paths (approx. 29.3 m²) were covered with linen sheets for the assessment of dead bees in the crop area. The crop area per tent was 170.72 m².
4. Environmental conditions during the exposure period (03 Sep – 13 Sep 2013):
Temperature (min/max): 10.1–29.7°C
Relative humidity (min/max): 39.1–100.0%
Photoperiod (exposure): natural light conditions

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
02-September-2013 to 04-October-2013
2. Experimental treatments
 - Indoxacarb 150 g/L EC treatment group T1 with one application of the test item in each replicate (tunnel tent). The application was performed during flowering (BBCH 63-65) on 05 September 2013, 3 days after set-up of the bee hives in the tunnels in the evening, after bee-flight. The application was carried out at a target rate of 37.5 g a.s./ha.
 - Indoxacarb 150 g/L EC treatment group T2 with one application of the test item in each replicate (tunnel tent). The application was performed during flowering (BBCH 63-65) on 06 September 2013, 4 days after set-up of the bee hives in the tunnels and during daily honey bee flight. The application was carried out at a target rate of 37.5 g a.s./ha.

- Reference item treatment group R with one application of BAS 152 11 I (a.s. dimethoate) at a target rate of 1000 mL product/ha (equivalent to 400 g dimethoate/ha nominal) during flowering and daily honey bee flight, on the same day as the application in the treatment group T2 and in the control.
- Control group C with one application of tap water during flowering (BBCH 63-65) of *Zea mays* and daily honey bee flight, on the same day as the applications in the treatment group T2 and the reference item treatment group R.

All applications were carried out with a target spray volume of 400 L water per ha.

3. Observations

The influence of the test item treatments was evaluated by comparing the results in the test item treatments T1 (applied after honey bee flight) and T2 (applied during honey bee flight) to the data in the control treatment as well as in the reference item treatment regarding the following observations:

- Number of dead honey bees within the crop area (on the linen sheets) and in the dead honey bee trap at the entrance of the hives,
- Flight intensity on the crop (number of forager honey bees per 30 plants),
- Counting of returning forager bees with maize pollen at the entrance of the hives,
- Condition of the colonies and development of the brood,
- Behaviour of the honey bees in the crop area and around the hives.

II. RESULTS AND DISCUSSION

A. MORTALITY

Pre-application mortality (3DBA to 1DBA in T1 and 3DBA to 0DBA in T2 and R) was similar in the four treatment groups. The daily mortality during this period was in the range from 4.7 to 81.0 dead honey bees/day in T1, from 11.3 to 97.3 dead honey bees/day in T2, from 6.3 to 94.7 dead honey bees/day in R and from 8.3 to 50.3 dead honey bees/day in the control. The highest value was recorded in the morning of 2DBA in all treatment groups. Higher mortality rates than usual may occur during the first days after set-up of the colonies in the tunnels, due to adaptation of the honey bees to the new environment. The mean pre-application mortality in T1 was 33.3 dead honey bees/day compared to a value of 26.1 dead honey bees/day in the control (adapted for comparison with T1). In T2 the mean pre-application mortality was 40.1 dead honey bees/day, 38.2 dead honey bees/day in R and 26.5 dead honey bees/day in the control (adapted for comparison with T2 and R). There were no significant differences of the daily mortality or mean pre-application mortality between the four treatment groups (Tukey's Studentized Range test, $p \leq 0.05$).

After the application in T1, daily mortality was higher than in the control (significantly different from the control on 0DAA; pooled t-test, $p \leq 0.05$). During the post-application period (0DBA to 7DAA), daily mortality in T1 was in the range from 6.3 dead honey bees/day to 66.3 dead honey bees/day (mean: 20.8 dead honey bees/day) compared to values from 4.3 to 15.3 dead honey bees/day (mean: 9.6 dead honey bees/day) in the control, adapted for comparison with T1. The mean post-application mortality in T1 was statistically significantly higher than control mortality (pooled t-test, $p \leq 0.05$).

The application of the test item in T2 had an effect on honey bee mortality from 0DAA to 3DAA. Post-application mortality (0DAA to 7DAA) was in the range from 7.0 to 114.3 dead honey bees/day (mean: 35.0 dead honey bees/day) in T2, compared to values from 4.3 to 15.0 dead honey bees/day (mean: 9.4 dead honey bees/day) in the control, adapted for comparison with T2 and R. These differences were statistically significant on 0DAA, 1DAA, 2DAA and 3DAA (pooled t-test, $p \leq 0.05$). Mean post-application mortality in T2 was statistically significantly higher than control mortality (pooled t-test, $p \leq 0.05$).

The application of the reference item in treatment R had a clear effect on honey bee mortality. During the post-application period (0DAA to 7DAA), mortality was in the range from 10.7 to 335.3 dead honey bees/day (mean: 79.8 dead honey bees/day) compared to 4.3 to 15.0 dead honey bees/day (mean: 9.4 dead honey bees/day) in the control. These differences were statistically significant from 0DAA to 6DAA and for the mean of the post-application period (pooled t-test, $p \leq 0.05$).

Overall, there was a slight increase in honey bee mortality in the Indoxacarb 150 g/L EC treatment T1 (application after bee flight) on the day after the application and during the post-application period from 0DAA to 7DAA. In the Indoxacarb 150 g/L EC treatment T2 (application during bee flight), mortality was increased from 0DAA to 3DAA as well as for the post-application period. There was a clear impact of the reference item treatment from 0DAA to 6DAA as well as for the post-application period.

Table 103
Honey bee mortality

Date	DBA/DAA	Mean number of dead honey bees, larvae and pupae				
		C		T1	T2	R
		For T1	For T2 and R			
03 Sep 2013	3DBA	8.3		4.7	11.3	6.3
04 Sep 2013	2DBA	50.3		81.0	97.3	94.7
05 Sep 2013	1DBA	28.3		30.7	27.3	30.7
05 Sep 2013	1DBAba	17.3	---	16.7	---	---
06 Sep 2013	0DBA ^a	---	19.0	---	24.3	21.0
Mean 3DBA to 1DBAba/0DBA		26.1	26.5	33.3	40.1	38.2
STD		5.2	5.1	17.6	8.7	11.3
06 Sep 2013	0DAA ^b	15.3	13.7	66.3*	114.3*	335.3*
07 Sep 2013	1DAA	4.3		16.0	35.3*	80.3*
08 Sep 2013	2DAA	8.7		29.7	45.3*	112.0*
09 Sep 2013	3DAA	15.0		26.3	34.7*	49.0*
10 Sep 2013	4DAA	9.3		8.0	19.3	23.0*
11 Sep 2013	5DAA	7.0		6.7	10.7	13.7*
12 Sep 2013	6DAA	8.0		6.3	13.0	14.7*
13 Sep 2013	7DAA	9.0		7.3	7.0	10.7
Mean 0DAA ^b to 7DAA		9.6	9.4	20.8*	35.0*	79.8*
STD		4.6	4.5	6.0	12.7	10.2

DBA = Days before application during honey bee flight

DAA = Days after application during honey bee flight

STD = Standard Deviation

ba = before application

--- = not applicable

^a Results of the assessments on 1DBA (before application in T1) and 0DBA (before applications in C, T2 and R) were summarised and counted as one value.

^b Including the value of 0DBA in C for T1 and T1

* Statistically significantly different compared to the control (pooled t-Test, Satterthwaite t-Test or Dunnett's T-Test, $p \leq 0.05$)

B. FLIGHT INTENSITY

On 3DBA (the first day after installation of the bee hives in the tunnels on 4DBA in the evening) low flight activity was observed in all treatment groups due to adverse weather conditions. On 2DBA and 1DBA

normal flight activity was recorded in all treatments (>5 honey bees/30 plants). Flight activity in T2 was slightly higher during this time compared to the other treatment groups. During the pre-application period there were 0.5 to 8.5 forager bees/30 plants in T1 (mean: 5.6 forager bees/30 plants), 0.5 to 11.0 forager bees/30 plants (mean 6.8 forager bees) in the control (adapted for comparison with T1), 1.7 to 32.5 forager bees/30 plants in T2 (mean: 19.8 forager bees/30 plants), 0.5 to 20.2 forager bees/30 plants in R (mean: 8.3 forager bees/30 plants) and 0.5 to 11.0 forager bees/30 plants (mean: 7.6 forager bees/30 plants) in the control (adapted for comparison with T2 and R). None of these slight differences were statistically significant.

After the application (0DBA to 7DAA), daily flight activity in T1 was in the range from 0.0 to 8.8 forager bees/30 plants in T1 compared to values ranging from 0.0 to 9.9 forager bees/30 plants in the control (adapted for comparison with T1). The mean post-application flight activity was 3.1 forager bees/30 plants in T1 compared to 3.8 forager bees/30 plants in the control. None of these differences were statistically significant.

In the treatment T2, post-application flight activity was in the range from 0.0 to 19.6 forager bees/30 plants compared to values ranging from 0.0 to 9.9 forager bees/30 plants in the control (adapted for comparison with T2 and R). The mean post-application flight activity was 6.9 forager bees/30 plants in T2 compared to 3.8 forager bees/30 plants in the control. In treatment T2, flight activity was higher after the application compared to the other treatment groups, on a similar elevated level as before the application. Therefore, no test item effect on flight activity seems to occur in this treatment group.

In the reference item treatment R, a repellence effect was observed after the application. Daily flight activity was significantly lower on 0DAA compared to the control (pooled t-test, $p \leq 0.0$). Daily flight activity during the exposure phase was in the range from 0.0 to 5.7 forager bees/30 plants with a mean value of 2.2 forager bees/30 plants.

From 4DAA to 6DAA, no or low flight activity was observed in all four treatments due to adverse weather conditions.

Overall, flight intensity in both Indoxacarb 150 g/L EC treatments (T1 and T2) was comparable to the control. There was no test item effect on flight activity during the post-application period. There was an effect of the reference item on flight activity on the day of application.

Table 104
Honey bee flight intensity

Date	DBA/DAA	Flight intensity (mean number of forager bees/30 plants)				
		C		T1	T2	R
		For T1	For T2 and R			
03 Sep 2013	3DBA	0.5		0.5	1.7	0.5
04 Sep 2013	2DBA	8.9		7.7	19.9	5.3
05 Sep 2013	1DBA	11.0		8.5	25.1	12.2
06 Sep 2013	0DBA	---	9.8	---	32.5	20.2
Mean 3DBA to 1DBA/0DBA		6.8	7.6	5.6	19.8	8.3
STD		3.5	3.1	1.5	11.1	4.4
06 Sep 2013	0DAA ^a	9.9	9.9	8.8	19.6	5.7*
07 Sep 2013	1DAA	7.0		7.1	14.7	5.6
08 Sep 2013	2DAA	9.3		4.1	12.5	5.2
09 Sep 2013	3DAA	4.2		3.7	6.1	1.2
10 Sep 2013	4DAA	0.0		0.0	0.3	0.0
11 Sep 2013	5DAA	0.0		0.0	0.1	0.0
12 Sep 2013	6DAA	0.0		0.0	0.0	0.0
13 Sep 2013	7DAA	0.2		0.7	1.6	0.2
Mean 0DAA ^a to 7DAA		3.8	3.8	3.1	6.9	2.2
STD		2.2	2.2	1.4	2.4	1.1

DBA = Days before application during honey bee flight

DAA = Days after application during honey bee flight

STD = Standard deviation

--- = not applicable

* = statistically significantly different compared to the control (pooled t-Test)

^a including the value of 0DBA in C for T1 and T1

C. BEHAVIOUR OF THE HONEY BEES

During the pre-application period (3DBA to 1DBA/0DBA) normal behaviour was observed in all 4 treatments. Cramping was only observed once in T1 and the control, and one motionless bee was seen once in T2 during exposure to the untreated crop.

In the treatment group T1 (application in the evening), two bees with intensive cleaning behaviour were found in two replicates on 0DAA+2 h. In total, 25 motionless bees were present in all replicates during the post-application period (0DBA to 7DAA). Additionally, 30 bees with locomotion problems, 26 cramping bees, 12 trembling bees and one hanging bee were found during this time period.

In the treatment T2, unusual behaviour like cramping, trembling, locomotion problems or motionless honey bees were observed mainly on the day of application (0DAA). A small number of bees with symptoms (locomotion problems, motionless honey bees, cramping, hanging bees or trembling) were observed on the following days (1DAA, 3DAA to 7DAA). Overall, there were 57 bees with locomotion problems, 31 inactive bees, 25 cramping bees and 84 trembling bees during the post-application period. Additionally, 1 hanging bee was observed and 6 bees were intensely self-grooming.

Few bees with unusual behaviour were observed in the control during the post-application period. Overall, there were 2 bees showing locomotion problems, 2 cramping bee, 22 inactive bees and 3 intensely self-grooming bees during the post-application period. Additionally, 40 bees were clustering on 6DAA.

In the reference item treatment R, a clear impact of the application was observed in numerous bees mainly on 0DAA and 1DAA and in few bees on 2DAA, 3DAA, 6DAA and 7DAA. Overall, there were 72 bees showing locomotion problems, 13 inactive bees, 58 cramping bees and 17 trembling bees. Additionally, 4 bees were flying without landing and 3 bees were intensely self-grooming.

Overall, the application of Indoxacarb 150 g/L EC after daily honey bee flight (T1), had a slight effect on honey bee behaviour, mainly on the day following the application (0DAA). The application of Indoxacarb 150 g/L EC during daily honey bee-flight (T2) had an effect on honey bee behaviour. There were only few bees showing unusual behaviour in the control while there was a clear effect of the reference item on honey bee behaviour.

D. COUNTING OF FORAGER BEES RETURNING WITH *ZEAMAYS* POLLEN

The assessment of forager bees returning with *Zea mays* pollen loads mainly served to demonstrate that the experimental bee colonies were actually foraging on *Zea mays* plants.

During the pre-application period (3DBA to 1DBA), rates of forager bees returning with *Zea mays* pollen were comparable in the four treatment groups. There were 0.0 to 6.7 forager bees/minute in T1, 0.3 to 4.0 forager bees/minute in T2, 0.0 to 3.3 forager bees/minute in R and 1.0 to 6.7 forager bees/minute in the control. Mean rates for this period were 4.0 forager bees/minute in T1, 2.5 forager bees/minute in T2, 1.8 forager bees/minute in R and 3.2 forager bees/minute in the control, showing that the experimental bee colonies had got used to foraging in the crop until start of the application.

During the post-application period, there was a temporary decrease of the number of forager bees returning with *Zea mays* pollen loads in R on 0DAA (1.2 forager bees/minute compared to 4.0 forager bees/minute in the control) but then returned to higher values comparable to the control. Mean post-application rates of returning forager bees loaded with maize pollen were 3.2 forager bees/minute in T1, 3.9 forager bees/minute in T2, 1.6 forager bees in R and 2.5 forager bees in the control.

From 4DAA to 7DAA, no forager bees returning with *Zea mays* pollen were observed, due to adverse weather conditions.

In general, the data show that bees from the experimental colonies were actively foraging on the crop and continued foraging in the treated crop after the application. The numbers of bees returning with maize pollen were similar in the treatment groups T1 and T2 compared to the control. In the reference item treatment group, there was a decrease of bees returning with maize pollen.

E. CONDITION OF THE COLONIES AND DEVELOPMENT OF THE BROOD

At the first assessment before set-up of the hives in the tunnels (4DBA), the mean colony sizes were similar in all treatments. There were 7146 bees in C, 6833 bees in T1, 7125 bees in T2 and 7417 bees in R.

At the second assessment, there were only marginal changes of the mean colony sizes compared to the last assessment. There were 7250 bees in C, 7292 bees in T1, 6959 bees in T2 and 7375 bees in R.

On the third assessment, the mean colony sizes slightly decreased in C and slightly increased in T1, T2 and R. There were 6646 bees in C, 7480 bees in T1, 7667 bees in T2 and 7625 bees in R.

On the fourth assessment, the mean colony sizes slightly decreased in all treatment groups. There were 6125 bees in C, 6521 bees in T1, 7063 bees in T2 and 5792 bees in R.

On the last assessment, a slight decline of the mean colony sizes was once again observed in all treatment groups (C: 5604 bees, T1: 5875 bees, T2: 6042 bees, R: 5479 bees).

Overall, no negative impact on the colony size was observed in both Indoxacarb 150 g/L EC treatments (T1 and T2) and the reference item group R.

At the first assessment before set-up of the hives in the tunnels, all brood stages (eggs, larvae, pupae) were present in all hives, indicating the presence of a healthy queen, and the mean size of the brood area was similar in all treatments. The mean number of brood cells on 4DBA was 11933 cells in C, 11533 cells in T1, 14933 cells in T2 and 13133 cells in R.

At the following assessment at the end of exposure (7DAA), a decline of the amount of brood was observed in both test item treatments T1 (5333 brood cells/hive) and T2 (5800 brood cells/hive) as well as in the control (3800 brood cells/hive) and the reference item treatment (3667 brood cells/hive). Since this decrease was also observed in the control, it is not due to the treatment but rather due to the confinement in the tunnels.

On the third assessment on 14DAA, the numbers of eggs and larvae had increased in all treatment groups, showing that all colonies were recovering from the confinement period. There were 7667 brood cells/hive in C, 7467 brood cells/hive in T1, 6000 brood cells/hive in T2 and 5133 brood cells/hive in R.

On the fourth assessment on 20DAA, there were no considerable differences in brood development. The mean number of brood cells per hive was 6400 in C, 7867 in T1, 6133 in T2 and 7267 in R.

On the last assessment on 28DAA, the amount of brood cells increased to 5867 brood cells/hive in the control, 6267 brood cells/hive in T1 and 6400 brood cells/hive in T2. In the reference item treatment R, the amount of brood also increased to 6267 brood cells/hive. Since this assessment was done on 04 Oct 2013, the amount of brood is most probably low due to seasonal effects.

All colonies had plenty amount of nectar throughout the experimental period. Feeding of all hives with 2.0 to 3.0 L maltose solution was done on 5DAA. Except for one colony in T1, there was no stored pollen available on 7DAA and 14 DAA in all treatment groups. On 20DAA and 28DAA, pollen storages were back to normal.

Overall, there was no negative impact of the test item treatments T1 and T2 on honey bee brood development and colony condition.

III CONCLUSION:

Indoxacarb 150 g/L EC, applied once during flowering of *Zea mays* in the evening after honey bee flight (treatment T1) or during daily honey bee flight (treatment T2) had no effect on honey bee flight activity, brood development and colony condition in both test item treatments T1 and T2.

There was a slight increase in honey bee mortality in the Indoxacarb 150 g/L EC treatment T1 on the day of application and considering the post-application period from 0DAA to 7DAA. There was an increased daily mortality from 0DAA to 3DAA and considering the whole post-application period in the Indoxacarb 150 g/L EC treatment T2.

Additionally, there was a slight effect on behaviour in the treatment group T1 and an effect on behaviour in the treatment group T2.

(Berg, C., 2013)

RMS comment

Study submitted to the EU for the first time in this submission.

Indoxacarb 150 g/L EC was applied once during flowering of *Zea mays*. The effects of Indoxacarb 150 g/L EC were tested when applied during and after bee flight (three tents per treatment group).

This study is considered valid according to validity criteria but to be used with caution. A deviation is reported: The distance to target during the application was partly not approx. 50 cm. Some of the plants in some of the tunnel were even higher than the boom height. The impact was considered minor. RMS also notes that rain occurred the day after application (42.8mm) and the following days. The impact of such rainfall on the actual exposure of bees is not known.

There was a slight increase in honey bee mortality in the Indoxacarb 150 g/L EC treatment T1 (application in evening) on the day of application and considering the post-application period from 0DAA to 7DAA. There was an increased daily mortality from 0DAA to 3DAA and considering the whole post-application period in the Indoxacarb 150 g/L EC treatment T2 (application during foraging).

Indoxacarb 150 g/L EC, applied in the evening after honey bee flight (treatment T1) or during daily honey bee flight (treatment T2) had no effect on honey bee flight activity, brood development and colony condition in both test item treatments T1 and T2.

Additionally, there was a effect on behaviour in the treatment group T1 and an effect on behaviour in the treatment group T2.

Report: Kleinhenz, M. (2014a); Indoxacarb (DPX-KN128) 150 g/L EC: A semi-field study to evaluate effects on the brood of honey bees (*Apis mellifera*; Hymenoptera, Apidae) in *Phacelia tanacetifolia* in Germany 2012

DuPont Report No.: DuPont-34108

Guidelines: OECD 75 (2007) **Deviations:** None

Testing Facility: Eurofins Agrosience Services GmbH, Niefern-Oeschelbronn, Germany

Testing Facility Report No.: S12-00228

GLP: Yes

Certifying Authority: Landesanstalt Fur Umwelt, Messungen Und Naturschutz Baden-Wurtemberg

Executive summary:

The effects of the test item Indoxacarb 150 g/L EC were tested on the honey bee (*Apis mellifera* L.) under semi-field conditions following the OECD guidance document No. 75 (2007). This study was conducted near Celle in northern Germany (region: Niedersachsen) from June to July 2012 and included a total of three treatment groups:

- Indoxacarb 150 g/L EC treatment group T with one application of the test item in each replicate (tunnel tent). The application was carried out during flowering (BBCH 65) and daily bee-flight (≥ 5 forager bees/m²). The application was carried out at a target rate of 50 g a.s./ha.
- Reference item treatment group R with one application of Insegar 25WG (fenoxycarb) at a target rate of 600 g product/ha (equivalent to 150 g a.s./ ha). The application was carried out during flowering of *P. tanacetifolia* and bee-flight (BBCH 64–65), on the same day as the application in the treatments T.
- Control group C with one application of tap water during flowering of *P. tanacetifolia* and bee-flight (BBCH 65), on the same day as the applications in the treatment T.

The applications in the test item treatment group T, in the reference item treatment R and in the control were carried out on 30 June 2012 (5 days after installation of the hives in the tunnels). All applications were carried out with a spray volume of 500 L water per ha. The effects of the test item treatment were examined on small honey bee colonies in tunnel tents (5.0 m × 16.0 m and a height of 3.5 m in the centre) placed over the plots of *P. tanacetifolia*. The semi-field test comprised 3 replicate tunnel tents in each of the treatment groups.

The influence of the application of Indoxacarb 150 g/L EC was evaluated by comparing the results in this treatment to the data in the control treatment as well as in the reference item treatment regarding the following observations:

-
- Mortality: number of dead honey bees at the edge of the treated area (linen sheets) and in the dead honey bee traps in front of the hives,
 - Foraging activity on the crop (number of forager honey bees/m² *P. tanacetifolia*),
 - Condition of the colonies and development of the brood,
 - Detailed observation of the brood development in ≥ 200 selected cells,
 - Behaviour of the honey bees in the crop area and around the hives.

It can be concluded that one application of Indoxacarb 150 g/L EC at a rate of 50 g a.s./ha applied during bee flight has a transient effect on honey bee mortality on DAA0aa and DAA1 and on behaviour on DAA0aa.

There was no effect on the amount of brood in the Indoxacarb 150 g/L EC treatment (T) except a slight reduction of the number of larvae on DAA9 based on whole colony assessment.

The termination rates of the individually marked cells were significantly higher in the Indoxacarb 150 g/L EC treatment (T) than in the control. The brood index and compensation index were significantly lower than in the control on BFD+11, BFD+15 and BFD+21.

Overall, an effect on the brood was detected on the level of individual cells during the whole monitoring period (BFD+11 to BFD+21) but there was only a transient effect (DAA9 = BFD+11) detectable on the level of the whole bee colony and no effect on pupal mortality.

There was no negative effect on honey bee flight activity and on the size of the colonies in the Indoxacarb 150 g/L EC treatment (T).

I. MATERIAL AND METHODS

A. MATERIALS:

1. Test material:
Lot/Batch#: DPX-KN128-311
Purity: 150 g a.s./L
CAS#: None for the formulation
173584-44-6 for the active substance
Stability of test compound: 98.2% of the indoxacarb remains in the delivery vehicle after one hour under agitation
Reference item: Insegar 25WG (fenoxycarb)
Batch: SMO1J414
Content of a.s., nominal: 25.0% (w/w)
CAS#: 72490-01-8
Stability in solution: not available
2. Vehicle and/or control: Tap water
3. Test organism
Species: *Apis mellifera* L.
Age at dosing: Direct exposure of adult honey bees; indirect exposure of all stages of development
Source: LAVES Institut für Bienenkunde, Celle
Diet: Nectar and pollen of flowering *Phacelia tanacetifolia*.
Tunnel tents (exposure): Within the test field 12 plots (each 5.0 m × 16.0 m) were marked and labelled before the first application. Paths (0.5 m) were made in each plot by removing the plants and smoothing the ground. Before set-up of the bee colonies, tunnel tents (5.0 m × 16.0 m and a height of 3.5 m in the centre) were installed over the marked plots. The tent frames were covered with light plastic gauze. The paths (approx. 12.5 m²) were covered with linen sheets for the assessment of dead bees in the crop area. The crop area per tent was approx. 67.5 m².
4. Environmental conditions during the exposure period (30 June – 07 July 2012):
Temperature (min/max): 8.0–28.2°C
Relative humidity: 41–100%
Photoperiod (exposure): natural light conditions

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
25-June-2014 to 26-July-2014
2. Experimental treatments
 - Indoxacarb 150 g/L EC treatment group T with one application of the test item in each replicate (tunnel tent, 3 replicates per treatment). The application was carried out 5 days after set-up of the bee colonies in the tunnel tents, during full flowering of *P. tanacetifolia* flowering (BBCH 65) and daily bee-flight. Each application was carried out at a target rate of 50 g a.s./ha.
 - Reference item treatment group R with one application of Insegar 25WG (fenoxycarb) at a rate of 600 g product/ha (equivalent to 150 g fenoxycarb/ ha) during full flowering of *P. tanacetifolia* and bee-flight, on the same day as the application in the treatment T and in the control.
 - Control group C with one application of tap water during flowering of *P. tanacetifolia* and bee-flight, on the same day as the application in the test item treatment T and in the reference item treatment R.

3. Observations

The influence of Indoxacarb 150 g/L EC was evaluated by comparing the results in this treatment (T) to the data in the control treatment (C) as well as in the reference item treatment (R) regarding the following observations:

Mortality: Number of dead honey bees at the edge of the treated area (linen sheets) and in the dead honey bee traps in front of the hives,

Foraging activity on the crop (number of forager honey bees/m² *P. tanacetifolia*),

Condition of the colonies and development of the brood,

Detailed observation of the brood development in >200 selected cells,

Behaviour of the honey bees in the crop area and around the hives.

II. RESULTS AND DISCUSSION

A. MORTALITY

Daily mortality after installation of the bee hives until start of the applications (DAA-4 to DAA0ba) was in the range from 55.7 to 447.3 dead honey bees/day in the control, from 28.0 to 332.7 dead honey bees/day in the test item treatment T and from 29.3 to 350.7 dead honey bees/day in the reference item treatment R. None of these differences was statistically significant. In general, mortality was rather high in all treatments including the control and resulted mainly from dead bees that were found on the linen sheets and only few bees in the dead bee traps. The highest values occurred during the period from DAA-3 to DAA-1. At the last assessment before the applications (DAA0ba), mortality was returning to a lower level, showing that the bees got used to the confinement in the tunnels. However, mortality was higher in T (142.3 dead honey bees) than in C (94.7 dead honey bees) and R (78.7 dead honey bees) but this was not related to the treatment since the crop was still untreated at this time.

In all treatments, the total number of dead pupae and young bees was low during the pre-application period (4 dead pupae and young bees in C, 2 dead pupae in T and 3 dead pupae in R).

The mean pre-application mortality from DAA-4 to DAA0ba was 254.7 dead honey bees/day in the control, 202.3 dead honey bees/day in T and 207.1 dead honey bees/day in R. These differences were statistically not significant.

After the application, mortality on DAA0aa and DAA1 were significantly higher in T (447.0 and 163.7 dead honey bees/day) than in the control (90.3 and 22.7 dead honey bees/day) (one-sided pooled t-test, $p \leq 0.05$). In the reference item treatment R, mortality was 51.7 and 15.3 dead honey bees on these two days.

On the following days until end of exposure in the tunnels (DAA2 to DAA7) mortality was in the range from 70.7 to 255.0 dead honey bees/day in the control, from 48.3 to 107.7 dead honey bees/day in T and from 71.0 to 184.7 dead honey bees/day in R. The differences were not significant and mortality in T was lower than in the control and R on all days except DAA2.

The mean post-application mortality during the exposure period (DAA0aa to DAA7) was 137.3 dead honey bees/day in the control, 143.5 dead honey bees/day in the test item treatment T and 112.1 dead honey bees/day in R. These differences were statistically not significant.

Table 105
Honey bee mortality

Date	DAA ^a	Mean number of dead honey bees/day per treatment group		
		C	T	R
26 Jun 2012	-4	55.7	28.0	29.3
27 Jun 2012	-3	341.0	198.0	244.0
28 Jun 2012	-2	334.7	332.7	332.7
29 Jun 2012	-11	447.3	310.3	350.7
30 Jun 2012	0ba	94.7	142.3	78.7
Mean DAA-4 to DAA0 ba ^c		254.7	202.3	207.1
STD ^b		214.0	64.0	139.0
30 Jun 2012	0aa ^d	90.3	446.0*	51.7
01 Jul 2012	1	22.7	163.7*	15.3
02 Jul 2012	2	92.0	107.0	84.7
03 Jul 2012	3	70.7	48.3	71.0
04 Jul 2012	41	181.7	99.7	175.3
05 Jul 2012	5	184.0	74.7	168.3
06 Jul 2012	6	201.7	99.7	145.7
07 Jul 2012	7	255.0	107.7	184.7
Mean DAA0aa to DAA7		137.3	143.3	112.1
STD		93.8	28.3	36.5
08 Jul 2012	8	1.0	3.0	1.0
09 Jul 2012	9	12.7	7.3	5.3
10 Jul 2012	10 ^e	58.7	35.0	54.3
11 Jul 2012	11	5.3	5.3	14.7
12 Jul 2012	12	1.7	4.3	13.7
13 Jul 2012	13	1.3	0.3	34.0
14 Jul 2012	14 ^e	6.0	8.7	67.7
15 Jul 2012	15	2.0	4.0	45.7
16 Jul 2012	16	4.0	4.3	37.7
17 Jul 2012	17	2.7	0.7	35.0
18 Jul 2012	18	2.0	2.3	7.0
19 Jul 2012	19	1.3	1.3	3.7
20 Jul 2012	20 ^e	117.3	71.0	67.0
21 Jul 2012	21	19.3	7.0	13.0
22 Jul 2012	22	6.7	4.7	2.7
23 Jul 2012	23	1.3	3.3	3.7
24 Jul 2012	24	2.3	2.0	7.0
25 Jul 2012	25	1.3	4.3	6.3
26 Jul 2012	26 ^e	134.0	31.3	44.3
Mean DAA0aa to DAA26		54.8	49.9	50.3
STD		16.0	9.0	3.8

Table 105
Honey bee mortality (continued)

Date	DAA ^a	Mean number of dead honey bees/day per treatment group		
		C	T	R
Dead pupae and malformed bees and pupae				
Sum per treatment (DAA-4 to DAA0ba)		4	2	3
Sum per treatment (DAA0aa to DAA26)		7	21	657
Mean per hive (DAA0aa to DAA26)		2.3	7.0	219.0
Mean per hive (DAA0aa to DAA26) [†]		0.7	3.7	219.0

^a DAA= Days after application

^b STD = Standard deviation

^c ba= before application

^d aa= after application

^e Potentially higher mortality due to a colony assessment that was done in all treatments on the day before

^f excluding chalk brood mummies

* significantly different from the control (one-sided pooled t-test, $p \leq 0.05$)

DAA-4 to DAA7: Dead bees from linen sheets and dead bee traps (exposure period inside tunnel)

DAA8 to DAA26: Dead bees from dead bee traps (monitoring period)

During the monitoring period after removal of the bee hives out of the tunnels, daily mortality (recorded in the dead bee traps only) was generally on a low level, except those days that were preceded by a colony assessment the day before (DAA10, DAA14, DAA20, DAA26). From DAA8 to DAA26, daily mortality was in the range from 1.0 to 134.0 dead honey bees/day in the control, from 0.3 to 71.0 dead honey bees/day in T and from 1.0 to 67.7 dead honey bees/day in R. None of these differences were statistically significant.

The number of dead young bees, dead pupae and dead malformed pupae and bees was low in the control (0.7 dead bees per hive during the observation period) and in T (3.7 dead bees per hive), but it was high in the reference item treatment R (219.0 dead bees per hive). The patchy occurrence of very few dead pupae in the treatment T (only 0-2 per day in most cases; not all replicates were affected) do not suggest an effect of the test item on pupal mortality. In R, the majority of dead pupae were observed between DAA11 and DAA18, an effect that could be expected from this reference item.

The mean post-application mortality (DAA0aa to DAA26) was 54.8 dead honey bees/day in the control, 49.9 dead honey bees/day in T and 50.3 dead honey bees/day in R (statistically not significant).

Overall, there was a transient increase in honey bee mortality on DAA0aa and DAA1 in the Indoxacarb 150 g/L EC treatment (T).

B. FLIGHT INTENSITY

Daily flight activity from installation of the colonies in the tunnels until the application during bee-flight (DAA-4 to 0ba) was in a range from 2.0 to 16.0 forager bees/m² in the control, from 1.0 to 27.0 forager bees/m² in test item treatment T and from 2.0 to 21.0 forager bees/m² in R. The daily mean flight activities were similar in the three treatments and statistically not significantly different from each other, except the record on DAA0ba when flight activity in T was significantly higher than in C and R (Tukey's Studentized Range test, $p \leq 0.05$).

The mean daily foraging activity during this period (DAA-4 to 0ba) was 8.7 forager bees/m² in the control, 10.5 forager bees/m² in T and 9.7 forager bees/m² in R. These differences were statistically not significant.

On the day of the application during bee-flight, the mean foraging activity after application (DAA0aa) was 20.1 forager bees/m² in the control, 21.0 forager bees/m² in T and 20.7 forager bees/m² in R (statistically not significantly different).

On the following day (DAA1) there were 14.3 forager bees/m² in T, 19.2 forager bees/m² in the control and 18.3 forager bees/m² in R. Flight activity in T was significantly lower than in the control (one-sided pooled t-test, $p \leq 0.05$) but still an acceptable high level and of no biological relevance.

On the following days (DAA2 to DAA7) flight activity was in the range from 2.7 to 16.3 forager bees/m² in the control, from 4.0 to 18.7 forager bees/m² in T and from 5.0 to 19.0 forager bees/m² in R. There were no statistically significant differences of the test item treatment or reference item treatment compared to the control except the record on DAA4 in the reference item treatment.

The mean post-application flight activity (DAA0aa to DAA7) was 11.9 forager bees/m² in the control, 12.1 forager bees/m² in T and 11.9 forager bees/m² in R. These differences were statistically not significant.

Overall, there was no negative impact with biological relevance of the application in the Indoxacarb 150 g/L EC treatment (T) on the flight activity of the honey bees.

Table 106
Honey bee flight intensity

Date	DAA	Flight intensity (mean number of forager bees/m ²)		
		C	T	R
26 Jun 2012	-4	6.7	5.3	5.3
27 Jun 2012	-3	2.0	1.0	2.0
28 Jun 2012	-2	5.3	7.0	8.7
29 Jun 2012	-1	13.7	12.3	11.7
30 Jun 2012	0ba	16.0	27.0 ^a	21.0
Mean DAA-4 to DAA0ba		8.7	10.5	9.7
STD		2.3	1.3	0.6
30 Jun 2012	0aa+15 min	16.7	26.7	18.0
	0aa+30 min	19.3	29.7	21.3
	0aa+45 min	21.3	26.7	20.3
	0aa+1 h	22.0	23.0	22.3
	0aa+2 h	18.3	14.7	25.7
	0aa+4 h	23.0	16.0	22.7
	0aa+6 h	20.3	10.3	14.3
Mean DAA0aa		20.1	21.0	20.7
STD		2.7	1.5	3.2
01 Jul 2012	1	19.7	13.7	16.7
	1	24.0	17.3	21.3
	1	14.0	12.0	17.0
Mean DAA1		19.2	14.3 ^b	18.3
STD		1.7	1.7	1.2
02 Jul 2012	2	14.7	18.7	19.0
03 Jul 2012	3	5.7	10.3	5.7
04 Jul 2012	4	16.3	13.0	11.3 ^b
05 Jul 2012	5	10.0	8.0	9.3
06 Jul 2012	6	2.7	4.0	5.0
07 Jul 2012	7	6.3	7.3	6.0
Mean DAA0aa to DAA7		11.9	12.1	11.9
STD		1.4	0.9	0.5

ba = before application, aa = after application

DAA = Days after application

STD = Standard deviation

^a significantly different from C and R (Tukey's Studentized Range test, $p \leq 0.05$)

^b Significantly lower than the control (one-sided pooled t-test, $p \leq 0.05$)

C. BEHAVIOUR OF THE HONEY BEES

Before the application, normal behaviour was recorded in all treatments.

After the application, no unusual behaviour was observed in the control.

In the test item treatment T, no unusual behaviour was observed except on DAA0aa when cramping bees and bees with locomotion problems were recorded in all three tunnels of this treatment.

In the reference item treatment R, no unusual behaviour was observed except for one record on DAA0aa when 2 cramping bees were observed.

Overall, there was a transient effect on behaviour in the Indoxacarb 150 g/L EC treatment (T) on the day of the application (DAA0aa).

D. BROOD DEVELOPMENT AND COLONY CONDITION

At the first assessment of colony condition before set-up in the tunnel tents (DAA-12) the mean colony sizes were comparable in the four treatment groups. Mean strength of the colonies (number of honey bees according to Liebefeld method) was 5363 honey bees in C, 5250 honey bees in T and 4932 honey bees in R.

In all treatments, an increase of the colony size was observed after installation of the hives in the tunnel tents. The maximum colony sizes were 9469 honey bees in the control (DAA9), 9506 honey bees in test item treatment T (DAA19) and 8419 honey bees in R (DAA19).

Also at the last assessment (DAA25), the mean colony sizes were similar in the three treatment groups: there were 7782 honey bees in C, 7838 honey bees in T and 7182 honey bees in R.

Overall, there was no negative effect of the Indoxacarb 150 g/L EC treatment (T) on the size of the colonies (number of honey bees).

Brood of all stages (eggs, larvae, capped brood) was present in all colonies at all assessments during the study. At the first assessment before installation of the hives (DAA-12), the mean total amount of brood cells (brood of all stages) was similar in the three treatments and increased in all treatments until the next assessment (DAA-2, before application), namely from 16260 to 20880 brood cells in the control, from 13800 to 25500 brood cells in T and from 14640 to 23460 brood cells in R.

At the assessments after application (DAA3, DAA9, DAA13) the amount of brood decreased in all treatments including the control. The minimum values were 11580 brood cells in the control, 10380 brood cells in T and 6540 brood cells in R. Although the total amount of brood in treatment T was comparable to the control on DAA9, a noticeable decrease of the amount of young brood (open brood cells containing eggs or larvae) was observed in T during this assessment.

On the following two assessments until the end of the assessment period, an increase of the amount of brood was observed in all treatments and at the last assessment (DAA25) there were 17040 brood cells in the control, 17400 brood cells in T and 14940 brood cells in R.

All colonies had sufficient amount of nectar and pollen stores except the temporary lack of pollen in hive Ta (DAA13) and Tb (DAA19).

Overall, honey bee brood development in Indoxacarb 150 g/L EC treatment (T) was similar to the control during the whole assessment period except a slight reduction of the amount of larvae on DAA9.

E. DEVELOPMENT OF THE HONEY BEE BROOD IN INDIVIDUAL CELLS

According to the development time of a worker honey bee from egg to imago (adult bee) which normally averages approximately 21 days it can be assumed that almost all young bees hatch until the assessment date BFD+21. Therefore, the study covered one complete development cycle of the honey bee brood.

Low levels of infestation with chalk brood were observed in all treatments (hives Ca, Cb, Cc, Tb, Tc, Rb) at the colony assessments before application (DAA-12: 13 cells in Ca, 2 cells in Cb, 6 cells in Cc, 4 cells in Tb; DAA-2: 4 cells in Cc, 1 cell in Tb, 2 cells in Tc, 12 cells in Rb) and also on some of the following assessments (DAA3: 1 cell in Ca, 6 cells in Cc; DAA9: 27 cells in Cc, 4 cells in Tb, 20 cells in Tc; DAA13: 14 cells in Cc, 6 cells in Tc; DAA19: 10 cells in Cc, 1 cell in Tb, 5 cells in Tc; DAA25: 29 cells in Cc, 5 cells in Tc, 1 cell in Rb). Although the symptoms are easily detectable, there were no signs of disease in the individually marked brood cells. Therefore, this low level of infestation is considered to have no influence on the results of these assessments.

The control colonies showed a successful development with rising brood index values over the entire assessment period. The brood indices on BFD+15 remained on almost the same level as on BFD+11, which is not unusual because a normal developing bee is expected to be in the same stage (pupa) at both assessments. The mean brood index in the control reached a final value of 3.76 and the mean compensation index was 3.80 on BFD+21. The termination rates on BFD+21 were between 14.08 and 31.90 with a mean value of 24.81.

In the test item treatment group T, the brood and compensation indices were on a low level throughout the assessment period. The low index values on BFD+5 (brood index: 1.09; compensation index: 1.10) indicate that a proportion of the observed brood was removed shortly after the application. On BFD+11, BFD+15 and BFD+21, both the brood index and the compensation index were significantly different from the control (one-sided pooled t-test, $p \leq 0.05$). At the last assessment on BFD+21, the termination rates were between 57.51 and 100.00 with a mean value of 73.17 (significantly different from the control; one-sided pooled t-test, $p \leq 0.05$).

In the treatment group R, the effect of the reference item was clearly detectable in all three replicates. At the assessment after application (BFD+5), the mean brood index decreased from 1.00 to 0.07 and the mean compensation index decreased to 0.12. Both indices further decreased during the following two assessments, to 0.04 at BFD+15, and increased only slightly to 0.05 at the end of the observation period (BFD+21). The brood index and compensation index were significantly different from the control at all assessments after the application (BFD+5, BFD+11, BFD+15, BFD+21). Consequently, very high termination rates between 97.27 and 100.00 (mean: 99.09) were calculated (BFD+21) in treatment R (significantly different from the control; one-sided pooled t-test, $p \leq 0.05$).

Table 107
Honey bee brood/compensation indices and termination rates

Treatment Group/Replicate	Brood/Compensation Indices and Termination Rates at \times days after brood area fixing day (BFD0)					Termination Rate
	BFD0	BFD+5	BFD+11	BFD+15	BFD+21	BFD+21
Ca	1.00/1.00	1.87/1.87	2.72/2.72	2.72/2.72	3.40/3.52	31.90
Cb	1.00/1.00	2.55/2.55	3.44/3.44	3.44/3.44	4.30/4.30	14.08
Cc	1.00/1.00	2.26/2.26	2.98/2.98	2.90/2.90	3.58/3.58	28.45
Mean C	1.00/1.00	2.23/2.23	3.05/3.05	3.02/3.02	3.76/3.80	24.81
STD	0.00/0.00	0.34/0.34	0.36/0.36	0.37/0.37	0.48/0.43	9.45
Ta	1.00/1.00	2.10/2.10	1.70/1.70	1.70/1.70	2.12/2.27	57.51
Tb	1.00/1.00	1.18/1.18	1.52/1.53	1.52/1.59	1.90/2.51	62.00
Tc	1.00/1.00	0.00/0.02	0.00/0.00	0.00/0.00	0.00/0.00	100.00
Mean T	1.00/1.00	1.09/1.10	1.07*/1.08*	1.07*/1.10*	1.34*/1.59*	73.17*
STD	0.00/0.00	1.05/1.04	0.93/0.94	0.93/0.95	1.17/1.39	23.34
Ra	1.00/1.00	0.21/0.28	0.11/0.11	0.11/0.11	0.14/0.14	97.27
Rb	1.00/1.00	0.00/0.06	0.00/0.06	0.00/0.00	0.00/0.00	100.00
Rc	1.00/1.00	0.00/0.02	0.00/0.00	0.00/0.00	0.00/0.00	100.00
Mean R	1.00/1.00	0.07*/0.12*	0.04*/0.06*	0.04*/0.04*	0.05*/0.05*	99.09*
STD	0.00/0.00	0.12/0.14	0.06/0.06	0.06/0.06	0.08/0.08	1.58

BFD = Brood area fixing day

STD = Standard deviation

* Significantly different from the control (one-sided pooled t-test, $p \leq 0.05$)

Overall, an effect on the brood was observed on the level of detailed cell assessments from BFD+11 to BFD+21 but there was only a slight transient effect on the level of the whole colony assessments (small

number of larvae observed on DAA9) and no effect on mortality and malformations of pupae that were found in front of the hives.

III. CONCLUSION

It can be concluded that one application of Indoxacarb 150 g/L EC at a rate of 50 g a.s./ha applied during bee flight has a transient effect on honey bee mortality on DAA0aa and DAA1 and on behaviour on DAA0aa.

There was no effect on the amount of brood in the Indoxacarb 150 g/L EC treatment (T) except a slight reduction of the number of larvae on DAA9 based on whole colony assessment.

The termination rates of the individually marked cells were significantly higher in the Indoxacarb 150 g/L EC treatment (T) than in the control. The brood index and compensation index were significantly lower than in the control on BFD+11, BFD+15 and BFD+21.

Overall, an effect on the brood was detected on the level of individual cells during the whole monitoring period (BFD+11 to BFD+21) but there was only a transient effect (DAA9 = BFD+11) detectable on the level of the whole bee colony and no effect on pupal mortality.

There was no negative effect on honey bee flight activity and on the size of the colonies in the Indoxacarb 150 g/L EC treatment (T).

(Kleinhenz, M., 2014a)

RMS comment

Study submitted to the EU for the first time in this submission.

This study is considered valid. The mean brood termination rate in the control group was $\leq 50\%$ at the end of the study (as required by the study plan). No analytical confirmation of the level to which the bees were exposed is available. There were three bee colonies per treatment group.

RMS notes that flight intensity decreased in the tunnels treated with Indoxacarb 150 g/L EC on the day of application and the day after (considered of no biological relevance by the study author). RMS also notes that the brood/compensation indices were lower than in control at all assessment (even if not significant at BFD+5).

Indoxacarb 150 g/L EC at a rate of 50 g a.s./ha applied during bee flight has a transient effect on honey bee mortality on DAA0aa and DAA1 and on behaviour on DAA0aa.

There was a transient decrease of flight activity on the day of application and the day after. No effect was observed on the size of the colonies in the Indoxacarb 150 g/L EC treatment (T).

There was no effect on the amount of brood in the Indoxacarb 150 g/L EC treatment (T) except a slight reduction of the number of larvae on DAA9 based on whole colony assessment.

The termination rates of the individually marked cells were significantly higher in the Indoxacarb 150 g/L EC treatment (T) than in the control. The brood index and compensation index were lower than in the control at all assessments.

Overall, an effect on the brood was detected on the level of individual cells during the whole monitoring period but there was only a transient effect (DAA9 = BFD+11) detectable on the level of the whole bee colony and no effect on pupal mortality.

Report: Kleinhenz, M. (2014b); Indoxacarb (DPX-KN128) 150 g/L EC: A semi-field study to evaluate effects on the honey bee (*Apis mellifera*; Hymenoptera, apidae) in *Phacelia tanacetifolia* in Germany 2013

DuPont Report No.: DuPont-36482

Guidelines: OEPP/EPPO Guideline No 170 (4), 2010 **Deviations:** None

Testing Facility: Eurofins Agrosience Services EcoChem GmbH, Niefern-Öschelbronn, Germany

Testing Facility Report No.: S13-00880

GLP: Yes

Certifying Authority: Landesanstalt Für Umwelt, Messungen Und Naturschutz Baden-Württemberg

Executive summary:

The effects of the test item Indoxacarb 150 g/L EC were tested on the honey bee (*Apis mellifera* L.) under semi-field conditions following the OEPP/EPPO Guideline No. 170(4), (2010). The field phase of this study was conducted in Bodelshausen near Tübingen, Germany (region: Baden-Württemberg) from June to July 2013 and included a total of four treatment groups:

- Indoxacarb 150 g/L EC treatment group T1 with one application of the test item in each replicate (tunnel tent). The application was performed on 30 June 2013 during full flowering (BBCH 65) in the evening after daily honey bee-flight, 5 days after installation of the honey bee colonies in the tunnel tents. The application was carried out at a target rate of 37.5 g a.s/ha.
- Indoxacarb 150 g/L EC treatment group T2 with one application of the test item in each replicate (tunnel tent). The application was performed on 01 July 2013 during flowering (BBCH 65) and daily honey bee-flight, 6 days after installation of the honey bee colonies in the tunnel tents. The application was carried out at a target rate of 37.5 g a.s/ha.
- Reference item treatment group R with one application of BAS 152 11 I (a.s. dimethoate) at a target rate of 1000 mL product/ha (equivalent to 400 g dimethoate/ha nominal) during flowering of *P. tanacetifolia* (BBCH 65) and daily honey bee-flight, on the same day as the application in the treatment group T2 and in the control.
- Control group C with one application of tap water during flowering of *P. tanacetifolia* (BBCH 65) and during daily honey bee-flight, on the same day as the applications in the treatment group T2 and the reference item treatment group R.

The application in the test item treatment group T1 was performed on 30 June 2013 in the evening and the applications in C, T2 and R were performed on the next day (01 July 2013). The honey bee colonies were placed in the tunnel tents at early flowering of *P. tanacetifolia* (BBCH 63) on 24 June 2013. All applications were carried out with a target spray volume of 400 L water per ha.

The effects of the test item treatments were examined on small honey bee colonies in tunnel tents (5.0 m × 16.0 m and a height of 3.5 m in the centre) placed over plots of *P. tanacetifolia*. The semi-field test comprised 4 replicate tunnel tents in each treatment group except reference item treatment R (3 tunnel tents). Three tunnel tents per treatment (replicates a, b and c) were used for biological assessments, the 4th tunnel tent (replicate d) in T1, T2 and in the control C was used for sampling for residue analysis.

The influence of the test item treatments was evaluated by comparing the results in the test item treatment groups to the control and reference item data from before and after the application during full flowering in view of the following observations:

- Mortality: Number of dead honey bees on the linen and in the bee traps;
- Flight intensity: Number of forager honey bees/m² of flowering *P. tanacetifolia*;
- Behaviour of the honey bees on the crop and around the hive;
- Condition of the colonies and development of the honey bee brood;

- Level of the residues in samples of hive products (nectar, pollen), honey stomach contents and pollen loads from forager bees.

Indoxacarb 150 g/L EC, applied once at 37.5 g a.s./ha during flowering in the evening after honey bee-flight (treatment T1) or during daily honey bee-flight (treatment T2) had no effect on honey bee flight activity, brood development and colony condition in both test item treatments T1 and T2.

There was no effect on honey bee mortality in T1.

In T2, there was a transient increase in honey bee mortality (0DAA to 2DAA).

A slight transient effect on behaviour was observed on 0DAA in T1 and T2.

The residue levels in nectar and pollen on 1DAA and 4DAA were as follows:

- Pollen from forager bees: there were 0.33 (1DAA) and 0.04 (4DAA) mg a.s./kg in T1, 0.25 (1DAA) and 0.02 (4DAA) mg a.s./kg in T2 and no detectable residues in the control.
- Pollen from hives: there were 0.83 (1DAA) and 0.66 (4DAA) mg a.s./kg in T1, 0.76 (1DAA) and 0.49 (4DAA) mg a.s./kg in T2 and no detectable residues in the control.
- Nectar from forager bees and nectar from hives: there were no detectable residues in T1, T2 and in the control.

I. MATERIAL AND METHODS**A. MATERIALS:**

1. Test material: Indoxacarb 150 g/L EC
Batch/Lot: DPX-KN128-311
Content of a.s., nominal: 150 g a.s./L
Description: Liquid
CAS#: None for the formulation
173584-44-6 for the active substance
Stability of test compound: 98.2% of the indoxacarb remains in the delivery vehicle after one hour under agitation
Reference item: Dimethoate
Batch: 0001017331
Content of a.s., nominal: 400 g/L
Stability in solution: Sufficient for test purpose (at least 1 hour)
2. Vehicle and/or control: Tap water
3. Test organism
Species: *Apis mellifera* L.
Age at dosing: Direct exposure of adult honey bees; indirect exposure of all stages of development
Source: Eurofins Agrosience EcoChem GmbH
Diet: Nectar and pollen of flowering *Phacelia tanacetifolia*.
Tunnel tents (exposure): Within the test field 15 plots (each 5.0 m × 16.0 m) were marked and labelled before set-up of the colonies in the tunnel tents. Paths (0.6 m) were made in each plot by removing the plants and smoothing the ground. Before set-up of the bee colonies, tunnel tents (5.0 m × 16.0 m and a height of 3.5 m in the centre) were installed over the marked plots. The tent frames were covered with light plastic gauze. The paths (approx. 14.9 m²) were covered with linen sheets for the assessment of dead bees in the crop area. In the tunnels used for sampling for residue analysis (replicates Cd, T1d, T2d), no linen sheets were spread on the ground. The crop area per tent was approx. 65.1 m².
4. Environmental conditions during the exposure period
Temperature (min/max): 5.0–27.5°C
Relative humidity: 43.3–100%
Photoperiod (exposure): Natural light conditions

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed:
24-June-2013 to 24-October-2014
2. Experimental treatments
 - Indoxacarb 150 g/L EC treatment group T1 with one application of the test item in each replicate (tunnel tent). The application was performed on 30 June 2013 during full flowering (BBCH 65) in the evening after daily honey bee-flight, 5 days after installation of the honey bee colonies in the tunnel tents. The application was carried out at a target rate of 37.5 g a.s./ha.
 - Indoxacarb 150 g/L EC treatment group T2 with one application of the test item in each replicate (tunnel tent). The application was performed on 01 July 2013 during flowering (BBCH 65) and daily honey bee-flight, 6 days after installation of the honey bee colonies in the tunnel tents. The application was carried out at a target rate of 37.5 g a.s./ha.

- Reference item treatment group R with one application of BAS 152 11 I (a.s. dimethoate) at a target rate of 1000 mL product/ha (equivalent to 400 g dimethoate/ha nominal) during flowering of *P. tanacetifolia* (BBCH 65) and daily honey bee-flight, on the same day as the application in the treatment group T2 and in the control.
- Control group C with one application of tap water during flowering of *P. tanacetifolia* (BBCH 65) and during daily honey bee-flight, on the same day as the applications in the treatment group T2 and the reference item treatment group R.

3. Observations

The influence of the test item treatments was evaluated by comparing the results in the test item treatment groups to the control and reference item data from before and after the application during full flowering in view of the following observations:

Mortality: Number of dead honey bees on the linen and in the bee traps;

Flight intensity: Number of forager honey bees/m² of flowering *P. tanacetifolia*;

Behaviour of the honey bees on the crop and around the hive;

Condition of the colonies and development of the honey bee brood;

Level of the residues in the samples of hive products and honey stomach contents and pollen loads from forager bees.

II. RESULTS AND DISCUSSION

A. MORTALITY

During the pre-application period (4DBA to 1DBA(ba) in T1 and 4DBA to 0DBA in T2 and R), mortality was similar in all four treatment groups. Mean daily mortality was in the range from 51.7 to 194.3 dead honey bees/day (mean: 97.9 dead honey bees/day) in T1 and from 33.0 to 84.7 dead honey bees/day (mean: 58.8 dead honey bees/day) in the control, adapted for comparison with T1. This adaptation and the calculation of two different control values for certain assessment periods was done in order to cover equal time spans for the comparison of the control with either T1 (where the application was done in the evening of 1DBA, and the first assessment on the next morning was assigned to the post-application period instead of being summarized with the assessment on 1DBA in the evening) or with T2 and R (where the application was done during daytime on 0DAA and the first assessment on that day (0DBA) was part of the pre-application period and summarized with the assessment on 1DBA in the evening and counted as one value).

In T2, daily mortality was in the range from 50.3 to 130.0 dead honey bees/day (mean: 79.7 dead honey bees/day) and in R there were 53.7 to 118.7 dead honey bees/day (mean: 87.8 dead honey bees/day). In the control (adapted for comparison with T2 and R) there were 36.0 to 84.7 dead honey bees/day (mean: 61.5 dead honey bees/day). Values in T1, T2 and R were slightly higher but still comparable to the control, and there were no significant differences of pre-application mortality in all four treatment groups (Tukey's Studentized Range test, $p \leq 0.05$).

After the application, there was no effect of the test item treatment on honey bee mortality in T1. During the post-application period (0DAA to 7DAA), daily mortality was in the range from 39.3 dead honey bees/day to 125.3 dead honey bees/day (mean: 78.8 dead honey bees/day) compared to values from 19.0 to 71.7 dead honey bees/day (mean: 43.7 dead honey bees/day) in the control, adapted for comparison with T1. Post-application mortality in T1 was slightly higher than control mortality but these differences were not statistically significant. Since these differences were already present during the pre-application period, they are not related to the test item treatment.

Table 108
Honey bee mortality

Date	DBA/DAA	Mean Mortality (mean number of dead honey bees, larvae and pupae) per day and treatment group				
		C		T1	T2	R (Dimethoate)
		for T1	for T2 and R	(indoxacarb, 37.5 g a.s./ha in the evening)	(indoxacarb, 37.5 g a.s./ha during bee-flight)	
27 Jun 2013	4DBA	81.7		107.3	74.0	103.3
28 Jun 2013	3DBA	84.7		194.3	130.0	118.7
29 Jun 2013	2DBA	58.7		83.3	75.0	85.0
30 Jun 2013	1DBA	36.0		51.7	50.3	53.7
30 Jun 2013	1DBA(ba) ^a	33.0	46.3	52.7	69.0	78.3
01 Jul 2013	0DBA ^{b,c}	---		---		
Mean pre-application (4DBA to 1DBA(ba)/0DBA)		58.8	61.5	97.9	79.7	87.8
STD		16.9	17.1	68.2	30.4	11.4
01 Jul 2013	0DAA	60.0	46.7	98.3	449.0*	1049.0*
02 Jul 2013	1DAA	19.7		39.3	142.3*	258.0*
03 Jul 2013	2DAA	19.0		51.3	89.7*	288.3*
04 Jul 2013	3DAA	26.3		59.7	35.7	78.3*
05 Jul 2013	4DAA	32.7		60.7	31.0	70.3*
06 Jul 2013	5DAA	69.3		125.3	83.7	154.3
07 Jul 2013	6DAA	71.7		115.0	77.7	151.3
08 Jul 2013	7DAA	50.7		80.7	33.0	48.7
Mean post-application (0DAA to 7DAA)		43.7	42.0	78.8	117.8*	262.3*
STD		15.1	13.6	26.4	34.3	41.5

4DBA to 7DAA: Dead honey bees from linen sheets and dead bee traps (pre-exposure period and exposure period inside tunnel)

The number of dead bees includes the number of adult worker bees, dead pupae and young bees

DBA = Days before application during honey bee-flight

DAA = Days after application during honey bee-flight

STD = Standard deviation

ba = before application

^a Assessment shortly before start of application in T1 in the evening after honey bee-flight

^b Assessment in the morning, before applications in C, T2 and R (part of the post-application period in T1 and part of the pre-application period in T2 and R)

^c Assessments at 1DBA(ba) and 0DBA were summarized and counted as one value in T2 and R and in the control (adapted for comparison with T2 and R),

* Significantly different from the control (one-sided Dunnett's t-test, $p \leq 0.05$, for the test item treatment T2 (1DAA and 2DAA); one-sided pooled t-test, $p \leq 0.05$, for the reference item treatment, 0DAA (T2) and mean post-application (T2).

The application of the test item in T2 had a significant effect on honey bee mortality on 0DAA to 2DAA. Post-application mortality was in the range from 31.0 to 449.0 dead honey bees/day (mean: 117.8 dead

honey bees/day) in T2, compared to values from 19.0 to 71.7 dead honey bees/day (mean: 42.0 dead honey bees/day) in the control, adapted for comparison with T2 and R. These differences were statistically significant on 0DAA, 1DAA and 2DAA and for the mean post-application mortality. During further observation (3DAA to 7DAA) mortality in T2 was on the same level as the control.

The application of the reference item in treatment R had a clear effect on honey bee mortality. During the post-application period (0DAA to 7DAA), mortality was in the range from 48.7 to 1049.0 dead honey bees/day (mean: 262.3 dead honey bees/day) compared to 19.0 to 71.7 dead honey bees/day (mean: 42.0 dead honey bees/day) in the control. These differences were statistically significant from 0DAA to 4DAA and for the mean post-application mortality (0DAA to 7DAA).

Overall, there was no effect of the treatment on honey bee mortality in the test item group T1. Mortality in treatment group T2 was increased from 0DAA to 2DAA, whereas there was a clear impact of the reference item treatment from 0DAA to 4DAA.

B FLIGHT INTENSITY

Flight intensity in all 4 treatment groups was comparable during the pre-application period except for 4DBA and 0DBA. There were 0.0 to 23.3 forager bees/m² in T1 (mean: 11.7 forager bees/m²), 0.0 to 19.7 forager bees/m² (mean 9.9 forager bees/m²) in the control (adapted for comparison with T1), 0.0 to 23.3 forager bees/m² in T2 (mean: 12.1 forager bees/m²), 0.0 to 20.3 forager bees/m² in R (mean: 11.5 forager bees/m²) and 0.0 to 19.7 forager bees/m² (mean: 10.1 forager bees/m²) in the control (adapted for comparison with T2 and R). None of these slight differences were statistically significant except on 4DBA and 0DBA (Tukey's Studentized Range test, $p \leq 0.05$). On 4DBA there was hardly any flight activity in all treatments due to adverse weather conditions and data in T1 and T2 were significantly different from each other. On 0DBA, the mean flight intensity in the control (C) was significantly lower than in T2 and R (Tukey's Studentized Range test, $p \leq 0.05$) because the assessment in C was done at an earlier time of the day (shortly before start of the applications in C) when daily flight activity was only about to start. These differences are not biologically relevant and flight activity in the control on 0DBA was sufficient to ensure direct exposure of the bees during the application.

On all days during the post-application period, flight activity in T1 and T2 was similar to the control and no test item effect could be discerned.

After the applications (0DAA to 7DAA), flight activity was in the range from 12.3 to 30.7 forager bees/m² in T1 (mean: 23.8 forager bees/m²), from 4.3 to 27.3 forager bees/m² in T2 (mean: 18.1 forager bees/m²) and from 11.3 to 32.3 forager bees/m² in the control (mean: 21.9 forager bees/m², adapted for comparison with T1, and 22.1 forager bees/m², adapted for comparison with T2 and R).

There were no statistically significant differences of the daily flight activity in T1 or T2 compared to the control. The slightly different mean post-application flight intensities in T2 (18.1 forager bees/m²) and in the control (22.1 forager bees/m²) were statistically significant (pooled t-test, $p \leq 0.05$) but both values are on an acceptable level and this slight difference is not of any biological relevance.

In the reference item treatment R, the application had a clear effect on honey bee flight intensity. Values were in a range from 0.0 to 4.3 forager bees/m² (mean: 1.6 forager bees/m²) compared to 11.3 to 32.3 forager bees/m² (mean: 22.1 forager bees/m²) in the control. Mean post-application flight activity and daily flight activity were significantly lower compared to the corresponding values in the control on all days from 0DAA to 7DAA (pooled t-test or Satterthwaite t-test, $p \leq 0.05$).

Overall, there was no effect of the treatments on flight activity in the test item groups T1 and T2 whereas there was a clear impact of the reference treatment over 7 days after the application.

Table 109
Honey bee flight intensity

Date	DBA/ DAA	Mean Flight Intensity (mean number of forager bees/m ²)				
		C		T1 (indoxacarb, 37.5 g a.s./ha in the evening)	T2 (indoxacarb, 37.5 g a.s./ha during bee-flight)	R (Dimethoate)
		For T1	For T2 and R			
27 Jun 2013	4DBA ^a	0.7		2.0 [#]	0.0 [#]	0.3
28 Jun 2013	3DBA	19.7		21.3	21.7	18.0
29 Jun 2013	2DBA ^a	0.0		0.0	0.0	0.0
30 Jun 2013	1DBA	19.3		23.3	15.7	19.0
01 Jul 2013	0DBA ^b	---	11.0	---	23.3 ^{##}	20.3 ^{##}
Mean pre-application (4DBA to 1DBA/0DBA)		9.9	10.1	11.7	12.1	11.5
STD		1.8	1.2	0.8	0.6	1.4
01 Jul 2013	0DAA	21.0	22.5	25.3	17.9	4.3*
02 Jul 2013	1DAA	24.2		24.8	19.3	0.1*
03 Jul 2013	2DAA	14.7		17.7	16.7	0.0*
04 Jul 2013	3DAA	11.3		12.3	4.3	0.0*
05 Jul 2013	4DAA	19.0		21.3	12.3	1.3*
06 Jul 2013	5DAA	26.0		28.7	24.7	2.0*
07 Jul 2013	6DAA	32.3		30.7	27.3	2.0*
08 Jul 2013	7DAA	26.3		29.7	22.0	2.7*
Mean post-application (0DAA to 7DAA)		21.9	22.1	23.8	18.1*	1.6*
STD		1.9	2.0	5.6	0.7	0.4

DBA = Days before application during honey bee flight

DAA = Days after application during honey bee-flight

STD = Standard deviation

^a No flight activity (2DBA) or hardly any flight activity (4DBA) in all treatments due to adverse weather conditions

^b Assessment in the morning, before applications in C, T2 and R (part of the post-application period in T1 and part of the pre-application period in T2 and R)

* Significantly different from the control (pooled t-test or Satterthwaite t-test, $p \leq 0.05$)

Significantly different from T1 or T2, respectively (Tukey's Studentized Range test, $p \leq 0.05$)

Significantly different from the control (Tukey's Studentized Range test, $p \leq 0.05$).

C BEHAVIOUR OF THE HONEY BEES

During the pre-application period (4DBA to 1DBA/0DBA) normal behaviour was observed in all 4 treatments. Clustering on the linen sheets in front of the hive was observed once in T1 and R during exposure to the untreated crop. The occurrence of this behaviour is not related to the treatments but rather due to external conditions.

After the application of indoxacarb in the evening after bee-flight (treatment T1) there was a slight and transient effect in this treatment: few motionless bees were present in all replicates and two bees with locomotion problems were observed in one replicate at the first assessment in the following morning (0DBA). The 2 cramping bees that were observed in one replicate on 7DAA are not considered to be of any biological relevance or treatment related, since no such behaviour or other unusual behaviour was observed in all replicates of this treatment on the days before (1DAA to 6DAA) but similar observations were also made in the control on the day before (6DAA).

In the treatment T2, symptoms (locomotion problems, cramping, trembling) were observed mainly on the day of application (0DAA). Bees flying over the crop without landing on the crop and bees intensely grooming themselves were observed mainly during the first 2 hours after the application. On the following days (1DAA, 5DAA, 6DAA) a very small number of bees (1-2 bees per assessment date) with symptoms (cramping or motionless) were observed but in few replicates. These observations are not considered of any biological relevance or related to the treatment since no unusual observations were made in T2 on 2DAA to 4DAA but similar observations were made also in the control on 6DAA in few bees.

Overall, the application of indoxacarb after (T1) or during (T2) daily honey bee-flight had a slight transient effect on honey bee behaviour on 0DAA.

Few bees with unusual behaviour were also observed in the control on 0DAA and on 6DAA but this was not due to the application in this treatment (only water was sprayed and different equipment was used). Since no such observations were made in the control on 0DAA (in parallel to the observations in T1) and the first occurrence of this behaviour (0DAA + 15 min) was recorded before the applications in T2 and R on this day, it can be excluded that this behaviour was related to the applications in the other tunnels.

In the reference item treatment R, a strong repellent effect and clear impact of the application was observed in numerous bees that displayed symptoms mainly on 0DAA and 1DAA, and in few bees from 4DAA to 7DAA. Behavioural effects included aggressiveness towards the observer, locomotion problems, cramping and trembling, as well as intensive self-grooming and flying without landing on the crop.

D BROOD DEVELOPMENT AND COLONY CONDITION

At the first assessment before installation of the hives in the tunnel tents (7DBA) all colonies were strong and the mean colony sizes were similar in the four treatments (7667 honey bees/hive in C, 7813 honey bees/hive in T1, 8292 honey bees/hive in T2 and 7313 honey bees/hive in R). During the exposure period in the tunnels until 7DAA, the colony sizes declined and were similar in the two test item treatments, the control and the reference item treatment (6167 honey bees/hive in C, 5271 honey bees/hive in T1 and 5688 honey bees/hive in T2, 5271 honey bees in R).

During further observation of the hives at the monitoring site the mean colony sizes initially (15DAA) increased to 8355 honey bees in C, 8875 honey bees in T1, 8917 honey bees in T2 and then (22DAA) decreased to 4250 honey bees in C, 4188 honey bees in T1 and 4938 honey bees in T2. At the last assessment (29DAA), the mean colony sizes were similar in the control (6271 honey bees), in T1 (6542 honey bees) and in T2 (6813 honey bees).

In the reference item treatment R, the mean colony size decreased from 5146 honey bees/hive on 15DAA to 3313 honey bees/hive on 22DAA and at the last assessment (29DAA) there were only 4229 honey bees/hive in this treatment.

Overall, there was no negative impact of the test item treatment on the colony sizes in the Indoxacarb 150 g/L EC treatments T1 and T2 while there was a clear impact in the reference item treatment R.

Brood of all stages (eggs, larvae, capped brood) was present in all colonies at the first assessment (7DBA) before installation of the bee colonies in the tunnels and at all following assessments during the study period until 29DAA, except for hive Rb in the reference item treatment group where the queen died during the exposure period (3DAA) and certain brood stages were missing at the following assessments, before a new queen was reared and started laying eggs.

The mean total amount of brood was on a high level in all treatments at the first assessment before installation of the hives in the tunnels (7DBA) with 18667 brood cells/hive in the control, 22667 brood cells/hive in T1, 24533 brood cells/hive in T2 and 22600 brood cells/hive in R.

At the following assessment at the end of exposure (7DAA), a slight decline of the amount of brood was observed in both test item treatments T1 (13400 brood cells/hive) and T2 (17533 brood cells/hive) as well as in the control (15067 brood cells/hive). Since this slight decrease was also observed in the control, it is

not due to the treatment but rather due to the confinement in the tunnels. Until the end of the study (29DAA) the amount of brood cells increased to 22533 brood cells/hive in the control, 21667 brood cells/hive in T1 and 23333 brood cells/hive in T2. In the reference item treatment R, the amount of brood decreased to 7200 brood cells/hive on 7DAA. Afterwards the amount of brood increased but was always on a lower level than in T1, T2 and the control (up to 15200 cells/hive on 29DAA). This decrease was mainly due to the death of the queen of hive Rb on 04 July 2013 (3DAA) that was followed by a temporary interruption of brood rearing in this hive until a new queen was reared and started laying eggs.

All colonies had plenty amount of nectar and pollen throughout the experimental period.

Overall, there was no negative impact of the test item treatments T1 and T2 on honey bee brood development and colony condition.

D RESIDUE ANALYSIS

The application of the test item resulted in residue levels in nectar and pollen taken on 1DAA and 4DAA as follows:

Pollen from forager bees: there were 0.33 (1DAA) and 0.04 (4DAA) mg a.s./kg in T1, 0.25 (1DAA) and 0.02 (4DAA) mg indoxacarb/kg in T2 and no detectable residues in the control.

Pollen from hives: there were 0.83 (1DAA) and 0.66 (4DAA) mg a.s./kg in T1, 0.76 (1DAA) and 0.49 (4DAA) mg indoxacarb/kg in T2 and no detectable residues in the control.

Nectar from forager bees and nectar from hives: there were no detectable residues in T1, T2 and in the control.

Table 110
Indoxacarb residue levels in pollen and nectar

Sample Type	Treatment	Timing	Sample code	Residue level (mg indoxacarb/kg)
Pollen from forager bees	C	1DAA	L13-00880-01-PFB-A	n.d.
		4DAA	L13-00880-04-PFB-A	n.d.
	T1	1DAA	L13-00880-02-PFB-A	0.33
		4DAA	L13-00880-05-PFB-A	0.04
	T2	1DAA	L13-00880-03-PFB-A	0.25
		4DAA	L13-00880-06-PFB-A	0.02
Pollen from hives (combs)	C	1DAA	L13-00880-07-PH-A	n.d.
		4DAA	L13-00880-10-PH-A	n.d.
	T1	1DAA	L13-00880-08-PH-A	0.83
		4DAA	L13-00880-11-PH-A	0.66
	T2	1DAA	L13-00880-09-PH-A	0.76
		4DAA	L13-00880-12-PH-A	0.49
Nectar from forager bees	C	1DAA	L13-00880-01-NFB-A	n.d.
		4DAA	L13-00880-04-NFB-A	n.d.
	T1	1DAA	L13-00880-02-NFB-A	n.d.
		4DAA	L13-00880-05-NFB-A	n.d.
	T2	1DAA	L13-00880-03-NFB-A	n.d.
		4DAA	L13-00880-06-NFB-A	n.d.
Nectar from hives (combs)	C	1DAA	L13-00880-13-NH-A	n.d.
		4DAA	L13-00880-16-NH-A	n.d.
	T1	1DAA	L13-00880-14-NH-A	n.d.
		4DAA	L13-00880-17-NH-A	n.d.
	T2	1DAA	L13-00880-15-NH-A	n.d.
		4DAA	L13-00880-18-NH-A	n.d.

n.d. = not detected (residues below the limit of detection (LOD), <0.003 mg/kg),

III. CONCLUSION

Indoxacarb 150 g/L EC, applied once at 37.5 g a.s./ha during flowering in the evening after honey bee-flight (treatment T1) or during daily honey bee-flight (treatment T2) had no effect on honey bee flight activity, brood development and colony condition in both test item treatments T1 and T2.

There was no effect on honey bee mortality in T1.

In T2, there was a transient increase in honey bee mortality (0DAA to 2DAA).

A slight transient effect on behaviour was observed on 0DAA in T1 and T2.

The residue levels in nectar and pollen on 1DAA and 4DAA were as follows:

Pollen from forager bees: there were 0.33 (1DAA) and 0.04 (4DAA) mg a.s./kg in T1, 0.25 (1DAA) and 0.02 (4DAA) mg indoxacarb/kg in T2 and no detectable residues in the control.

Pollen from hives: there were 0.83 (1DAA) and 0.66 (4DAA) mg a.s./kg in T1, 0.76 (1DAA) and 0.49 (4DAA) mg indoxacarb/kg in T2 and no detectable residues in the control.

Nectar from forager bees and nectar from hives: there were no detectable residues in T1, T2 and in the control.

(Kleinhenz, M., 2014b)

RMS comment:

Study submitted to the EU for the first time in this submission.

This study is considered valid. There were three bee colonies per treatment group (4th tunnel tent was for residue analysis).

Indoxacarb 150 g/L EC, applied once at 37.5 g a.s./ha during flowering in the evening after honey bee-flight (treatment T1) or during daily honey bee-flight (treatment T2) had no effect on brood development and colony condition in both test item treatments T1 and T2.

RMS notes that flight intensity slightly decreased in the tunnels treated with Indoxacarb 150 g/L EC during bee flight during all post-application period (considered of no biological relevance by the study author). The reason of this decrease is not known but RMS agrees that the difference is slight. No effect on flight intensity was observed when Indoxacarb 150 g/L EC is applied in the evening after honey bee-flight (treatment T1).

There was no effect on honey bee mortality in T1.

In T2, there was a transient increase in honey bee mortality (0DAA to 2DAA).

A slight transient effect on behaviour was observed on 0DAA in T1 and T2.

The residue levels in nectar and pollen on 1DAA and 4DAA were as follows:

Pollen from forager bees: there were 0.33 (1DAA) and 0.04 (4DAA) mg a.s./kg in T1, 0.25 (1DAA) and 0.02 (4DAA) mg indoxacarb/kg in T2 and no detectable residues in the control.

Pollen from hives: there were 0.83 (1DAA) and 0.66 (4DAA) mg a.s./kg in T1, 0.76 (1DAA) and 0.49 (4DAA) mg indoxacarb/kg in T2 and no detectable residues in the control.

Nectar from forager bees and nectar from hives: there were no detectable residues in T1, T2 and in the control.

Report: Berg, C. (2014); Indoxacarb (DPX-KN128) 150 g/L EC: A semi-field study to evaluate effects on the brood of honey bees (*Apis mellifera*; Hymenoptera, Apidae) in *Phacelia tanacetifolia* in Germany 2014

DuPont Report No.: DuPont-38405

Guidelines: OECD 75 (2007) **Deviations:** None

Testing Facility: Eurofins Agrosience Services GmbH, Niefern-Oeschelbronn, Germany

Testing Facility Report No.: S14-03576

GLP: Yes

Certifying Authority: Landesanstalt Für Umwelt, Messungen Und Naturschutz Baden-Württemberg

Executive summary:

The effects of the insecticide Indoxacarb 150 g/L EC applied during flowering were tested on the honey bee (*Apis mellifera* L.) under semi-field conditions following the OECD guidance document No. 75 (2007). This study was conducted near Karlsruhe in Southern Germany (region: Baden-Württemberg) from June to July 2014 and included a total of three treatment groups:

- Indoxacarb 150 g/L EC treatment group S with one application of the test item in each replicate (tunnel tent). The application was carried out during flowering of *P. tanacetifolia* (BBCH 65) on 1DBA in the

evening after bee-flight had stopped (0 honey bees/m² flying inside the tunnels). The application was carried out at a target rate of 50.0 g a.s./ha.

- Reference item treatment group R with one application of Insegar (fenoxycarb) at a target rate of 1200 g product/ha (equivalent to 300 g fenoxycarb/ha). The application was carried out during flowering of *P. tanacetifolia* (BBCH 65-67) and bee-flight (≥ 10 honey bees/m² flying inside the tunnels), on the same day as the application in the control.
- Control group C with one application of tap water during flowering of *P. tanacetifolia* (BBCH 65-67) and bee-flight (≥ 10 honey bees/m² flying inside the tunnels), one day after the application in the treatment group S.

Data from the control group C and reference item group R were shared another study running on the same field at the same time (Dupont-37489). The applications in the reference item treatment R and in the control were carried out on 13 June 2014 (6 days after installation of the hives in the tunnels). In treatment group S, the application was done on 12 July 2014. All applications were carried out with a spray volume of 400L water per ha. The effects of the test item treatment were examined on honey bee colonies in tunnel tents (5.0 m \times 18.0 m and a height of 3.5 m in the centre) placed over the plots of *P. tanacetifolia*. The semi-field test comprised 4 replicate tunnel tents in each of the treatment groups for biological assessments and additionally one tunnel tent (replicate e) for sampling in C and S for residue analysis.

The influence of the application of Indoxacarb 150 g/L EC was evaluated by comparing the results in this treatment to the data in the control treatment as well as in the reference item treatment regarding the following observations:

- Mortality: number of dead honey bees at the edge of the treated area (linen sheets), in the dead honey bee traps in front of the hives and in the bottom drawers.
- Flight intensity on the crop (number of forager honey bees/m² *P. tanacetifolia*),
- Condition of the colonies and development of the brood,
- Detailed observation of the brood development of eggs and young larvae in ≥ 200 selected cells and of old larvae in ≥ 160 selected cells,
- Behaviour of the honey bees in the crop area and around the hives.
- Level of residues in the samples of hive products and nectar stomach contents and pollen loads from forager bees

It can be concluded that the test item Indoxacarb 150 g/L EC applied after bee flight had a very slight effect on honeybee mortality compared to the control. Considering the non-test item related slightly increased numbers of dead bees/day that already occurred in the pre-application period of the treatment group S and the values after the application, which were similar, no biologically relevant effect can be assumed.

The application of Indoxacarb 150 g/L EC after bee flight had a slight effect on the flight intensity on the day after the application. After this day, the flight intensity was at the same level as in the control. The slight and transient effect can be considered as not biologically relevant.

The application of Indoxacarb 150 g/L EC had a slight effect on the behaviour of the honey bees shortly after the applications. Since the numbers of honey bees showing unusual behaviour are generally low, this effect can be considered as not biologically relevant.

There was no negative effect on the colony size relating to the application of Indoxacarb 150 g/L EC in treatment group S.

There was only a slight and transient effect on the amount of brood cells in the treatment group S shortly after the application of Indoxacarb 150 g/L EC.

The application of Indoxacarb 150 g/L EC carried out after bee flight had a slight effect on the development of the marked eggs, whereas no effect occurred on the development of young larvae and old larvae.

The spray application of Indoxacarb 150 g/L EC carried out at 50.0 g a.s./ha during flowering and after bee flight (S) caused slight effects of minor biological relevance on honeybee colonies.

No residues of indoxacarb could be found in the nectar samples prepared from forager bees or in nectar samples which were taken directly from the hive in the control group as well as in the treatment group S.

All pollen samples of the control group were free of residues of indoxacarb. In the sample taken from the forager bees in the treatment group S, 0.059 mg/kg could be found two days after the application and 0.032 mg/kg four days after the application. In the pollen sample which was taken directly from the hive, 0.046 mg/kg could be found two days after the application and 0.017 mg/kg could be found four days after the application.

I. MATERIAL AND METHODS

A. MATERIALS:

1. Test material:
Lot/Batch: DPX-KN128-434
Purity: 150 g a.s./L
Description: Liquid
CAS#: None for the formulation
173584-44-6 for the active substance
Stability of test compound: 98.2% of the indoxacarb remains in the delivery vehicle after one hour under agitation
Reference item: Insegar (fenoxycarb)
Purity: 25.0% (w/w)
CAS#: 72490-01-8
2. Vehicle and/or control: Tap water
3. Test organism
Species: *Apis mellifera* L.
Age at dosing: Direct exposure of adult honey bees; indirect exposure of all stages of development
Source: Eurofins Agrosience Services EcoChem GmbH
Diet: Nectar and pollen of flowering *Phacelia tanacetifolia*,
1 feeding of Api-Invert on 12DAA
Tunnel tents (exposure): Within the test field, plots (5.0 m × 18.0 m) were marked. Tunnel tents (5.0 m × 18.0 m and a height of 3.5 m in the centre) covering the marked plots were installed before full flowering of *P. tanacetifolia* and before installation of the bee hives. The tunnel frames were covered with light plastic gauze. Paths (0.6 m) were made in the tunnels by removing the plants and smoothing the ground. Subsequently, the paths (approx. 16.1 m²) in the tunnels intended for the biological assessments (replicates a, b, c and d) were covered with linen sheets for the assessment of dead bees in the crop area. The crop area per tunnel was approx. 73.9 m². In the tunnels used for sampling for residue analysis (replicates Ce and Se) linen sheets were also spread on the ground.
4. Environmental conditions during the experimental phase
Temperature (min/max): 7.0–37.2°C
Relative humidity: 18.2–100%
Photoperiod (exposure): natural light conditions

B STUDY DESIGN AND METHODS

1. In-life initiated/completed
06-June-2014 to 05-November-2014
2. Experimental treatments
Indoxacarb 150 g/L EC treatment group S with one application of the test item in each replicate (tunnel tent). The application was carried out during flowering of *P. tanacetifolia* (BBCH 65) on 1DBA in the evening after bee-flight has stopped (0 honey bees/m² flying inside the tunnels). The application was carried out at a target rate of 50.0 g a.s./ha.

Reference item treatment group R with one application of Insegar (fenoxycarb) at a target rate of 1200 g product/ha (equivalent to 300 g a.s./ ha). The application was carried out during flowering of *P. tanacetifolia* (BBCH 65-67) and bee-flight (≥10 honey bees/m² flying inside the tunnels), on the same day as the application in the control.

Control group C with one application of tap water during flowering of *P. tanacetifolia* (BBCH 65-67) and bee-flight (≥ 10 honey bees/m² flying inside the tunnels), one day after the application in the treatment group S.

3. Observations

The influence of the application of Indoxacarb 150 g/L EC was evaluated by comparing the results in this treatment to the data in the control treatment as well as in the reference item treatment regarding the following observations:

Mortality: number of dead honey bees at the edge of the treated area (linen sheets) and in the dead honey bee traps in front of the hives,

Foraging activity on the crop (number of forager honey bees/m² *P. tanacetifolia*),

Condition of the colonies and development of the brood,

Detailed observation of the brood development of eggs, young larvae and old larvae in ≥ 160 selected cells

Behaviour of the honey bees in the crop area and around the hives.

II. RESULTS AND DISCUSSION

A. MORTALITY

Mortality after installation of the colonies until the application (4DBA to 1DBA for S or 0DBA for R) was comparable in all treatment groups, but with a trend towards higher values for S and R versus the control. The mean daily mortality was in the range from 22.3 to 194.0 dead honey bees/day in the control, from 36.5 to 268.8 dead honey bees/day in test item treatment group S and from 26.5 to 248.0 dead honey bees/day in the reference item treatment group R. The unusually high numbers of dead bees which were found on the linen on 3DBA in all treatment groups are probably due to the very high temperatures and bees which could not adapt quickly enough to the confined situation in the tunnel tents. Mean daily mortality in the control over the whole period was 63.7 (for S) or 65.3 (for R) dead honey bees/day, 89.0 dead honey bees/day in S and 100.6 dead honey bees/day in R. The numbers of dead bees in the treatment groups S and R during the pre-application period are tangentially higher. Significant differences are found in R on 4DBA, 1DBA and 0DBA, whereas the numbers of dead honey bees/day in the treatment group S are not statistically significant compared to the control. (Tukey's Studentized Range test, $p \leq 0.05$). For the mean number of dead bees/day of the entire period before the application, there were no statistically significant results between the treatment groups.

Daily mortality values during the period after the application until the removal of the hives from the tunnel tents (0DAA to 7DAA) were in the range from 13.8 to 63.3 dead honey bees/day in the control, from 59.8 to 127.0 dead honey bees/day in S and from 35.3 to 143.8 dead honey bees/day in R.

At the application day (0DAA), after the application during bee flight in the control and R, the mortality of the test item treatment group S was slightly, but statistically significantly higher compared to the mortality of the control group (pooled t-test, $p \leq 0.05$). The numbers of dead bees in S were significantly higher on 2DAA, 3DAA, 5DAA and when considering the whole period from 0DAA to 7DAA (pooled t-Test, $p \leq 0.05$). The mean value of the control during that period was 53.9 (for S) or 52.9 (for R) dead honey bees/day, compared to values of 76.5 dead honey bees/day in S and 111.7 dead honey bees in R. Considering the non-test item related slightly increased numbers of dead bees/day that already occurred during the pre-application period of the treatment groups S and the values after the application, which is lower, no biologically relevant effect can be assumed.

The mortality of the reference item treatment group R was statistically significantly increased on every day during the period from 0DAA to 7DAA (pooled t-Test, Satterthwaite t-Test, $p \leq 0.05$) with the exception of

1DAA, where no significant difference occurred. Considering the whole period from 0DAA to 7DAA, the mortality in R was significantly increased versus the control (pooled t-test, $p \leq 0.05$).

During further observation at the monitoring site from 8DAA to 28DAA, daily mortality values were in the range from 4.3 to 101.0 dead honey bees/day in the control, from 8.3 to 33.3 dead honey bees/day in S and from 15.3 to 101.8 dead honey bees/day in R. The mean values during that period were 16.6 dead honey bees/day in the control, 17.5 dead honey bees in S, and 54.6 dead honey bees in R. In S, the mean daily mortality was significantly higher on 8DAA and 22DAA. The values during that period are on the same level as in the control.

The mortality of the reference item treatment group R during the period on the monitoring site was statistically significantly increased in comparison to the control on every day from 9DAA to 27DAA (pooled t-test, Satterthwaite t-Test or Mann Whitney Exact test, $p \leq 0.05$), with the exception of 14DAA, 20DAA and 26DAA. Considering the whole period from 8DAA to 28DAA, the mortality was significantly higher in R (pooled t-Test, $p \leq 0.05$).

Table 111
Honey bee mortality

Date	DBA/ DAA	Mean number of dead honey bees/day per treatment group			
		C		S	R
		For S	For R		
09 Jun 2014	4DBA	44.3		51.3	98.0*
10 Jun 2014	3DBA	194.0		268.8	248.0
11 Jun 2014	2DBA	30.5		36.5	26.5
12 Jun 2014	1DBA	22.3		44.8	50.3*
12 Jun 2014	1DBAba	27.3	---	43.8	---
13 Jun 2014	0DBA	---	35.5 ^a	---	80.3* ^a
Mean 4DBA to 1DBAba or 0DBA		63.7	65.3	89.0	100.6
STD		36.1	36.3	20.4	11.6
13 Jun 2014	0DAA	55.3 ^b	47.0	105.8* ^b	126.5*
14 Jun 2014	1DAA	13.8		21.5	35.3
15 Jun 2014	2DAA	72.3		127.0*	143.8*
16 Jun 2014	3DAA	63.3		94.5*	142.8*
17 Jun 2014	4DAA	60.8		61.8	118.8*
18 Jun 2014	5DAA ^c	51.5		71.3*	100.8*
19 Jun 2014	6DAA	60.8		70.8	124.3*
20 Jun 2014	7DAA	53.5		59.8	101.3*
Mean 0DBA or 0DAA to 7DAA		53.9	52.9	76.5*	111.7*
STD		12.8	12.2	4.7	41.4

Table 111
Honey bee mortality (continued)

Date	DAA	Mean number of dead honey bees/day per treatment group		
		C	S	R
21 Jun 2014	8DAA	4.3	18.3*	15.3
22 Jun 2014	9DAA	7.8	15.5	33.3*
23 Jun 2014	10DAA	8.0	16.0	68.0*
24 Jun 2014	11DAA ^c	15.0	27.8	87.8*
25 Jun 2014	12DAA	9.5	15.0	72.0*
26 Jun 2014	13DAA	12.3	22.3	96.3*
27 Jun 2014	14DAA	25.0	8.5	46.8
28 Jun 2014	15DAA	17.8	14.3	50.5*
29 Jun 2014	16DAA ^c	20.3	22.0	71.0*
30 Jun 2014	17DAA	9.5	23.3	70.8*
01 Jul 2014	18DAA	9.5	17.0	58.5*
02 Jul 2014	19DAA	16.3	14.3	55.3*
03 Jul 2014	20DAA	15.3	8.3	37.8
04 Jul 2014	21DAA	11.8	16.8	59.8*
05 Jul 2014	22DAA ^c	10.5	21.3*	38.0*
06 Jul 2014	23DAA	9.3	14.3	49.8*
07 Jul 2014	24DAA	5.3	10.5	22.5*
08 Jul 2014	25DAA	5.0	8.5	28.3*
09 Jul 2014	26DAA	18.3	19.0	32.8
10 Jul 2014	27DAA	16.3	22.3	50.3*
11 Jul 2014	28DAA	101.0	33.3	101.8
Mean 8DAA to 28DAA		16.6	17.5	54.6*
STD		4.5	6.4	23.1

DBA = Days before application

DAA = Days after application

ba = before application

STD = Standard deviation

4DBA to 7DAA: Dead bees from linen sheets, dead bee traps and bottom drawers (exposure period inside tunnel)

8DAA to 28DAA: Dead bees from dead bee traps and bottom drawers (monitoring period)

^a = Results of the assessments on 1DBAba (before application in S) and 0DBA (before applications in C and R) were summarized and counted as one value.

^b = Results of the assessments on 0DBA (before applications in C and R) and 0DAA were summarized and counted as one value.

^c = potentially higher mortality due to a colony assessment that was done in all treatments on the day before

* = significantly different to the control (Tukey's Studentized Range test or pooled t-test, $p \leq 0.05$)

In the pre-application period from 4DBA to 1DBA (for S) or 0DBA (for R), the total number of dead pupae and dead malformed pupae was 3 in the control, 14 in the test item treatment group S and 18 in the reference item treatment group R. In the post exposure period in the tunnel tents from 0DAA to 7DAA there were 7 dead pupae and dead malformed pupae in C, 97 in S and 45 in R. In the monitoring period from 8DAA to 28DAA, there were 22 dead pupae and dead malformed pupae in C, 122 in S and 1063 in R. 38 of the dead pupae in S were found in one replicate on 28DAA, one day after the last colony assessment. Since no more than 5 pupae per day were found until that day in the period after the application (with the exception of replicate Sb, where 20 dead pupae and dead malformed pupae were found on 8DAA), the slightly increased numbers of dead pupae and dead malformed pupae in the treatment groups S can be considered as non-test item related. There are no considerable differences of biological relevance in the mortality of dead young bees, malformed honey bees, dead pupae and dead malformed pupae between the treatment group S compared to the control.

Overall, there was no increase in honey bee mortality in the Indoxacarb 150 g/L EC treatment group S after the application. The differences of the treatment group S compared to the control are considered as a very slight effect of the test item, which has no biological relevance. There was no considerable impact of the Indoxacarb 150 g/L EC treatment in S on honey bee pupal mortality. The toxic reference treatment resulted in a clear impact on honey bee mortality, mainly resulting in high numbers of dead pupae.

B. FLIGHT INTENSITY

Daily flight intensity from installation of the colonies in the tunnels until the application during bee-flight from 4DBA to 1DBA (for S) or 0DBA (for R) was in the range from 11.7 to 21.8 forager bees/m² in the control, from 14.9 to 22.6 forager bees/m² in the test item treatment group S and from 19.6 to 25.1 forager bees/m² in the reference item treatment group R. The mean daily flight intensity during this period was 15.6 (for S) or 14.8 (for R) forager bees/m² in the control, 18.5 forager bees/m² for S and 22.8 forager bees/m² for R. The reference item group R was statistically different from the control at 4DBA, 2DBA, 0DBA and when considering the whole period from 4DBA to 0DBA (Tukey's Studentized Range test, $p \leq 0.05$). The treatment group S was on the same level as the control during that period and there were no statistically significant differences between S and C.

On the day of application, the number of forager bees/m² was statistically significantly lower in S (11.5 forager bees/m²) compared to the control (18.5 forager bees/m² for S; pooled t-test, $p \leq 0.05$). R (23.3 forager bees/m²) was not statistically significant different to the control (19.8 forager bees/m² for R).

During the period from 1DAA to 7DAA, the mean number of forager bees/m² was on the same level in all treatment groups and was in a range from 5.2 to 14.0 forager bees/m² in the control, from 5.2 to 10.7 in S and from 7.9 to 16.2 forager bees/m² in R. The mean number of forager bees/m² was 10.2 (for S) or 10.4 (for R) in C, 8.1 in S and 13.0 forager bees/m² in R. There were no statistically significant results in any of the treatment groups considering the whole period from 0DAA to 7DAA.

Table 112
Honey bee flight intensity

Date	DAA	Flight intensity (mean number of forager bees/m ²)			
		C		S	R
		For S	For R		
09 Jun 2014	4DBA	15.5		16.1	22.4*
10 Jun 2014	3DBA	13.3		20.4	19.6
11 Jun 2014	2DBA	11.7		14.9	22.1*
12 Jun 2014	1DBA	21.8		22.6	25.1
13 Jun 2014	0DBA	---	11.9	---	24.5*
Mean 4DBA to 1DBA or 0DBA		15.6	14.8	18.5	22.8*
STD		3.5	2.9	3.0	4.0
13 Jun 2014	0DAA	18.5 ^a	19.8	11.5*	23.3
14 Jun 2014	1DAA	10.1		10.7	13.5
15 Jun 2014	2DAA	14.0		10.4	16.2
16 Jun 2014	3DAA	11.9		7.7	11.8
17 Jun 2014	4DAA	6.5		5.2	9.4
18 Jun 2014	5DAA	10.1		7.9	13.5
19 Jun 2014	6DAA	5.2		5.3	8.3
20 Jun 2014	7DAA	5.3		6.0	7.9
Mean 0DAA to 7DAA		10.2^a	10.4	8.1	13.0
STD		2.1	2.1	0.6	2.3

DAA = Days after application

DBA = Days before application

STD = Standard deviation

^a Includes the data of the assessment on 0DBA (part of the post-application period in S)

* Significantly different to the control (Tukey's Studentized Range test or pooled t-test, $p \leq 0.05$)

Overall, there was a slight reduction of foraging activity on the day after the application in the test item treatment group S. After this day, the flight intensity was back on the same level as in the control during the complete period in the tunnel tents.

C. BEHAVIOUR OF THE HONEY BEES

In the control, the treatment group S and in the reference item group R, normal behaviour was recorded in the period before the applications (4DBA to 1DBA or 0DBA). Only very few bees showed unusual behaviour: In the control, there were 7 cramping bees, 12 bees with locomotion problems, 2 inactive bees and 5 hanging bees. In S, there were 5 cramping bees, 2 trembling bees, 7 bees with locomotion problems and 13 inactive bees. In R, there were 15 cramping bees, 8 trembling bees, 8 bees with locomotion problems and 2 inactive bees. The observed behaviour during this period before the applications is showing the normal background level of unusual behaviour.

In the tunnel period after the application in the treatment groups (0DBA or 0DAA to 7DAA), the numbers of bees showing unusual behaviour in the treatment groups S and R were slightly elevated compared to the control, but the total number of bees showing unusual behaviour remained on a low level in the test item treated group S as well as in the reference item treatment group. There were 43 cramping bees in S and 35 cramping bees in R compared to 15 cramping bees in the control. The numbers of trembling bees were 10 in S and 25 in R compared to 0 in the control. There were 66 bees showing locomotion problems in S, 79 in R and 24 in the control. The numbers of inactive bees were on a similar level in the treatment groups and the reference group compared to the control, there were 7 in S, 25 in R and 27 in the control.

The numbers of bees which were clustering, hanging on the crop or intensely self-grooming are marginal and do not show any significance.

After the tunnel period on the monitoring site, the numbers of bees showing unusual behaviour in the treatment group S is on the same level as in the control, whereas the number of bees showing unusual behaviour in R is very low, although very slightly elevated compared to the control. There were 7 cramping bees in S and 45 cramping bees in R compared to 17 cramping bees in the control. There were 10 bees with locomotion problems in S, 33 in R and 1 in the control. The numbers of inactive bees were again on a similar level in the treatment groups compared to the control, there were 51 in S, 72 in R and 95 in the control. The numbers of bees which were showing any other unusual behaviour are marginal and do not show any significance.

Overall, a very slight effect on the behaviour could be observed, with only very few bees showing unusual behaviour at each of the assessments in the test item treatment group S and the control. After the tunnel period, the numbers of bees showing unusual behaviour is on the same level as in the control, showing that the slight effect on behaviour is transient and disappears after approximately 7 days. There does not seem to be an effect of the test item on the behaviour of the bees, which is biologically relevant.

D, BROOD DEVELOPMENT AND COLONY CONDITION

At the first assessment, there were 6313 to 7500 honey bees/hive with a mean of 6703 honey bees per hive in the control. The treatment group S had 6313 to 8625 honey bees/hive with a mean of 7157 honeybees per hive. In the reference item treatment group, there were 7250 to 9313 honey bees/hive with a mean of 8453 honey bees per hive at the first assessment date.

At the next assessment on 1DBA, shortly before the brood fixing, the mean colony size stayed on a similar level in all treatment groups. The mean colony size was 6266 in the control, 7469 in S and 7578 in R.

At the first colony assessment after the application on 4DAA, the mean colony size was slightly increasing in all treatment groups. The mean colony size was 8203 in the control, 8641 in S and 8969 in R.

At the fourth assessment on 10DAA, the first on the monitoring site after the removal from the tunnel tents, the mean colony size strongly increased in all treatment groups. The mean colony size was 11000 in the control, 12797 in S and 12578 in R.

Five days later on 15DAA, the mean colony size of all treatment groups had only slightly changed. The mean colony size was 10844 in the control, 11594 in S and 12578 in R.

At the sixth colony assessment on 21DAA, the mean colony size was slightly decreasing in all treatment groups. There were 10391 honey bees in the control, 11172 honey bees in S and 10125 honey bees in R.

At the last colony assessment on 27DAA, the mean colony size was decreasing in all treatment groups. The mean colony size was 7641 in the control, 8204 in S and 8344 in R.

The development of the colony size in the treatment group S is very similar to the development in the control group. There does not seem to be an effect on the colony size following the application of the test item.

At the first colony assessment on 7DBA, high numbers of brood cells, which contain eggs, larvae and pupae, were observed in all treatment groups. The mean number of brood cells was 23050 in the control, 31100 in S and 31400 in R.

During the next assessments, the number of brood cells constantly decreased in all treatment groups. The minimum mean quantity of brood cells was reached at the fourth assessment on 10DAA. At this assessment, there were 13800 brood cells in C, 10200 in S and 9350 in R. There are very slightly lower numbers of brood cells on 4DAA and 10DAA in the treatment group S compared to the control. In R, there

was a clear effect on honey bee brood, where the mean number of larvae was only 550 on 4DAA. At 10DAA, the mean number of eggs per colony had already increased in all treatment groups.

At the following two assessments, the mean total number of brood cells increased up to 21000 brood cells in C, 22450 in S and 26500 in R on 21DAA.

On the last assessment, the mean number of brood cells stayed on the same level. There were 19700 brood cells in C, 21300 brood cells in S and 24650 in R. The number of food cells was sufficient throughout the study with small reductions of the total amount of food cells in the tunnel period.

Overall, no effect of the test item treatment on honey bee development and colony condition in the treatment group S could be discerned. The colony sizes and the numbers of brood cells of the treatment group S were comparable to the control and the development of the colonies showed the typical characteristics of colonies which are confined in a tunnel tent.

E. DEVELOPMENT OF THE HONEY BEE BROOD IN INDIVIDUAL CELLS

Development of eggs in the marked cells:

The brood indices of S are lower than in the control, statistically significant on BFD+5 (5 days after the “Brood Area Fixing Day” = BFD, pooled t-Test, $p \leq 0.05$), whereas there were no statistically significant differences between S compared to the control on BFD+11, BFD+16 and BFD+22. The final values were 3.19 in the control and 1.35 in S.

The compensation index was statistically significantly lower in the test item group S on BFD+5 compared to the control (pooled t-Test, $p \leq 0.05$). Afterwards, the compensation index was increasing in S up to the last assessment on BFD+22, reaching almost the value of the control. The final values on BFD+22 were 3.87 in C and 3.57 in S. There were no statistically significant differences between S compared to the control on BFD+11, BFD+16 and BFD+22.

The termination rate in S was slightly higher than in the control. The values on BFD+22 were 36.17% in C and 73.10% in S. The termination rate was statistically significantly higher on BFD+5 (pooled t-Test, $p \leq 0.05$), whereas there were no statistically significant differences between the test item treatment groups and the water-treated control on BFD+11, BFD+16 and BFD+22 (pooled t-Test, Satterthwaite t-Test or Mann Whitney Exact Test, $p \leq 0.05$).

In the reference item group, the brood index reached a final value of 0.14 on BFD+22. There were only very few marked cells in which a successful development took place. The brood index was statistically significantly lower on BFD+5, BFD+11, BFD+16 and BFD+22 (pooled t-Test, $p \leq 0.05$). The compensation index was statistically significantly lower on BFD+5, BFD+11 and BFD+16 (pooled t-Test, $p \leq 0.05$). The termination rates in the reference item group R reached a mean value of 97.12 on BFD+22, showing a clear effect on the brood development. It was statistically significantly different on BFD+5, BFD+11, BFD+15 and BFD+22 compared to the control (pooled t-Test, $p \leq 0.05$).

Table 113
Honey bee egg brood/compensation indices and termination rate

Treatment group/ replicate	Brood/Compensation Indices at × days after brood area fixing day (BFD0) for marked cells containing eggs at the first assessment					Termination rate %
	BFD0	BFD+5	BFD+11	BFD+16	BFD+22	BFD+22
Ca	1.00/1.00	1.16/1.19	0.92/0.92	0.92/1.03	1.15/2.35	77.06
Cb	1.00/1.00	2.36/2.46	2.53/2.60	2.53/2.73	3.14/4.05	37.13
Cc	1.00/1.00	2.85/2.85	3.41/3.41	3.33/3.36	4.16/4.35	16.75
Cd	1.00/1.00	2.58/2.58	3.47/3.47	3.45/3.57	4.31/4.73	13.73
Mean C	1.00/1.00	2.24/2.27	2.58/2.60	2.56/2.67	3.19/3.87	36.17
STD	0.00/0.00	0.75/0.74	1.19/1.19	1.17/1.15	1.46/1.05	29.18
Sa	1.00/1.00	1.56/1.56	2.06/2.06	2.06/2.21	2.57/3.63	48.61
Sb	1.00/1.00	0.08/0.08	0.08/0.10	0.08/1.01	0.11/2.96	97.88
Sc	1.00/1.00	0.33/1.00	0.13/1.16	0.13/2.84	0.14/3.67	97.13
Sd	1.00/1.00	1.64/1.66	2.05/2.06	2.05/2.63	2.56/4.00	48.76
Mean S	1.00/1.00	0.90*/1.08*	1.08/1.35	1.08/2.17	1.35/3.57	73.10
STD	0.00/0.00	0.81/0.72	1.13/0.93	1.13/0.82	1.41/0.44	28.19
Ra	1.00/1.00	0.29/0.30	0.17/0.18	0.16/0.65	0.19/2.25	96.11
Rb	1.00/1.00	0.69/0.74	0.22/0.60	0.20/2.08	0.25/3.61	94.98
Rc	1.00/1.00	0.24/0.30	0.16/0.20	0.10/0.67	0.13/2.65	97.39
Rd	1.00/1.00	0.04/0.23	0.00/0.07	0.00/1.35	0.00/3.36	100.00
Mean R	1.00/1.00	0.32*/0.39*	0.14*/0.26*	0.12*/1.19*	0.14*/2.97	97.12*
STD	0.00/0.00	0.27/0.23	0.10/0.23	0.09/0.68	0.11/0.63	2.16

BFD0 = Brood area fixing day

STD = standard deviation

* = significantly different compared to the control (pooled Test, $p \leq 0.05$)

E. DEVELOPMENT OF YOUNG LARVAE IN THE MARKED CELLS:

The brood index of the control group C reached a final level of 2.61. In the test item treatment group S, the brood index reached a final level of 2.12. In the reference item treatment group, the final brood index was 1.69. There were no statistically significant differences between the treatment groups (pooled t-Test, Satterthwaite t-Test, Mann Whitney Exact Test, $p \leq 0.05$).

The compensation index was 2.68 in the control group, 3.33 in the treatment group S and 2.90 in R on the last photographic assessment of the brood on BFD+22. There were no statistically significant differences between the treatment groups (pooled t-Test, Satterthwaite t-Test, Mann Whitney Exact Test or pooled t-Test, $p \leq 0.05$).

The final values of the termination rates were 47.84 % in the control, 57.68 % in S and 66.26 % in R. There were no statistically significant differences between the treatment groups (pooled t-Test, Satterthwaite t-Test, Mann Whitney Exact Test or pooled t-Test, $p \leq 0.05$).

Table 114
Honey bee young larvae brood/compensation indices and termination rate

Treatment group/ replicate	Brood/Compensation Indices at x days after brood area fixing day (BFD0) for marked cells containing young larvae at the first assessment					Termination rate %
	BFD0	BFD+5	BFD+11	BFD+16	BFD+22	BFD+22
Ca	2.00/2.00	0.82/0.90	0.82/0.86	0.91/1.30	1.03/2.71	79.39
Cb	2.00/2.00	0.40/1.05	0.30/0.76	0.36/1.18	0.38/2.49	92.40
Cc	2.00/2.00	3.60/3.60	3.38/3.38	4.09/4.11	4.22/2.08	15.52
Cd	2.00/2.00	3.92/3.93	3.84/3.84	4.32/4.36	4.80/3.43	4.04
Mean C	2.00/2.00	2.19/2.37	2.09/2.21	2.42/2.74	2.61/2.68	47.84
STD	0.00/0.00	1.83/1.62	1.78/1.63	2.08/1.73	2.23/0.57	44.51
Sa	2.00/2.00	3.39/3.40	3.30/3.30	3.90/3.95	4.08/2.67	18.40
Sb	2.00/2.00	0.34/0.34	0.30/0.30	0.38/1.25	0.38/2.57	92.41
Sc	2.00/2.00	0.07/1.08	0.07/0.95	0.08/2.66	0.08/3.59	98.37
Sd	2.00/2.00	3.41/3.47	3.14/3.15	3.15/3.37	3.92/4.50	21.54
Mean S	2.00/2.00	1.80/2.07	1.70/1.93	1.88/2.81	2.12/3.33	57.68
STD	0.00/0.00	1.85/1.60	1.76/1.53	1.93/1.16	2.18/0.90	43.63
Ra	2.00/2.00	1.36/1.37	1.21/1.21	1.45/2.03	1.51/2.69	69.79
Rb	2.00/2.00	2.98/3.05	2.74/2.75	3.12/3.56	3.11/2.68	37.90
Rc	2.00/2.00	1.99/2.02	1.74/1.76	2.15/2.55	2.13/2.81	57.35
Rd	2.00/2.00	0.00/0.23	0.00/0.11	0.00/1.55	0.00/3.40	100.00
Mean R	2.00/2.00	1.58/1.67	1.42/1.46	1.68/2.42	1.69/2.90	66.26
STD	0.00/0.00	1.25/1.18	1.14/1.10	1.31/0.86	1.30/0.34	26.04

BFD0 = Brood area fixing day

STD = standard deviation

F. DEVELOPMENT OF OLD LARVAE IN THE MARKED CELLS:

The final brood indices were on a similar level in all treatment groups at the final relevant photographic assessment concerning the old larvae on BFD+16. It was 4.34 in the control, 3.85 in S and 3.73 in R. There were no statistically significant differences between the treatment groups (pooled t-Test, Satterthwaite t-Test, Mann Whitney Exact Test, $p \leq 0.05$).

The compensation indices on BFD+16 were 4.40 in C, 2.17 in S and 4.04 in R. There were no statistically significant differences between the treatment groups (pooled t-Test, Satterthwaite t-Test, Mann Whitney Exact Test or pooled t-Test, $p \leq 0.05$).

The termination rates on BFD+16 were 13.25% in C, 23.04% in S and 25.51 in R. There were no statistically significant differences between the treatment groups (pooled t-Test, Satterthwaite t-Test, Mann Whitney Exact Test, $p \leq 0.05$).

Table 115
Honey bee old larvae brood/compensation indices and termination rate

Treatment group/replicate	Brood/Compensation Indices at x days after brood area fixing day (BFD0) for marked cells containing old larvae at the first assessment				Termination rate
	BFD0	BFD+5	BFD+11	BFD+16	BFD+16
Ca	3.00/3.00	3.84/3.84	3.80/3.80	4.75/4.78	5.08
Cb	3.00/3.00	2.32/2.32	2.22/2.22	2.78/2.96	44.40
Cc	3.00/3.00	3.93/3.93	3.88/3.88	4.85/4.86	3.04
Cd	3.00/3.00	3.98/3.98	3.98/3.98	4.98/4.98	0.47
Mean C	3.00/3.00	3.52/3.52	3.47/3.47	4.34/4.40	13.25
STD	0.00/0.00	0.80/0.80	0.84/0.84	1.04/0.96	20.85
Sa	3.00/3.00	3.97/3.97	3.94/3.94	4.92/4.93	1.61
Sb	3.00/3.00	3.98/3.98	3.88/3.88	4.85/4.90	2.94
Sc	3.00/3.00	1.99/2.04	1.72/1.72	2.15/2.26	57.06
Sd	3.00/3.00	3.76/3.77	2.78/2.78	3.47/3.69	30.56
Mean S	3.00/3.00	3.43/3.44	3.08/3.08	3.85/3.95	23.04
STD	0.00/0.00	0.96/0.94	1.05/1.05	1.31/1.26	26.31
Ra	3.00/3.00	3.57/3.57	3.23/3.23	4.04/4.21	19.25
Rb	3.00/3.00	3.62/3.67	3.09/3.10	3.86/4.19	22.75
Rc	3.00/3.00	3.66/3.68	2.90/2.90	3.63/3.83	27.43
Rd	3.00/3.00	3.27/3.27	2.70/2.74	3.37/3.93	32.61
Mean R	3.00/3.00	3.53/3.55	2.98/2.99	3.73/4.04	25.51
STD	0.00/0.00	0.18/0.19	0.23/0.22	0.29/0.19	5.80

BFD0 = Brood area fixing day

STD = standard deviation

Overall, a very slight effect on the brood development of eggs could be detected in the treatment group S. The development of the young and old larvae in the treatment group S is comparable to the control; no test item effect could be discerned. In the reference item group, a clear effect could be seen in the development of the eggs.

G. RESIDUES IN NECTAR AND POLLEN

No residues of indoxacarb could be found in the nectar samples prepared from forager bees or in nectar samples that were taken directly from the hive in the control group as well as in the treatment group S taken two and four days after the application.

All pollen samples of the control group were free of residues of indoxacarb. In the pollen samples taken from the forager bees in the treatment group S, 0.059 mg a.s./kg could be found two days after the application and 0.032 mg a.s./kg four days after the application. In the pollen samples which was taken directly from the hive, 0.046 mg a.s./kg could be found two days after the application and 0.017 mg a.s./kg could be found four days after the application.

Table 116
Indoxacarb residue levels in nectar

Sample type	Treatment	Timing	Residue (mg a.s./kg)
Nectar from forager bees	Ce	2 DAA	n. d.
Nectar from forager bees	Ce	4 DAA	n. d.
Nectar taken from the hive	Ce	2 DAA	n. d.
Nectar taken from the hive	Ce	4 DAA	n. d.
Nectar from forager bees	Se	2 DAA	n. d.
Nectar from forager bees	Se	4 DAA	n. d.
Nectar taken from the hive	Se	2 DAA	n. d.
Nectar taken from the hive	Se	4 DAA	n. d.

DAA: Days after application

LOQ: 0.010 mg/kg,

n. d.: not detectable (<LOD = 0.003 mg/kg).

Ce: Control samples, Se: Treated samples.

Table 117
Indoxacarb residue levels in pollen

Sample type	Treatment	Timing	Residue (mg a.s./kg)
Pollen from forager bees	Ce	2 DAA	n. d.
Pollen from forager bees	Ce	4 DAA	n. d.
Pollen taken from the hive	Ce	2 DAA	n. d.
Pollen taken from the hive	Ce	4 DAA	n. d.
Pollen from forager bees	Se	2 DAA	0.059
Pollen from forager bees	Se	4 DAA	0.032
Pollen taken from the hive	Se	2 DAA	0.046
Pollen taken from the hive	Se	4 DAA	0.017

DAA: Days after application

LOQ: 0.010 mg/kg,

n. d.: not detectable (<LOD = 0.003 mg/kg).

Ce: Control samples, Se: Treated samples

III. CONCLUSION

It can be concluded that the test item Indoxacarb 150 g/L EC applied after bee flight had a very slight effect on honeybee mortality compared to the control. Considering the non-test item related slightly increased numbers of dead bees/day that already occurred in the pre-application period of the treatment group S and the values after the application, which were similar, no biologically relevant effect can be assumed.

The application of Indoxacarb 150 g/L EC after bee flight had a slight effect on the flight intensity on the day after the application. After this day, the flight intensity was at the same level as in the control. The slight and transient effect can be considered as not biologically relevant.

The application of Indoxacarb 150 g/L EC had a slight effect on the behaviour of the honey bees shortly after the applications. Since the numbers of honey bees showing unusual behaviour are generally low, this effect can be considered as not biologically relevant.

There was no negative effect on the colony size relating to the application of Indoxacarb 150 g/L EC in treatment group S.

There was only a slight and transient effect on the amount of brood cells in the treatment group S shortly after the application of Indoxacarb 150 g/L EC.

The application of Indoxacarb 150 g/L EC carried out after bee flight had a slight effect on the development of the marked eggs, whereas no effect occurred on the development of young larvae and old larvae.

The spray application of Indoxacarb 150 g/L EC carried out at 50.0 g a.s./ha during flowering and after bee flight (S) caused slight effects of minor biological relevance on honeybee colonies.

No residues of indoxacarb could be found in the nectar samples prepared from forager bees or in nectar samples which were taken directly from the hive in the control group as well as in the treatment group S.

All pollen samples of the control group were free of residues of indoxacarb. In the sample taken from the forager bees in the treatment group S, 0.059 mg/kg could be found two days after the application and 0.032 mg/kg four days after the application. In the pollen sample which was taken directly from the hive, 0.046 mg/kg could be found two days after the application and 0.017 mg/kg could be found four days after the application.

(Berg, C., 2014)

RMS comment:

Study submitted to the EU for the first time in this submission.

RMS notes that the application rate is of 50 g a.s./ha.

This study is considered valid. The mean brood termination rate for eggs, young larvae and old larvae in the control group was $\leq 50\%$ at the end of the study (as required by the study plan). There were four bee colonies per treatment group (+ 1 tunnel tent for C and S for residue analysis).

RMS considers that the mortality in the tunnels treated with Indoxacarb 150 g/L EC until DAA3 might be treatment related (considered of no biological relevance by the study author). Afterwards (from DAA4 to the end of the study), RMS agrees that the mortality is no longer treatment related. It is also reported that a higher number of dead pupae and dead malformed pupae was found in tunnels treated with Indoxacarb 150 g/L EC (considered of no biological relevance by the study author). RMS cannot ascertain if it is treatment related or not. This should be considered in conjunction with other studies.

The application of Indoxacarb 150 g/L EC after bee flight had a slight effect on the flight intensity on the day after the application. After this day, the flight intensity was at the same level as in the control.

The application of Indoxacarb 150 g/L EC had a slight effect on the behaviour of the honey bees shortly after the applications. The number of honey bees showing unusual behaviour was low.

There was no negative effect on the colony size relating to the application of Indoxacarb 150 g/L EC in treatment group S.

There was only a slight and transient effect on the amount of brood cells in the treatment group S shortly after the application of Indoxacarb 150 g/L EC.

RMS notes that the brood/compensation indices were much lower than in control at all assessment (even if not significant after BFD+5). The termination rates also appear higher than in the control (more than twice higher even if not significant according to the study report). RMS considers that it is treatment related.

Lower brood/compensation indices and higher termination rates were also observed for young larvae and old larvae but the difference was lesser. RMS nevertheless considers that it is treatment related.

No residues of indoxacarb could be found in the nectar samples prepared from forager bees or in nectar samples which were taken directly from the hive in the control group as well as in the treatment group S.

All pollen samples of the control group were free of residues of indoxacarb. In the sample taken from the forager bees in the treatment group S, 0.059 mg/kg could be found two days after the application and 0.032 mg/kg four days after the application. In the pollen sample which was taken directly from the hive, 0.046 mg/kg could be found two days after the application and 0.017 mg/kg could be found four days after the application. RMS however notes that the origin of the samples taken from the hive cannot be verified. It is not known if the samples are taken from newly deposited nectar and pollen or not (even if it is reported in the study report that the samples were preferably taken from newly deposits).

Report: Rentschler, S. (2014); Indoxacarb (DPX-KN128) 150 g/L EC: A semi-field study to evaluate effects on the honey bee (*Apis mellifera*; Hymenoptera, Apidae) in *Phacelia tanacetifolia* in Germany 2014

DuPont Report No.: DuPont-41668

Guidelines: OEPP/EPPO Guideline No 170 (4), 2010 **Deviations:** None

Testing Facility: Eurofins Agrosience Services EcoChem GmbH, Niefern-Öschelbronn, Germany

Testing Facility Report No.: S14-01619

GLP: Yes

Certifying Authority: Landesanstalt Für Umwelt, Messungen Und Naturschutz Baden-Württemberg

Executive summary:

The effects of the test item Indoxacarb 150 g/L EC were tested on the honey bee (*Apis mellifera* L.) under semi-field conditions following the OEPP/EPPO guideline 170 (4), 2010. This study was conducted near Pforzheim in Southern Germany (region: Baden-Württemberg) in August 2014 and included four treatment groups:

- Indoxacarb 150 g/L EC treatment group T1 with one application of the test item in each replicate (tunnel). The application was performed on 08 August 2014, 4 days after installation of the bee hives in the tunnels, during flowering of *Phacelia tanacetifolia* (BBCH 65) in the evening, after bee-flight. The application was carried out at a rate of 232.1 g formulated product/ha, equivalent to 37.5 g a.s./ha.
- Indoxacarb 150 g/L EC treatment group T2 with one application of the test item in each replicate (tunnel). The application was performed on 09 August 2014, 5 days after installation of the bee hives in the tunnels, during flowering of *P. tanacetifolia* (BBCH 65) and during daily bee-flight. The application was carried out at a rate of 232.1 g formulated product/ha, equivalent to 37.5 g a.s./ha.
- Reference item treatment group R with one application of BAS 152 11 I (dimethoate) at a rate of 1000 mL product/ha (equivalent to 400 g dimethoate/ha), during flowering of *P. tanacetifolia* (BBCH 65) and during bee-flight, on the same day as the application in the treatment T2 and in the control (09 August 2014).
- Control group C with one application of tap water during flowering of *P. tanacetifolia* and during bee-flight, on the same day as the application in the treatment groups T2 and R (09 August 2014).

All applications were carried out with a spray volume of 400 L water/ha.

The effects of the test item treatment were examined on honey bee colonies in tunnel (5.0 m × 16.0 m and a height of 3.5 m in the centre) placed over plots of flowering *Phacelia tanacetifolia*. The semi-field test comprised 4 replicate tunnel in the treatment groups T1, T2 and C (= 3 replicates for biological assessments, 1 for residue sampling) and 3 replicates in the treatment group R.

The influence of Indoxacarb 150 g/L EC was evaluated by comparing the results in the test item treatments T1 (applied after bee-flight) and T2 (applied during bee-flight) to the data in the control treatment as well as in the reference item treatment regarding the following observations:

- Number of dead honey bees at the edge of the treated area (linen sheets) and in the dead honey bee traps in front of the hives,
- Flight activity on the crop (number of forager honey bees/m² flowering *P. tanacetifolia*),
- Condition of the colonies and development of the brood,
- Behaviour of the honey bees in the crop area and around the hives.

The Indoxacarb 150 g/L EC treatments had no effect on the mortality, flight intensity, colony strength and brood development.

There was a slight and transient effect on the behaviour of the honey bees at the first two days after application of Indoxacarb 150 g/L EC in the evening after bee flight and also after application of Indoxacarb 150 g/L EC during bee flight at the application day.

Overall, Indoxacarb 150 g/L EC applied after honey bee flight and during honey bee flight seems to have only a slight and transient effect on the behaviour of honey bees and no other negative impact.

The application of the test item resulted in residue levels in nectar and pollen taken on 2DAA and 5DAA as follows:

Pollen_from forager bees: There were 0.011 mg indoxacarb/kg in T1 on 2DAA, 0.038 mg indoxacarb/kg in T2 on 2DAA and no detectable residues in the control.

Pollen from hives: There were 0.053 mg a.s./kg in T1 on 2DAA, 0.042 mg a.s./kg in T1 on 5DAA, 0.102 mg a.s./kg in T2 on 2DAA and 0.052 mg a.s./kg in T2 on 5DAA. No residues could be detected in the control.

Nectar_from forager bees: There were 0.022 mg a.s./kg in T2 on 2DAA and no residues in T1, and in the control.

Nectar from hives: There were no residues of indoxacarb in T1, T2 and in the control.

I. MATERIAL AND METHODS

A. MATERIALS:

1. Test material: Indoxacarb 150 g/L EC
Lot/Batch#: DPX-KN128-434
Purity: 150 g a.s./L
Description: Liquid
CAS#: None for the formulation
173584-44-6 for the active substance
Stability of test compound: 98.2% of indoxacarb remains in the delivery vehicle after one hour under agitation
Reference item: BAS 152 11 I (dimethoate)
Lot/Batch: FRE-000926
Purity: 400 g/L
Description: Liquid
CAS#: 60-51-5
Stability in solution: Sufficient for test purpose (at least 1 hour)
2. Vehicle and/or control: Tap water
3. Test organism
Species: *Apis mellifera* L.
Age at dosing: Direct exposure of adult honey bees; indirect exposure of all stages of development.
Source: Eurofins Agrosience Services EcoChem GmbH, Eutinger Str. 24, 75223 Niefern-Öschelbronn.
Diet: Nectar and pollen of flowering *Phacelia tanacetifolia*.
Tunnel (exposure): Within the test field tunnels (5.0 m × 16.0 m and a height of 3.5 m in the centre) were installed before full flowering of *P. tanacetifolia*. The tunnel frames were covered with light plastic gauze. Linen sheets (approx. 14.9 m²) were spread along the middle of the crop area and along the tunnel walls for the assessment of dead honey bees in the crop area.
4. Environmental conditions during the exposure period (06 Aug 2014 to 16 Aug 2014):
Temperature (min/max): 7.8–29.5°C
Relative humidity: (min/max) 41.3–100%
Photoperiod (exposure): natural light conditions

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
04-August-2014 to 29-November-2014

2. Experimental treatments

Indoxacarb 150 g/L EC treatment group T1 with one application of the test item in each replicate (tunnel). The application was performed on 08-August-2014, 4 days after installation of the bee hives in the tunnels, during flowering of *P. tanacetifolia* (BBCH 65) in the evening, after bee-flight. The application was carried out at a rate of 232.1 g formulated product/ha, equivalent to 37.5 g a.s./ha.

Indoxacarb 150 g/L EC treatment group T1 with one application of the test item in each replicate (tunnel). The application was performed on 08-August-2014, 4 days after installation of the bee hives in the tunnels, during flowering of *P. tanacetifolia* (BBCH 65) in the evening, after bee-flight. The application was carried out at a rate of 232.1 g formulated product/ha, equivalent to 37.5 g a.s./ha.

Indoxacarb 150 g/L EC treatment group T2 with one application of the test item in each replicate (tunnel tent). The application was performed on 09-August- 2014, 5 days after installation of the bee hives in the tunnels, during flowering of *P. tanacetifolia* (BBCH 65) and during daily bee-flight. The application was carried out at a rate of 232.1 g formulated product/ha, equivalent to 37.5 g a.s./ha.

Reference item treatment group R with one application of BAS 152 11 I (dimethoate) at a rate of 1000 mL product/ha (equivalent to 400 g a.s./ha), during flowering of *P. tanacetifolia* (BBCH 65) and during bee-flight, on the same day as the application in the treatment T2 and in the control (09-August-2014).

Control group C with one application of tap water during flowering of *P. tanacetifolia* and during bee-flight, on the same day as the application in the treatment groups T2 and R (09-August-2014).

All applications were carried out with a spray volume of 400 L water/ha.

3. Observations

The influence of Indoxacarb (150 g/L EC) was evaluated by comparing the results in the test item treatments T1 (applied after bee-flight) and T2 (applied during bee-flight) to the data in the control treatment as well as in the reference item treatment regarding the following observations:

Mortality: Number of dead honey bees at the edge of the treated area (linen sheets) and in the dead honey bee traps in front of the hives,

Flight intensity: Number of forager honey bees/m² flowering *P. tanacetifolia*,

Condition of the colonies and development of the brood,

Behaviour of the honey bees in the crop area and around the hives.

Level of residues in the samples of hive products and nectar stomach contents and pollen loads from forager bees

II. RESULTS AND DISCUSSION

A. MORTALITY

The mortality during the period before the application was comparable in all treatment groups. Daily mortality in this period was in the range from 57.3 to 185.0 dead honey bees/day in the control (adapted for comparison with T1), from 83.0 to 185.0 dead honey bees/day in the control adapted for comparison with T2 and R), from 45.7 to 154.7 dead honey bees/day in T1, from 58.3 to 198.3 dead honey bees/day in T2 and from 60.3 to 164.0 dead honey bees/day in the reference item treatment group R.

At 0DAA the mean daily mortality was 116.7 dead honey bees/day in the control (adapted for comparison with T1), 91.0 (adapted for comparison with T2 and R), 174.7 dead honey bees/day in T1, 109.3 dead honey bees/day in T2 and 532.7 dead honey bees/day in R. The mortality of the reference item treatment

group was statistically significant higher at this day than the mortality of the control and also at 1DAA and 2DAA (pooled t-test, $p \leq 0.05$).

In the period after the application (0DAA to 7DAA) the mean daily mortality was 87.4 dead honey bees/day in the control (adapted for comparison with T1), 84.2 dead honey bees/day in the control (adapted for comparison with T2 and R), 62.8 dead honey bees/day in T1, 53.2 dead honey bees/day in T2 and 168.0 dead honey bees/day in R. Over this period the mortality of the reference item treatment group was statistically significant higher than the mortality in the control group (pooled t-test, $p \leq 0.05$).

Daily mortality was in the range from 19.7 to 143.3 dead honey bees/day in the control, from 28.0 to 174.7 dead honey bees/day in T1, from 21.0 to 109.3 dead honey bees/day in T2 and from 33.3 to 532.7 dead honey bees/day in R.

Table 118
Honey bee mortality

Date	DBA/DAA	Mean number of dead honey bees, larvae and pupae				
		C		T1	T2	R
		For T1	For T2 and R			
06 Aug 2014	3DBA	185.0		154.7	198.3	164.0
07 Aug 2014	2DBA	127.7		60.0	166.3	141.3
08 Aug 2014	1DBA	83.7		51.0	98.7	73.7
08 Aug 2014	1DBA(ba)	57.3	45.7 ^a	45.7	---	---
09 Aug 2014	0DBA ^b	---		---	58.3	60.3
Mean DAA-3 to DAA-1ba/0ba		113.4	119.9	77.8	118.0	109.8
STD		36.3	38.8	27.7	82.8	21.6
09 Aug 2014	0DAA	116.7	91.0	174.7	109.3	532.7*
10 Aug 2014	1DAA	19.7		31.0	29.7	100.7*
11 Aug 2014	2DAA	143.3		96.7	87.0	345.3*
12 Aug 2014	3DAA	105.7		63.3	72.3	143.3
13 Aug 2014	4DAA	96.7		44.0	52.7	97.3
14 Aug 2014	5DAA	72.3		35.0	32.7	43.3
15 Aug 2014	6DAA	89.7		29.3	21.0	48.3
16 Aug 2014	7DAA	55.3		28.0	21.0	33.3
Mean DAA0/0aa to DAA7		87.4	84.2	62.8	53.2	168.0*
STD		39.8	37.5	26.9	24.9	12.6

DAA = Days after application during bee-flight

DBA = Days before application during bee-flight

STD = Standard deviation

ba = before application

--- = not applicable

* Significantly different from the control (pooled t-test, $p \leq 0.05$)

^a The values of 0DBA and 1DBA(ba) were added and treated as one value (pre-application)

^b Assessment shortly before start of application in the control (part of the post-application period in treatment T1)

Note: For calculation of mean values and STD the numbers of dead bees on the linen sheets and in the dead bee traps were summarized per hive and counted as one value.

Overall, there was no significant effect of the test item Indoxacarb 150 g/L EC on the mortality of honey bees. In the reference item treatment group R a clear impact on honey bee mortality after the application was observed.

B. FLIGHT ACTIVITY

Flight activity was comparable during the period from 3DBA to 0DBA(ba) in all four treatment groups and there were no significant differences of the flight activity in the test item treatments T1, T2 and R compared to the control in the pre-application period (3DBA to 0DBA(ba)) flight activity was in the range from 9.8 to 14.8 forager bees/m² in the control, from 9.3 to 18.5 forager bees/m² in T1, from 10.9 to 12.7 forager bees/m² in T2 and from 10.8 to 12.2 forager bees/m² in the reference item group.

On the day of the application (0DAA), after the application, flight activity was similar for all treatment groups except for the reference item treatment group which had lower flight activity (pooled t-test, $p \leq 0.05$). The mean flight activity was 11.5 forager bees/m² in the control (adapted for comparison with T1), 11.8 forager bees/m² in the control (adapted for comparison with T2 and R), 10.2 forager bees/m² in T1, 11.0 forager bees/m² in T2 and 4.1 forager bees/m² in R. During the post application period 0DAA to 7DAA the mean flight activity was in the range from 0.0 to 14.9 forager bees/m² in the control, from 0.0 to 14.5 forager bees/m² in T1, from 0.0 to 12.5 forager bees/m² in T2 and from 0.0 to 4.1 forager bees/m² in the reference item treatment group. The flight activity of the test item treatment groups T1 and T2 was comparable to the control over the post application period (0DAA to 7DAA). At 6DAA no flight activity was observed in all treatment groups because of rain.

Flight activity after the application in R was significantly reduced from 0DAA to 5DAA as well as for the complete post-application period from 0DAA to 7DAA (pooled t-test or Satterthwaite t-test, $p \leq 0.05$).

Overall, there was no effect on flight activity in both test item Indoxacarb 150 g/L EC treatments (applied after flight (T1) or during bee flight (T2)) while there was a clear impact in the reference item treatment after the application.

Table 119
Honey bee flight intensity

Date	DBA/DAA	Flight intensity (mean number of forager bees/m ²)				
		C		T1	T2	R
		for T1	for T2 and R			
06 Aug 2014	3DBA	11.5		9.3	11.8	10.8
07 Aug 2014	2DBA	14.0		18.5	12.7	11.4
08 Aug 2014	1DBA	14.8		15.0	10.9	12.2
09 Aug 2014	0DBA(ba)	---	9.8	---	11.8	11.3
Mean 3DBA to 0DBA(ba)		13.4	12.5	14.3	11.8	11.4
STD		1.9	1.4	0.6	0.6	1.6
09 Aug 2014	0DAA	11.5	11.8	10.2	11.0	4.1*
10 Aug 2014	1DAA	14.9		14.5	12.5	0.6*
11 Aug 2014	2DAA	9.2		9.6	7.3	0.9*
12 Aug 2014	3DAA	11.9		12.2	10.4	1.6*
13 Aug 2014	4DAA ^a	2.0		1.2	0.7*	0.3*
14 Aug 2014	5DAA	4.3		2.4	3.2	0.0*
15 Aug 2014	6DAA ^a	0.0		0.0	0.0	0.0
16 Aug 2014	7DAA	4.0		4.6	3.6	0.2
Mean 0DAA to 7DAA		7.2	7.2	6.9	6.1	1.0*
STD		1.1	1.1	0.5	0.5	0.1

DBA/DAA= Days before/after application during bee-flight; STD = Standard deviation

(ba) = before application

--- = not applicable

* statistically significant different to the control (pooled t-test and t-test Satterthwaite $p \leq 0.05$)

^a No or only little flight activity in all treatments due to adverse weather conditions

C. BEHAVIOUR OF THE HONEY BEES

In period before the application in the control 25 honey bees showing abnormal behaviour, in the treatment group T1 seven honey bees, in T2 ten honey bees and fifteen in the reference item treatment group were observed. Mostly single bees in one replicate showed intoxication symptoms like cramping in all treatment groups. It can be concluded that there was no relevant difference concerning the behaviour between the treatment groups.

At 0DAA after the application six honey bees had locomotion problems in the control. In the treatment group T1 at the day after the application in the evening (0DBA + 0DAA) 105 cramping honey bees, 63 with locomotion problems, 33 inactive ones and 28 trembling honey bees were recorded. Seventeen honey bees showed intoxication symptoms like locomotion problems or flying without landing on crop (indication for a repellent effect of the test item) in the treatment group T2. In the reference item treatment group 38 honey bees were cramping, 143 had locomotion problems, 28 were inactive and 16 trembling at 0DAA. At this day in the treatment groups T1 and R an effect to the behaviour of the honey bees was observed with 229 honey bees (T1) and 225 honey bees (R) with intoxication symptoms in contrast to six (control) and 17 (T2) honey bees.

At the day after the application (1DAA) the honey bees in the control showed normal behaviour and also in T2 with the exception of single bees for e.g. one hanging bee. In the treatment group T1 nineteen honey

bees showed intoxication symptoms like cramping and 50 in the reference. In the reference and T1 the amount of honey bees having intoxication symptoms was slightly elevated.

In the period from 2DAA to 7DAA behavioural observations were on a normal level like before the application in all treatment groups.

Overall, there was a slight effect of the Indoxacarb 150 g/L EC treatment T1 on the behaviour of the honey bees at the day after the evening application and the following day. In the treatment group T2 a very slight and transient effect on the behaviour of the honey bees was noticed only at the day of the application during bee flight. The reference item had an effect on the behaviour of the honey bees at the application day and the first day after.

D. CONDITION OF COLONIES, DEVELOPMENT OF THE BROOD

At the first assessment of colony condition before set-up in the tunnel tents (5DBA) the mean colony size was comparable in the four treatment groups. Mean strength of the colonies (number of honey bees according to Liebefeld method) was 5625 honey bees/hive in the control, 5729 honey bees/hive in the treatment group T1, 5417 honey bees/hive in T2 and 5104 honey bees/hive in the reference item treatment group R.

From the first to the third assessment of colony condition an increase of the total number of honey-bees was observed in all treatment groups. The mean colony size at the third assessment was 7396 honey bees/hive in the control, 8583 honey bees/hive in T1, 7917 honey bees/hive in T2 and 7000 honey bees/hive in R.

In all treatment groups a decrease of colony size was observed at the fourth assessment of colony condition. There were 5959 honey bees/hive in the control, 8292 honey bees/hive in T1, 7084 honey bees/hive in T2 and 5646 honey bees in the reference item group R.

In the treatment groups C, T2 and R a recovery of the colony size was observed at the last assessment of colony condition, whereas in the treatment group T1a further decrease was observed. The mean colony size was 6667 honey bees/hive in the control, 7708 honey bees in T1, 8063 honey bees/hive in T2 and 6979 honey bees/hive in R.

Overall, there was no negative effect of the Indoxacarb 150 g/L EC treatment on the colony size (number of honey bees).

Brood of all stages was present in all colonies at the first assessment before set up in the tunnel. At the first assessment the mean amount of cells containing brood was 16333 brood cells/hive in the control, 17400 brood cells/hive in T1, 15400 brood cells/hive in T2 and 14733 brood cells/hive in R.

In the control only at the last assessment (27DAA) in replicate Cc no larvae were present. At the second assessment in T1c no larvae and at the last assessment no larvae and capped brood were present. In the treatment group T2 no replicate had larvae at the last assessment and replicate T2a also had no capped brood at 27DAA resulting from a lack of larvae in this replicate at 20DAA. In the reference item group the replicate Rc had no larvae at 13DAA and on 27DAA the replicates Ra and Rc. A *Varroa* treatment was made within all colonies after the fourth assessment because of *Varroa destructor* mites had been seen in the colonies at the second and third assessment of the colony condition. So the lack of larvae at 27DAA in some of the colonies can be traced back to the *Varroa* treatment.

At the second assessment directly after the exposure period in the tunnel (8DAA) in all treatments groups including the control a sharp reduction of brood cells was observed. This can be related to the confined conditions in the tunnel and the changing weather during this time resulting in reduced number of pollen cells at the third assessment. On 8DAA there were a mean of 6467 brood cells/hive in the control, 4933 brood cells/hive in T1, 6733 brood cells/hive in T2 and 7600 brood cells/hive in R.

All treatments showed a decrease in the amount of brood cells in the following assessments especially the treatment group T2. This is mainly related to the replicate T2a. At the fourth assessment (20DAA) there

was a mean of 5933 brood cells/hive in the control, 3333 brood cells/hive in T1, 2667 brood cells/hive in T2 and 6333 brood cells/hive in R.

At the last assessment there were a mean amount of 4933 brood cells/hive in the control, 4867 brood cells/hive in T1, 2267 brood cells in T2 and 5933 brood cells in R. Only in the treatment group T1 a slight increase in the amount of brood cells was observed at 27DAA.

The amount of food was comparable in all treatment groups at the beginning. There was no pollen at 8DAA in T2a and at 13 DAA in Cb. The lack of pollen in Cb was transient and had no effect of the conduct of the study. The longer lasting reduced number of pollen cells in T2a seemed to have an effect on the amount of larvae and capped brood in the subsequent assessments at 20DAA and 27DAA. As all replicates in T2 were affected by a lack of larvae at the last assessment it was not only an effect of the *Varroa* treatment and a lack of pollen. The amount of eggs in T2 was marginally reduced in all replicates after the application at 13DAA and 20DAA but the amount of eggs was increased on the last assessment (27DAA) and so a very slight, transient effect of the test item may be assumed.

Overall, there was no negative effect of the Indoxacarb 150 g/L EC treatment on the amount of brood in the test item treatment groups.

E. RESIDUES

The application of the test item resulted in residue levels in nectar and pollen taken on 2DAA and 5DAA as follows:

Pollen from forager bees: There were 0.011 mg indoxacarb/kg in T1 on 2DAA, 0.038 mg indoxacarb/kg in T2 on 2DAA and no detectable residues in the control.

Pollen from hives: There were 0.053 mg indoxacarb/kg in T1 on 2DAA, 0.042 mg indoxacarb/kg in T1 on 5DAA, 0.102 mg indoxacarb/kg in T2 on 2DAA and 0.052 mg indoxacarb/kg in T2 on 5DAA. No residues could be detected in the control.

Nectar from forager bees: There were 0.022 mg indoxacarb/kg in T2 on 2DAA and no residues in T1, and in the control.

Nectar from hives: There were no detectable residues of indoxacarb in T1, T2 and in the control.

III. CONCLUSION

The Indoxacarb 150 g/L EC treatments had no effect on the mortality, flight intensity, colony strength, and brood development.

There was a slight and transient effect on the behaviour of the honey bees at the first two days after application of Indoxacarb 150 g/L EC in the evening after bee flight and also after application of Indoxacarb 150 g/L EC during bee flight at the application day.

Overall, Indoxacarb 150 g/L EC applied after honey bee flight and during honey bee flight seems to have only a slight and transient effect on the behaviour of honey bees and no other negative impact.

The application of the test item resulted in residue levels in nectar and pollen taken on 2DAA and 5DAA as follows:

Pollen from forager bees: There were 0.011 mg indoxacarb/kg in T1 on 2DAA, 0.038 mg indoxacarb/kg in T2 on 2DAA and no detectable residues in the control.

Pollen from hives: There were 0.053 mg indoxacarb/kg in T1 on 2DAA, 0.042 mg indoxacarb/kg in T1 on 5DAA, 0.102 mg indoxacarb/kg in T2 on 2DAA and 0.052 mg indoxacarb/kg in T2 on 5DAA. No residues could be detected in the control.

Nectar from forager bees: There were 0.022 mg indoxacarb/kg in T2 on 2DAA and no residues in T1, and in the control.

Nectar from hives: There were no residues of indoxacarb in T1, T2 and in the control.

(Rentschler, S., 2014)

RMS comment

Study submitted to the EU for the first time in this submission.

RMS notes that rain occurred the day after treatment (10.8 mm). Besides a deviation was reported concerning the populations of the beehives used in the test. Two hives in control, two hives in T1, two hives in T2 and three hives of the toxic reference R had only 4000 to 5750 honey bees and not the required 6000-10000 honey bees according to the study plan.

This study is considered valid but results have to be used with caution.

There were three bee colonies per treatment group (4th tunnel tent was for residue analysis).

RMS considers that a slight effect on mortality cannot be discarded the day after treatment when Indoxacarb 150 g/L EC is applied in the evening after bee flight (not significant). Indoxacarb 150 g/L EC applied during bee flight had no effect on mortality.

The Indoxacarb 150 g/L EC treatments had no effect on the flight intensity and colony strength.

There was a slight and transient effect on the behaviour of the honey bees at the first two days after application of Indoxacarb 150 g/L EC in the evening after bee flight and also after application of Indoxacarb 150 g/L EC during bee flight at the application day.

RMS notes that effects on brood were observed which might be due to the presence of Varroa and the use of a Varroa treatment. No larvae or capped brood was observed in several tunnels including one replicate of the control (no larvae in T2 in all tunnels at the last assessment). Besides, all treatments showed a decrease in the amount of brood cells. According to the study report the reduction of the brood cells in all groups can be related to the confined conditions and the changing weather. The decrease of brood cells was more important in tunnels where Indoxacarb 150 g/L EC was applied during honey bee flight. RMS consider that the observations on brood are inconclusive and an effect of the treatment cannot be discarded.

The application of the test item resulted in residue levels in nectar and pollen taken on 2DAA and 5DAA as follows:

Pollen from forager bees: There were 0.011 mg indoxacarb/kg in T1 on 2DAA, 0.038 mg indoxacarb/kg in T2 on 2DAA and no detectable residues in the control.

Pollen from hives: There were 0.053 mg indoxacarb/kg in T1 on 2DAA, 0.042 mg indoxacarb/kg in T1 on 5DAA, 0.102 mg indoxacarb/kg in T2 on 2DAA and 0.052 mg indoxacarb/kg in T2 on 5DAA. No residues could be detected in the control.

Nectar from forager bees: There were 0.022 mg indoxacarb/kg in T2 on 2DAA and no residues in T1, and in the control.

Nectar from hives: There were no residues of indoxacarb in T1, T2 and in the control.

Report: Klein, O. (2014); Indoxacarb (DPX-KN128) 150 g/L EC: A semi-field study to evaluate effects on the bumble bee (*Bombus terrestris* L; Hymenoptera, Apidae) in *Phacelia tanacetifolia* in Germany in 2013

DuPont Report No.: DuPont-38419

Guidelines: OEPP/EPPO 170 (3) 2001 **Deviations:** None

Testing Facility: Eurofins Agrosience Services GmbH, Niefern-Oeschelbronn, Germany

Testing Facility Report No.: S13-03868

GLP: Yes

Certifying Authority: Landesanstalt Fur Umwelt, Messungen Und Naturschutz Baden-Wurtemberg

Executive summary:

The objective of the study was to determine the effects of the insecticide Indoxacarb 150 g/L EC on the bumble bee *Bombus terrestris* L. (Hymenoptera Apidae) applied as spray solution under semi-field conditions on *Phacelia tanacetifolia* in Germany based on general SETAC/ESCORT recommendations (BARRETT *et al.*, 1994) and OEPP/EPPO Guideline No. 170 (4), (2010). Additionally, nectar and pollen from forager honey bees and from honey bee combs and nectar from bumble bee colonies were taken for residue analysis.

Four replicate tunnels per test item treatment (T) control treatment (C) and toxic reference treatment (R) with one bumble bee hive each for biological assessment were set up with a tunnel size of 60 m². Additional 2 replicate tunnels of 100 m² per test item treatment (T) and control treatment (C) with two bumble bee hives and one honey bee hive together per tunnel were used for residue sampling. Bumble bee and honey bee hives in the residue tunnels were used for residue sampling only.

The application of the test item Indoxacarb 150 g/L EC applied as spray solution was performed in gauze tunnels with flowering *Phacelia* plants at growth stage BBCH 63. The test item Indoxacarb 150 g/L EC was applied once after daily bumble bee flight in the evening at a rate of 37.5 g formulated product/ha (equivalent to 249.1 g a.s./ha, based on measured concentration and density). The effects of the test item treatment were examined on bumble bee colonies. Additionally, a toxic reference (dimethoate applied once at a nominal rate of 2000 g/ha) to prove the test system and the setup was included. The application of the test item treatment (T), the toxic reference item treatment (R) and the control (C) in the tunnel tents was performed during early flowering of *P. tanacetifolia* and during bee flight after installation of the bumble bee colonies.

After the initial brood assessment the bumble bee colonies were set up in the tunnels and left for 2 days before exposition to get used to the new environment. The bumble bee colonies were exposed to the treated flowering *Phacelia* crop for 29 days in the tunnel tents. The colonies were assessed for mortality, flight activity, consumption of sugar solution, condition of colonies and development of bumble bee brood.

For each treatment group, 4 replicate tunnels were set up with one bumble bee colony each. The study was located in Southern Germany near Pforzheim.

The influence of the test item Indoxacarb 150 g/L EC and the toxic reference item dimethoate was evaluated by comparing the results of the test item and toxic reference item treatments to the data in the control treatment regarding the following observations:

- Number of living worker bumble bees and larvae
- Mortality of bumble bees (workers, queens, and larvae)
- Flight activity within the crop
- Consumption of sugar solution
- Development of the bumble bee brood
- Condition of bumble bee colonies
- Residue levels of the different analysed matrices

The test item Indoxacarb 150 g/L EC was applied once *via* spray application on flowering *Phacelia tanacetifolia* at an application rate of 37.5 g a.s./ha in 400 L water/ha after daily bumble bee flight in the evening.

No residues above the LOQ level of 0.01 mg/kg were found in any of the control samples. Residues of indoxacarb above the LOQ level were found for pollen samples after application in the test item treatment. Residues in nectar samples were below LOD.

Indoxacarb 150 g/L EC applied once *via* spray application on flowering *Phacelia* after bumble bee flight (evening application) did not have any effects regarding all parameters assessed *i.e.* mortality, flight activity, consumption of sugar solution, hive weight, condition of colonies, development of bumble bee brood, production of young queen offspring and vigour relative to the water treated control.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Indoxacarb 150 g/L EC
 Lot/Batch #: DPX-KN128-311
 Purity: 150 g a.s./L
 Description: Liquid/light amber
 CAS #: None for the formulation
 173584-44-6 for active substance
 Stability of test compound: 98.2% of indoxacarb remains in the delivery vehicle after one hour under agitation
2. Toxic reference: Perfekthion/BAS 152 11 I
 Lot/Batch #: 0001017331
 Concentration of a.s. analysed: 411.7 g/L dimethoate
 Description: Liquid/blue
 CAS #: 60-51-5
 Stability of test compound: Sufficient for test purpose (at least 1 hour)
3. Vehicle and/or positive control: Tap water
4. Test organism
 Species: *Bombus terrestris* L.
 Age at dosing: Adults; indirect exposure of all stages of development
 Source: Koppert BV.
 Postbus 155
 2650 AD Berkel en Rodenrijs
 The Netherlands
 Diet: Partially supplied with auxiliary food (pollen, sugar solution) inside the colony
 Test design: 4 replicate tunnels of 60 m² per test item, control and toxic reference treatment with one bumble bee hive each for biological assessments. Additional 2 replicate tunnels of 100 m² per test item and control treatment with two bumble bee hives and one honey bee hive together per tunnel for residue sampling.
 Test age: Indirect exposure of all stages of development
5. Environmental conditions during exposure of the bumble bees (measured inside tunnels)
 Temperature: Mean: 17.9°C, minimum: 5.9°C, maximum: 40.2°C
 Relative humidity: Mean: 72.5%, minimum: 21.9%, maximum: 99.8%

B. STUDY DESIGN AND METHODS:

1. In-life initiated/completed
 09-August-2013 to 16-November-2013

2. Experimental treatments

This study was conducted in Southern Germany in August to September 2013 and included three treatment groups (each with four replicates in tunnel tents). One test item treatment, a control, and a toxic reference were investigated: T = test item Indoxacarb 150 g/L EC applied after daily bumble bee flight in the evening at a rate of 37.5 g a.s. per ha (equivalent to 249.1 g formulated product/ha, based on measured concentration and density), C = water treated control applied twice and R = toxic reference applied once at a rate of 2000 g a.s. dimethoate/ha. All treatment groups were applied per spray application and were carried out with a spray volume of 400 L/ha.

3. Observations

The influence of the test item Indoxacarb 150 g/L EC and the toxic reference item dimethoate was evaluated by comparing the results of the test item and toxic reference treatments to the data in the control treatment regarding the following observations:

Bumble bee mortality (larvae and adults)

Flight activity within the crop

Sugar solution consumption

Development of the bumble bee colonies (measured by colony weight)

Condition of the colonies and development of bumble bee brood

Photographic evaluation of brood stages and brood development

Residue levels of the different analysed matrices

II. RESULTS AND DISCUSSION

A. FINDINGS:

Total mortality including dead adult bumble bees and larvae observed in the tunnels, in front of the bumble bee hives and inside the hives (mean values per day) for the control and the test item (T) values were generally low. There was no difference between the test item treatment and the control with regard to total mortality.

Total mortality was higher in the toxic reference group (R) with a maximum at DAA +1. Mortality of bumblebee workers was significantly higher ($p \leq 0.05$, t-test, Mann Whitney exact) at DAA 0, DAA +1, DAA +2, DAA +3, DAA +5 and DAA +7 compared to the control. A total mean mortality of adult bumble bees of 188 was observed for the toxic reference compared to 72 in the control hives and 33 in the test item hives (from DAA 0 to +31). Queen mortality (original queens) was observed in all four replicate hives of the toxic reference after several days (DAA + 2, DAA +3, DAA +5 and DAA +15). No mortality of queens (original queens) was observed in the control and the test item treatments.

Table 120
Mean number of dead workers and larvae per day and per bumble bee hive (in the tunnel, in front of and inside the bumble bee hives)

Date	DAA	Treatment groups					
		C		T		R	
		Workers	Larvae	Workers	Larvae	Workers	Larvae
09 Aug 2013	-3	1.25	1.00	0.00	0.00	0.00	0.00
10 Aug 2013	-2	0.25	0.50	0.25	0.00	0.00	0.00
11 Aug 2013	-1	1.75	0.25	1.00	0.00	1.00	0.00
12 Aug 2013	0	0.50	0.00	0.50	0.75	4.75* ^a	0.50
13 Aug 2013	+1	0.00	2.00	1.25	0.50	92.50* ^b	3.25
14 Aug 2013	+2	0.75	5.50	1.50	1.25	20.75* ^a	0.75
15 Aug 2013	+3	0.75	2.50	0.50	0.00	5.25* ^b	0.00
17 Aug 2013	+5	0.63	1.50	1.00	1.25	6.25* ^b	0.13* ^a
19 Aug 2013	+7	0.88	0.75	0.38	0.38	4.88* ^a	4.13
20 Aug 2013	+8	0.00	1.00	0.25	1.75	0.50	5.00
23 Aug 2013	+11	1.58	3.17	0.25	2.67	3.08	0.42* ^a
27 Aug 2013	+15	1.63	3.44	0.81	4.13	2.31	3.38
29 Aug 2013	+17	3.25	7.00	1.13	8.75	1.50	0.25
30 Aug 2013	+18	2.25 ^c	5.50	0.25	13.25	0.25	4.25
02 Sep 2013	+21	3.42	2.50	1.33	1.75	1.33	2.83
04 Sep 2013	+23	1.75	1.38	1.88	2.13	0.75	0.00* ^a
06 Sep 2013	+25	2.63	1.25	0.50	0.88	2.00	0.00* ^b
09 Sep 2013	+28	3.42	0.33	1.50	0.50	1.83	1.42
12 Sep 2013	+31	6.00	1.08	2.17	0.08	1.75	0.92
Mean per day and hive after application (DAA 0 to DAA +31)		1.82	2.43	0.95	2.50	9.36	1.70
		4.25		3.45		11.06	

DAA = days after application (**bold** indicates date of application)

Calculations based on unrounded values

* Statistically significant increase in mortality compared to control ($p \leq 0.05$)

^a t-test

^b Mann Whitney exact

^c Including 1 dead young queen

Flight activity within the crop:

The bumble bee hives were placed in the tunnels 2 days before application in order to get the bumble bees used to their new environment. In all treatment groups the bumble bees started immediately foraging the crop. The flight intensity increased to approx. 5 bumble bees per 4 m² at the application day (control value). In the control and the test item treatment a more or less continuous increase of the foraging activity was observed during the course of the study up to DAA +17 when a maximum of flight activity was reached (>20 bumble bees/4 m²). A significant difference ($p \leq 0.05$, t-test) in the flight activity of the test item group was observed at DAA +25 (increase). Decreasing flight activity in control tunnels was mainly due to the weather conditions as *i.e.* at DAA +15 with a clouding of 100 %.

The flight activity of the toxic reference was significantly ($p \leq 0.05$, t-test, Mann Whitney exact) reduced for all assessments after application, resulting in very low flight activities several days after application and reaching maximum values of approximately 5 bumble bees/4 m² at the end.

Table 121
Flight activity: Mean number and standard deviation of bumble bees per 4 m² (n = 4 tents/treatment)

Date	DAA	Treatment groups					
		C		T		R	
		Mean	STD	Mean	STD	Mean	STD
10 Aug 2013	-2	2.2	1.6	1.7	1.2	1.5	0.4
11 Aug 2013	-1	3.7	1.2	3.0	1.5	2.0	1.6
12 Aug 2013	0	4.9	2.4	4.0	1.0	0.8* ^a	0.4
13 Aug 2013	+1	4.9	1.0	4.5	1.2	0.0* ^a	0.0
14 Aug 2013	+2	6.5	1.0	6.1	1.2	0.0* ^a	0.0
15 Aug 2013	+3	7.1	1.0	6.7	1.4	0.1* ^b	0.2
17 Aug 2013	+5	6.6	1.5	8.8	3.6	0.3* ^a	0.4
19 Aug 2013	+7	5.5	1.5	5.9	0.9	0.0* ^b	0.0
20 Aug 2013	+8	9.2	1.5	10.0	1.0	0.3* ^a	0.3
23 Aug 2013	+11	14.9	2.3	15.6	2.9	0.4* ^a	0.3
27 Aug 2013	+15	10.2	1.0	11.8	2.9	1.9* ^a	1.4
29 Aug 2013	+17	20.6	3.5	19.0	3.0	1.7* ^a	1.2
30 Aug 2013	+18	17.8	3.7	22.2	2.9	1.4* ^a	1.5
02 Sep 2013	+21	14.9	2.7	17.4	5.7	5.4* ^a	3.8
04 Sep 2013	+23	15.5	3.2	21.8	7.4	5.3* ^a	5.5
06 Sep 2013	+25	13.6	3.1	23.9* ^a	5.7	5.2* ^a	4.5
09 Sep 2013	+28	18.1	5.1	15.2	7.0	4.8* ^a	4.6

DAA = days after application (**bold** indicates date of application); STD = standard deviation

Calculations based on unrounded values

* Statistically significant difference to control ($p \leq 0.05$)

^a t-test

^b Mann Whitney exact

Consumption of sugar solution:

The bumble bees had access to the sugar solution during the following time periods from 09 to 12 August 2013 (-3 to 0 DAA), from 17 to 20 August 2013 (5 to 8 DAA) and from 30 August to 12 September 2013 (18 to 31 DAA). The mean sugar solution uptake of the bumble bees was similar in the control treatment and in the test item treatment in the beginning. At DAA -1 and DAA +1 the sugar consumption in the test item treatment was significantly ($p \leq 0.05$, t-test, Mann Whitney exact) higher compared to the control treatment. Over the whole study period a higher sugar solution consumption (+0.40 kg) was observed for the test item treatment group. The higher sugar consumption in the test item hives is in accordance with the hive weight increase observed at the same timing.

The sugar consumption in the toxic reference hives was lower compared to the control treatment with significant ($p \leq 0.05$, t-test) lower consumption detected at DAA -1, DAA +5, DAA +25, DAA +28, DAA +30 and DAA +31.

Table 122
Mean weight [kg] and standard deviation of the sugar solution bags (n = 4 colonies/treatment)

Date	DAA	Treatment groups					
		C		T		R	
		Mean	STD	Mean	STD	Mean	STD
09 Aug 2013	-3 [#]	2.22	0.08	2.20	0.05	2.19	0.03
10 Aug 2013	-2 [#]	2.18	0.03	2.14	0.04	2.14	0.04
11 Aug 2013	-1 [#]	2.15	0.01	2.08* ^b	0.05	2.10* ^a	0.04
12 Aug 2013	0 [#]	2.13	0.03	2.05	0.06	2.07	0.05
13 Aug 2013	+1	2.16	0.04	2.09* ^a	0.04	2.09	0.06
14 Aug 2013	+2	2.16	0.03	2.08	0.05	2.08	0.05
15 Aug 2013	+3	2.16	0.03	2.08	0.05	2.09	0.04
17 Aug 2013	+5 [#]	2.14	0.03	2.08	0.05	2.08* ^a	0.04
19 Aug 2013	+7 [#]	2.07	0.11	1.94	0.09	2.06	0.06
20 Aug 2013	+8 [#]	2.03	0.14	1.87	0.10	2.06	0.07
23 Aug 2013	+11	2.02	0.15	1.88	0.11	2.05	0.06
27 Aug 2013	+15	2.07	0.14	1.81	0.32	2.08	0.07
29 Aug 2013	+17	2.07	0.14	1.81	0.33	2.07	0.06
30 Aug 2013	+18 [#]	2.06	0.14	1.80	0.31	2.06	0.06
02 Sep 2013	+21 [#]	1.84	0.21	1.42	0.33	2.00	0.12
04 Sep 2013	+23 [#]	1.63	0.22	1.26	0.37	1.95	0.17
06 Sep 2013	+25 [#]	1.43	0.27	1.08	0.42	1.91* ^a	0.20
09 Sep 2013	+28 [#]	1.17	0.34	0.76	0.34	1.85* ^a	0.30
11 Sep 2013	+30 [#]	1.06	0.37	0.64	0.28	1.80* ^a	0.34
12 Sep 2013	+31 [#]	1.03	0.37	0.62	0.26	1.75* ^a	0.32
Total weight loss from DAA -3 to DAA +31 = consumption of sugar solution		-1.19	0.42	-1.59	0.23	-0.44	0.29

DAA = days after application; STD = standard deviation

Calculations based on unrounded values

* Statistically significant difference to control ($p \leq 0.05$)

Indicates dates bags were opened

^a t-test

^b Mann Whitney exact

Development of bumble bee colonies (measured by colony weight):

The weight (including hive box) of the bumble bee colonies in the water-treated control, the test item treatment, and the toxic reference item treatment was observed in short intervals of one or a few days. Strong increases in weight of the hives occurred when the sugar solution supply was opened (from 09 to 12 August 2013 (-3 to 0 DAA), from 17 to 20 August 2013 (5 to 8 DAA) and from 30 August to 12 September 2013 (18 to 31 DAA)). The weight development of the control and treatment group hives was very similar. Significant differences ($p \leq 0.05$, t-test) were detected between the control and the test item at DAA +15 to DAA +21 with higher weight increases for the test item compared to the control. From DAA +1 until the last assessment date on DAA +31 the mean weight in the colonies of the control and test item increased clearly. In view of the total observation period from DAA -3 until DAA +31, the colonies increased their mean weight by 558 g in the control and 683 g in the test-item treatment.

In contrast, the weight development of the toxic reference showed a decrease in weight starting from the application on with significant differences ($p \leq 0.05$, t-test) compared to the control from Day 2 after

application onwards. In view of the total observation period from DAA -3 until DAA +31 the toxic reference colonies increased their mean weight by 146 g only.

Table 123
Mean weight (g) and standard deviation of the four colonies per treatment group

Date	DAA	Treatment groups					
		C		T		R	
		Mean	STD	Mean	STD	Mean	STD
09 Aug 2013	-3[#]	965	9	982	31	1007*	25
10 Aug 2013	-2[#]	974	8	997	32	1016*	29
11 Aug 2013	-1[#]	1042	20	1053	35	1067	24
12 Aug 2013	0[#]	973	27	1005	22	1011	35
13 Aug 2013	+1	991	17	994	25	995	29
14 Aug 2013	+2	997	18	1005	19	951*	28
15 Aug 2013	+3	1000	15	1009	18	936*	26
17 Aug 2013	+5[#]	1072	20	1057	36	910*	23
19 Aug 2013	+7[#]	1155	57	1224	39	915*	27
20 Aug 2013	+8[#]	1196	66	1249	33	909*	24
23 Aug 2013	+11	1257	46	1292	45	889*	19
27 Aug 2013	+15	1272	45	1375*	69	904*	26
29 Aug 2013	+17	1267	43	1355*	40	913*	36
30 Aug 2013	+18[#]	1247	38	1343*	44	919*	41
02 Sep 2013	+21[#]	1305	124	1511*	97	966*	82
04 Sep 2013	+23[#]	1345	83	1517	140	982*	97
06 Sep 2013	+25[#]	1423	103	1591	111	995*	113
09 Sep 2013	+28[#]	1505	166	1675	88	1026*	141
11 Sep 2013	+30[#]	1480	190	1625	53	1023*	155
12 Sep 2013	+31[#]	1523	118	1665	43	1154*	139
Total hive weight increase from DAA -3 to DAA +31		558	116	683	22	146	117

DAA = days after application (**bold#** indicates dates when sugar solution bags were open); STD = standard deviation

Calculations based on unrounded values

* Statistically significant difference to control ($p \leq 0.05$)

Condition of the colonies and development of bumble bee brood:

The initial brood assessment revealed that the bumble bee hives were all queenright and in good condition with a mean number of 158 workers per hive. Additionally, the hives of the different treatment groups showed similar strength with regard to the number of workers, brood, and food storage. Only the weight of the hives was significantly ($p \leq 0.05$, t-test) higher in the hives of the toxic reference due to the higher amount of filled nectar cells.

Table 124
Condition of the bumble bee colonies

Initial brood assessment: 09 August 2013						
Treatment group	C		T		R	
	Mean	STD	Mean	STD	Mean	STD
Living queen	1	-	1	-	1	-
Number of alive worker bees	151.8	17.2	157.3	29.2	163.8	18.4
Number of brood cells with eggs	18.3	4.0	21.5	2.9	17.5	5.3
Number of brood cells with larvae (workers)	152.3	8.8	135.8	37.2	151.0	44.0
Number of alive pupae (workers)	150.8	33.0	162.5	7.6	153.8	62.2
Number of filled nectar cells	48.8	13.8	54.0	17.2	62.0	11.3
Number of filled pollen cells	0	-	0	-	0	-
Weight of hive (without hive box) [g]	317.7	8.6	335.4	31.3	360.3* ^a	24.6
Total number of alive brood stages (eggs, larvae, pupae)	321.3	34.6	319.8	33.5	322.3	54.9
Total number of alive stages (alive brood and adult bees)	473.0	20.6	477.0	46.0	486.0	66.9
Final brood assessment: 12 September 2013^c						
Treatment group	C		T		R	
	Mean	STD	Mean	STD	Mean	STD
Number of alive young queens	113.0	32.2	97.8	23.4	0.0* ^a	0.0
Weight of alive young queens [g]	107.7	31.4	99.3	22.1	0.0* ^a	0.0
Number of alive workers	239.5	121.9	276.8	119.3	126.8	35.1
Number of alive males	60.5	12.8	79.5	33.6	0.0* ^a	0.0
Number of brood cells with eggs	16.3	7.3	24.0	20.9	8.0	4.5
Number of brood cells with larvae (workers/males)	67.0	46.9	42.0	31.8	61.5	53.0
Number of brood cells with larvae (queens)	2.5	2.1	6.8	5.9	0.0	0.0
Number of pupae (workers/males)	138.3	30.0	136.5	87.6	40.5* ^a	42.6
Number of pupae (queens)	28.8	26.9	41.8	22.2	0.0	0.0
Number of filled nectar cells	255.8	89.1	243.3	98.1	107.0	104.4
Number of filled pollen cells	5.8	3.5	2.3	3.2	8.0	9.1
Weight of hive (without hive box) [g]	773.0	162.3	882.6	51.1	383.5* ^a	143.2
Total number of alive brood stages (eggs, larvae, pupae)	252.8	41.6	251.0	74.6	110.0* ^a	68.5
Total number of alive adult bees (alive young queens, workers, males)	413.0	106.1	454.0	128.1	126.8* ^b	35.1
Total number of alive stages (alive brood and adult bees)	665.8	118.3	705.0	83.4	236.8* ^a	88.8
Weight/young alive queen [g]	0.95	0.04	1.03	0.15	-	-

Mean = mean values of all 4 replicates (hives) per treatment group

STD = standard deviation

NOTE: Calculations based on unrounded values

* Statistically significant difference to control ($p \leq 0.05$)

^a t-test

^b Mann Whitney exact

^c Deep-freezing of hives at 12 Sep 2013, brood assessments of deep-frozen hives from 16 Sep to 15 Oct 2013

The final brood assessment revealed that all hives of the control group C and the test item treatment group T had still their original living queens. In the toxic reference R all queens were dead.

The mean numbers of young queens, workers and males produced in the control and the treatment group did not show significant differences ($p \leq 0.05$, t-test, Mann Whitney exact). However, the number of young queens and males differed significantly ($p \leq 0.05$, t-test) between control and toxic reference, where no males and no young queens were found. The number of young queens, workers, and males was 113.0, 239.5 and 60.5 in the control and 97.8, 276.8 and 79.5 in the treatment group, respectively. Considering the total number of adult bees and brood stages, there was no difference between the test-item treatment group and the control with 454.0 adult bees, 251.0 brood stages and a total of alive stages of 705.0 in the test treatment group compared to 413.0 adults, 252.8 brood stages and 665.8 total alive stages in the control treatment. Significant reductions ($p \leq 0.05$, t-test, Mann Whitney exact) were found for the toxic reference compared to the control. Only 126.8 adults, 110.0 brood stages resulting in a total of 236.8 total alive stages were counted in the toxic reference.

Also with regard to the individual brood stages, the final brood assessment did not show significant differences ($p \leq 0.05$, t-test, Mann Whitney exact) between the control and the treatment group. The production of pupae was significantly ($p \leq 0.05$, t-test) reduced in the toxic reference.

The weight per adult young queen was approximately the same for the control and the treatment group. The mean weight of the hives was slightly higher in the treatment group compared to the control, but significantly ($p \leq 0.05$, t-test) lower in the toxic reference compared to the control.

Photographic evaluation of brood stages and brood development

Before exposure, several times during exposure and at the end of the study the brood stages were documented by photographs. For the photographs before exposure and at the end of the study all adult bumble bees were removed in order to have direct view on the brood nest. These photographs were used for documentation of the brood stages and the brood development.

Residue analysis

Bumble bee and honey bee hives in the residue tunnels were used for residue sampling. Honey bees were used to sample nectar and pollen from *Phacelia* flowers. Analysis of residues of indoxacarb were carried out for honey bee nectar sampled directly from combs and prepared from forager honey bees for honey bee pollen sampled directly from combs and prepared from forager bees and for bumble bee nectar sampled from nectar cells in their hives. Residue samples were taken from control and treated replicates at 5 dates (-1, +1, +3, +8 and +17 days after application). No residues above the LOD level of 0.003 mg/kg were found in any of the control samples. Residues of indoxacarb above the LOQ level were found for pollen samples after application in the test item treatment. No residues of indoxacarb above the LOQ level were found for any of the nectar samples. Residues in pollen reached maximum values just after application ranging from 0.044 to 0.062 mg/kg.

Table 125
Residues in pollen and nectar

DAA	Mean residues of indoxacarb in pollen and nectar samples (mg/kg, n=2)	
	C	T
	Residues of indoxacarb in pollen samples of forager honey bees (mg/kg)	
-1	n.d.	n.d.
+1	n.d.	0.044
+3	n.d.	0.025
+8	n.d.	0.011
+17	n.d.	n.d.
	Residues of indoxacarb in pollen samples of honey bee combs (mg/kg)	
-1	n.d.	n.d.
+1	n.d.	0.062
+3	n.d.	0.036
+8	n.d.	0.042
+17	n.d.	0.008
	Residues of indoxacarb in nectar samples of forager honey bees (mg/kg)	
-1	n.d.	n.d.
+1	n.d.	n.d.
+3	n.d.	n.d.
+8	n.d.	n.d.
+17	n.d.	n.d.
	Residues of indoxacarb in nectar samples of honey bee combs (mg/kg)	
-1	n.d.	n.d.
+1	n.d.	n.d.
+3	n.d.	n.d.
+8	n.d.	n.d.
+17	n.d.	n.d.
	Residues of indoxacarb in nectar samples of bumble bee hives (mg/kg)	
-1	n.d.	n.d.
+1	n.d.	n.d.
+3	n.d.	n.d.
+8	n.d.	n.d.
+17	n.d.	n.d.

n.d. = not detectable (below LOD of 0.003 mg/kg)

LOQ = 0.01 mg/kg (in some cases of pollen samples from forager honey bees with low sample amount LOQ = 0.015 to 0.21)

DAA = days after application

NOTE: If single values were <LOQ, mean values were calculated with 0.003 mg/kg; if single values were <LOD, mean values were calculated with 0.000 mg/kg

III. CONCLUSIONS

Indoxacarb 150 g/L EC was applied once *via* spray application on flowering *Phacelia tanacetifolia* at an application rate of 37.5 g a.s./ha in 400 L water/ha after daily bumble bee flight in the evening.

No residues above the LOQ level of 0.01 mg/kg were found in any of the control samples. Residues of indoxacarb above the LOQ level were found for pollen samples after application in the test item treatment. Residues in nectar samples were below LOD.

Indoxacarb 150 g/L EC applied once *via* spray application on flowering *Phacelia* after bumble bee flight (evening application) did not have any effects regarding all parameters assessed *i.e.* mortality, flight activity, consumption of sugar solution, hive weight, condition of colonies, development of bumble bee brood, production of young queen offspring and vigour relative to the water treated control.

(Klein, O., 2014)

RMS comment

Study submitted to the EU for the first time in this submission.

No specific guideline is available for this test.

4 replicates per treatment group with one bumble bee hive each + 2 additional replicates for residue samplings for Indoxacarb 150 g/L EC and control with 2 bumble bee hives and 1 honey bee hive together. The test item Indoxacarb 150 g/L EC was applied in the evening after bumble bee flight.

RMS notes that the mortality data reported in the summary above are the mean numbers of dead workers and larvae as normalized values per day. In the study report, the raw data in the appendices are the actual and mean numbers of dead workers and larvae found on the day of assessment (not normalized per day). The normalization takes into account the number of days that have passed since the last check on the colony.

RMS notes that bumble bees were fed with sugar solution during the test. Bees were fed during the test to combat the effects of abnormally high temperatures on the colonies' feeding patterns. RMS is of the opinion that this feeding might have affected the results. Colonies would be strengthened and adverse effects due to the test item might have been underestimated as artificial feeding does not represent natural conditions. RMS also remarks that the sugar consumption seems higher in the tunnels treated with the test item (even if not significantly different with control). It is noted in the study report that higher sugar consumption in the test item hives is in accordance with the hives weight increase observed at the same timing. Besides the presence of syrup makes difficult to force the bumble bees to forage on the crop. The actual exposure of the bumble bees and the brood is uncertain. The applicant feels that it is a valid study as all colonies were fed, control as well as the treated colonies. RMS however, doubts the reliability of the study.

It is noted in the study report that rainfall occurred on the day of application (12.8 mm) however the rain occurred before application and application took place after drying up on the target crop. Then RMS considers that the rain had no impact on the exposure of foraging bumble bees. RMS however notes that the max temperature recorded were particularly high in the tunnels (max 40.2°C). High temperatures were recorded for several days in the tunnels. The temperature was much lower outside the tunnels. It is not known if this high temperature might affect the behaviour of the substance on plants or the behaviour of the bumble bees but the representativeness of the study for normal climate conditions is wondered by RMS.

RMS also notes an increase of larval mortality on DAA17 and DAA18 (not significant). A decrease of this larval mortality was observed as soon as the sugar solution supply was opened again.

RMS considers the study inconclusive. RMS considers that the absence of effect of the test item Indoxacarb 150 g/L EC on bumble bees in more realistic conditions (natural conditions without feeding) cannot be proven on the basis of this study.

Report: Berg, C. (2015); Indoxacarb (DPX-KN128) 150 g/L EC: A semi-field study to evaluate effects on the brood of honey bees (*Apis mellifera*; Hymenoptera, Apidae) in *Phacelia tanacetifolia* in Germany 2014

DuPont Report No.: DuPont-37489, Revision No. 1

Guidelines: OECD 75 (2007) **Deviations:** None

Testing Facility: Eurofins Agrosience Services GmbH, Niefern-Oeschelbronn, Germany

Testing Facility Report No.: S14-03575

GLP: Yes

Certifying Authority: Landesanstalt Für Umwelt, Messungen Und Naturschutz Baden-Württemberg

Executive summary:

The effects of the insecticide Indoxacarb 150 g/L EC applied during flowering were tested on the honey bee (*Apis mellifera* L.) under semi-field conditions following the OECD guidance document No. 75 (2007). This study was conducted near Karlsruhe in Southern Germany (region: Baden-Württemberg) from June to July 2014 and included a total of four treatment groups:

- Indoxacarb 150 g/L EC treatment group T1 with one application of the test item in each replicate (tunnel tent). The application was carried out during flowering of *P. tanacetifolia* (BBCH 65) on 1DBA in the evening after bee-flight has stopped (0 honey bees/ m² flying inside the tunnels). The application was carried out at a target rate of 37.5 g a.s./ha.
- Indoxacarb 150 g/L EC treatment group T2 with one application of the test item in each replicate (tunnel tent). The application was carried out during flowering of *P. tanacetifolia* (BBCH 65-67) at the next day during bee flight and foraging activity of bees (≥10 honey bees/m² flying inside the tunnels). The application was carried out at a target rate of 37.5 g a.s./ha.
- Reference item treatment group R with one application of Insegar (fenoxycarb) at a target rate of 1200 g product/ha (equivalent to 300 g fenoxycarb/ha). The application was carried out during flowering of *P. tanacetifolia* (BBCH 65-67) and bee-flight (≥10 honey bees/m² flying inside the tunnels), on the same day as the application in the treatment T2.
- Control group C with one application of tap water during flowering of *P. tanacetifolia* during flowering of *P. tanacetifolia* (BBCH 65-67) and bee-flight (≥10 honey bees/m² flying inside the tunnels), on the same day as the application in the treatment T2.

Data from the control group C and reference item group R were shared with another study running on the same field at the same time (Dupont-38405). The applications in the test-item treatment group T2, in the reference item treatment R and in the control were carried out on 13 June 2014 (6 days after installation of the hives in the tunnels). In treatment group T1 the application was done on 12 July 2014 (5 days after the installation of the hives in the tunnels). All applications were carried out with a spray volume of 400 L water per ha. The effects of the test item treatment were examined on honey bee colonies in tunnel tents (5.0 m × 18.0 m and a height of 3.5 m in the centre) placed over the plots of *P. tanacetifolia*. The semi-field test comprised 4 replicate tunnel tents in each of the treatment groups for biological assessments and additionally one tunnel tent (replicate e) for sampling in C, T1, and T2 for residue analysis.

The influence of the application of Indoxacarb 150 g/L EC was evaluated by comparing the results in this treatment to the data in the control treatment as well as in the reference item treatment regarding the following observations:

- Mortality: Number of dead honey bees at the edge of the treated area (linen sheets), in the dead honey bee traps in front of the hives and in the bottom drawers.
- Flight intensity on the crop (number of forager honey bees/m² *P. tanacetifolia*),
- Condition of the colonies and development of the brood,
- Detailed observation of the brood development of eggs and young larvae in ≥200 selected cells and of old larvae in ≥160 selected cells,
- Behaviour of the honey bees in the crop area and around the hives,
- Level of residues in the samples of hive products and nectar stomach contents and pollen loads from forager bees.

It can be concluded that the test item Indoxacarb 150 g/L EC applied after bee flight had no effect on honeybee mortality of biological relevance. Since slightly increased numbers of dead bees/day occurred in the

pre-application period of the treatment groups T1 and T2 and the values after the application were similar, these are not indicating any biologically relevant effect.

The application of Indoxacarb 150 g/L EC after bee flight or during bee flight had a slight effect on the flight intensity shortly after the applications in T1 and T2. One day after the applications, the flight activity in T1 and T2 was at the same level as in the control.

The application of Indoxacarb 150 g/L EC in T1 and T2 had a slight effect on the behaviour of the honey bees shortly after the applications. Since the numbers of honey bees showing unusual behaviour were generally low, this effect can be considered as not biologically relevant.

There was no effect on the colony size relating to the application of Indoxacarb 150 g/L EC in T1 and T2.

There was only a slight effect on the amount of brood cells with reduced numbers of larvae cells in the treatment group T2 shortly after the application of Indoxacarb 150 g/L EC. There was no test item-related effect regarding the amount of brood cells in the treatment group T1.

Indoxacarb 150 g/L EC applications carried out after bee flight (T1) caused no harmful effects on the development of the marked eggs, young larvae, or old larvae (brood termination rate). The application of Indoxacarb 150 g/L EC carried out during bee flight (T2) had a slight effect on the development of the marked eggs, whereas no effect occurred on the development of young larvae and old larvae.

Overall, the spray application of Indoxacarb 150 g/L EC carried out at 37.5 g a.s./ha during flowering and after bee flight (T1) caused no biologically relevant effect on honey bee colonies. The spray application of 150 g/L EC) carried out at 37.5 g a.s./ha during flowering and during bee flight (T2) caused slight effects on honey bee colonies.

No residues of indoxacarb could be found in the nectar samples prepared from forager bees or in nectar samples which were taken directly from the hive in the control group as well as in the treatment groups T1 and T2 taken two and four days after the application.

All pollen samples of the control group were free of residues of indoxacarb. In the pollen samples taken from the forager bees in the treatment group T1, the maximum value of 0.095 mg a.s./kg was found two days after the application. In the pollen samples which were taken directly from the hive, the maximum value of 0.047 mg a.s./kg was found two days after the application.

In the pollen samples taken from the forager bees in the treatment group T2, the maximum value of 0.063 mg a.s./kg was found two days after the application. In the pollen samples taken from the hive, no residues of indoxacarb above the level of quantification (<0.010 mg a.s./kg) were detected.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test material: Indoxacarb 150 g/L EC
Lot/Batch#: DPX-KN128-434
Purity: 150 g a.s./L
CAS#: None for the formulation
173584-44-6 for the active substance
Description: Liquid
Stability of test compound: 98.2% of the indoxacarb remains in the delivery vehicle after one hour under agitation
Reference item: Insegar (fenoxycarb)
Content of a.s., nominal: 25.0% (w/w)
CAS#: 72490-01-8
2. Vehicle and/or control: Tap water
3. Test organism: Honey bee
Species: *Apis mellifera* L.
Age at dosing: Direct exposure of adult honey bees; indirect exposure of all stages of development
Source: Eurofins Agrosience Services EcoChem GmbH
Diet: Nectar and pollen of flowering *Phacelia tanacetifolia*,
1 feeding of Api-Invert on 12DAA
Tunnel tents (exposure): Within the test field, plots (5.0 m × 18.0 m) were marked. Tunnel tents (5.0 m × 18.0 m and a height of 3.5 m in the centre) covering the marked plots were installed before full flowering of *P. tanacetifolia* and before installation of the bee hives. The tunnel frames were covered with light plastic gauze. Paths (0.6 m) were made in the tunnels by removing the plants and smoothing the ground. Subsequently, the paths (approx. 16.1 m²) in the tunnels intended for the biological assessments (replicates a, b, c and d) were covered with linen sheets for the assessment of dead bees in the crop area. The crop area per tunnel was approx. 73.9 m². In the tunnels used for sampling for residue analysis (replicates Ce, T1e and T2e) linen sheets were also spread on the ground.
4. Environmental conditions during the experimental phase:
Temperature (min/max): 7.0–37.2 °C
Relative humidity: 18.2–100%
Photoperiod (exposure): natural light conditions

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
06-June-2014 to 05-November-2014
2. Experimental treatments
 - Indoxacarb 150 g/L EC treatment group T1 with one application of the test item in each replicate (tunnel tent). The application was carried out during flowering of *P. tanacetifolia* (BBCH 65) on 1DBA in the evening after bee-flight has stopped (0 honey bees/m² flying inside the tunnels). The application was carried out at a target rate of 37.5 g a.s./ ha.
 - Indoxacarb 150 g/L EC treatment group T2 with one application of the test item in each replicate (tunnel tent). The application was carried out during flowering of *P. tanacetifolia* (BBCH 65-67) at the next day during bee flight and foraging activity of bees (≥10 honey bees/m² flying inside the tunnels). The application was carried out at a target rate of 37.5 g a.s./ ha.

- Reference item treatment group R with one application of Insegar (fenoxycarb) at a target rate of 1200 g product/ha (equivalent to 300 g a.s./ ha). The application was carried out during flowering of *P. tanacetifolia* (BBCH 65-67) and bee-flight (≥ 10 honey bees/m² flying inside the tunnels), on the same day as the application in the treatment T2.
- Control group C with one application of tap water during flowering of *P. tanacetifolia* during flowering of *P. tanacetifolia* (BBCH 65-67) and bee-flight (≥ 10 honey bees/m² flying inside the tunnels), on the same day as the application in the treatment T2.

3. Observations

The influence of the application of Indoxacarb 150 g/L EC was evaluated by comparing the results in this treatment to the data in the control treatment as well as in the reference item treatment regarding the following observations:

- Mortality: Number of dead honey bees at the edge of the treated area (linen sheets), in the dead honey bee traps in front of the hives and in the bottom drawers.
- Flight intensity on the crop (number of forager honey bees/m² *P. tanacetifolia*),
- Condition of the colonies and development of the brood,
- Detailed observation of the brood development of eggs and young larvae in ≥ 200 selected cells and of old larvae in ≥ 160 selected cells,
- Behaviour of the honey bees in the crop area and around the hives,
- Level of residues in the samples of hive products and nectar stomach contents and pollen loads from forager bees.

II. RESULTS AND DISCUSSION

A. MORTALITY

Mortality after installation of the colonies until the application (4DBA to 1DBA for T1 or 0DBA for T2 and R) was comparable in all treatment groups, but with a trend towards higher values (almost double) for T1 and T2 versus the control. The mean daily mortality was in the range from 22.3 to 194.0 dead honey bees/day in the control, from 49.0 to 322.8 dead honey bees/day in test item treatment group T1, from 29.3 to 359.3 dead honey bees/day in the test item treatment group T2 and from 26.5 to 248.0 dead honey bees/day in the reference item treatment group R. The unusually high numbers of dead bees, which were found on the linen on 3DBA in all treatment groups, are probably due to the very high temperatures and bees which could not adapt quickly enough to the confined situation in the tunnel tents. Mean daily mortality in the control over the whole period was 63.7 (for T1) or 65.3 (for T2 and R) dead honey bees/day, 116.8 dead honey bees/day in T1, 118.8 dead honey bees in T2 and 100.6 dead honey bees/day in R. Although the numbers of dead bees in the treatment groups T1, T2 and R during the pre-application period are tangentially higher, there were no statistically significant differences, with the exception of 0DBA, where the number of dead honey bees/day was significantly higher in the treatment groups T2 and R compared to the control (Tukey's Studentized Range test, $p \leq 0.05$). For the mean number of dead bees/day of the entire period before the application, there were no statistically significant results between the treatment groups.

Daily mortality values during the period after the application until the removal of the hives from the tunnel tents (0DAA to 7DAA) were in the range from 13.8 to 72.3 dead honey bees/day in the control, from 14.5 to 120.3 dead honey bees/day in T1, from 74.5 to 128.5 dead honey bees/day in T2 and from 35.3 to 143.8 dead honey bees/day in R.

After the application, the numbers of dead bees in T1 were significantly higher on 2DAA, 3DAA, 5DAA and considering the whole period from 0DAA to 7DAA (Dunnett's t-Test, $p \leq 0.05$). In T2, the mortality

was significantly increased on 0DAA, 1DAA, 2DAA and considering the whole period from 0DAA to 7DAA (pooled t-test, Dunnetts t-test or Welch Bonferroni-Holms correlation, $p \leq 0.05$). The mean value of the control during that period was 53.9 dead honey bees/day, compared to values of 83.1 dead honey bees/day in T1 and 52.9 compared to 90.4 dead honey bees/day in T2 and versus 111.7 dead honey bees/day in R. Considering the non-test item related slightly increased numbers of dead bees/day that already occurred during the pre-application period of the treatment groups T1 and T2 and the values after the application, which are lower than in the pre-application period, no biologically relevant effect can be assumed.

The mortality of the reference item treatment group R was statistically significantly increased on every day during the period from 0DAA to 7DAA (pooled t-test, Satterthwaite t-test, $p \leq 0.05$) with the exception of 1DAA, where no significant difference occurred. Considering the whole period from 0DAA to 7DAA, the mortality in R was significantly increased compared to the control (pooled t-test, $p \leq 0.05$).

During further observation at the monitoring site from 8DAA to 28DAA, daily mortality values were in the range from 4.3 to 101.0 dead honey bees/day in the control, from 8.3 to 158.3 dead honey bees/day in T1, from 9.3 to 65.3 dead honey bees in T2 and from 15.3 to 101.8 dead honey bees/day in R. The mean values during that period were 16.6 dead honey bees/day in the control, 30.7 dead honey bees/day in T1, 21.3 dead honey bees/day in T2 and 54.6 dead honey bees/day in R.

In T1, the mean daily mortality was significantly higher on 9DAA, 12DAA, 18DAA, 20DAA, and considering the whole period from 8DAA to 28DAA. In T2, there were significantly higher numbers of dead bees/day on 8DAA and 24DAA. The slightly increased values in T1 and T2 during that period are not indicating a biologically relevant effect.

The mortality of the reference item treatment group R during the period on the monitoring site was statistically significantly increased in comparison to the control on every day from 9DAA to 27DAA (pooled t-test, Satterthwaite t-Test or Mann Whitney Exact test, $p \leq 0.05$), with the exception of 14DAA, 20DAA, and 26DAA. Considering the whole period from 8DAA to 28DAA, the mortality was significantly higher in R (pooled t-test, $p \leq 0.05$).

Table 126
Honey bee mortality

Date	DBA/ DAA	Mean number of dead honey bees/day per treatment group				
		C		T1	T2	R
		For T1	For T2 and R			
09 Jun 2014	4DBA	44.3		103.8	75.5	98.0
10 Jun 2014	3DBA	194.0		322.8	359.3	248.0
11 Jun 2014	2DBA	30.5		52.0	29.3	26.5
12 Jun 2014	1DBA	22.3		49.0	51.3	50.3
12 Jun 2014	1DBAba	27.3	---	56.5	---	---
13 Jun 2014	0DBA	---	35.5 ^a	---	78.8* ^a	80.3* ^a
Mean 4DBA to 1DBAba or 0DBA		63.7	65.3	116.8	118.8	100.6
STD		36.1	36.3	56.2	61.8	11.6
13 Jun 2014	0DAA	55.3 ^b	47.0	72.8 ^b	102.5*	126.5*
14 Jun 2014	1DAA	13.8		14.5	77.5*	35.3
15 Jun 2014	2DAA	72.3		103.8*	128.5*	143.8*
16 Jun 2014	3DAA	63.3		120.3*	106.3	142.8*
17 Jun 2014	4DAA	60.8		87.3	77.5	118.8*
18 Jun 2014	5DAA ^c	51.5		90.5*	74.5	100.8*
19 Jun 2014	6DAA	60.8		89.5	76.3	124.3*
20 Jun 2014	7DAA	53.5		86.3	79.8	101.3*
Mean 0DBA or 0DAA to 7DAA		53.9	52.9	83.1*	90.4*	111.7*
STD		12.8	12.2	19.2	2.9	41.4

Table 126
Honey bee mortality (continued)

Date	DAA	Mean number of dead honey bees/day per treatment group			
		C	T1	T2	R
21 Jun 2014	8DAA	4.3	12.5	14.5*	15.3
22 Jun 2014	9DAA	7.8	28.5*	9.3	33.3*
23 Jun 2014	10DAA	8.0	35.8	12.3	68.0*
24 Jun 2014	11DAA ^c	15.0	31.3	29.8	87.8*
25 Jun 2014	12DAA	9.5	31.0*	20.5	72.0*
26 Jun 2014	13DAA	12.3	20.5	13.8	96.3*
27 Jun 2014	14DAA	25.0	23.3	15.8	46.8
28 Jun 2014	15DAA	17.8	18.8	30.0	50.5*
29 Jun 2014	16DAA ^c	20.3	42.8	31.5	71.0*
30 Jun 2014	17DAA	9.5	27.8	24.5	70.8*
01 Jul 2014	18DAA	9.5	24.8*	15.0	58.5*
02 Jul 2014	19DAA	16.3	47.5	23.3	55.3*
03 Jul 2014	20DAA	15.3	33.8*	20.3	37.8
04 Jul 2014	21DAA	11.8	24.3	26.3	59.8*
05 Jul 2014	22DAA ³	10.5	24.0	26.0	38.0*
06 Jul 2014	23DAA	9.3	13.0	11.8	49.8*
07 Jul 2014	24DAA	5.3	8.3	11.5*	22.5*
08 Jul 2014	25DAA	5.0	14.5	10.3	28.3*
09 Jul 2014	26DAA	18.3	10.0	14.0	32.8
10 Jul 2014	27DAA	16.3	14.0	22.5	50.3*
11 Jul 2014	28DAA	101.0	158.3	65.3	101.8
Mean 8DAA to 28DAA		16.6	30.7*	21.3	54.6*
STD		4.5	9.8	4.5	23.1

DBA = Days before application

DAA = Days after application

STD = Standard deviation

ba = before application

4DBA to 7DAA: Dead bees from linen sheets and dead bee traps (exposure period inside tunnel)

8DAA to 28DAA: Dead bees from dead bee traps (monitoring period)

^a = Results of the assessments on 1DBAba (before application in T1) and 0DBA (before applications in C, T2 and R) were summarized and counted as one value.

^b = Results of the assessments on 0DBA (before applications in C, T2 and R) and 0DAA were summarized and counted as one value.

^c = Potentially higher mortality due to a colony assessment that was done in all treatments on the day before

* = Significantly different to the control (pooled t-test, Dunnetts t-test or Welch Bonferroni-Holms correlation, $p \leq 0.05$)

In the pre-application period from 4DBA to 1DBA (for T1) or 0DBA (for T2 and R), the total number of dead pupae and dead malformed pupae was 3 in the control, 13 in the test item treatment group T1, 18 in the test item treatment group T2 and 18 in the reference item treatment group R. In the post exposure period in the tunnel tents from 0DAA to 7DAA there were 7 dead pupae and dead malformed pupae in C, 21 in T1, 103 in T2 and 45 in R. In the monitoring period from 8DAA to 28DAA, there were 22 dead pupae and dead malformed pupae in C, 42 in T1, 26 in T2 and 1063 in R. There are no differences of biological relevance in the mortality of dead pupae and dead malformed pupae in the treatment groups T1 compared to the control. In the treatment group T2, a slight effect on the mortality of pupae can be assumed.

Overall, there was no increase in honey bee mortality in the Indoxacarb 150 g/L EC treatment groups T1 and T2 after application. The differences in T1 and T2 relative to the control are considered as a very slight effect of the test item, which is not considered as biologically relevant. There was no considerable impact of the Indoxacarb 150 g/L EC treatments on honey bee pupal mortality in the treatment group T1, whereas a slight impact on pupae mortality in T2 occurred. The toxic reference treatment resulted in a clear impact on honey bee mortality, mainly resulting in high numbers of dead pupae.

B FLIGHT INTENSITY

Daily flight activity from installation of the colonies in the tunnels until the application during bee-flight from 4DBA to 1DBA (for T1) or 0DBA (for T2 and R) was in the range from 11.7 to 21.8 forager bees/m² in the control, from 15.1 to 19.7 forager bees/m² in the test item treatment group T1, from 12.1 to 23.3 forager bees/m² in the test item treatment group T2 and from 19.6 to 25.1 forager bees/m² in the reference item treatment group R. The mean daily flight activity during this period was 15.6 (for T1) or 14.8 (for T and R) forager bees/m² in the control, 18.2 forager bees/m² for T1, 18.6 foragers bees/m² for T2 and 22.8 forager bees/m² for R. The reference item group R was statistically different from the control at 2DBA, 0DBA and considering the whole period from 4DBA to 1DBA (Tukey's Studentized Range test, $p \leq 0.05$). The treatment groups T1 and T2 were on the same level as the control during that period and there were no statistically significant differences between those groups.

On the day of application (0DAA), the number of forager bees/m² was statistically significantly lower in T1 (13.1 forager bees/m²) and T2 (12.7 forager bees/m²) compared to the control (18.5 forager bees/m² for T1 and 19.8 forager bees/m² for T2 and R; pooled t-test, $p \leq 0.05$). R (23.3 forager bees/m²) was not statistically significant different to the control.

During the period from 1DAA to 7DAA, the mean number of forager bees/m² was on the same level in all treatment groups and was in a range from 5.2 to 14.0 forager bees/m² in the control, from 5.9 to 12.4 in T1, from 5.9 to 11.4 forager bees/m² in T2 and from 7.9 to 16.2 forager bees/m² in R. The mean number of forager bees/m² was 10.2 (for T1) or 10.4 (for T2 and R) in C, 9.6 in T1, 9.1 in T2 and 13.0 forager bees/m² in R. There were no statistically significant results in any of the treatment groups during this period.

Table 127
Honey bee flight intensity

Date	DAA	Flight intensity (mean number of forager bees/m ²)				
		C		T1	T2	R
		For T1	For T2 and R			
09 Jun 2014	4DBA	15.5		19.6	20.0	22.4
10 Jun 2014	3DBA	13.3		19.7	19.6	19.6
11 Jun 2014	2DBA	11.7		15.1	17.5	22.1*
12 Jun 2014	1DBA	21.8		18.3	23.3	25.1
13 Jun 2014	0DBA	---	11.9	---	12.1	24.5*
Mean 4DBA to 1DBA or 0DBA		15.6	14.8	18.2	18.6	22.8*
STD		3.5	2.9	2.9	2.5	4.0
13 Jun 2014	0DAA	18.5 ^a	19.8	13.1 ^{*a}	12.7*	23.3
14 Jun 2014	1DAA	10.1		9.8	9.9	13.5
15 Jun 2014	2DAA	14.0		10.9	11.4	16.2
16 Jun 2014	3DAA	11.9		12.4	9.5	11.8
17 Jun 2014	4DAA	6.5		7.2	7.5	9.4
18 Jun 2014	5DAA	10.1		10.5	9.9	13.5
19 Jun 2014	6DAA	5.2		5.9	5.9	8.3
20 Jun 2014	7DAA	5.3		6.7	6.0	7.9
Mean 0DAA to 7DAA		10.2^b	10.4	9.6^b	9.1	13.0
STD		2.1	2.1	3.3	3.0	2.3

DAA Days after application

DBA Days before application

STD Standard deviation

^a Includes the data of the assessment on 0DBA (part of the post-application period in T1)\

^b Results of the assessments on 1DBA (before application in T1) and 0DBA (before applications in C, T2 and R) were summarized and counted as one value.

* Significantly different to the control (pooled t-test, p≤0.05)

Overall, there was a slight reduction of the foraging activity on the day of application in the test item treatment groups T1 and T2, without biological relevance. After this day, the flight intensity was back on the same level as in the control during the complete period in the tunnel tents.

C. BEHAVIOUR OF THE HONEY BEES

In the control, the treatment groups T1 and T2 and in the reference item group R, normal behaviour was recorded in the period before the applications (4DBA to 1DBA or 0DBA). Only very few bees showed unusual behaviour: In the control, there were 7 cramping bees, 12 bees with locomotion problems, 2 inactive bees, and 5 hanging bees. In T1, there were 9 cramping bees, 5 trembling bees 10 bees with locomotion problems, 4 inactive bees, and 2 hanging bees. In T2, there were 29 cramping bees, 3 trembling bees, 16 bees with locomotion problems, and 18 inactive bees. In R, there were 15 cramping bees, 8 trembling bees, 8 bees with locomotion problems, and 2 inactive bees. The observed behaviour during this period before the applications is showing the normal background level of unusual behaviour.

In the tunnel period after the application in the treatment groups (0DBA or 0DAA to 7DAA), the numbers of bees showing unusual behaviour in the treatment groups T1, T2, and R are very slightly elevated compared to the control, but the total number of bees showing unusual behaviour remains on a low level in

the test item treated groups as well as in the reference item treatment group. There were 37 cramping bees in T1, 35 cramping bees in T2, and 35 cramping bees in R compared to 15 cramping bees in the control. The numbers of trembling bees were 8 in T1, 10 in T2, and 25 in R compared to 0 in the control. There were 48 bees showing locomotion problems in T1, 62 in T2, 79 in R, and 24 in the control. The numbers of inactive bees were on a similar level in the treatment groups and the reference group compared to the control, there were 21 in T1, 45 in T2, 25 in R, and 27 in the control. The numbers of bees which were clustering, hanging on the crop or intensely self-grooming are marginal and do not show any significance.

After the tunnel period on the monitoring site, the numbers of bees showing unusual behaviour in the treatment groups T1, T2 and R are very low, although very slightly elevated compared to the control. There were 30 cramping bees in T1, 17 cramping bees in T2 and 45 cramping bees in R compared to 17 cramping bees in the control. There were 17 bees with locomotion problems in T1, 19 in T2, 33 in R and 1 in the control. The numbers of inactive bees were again on a similar level in the treatment groups compared to the control, there were 71 in T1, 58 in T2, 72 in R, and 95 in the control. The numbers of bees that were showing any other unusual behaviour are marginal and do not show any significance.

Overall, a very slight effect on the behaviour could be observed, with only very few bees showing unusual behaviour at each of the assessments in the test item treatment groups and in the control. Therefore, there does not seem to be a biologically relevant effect of the test item on the behaviour.

D. BROOD DEVELOPMENT AND COLONY CONDITION

At the first assessment, there were 6313 to 7500 honey bees/hive with a mean of 6703 honey bees per hive in the control. The treatment group T1 had 5688 to 7813 honey bees/hive with a mean of 6610 honeybees per hive and the treatment group T2 had 6125 to 9313 honey bees/hive with a mean of 7329 honey bees per hive. In the reference item treatment group, there were 7250 to 9313 honey bees/hive with a mean of 8453 honey bees per hive at the first assessment date.

At the next assessment on 1DBA shortly before the brood fixing, the mean colony size stayed on a similar level in all treatment groups. The mean colony size was 6266 in the control, 6516 in T1, 7313 in T2, and 7578 in R.

At the first colony assessment after the application on 4DAA, the mean colony size was slightly increasing in all treatment groups. The mean colony size was 8203 in the control, 7594 in T1, 8985 in T2 and 8969 in R.

At the fourth assessment on 10DAA, the first on the monitoring site after the removal from the tunnel tents, the mean colony size strongly increased in all treatment groups. The mean colony size was 11000 in the control, 10828 in T1, 13375 in T2, and 12578 in R.

Five days later on 15DAA, the mean colony size of all treatment groups had only marginally changed. The mean colony size was 10844 in the control, 10016 in T1, 13375 in T2, and 12578 in R.

At the sixth colony assessment on 21DAA, the mean colony size was slightly decreasing in all treatment groups. There were 10391 honey bees in the control, 8391 honey bees in T1, 12235 honey bees in T2 and 10125 honey bees in R.

At the last colony assessment on 27DAA, the mean colony size was decreasing in all treatment groups. The mean colony size was 7641 in the control, 7125 in T1, 8797 in T2, and 8344 in R.

The development of the colony size in the treatment groups T1 and T2 is very similar to the development in the control group. There does not seem to be an effect on the colony size following the application of the test item.

At the first colony assessment on 7DBA, high numbers of brood cells, which contain eggs, larvae and pupae, were observed in all treatment groups. The mean number of brood cells was 23050 in the control, 24100 in T1, 33400 in T2 and 31400 in R.

During the next assessments, the number of brood cells constantly decreased in all treatment groups. The minimum mean quantity of brood cells was reached at the fourth assessment on 10DAA. At this assessment, there were 13800 brood cells in C, 9600 in T1, 10450 in T2 and 9350 in R. There were slightly lower numbers of brood cells on 4DAA and 10DAA in the treatment group T2, especially considering the larvae shortly after the application. This indicates a slight effect on honey bee brood in T2, but not in T1. In R, there was a clear effect on honey bee brood, where the mean number of larvae was only 550 on 4DAA. At 10DAA, the mean number of eggs per colony had already increased in all treatment groups.

At the following two assessments, the mean total number of brood cells increased up to 21000 brood cells in C, 14950 in T1, 28350 in T2, and 26500 in R on 21DAA. In two replicates in the treatment group T1, the original queens were lost during the period on the monitoring site due to unknown reasons. In T1a, there was mainly male progeny left in the colony whereas in T1b, no eggs were found in the colony.

On the last assessment, the mean number of brood cells stayed on the same level. There were 19700 brood cells in C, 14800 brood cells in T1, 24750 brood cells in T2 and 24650 in R. The two queenless colonies in T1 did not recover at this time, which results in a lower mean number of brood cells as in the other treatment groups.

The number of food cells was sufficient throughout the study with small reductions of the total amount of food cells in the tunnel period.

Overall, there was no effect of the test item treatments T1 and T2 on colony size. There was a slight effect on honeybee brood in the treatment group T2 shortly after the application. After the tunnel period, the colony sizes and the numbers of brood cells of the treatment group T2 were fully recovered and were on the same level as the control, whereas two colonies in T1 were not queen-right, which results in lower numbers of brood cells. There was a clear effect on the honey bee brood in the reference item group R.

E. DEVELOPMENT OF THE HONEY BEE BROOD IN INDIVIDUAL CELLS

Development of eggs in the marked cells:

Although the brood indices of T1 and T2 are lower as in the control, these differences were not statistically different with the exception of T2, which had a lower brood index on BFD+5 (5 days after the “Brood Area Fixing Day” = BFD, Dunnetts t-Test, $p \leq 0.05$). The final values were 3.19 in the control, 1.61 in T1 and 1.02 in T2.

The compensation indices were lower in the test item groups T1 and T2 on BFD+5, with T2 being statistically significantly different compared to the control (Dunnetts t-Test, $p \leq 0.05$). Afterwards, the compensation indices were increasing in T1 and T2 up to the last assessment on BFD+22. The final values on BFD+22 were 3.87 in C, 2.78 in T1 and 2.69 in T2. There were no statistically significant differences between T1 and T2 compared to the control on BFD+11, BFD+16 and BFD+22.

The termination rates in T1 and T2 are slightly higher but comparable to the control. The values on BFD+22 were 36.17% in C, 67.54% in T1 and 79.64% in T2. There were no statistically significant differences between the test item treatment groups and the water-treated control (Dunnetts t-test or Welch Bonferroni-Holms correlation, $p \leq 0.05$).

In the reference item group, the brood index reached a final value of 0.14 on BFD+22. There were only very few marked cells in which a successful development took place. The brood index was statistically significantly lower on BFD+5, BFD+11, BFD+16 and BFD+22 (pooled t-Test, $p \leq 0.05$). The compensation index was statistically significantly lower on BFD+5, BFD+11 and BFD+16 (pooled t-Test, $p \leq 0.05$). The termination rates in the reference item group R reached a mean value of 97.12 on BFD+22, showing a clear effect on the brood development. It was statistically significantly different on BFD+5, BFD+11, BFD+15 and BFD+22 compared to the control (pooled t-Test, $p \leq 0.05$).

Table 128
Honey bee egg brood/compensation indices and termination rate

Treatment group/ replicate	Brood/Compensation Indices at x days after brood area fixing day (BFD0) for marked cells containing eggs at the first assessment					Termination rate (%)
	BFD0	BFD+5	BFD+11	BFD+16	BFD+22	BFD+22
Ca	1.00/1.00	1.16/1.19	0.92/0.92	0.92/1.03	1.15/2.35	77.06
Cb	1.00/1.00	2.36/2.46	2.53/2.60	2.53/2.73	3.14/4.05	37.13
Cc	1.00/1.00	2.85/2.85	3.41/3.41	3.33/3.36	4.16/4.35	16.75
Cd	1.00/1.00	2.58/2.58	3.47/3.47	3.45/3.57	4.31/4.73	13.73
Mean C	1.00/1.00	2.24/2.27	2.58/2.60	2.56/2.67	3.19/3.87	36.17
STD	0.00/0.00	0.75/0.74	1.19/1.19	1.17/1.15	1.46/1.05	29.18
T1a	1.00/1.00	2.74/2.75	2.77/2.79	2.74/2.76	3.38/3.56	32.39
T1b	1.00/1.00	1.59/1.59	1.97/2.00	1.91/1.95	2.38/2.41	52.30
T1c	1.00/1.00	0.09/0.09	0.11/0.11	0.11/0.73	0.14/2.35	97.15
T1d	1.00/1.00	0.40/0.44	0.44/0.46	0.44/0.96	0.54/2.81	89.12
Mean T1	1.00/1.00	1.21/1.22	1.32/1.34	1.30/1.60	1.61/2.78	67.74
STD	0.00/0.00	1.21/1.21	1.26/1.27	1.24/0.94	1.53/0.56	30.61
T2a	1.00/1.00	0.38/0.45	0.32/0.38	0.28/0.90	0.35/2.29	92.98
T2b	1.00/1.00	1.95/2.00	2.50/2.91	2.50/3.44	3.13/4.30	37.40
T2c	1.00/1.00	0.36/0.37	0.41/0.41	0.41/1.07	0.51/2.87	89.80
T2d	1.00/1.00	0.14/0.16	0.07/0.07	0.07/0.18	0.08/1.31	98.36
Mean T2	1.00/1.00	0.71*/0.75*	0.83/0.94	0.82/1.40	1.02/2.69	79.64
STD	0.00/0.00	0.84/0.85	1.13/1.32	1.13/1.42	1.42/1.25	28.38
Ra	1.00/1.00	0.29/0.30	0.17/0.18	0.16/0.65	0.19/2.25	96.11
Rb	1.00/1.00	0.69/0.74	0.22/0.60	0.20/2.08	0.25/3.61	94.98
Rc	1.00/1.00	0.24/0.30	0.16/0.20	0.10/0.67	0.13/2.65	97.39
Rd	1.00/ 1.00	0.04/0.23	0.00/0.07	0.00/1.35	0.00/3.36	100.00
Mean R	1.00/ 1.00	0.32*/0.39*	0.14*/0.26*	0.12*/1.19*	0.14*/2.97	97.12
STD	0.00/ 0.00	0.27/0.23	0.10/0.23	0.09/0.68	0.11/0.63	2.16

BFD0 Brood area fixing day

STD = standard deviation

* Significantly different compared to the control (Dunnetts t-Test, t-pooled Test or Satterthwaite t-Test, $p \leq 0.05$)

F. DEVELOPMENT OF YOUNG LARVAE IN THE MARKED CELLS:

The brood index of the control group C reached a final level of 2.61. In the test item treatment groups T1, the brood index reached a final level of 2.78, whereas in the treatment group T2, the brood index was 1.27. In the reference item treatment group, the final brood index was 1.69. There were no statistically significant differences between the treatment groups (pooled t-Test, Satterthwaite t-Test, Mann Whitney Exact Test or pooled t-Test, $p \leq 0.05$).

The compensation index of the control group was 2.68 on the last photographic assessment of the brood on BFD+22. In T1, the final value was 2.29 and in T2, it was 2.55. There were no statistically significant differences between the treatment groups (pooled t-Test, Satterthwaite t-Test, Mann Whitney Exact Test or pooled t-Test, $p \leq 0.05$).

The final values of the termination rates were 47.84% in the control, 44.50% in T1, 74.72% in T2, and 66.26% in R. There were no statistically significant differences between the treatment groups (pooled t-Test, Satterthwaite t-Test, Mann Whitney Exact Test or pooled t-Test, $p \leq 0.05$).

Table 129
Honey bee young larvae brood/compensation indices and termination rate

Treatment group/ replicate	Brood/Compensation Indices at × days after brood area fixing day (BFD0) for marked cells containing young larvae at the first assessment					Termination rate (%)
	BFD0	BFD+5	BFD+11	BFD+16	BFD+22	BFD+22
Ca	2.00/2.00	0.82/0.90	0.82/0.86	0.91/1.30	1.03/2.71	79.39
Cb	2.00/2.00	0.40/1.05	0.30/0.76	0.36/1.18	0.38/2.49	92.40
Cc	2.00/2.00	3.60/3.60	3.38/3.38	4.09/4.11	4.22/2.08	15.52
Cd	2.00/2.00	3.92/3.93	3.84/3.84	4.32/4.36	4.80/3.43	4.04
Mean C	2.00/2.00	2.19/2.37	2.09/2.21	2.42/2.74	2.61/2.68	47.84
STD	0.00/0.00	1.83/1.62	1.78/1.63	2.08/1.73	2.23/0.57	44.51
T1a	2.00/2.00	3.54/3.54	2.71/2.71	3.10/3.10	3.39/1.70	32.22
T1b	2.00/2.00	3.89/3.89	3.68/3.68	4.28/4.31	4.60/2.10	8.00
T1c	2.00/2.00	1.80/1.80	1.80/1.80	2.22/2.74	2.25/2.49	55.06
T1d	2.00/2.00	0.69/0.80	0.69/0.69	0.70/1.09	0.86/2.85	82.70
Mean T	2.00/2.00	2.48/2.51	2.22/2.22	2.58/2.81	2.78/2.29	44.50
STD	0.00/0.00	1.50/1.46	1.28/1.28	1.51/1.33	1.60/0.50	31.91
T2a	2.00/2.00	2.11/2.24	2.09/2.10	2.59/2.93	2.61/2.67	47.72
T2b	2.00/2.00	0.69/1.83	0.68/1.48	0.71/2.81	0.85/3.33	83.05
T2c	2.00/2.00	1.43/1.50	1.28/1.29	1.51/2.19	1.60/2.62	68.09
T2d	2.00/2.00	0.17/0.24	0.00/0.00	0.00/0.14	0.00/1.57	100.00
Mean T2	2.00/2.00	1.10/1.45	1.01/1.22	1.20/2.02	1.27/2.55	74.72
STD	0.00/0.00	0.85/0.86	0.89/0.88	1.11/1.29	1.11/0.73	22.22
Ra	2.00/2.00	1.36/1.37	1.21/1.21	1.45/2.03	1.51/2.69	69.79
Rb	2.00/2.00	2.98/3.05	2.74/2.75	3.12/3.56	3.11/2.68	37.90
Rc	2.00/2.00	1.99/2.02	1.74/1.76	2.15/2.55	2.13/2.81	57.35
Rd	2.00/2.00	0.00/0.23	0.00/0.11	0.00/1.55	0.00/3.40	100.00
Mean R	2.00/2.00	1.58/1.67	1.42/1.46	1.68/2.42	1.69/2.90	66.26
STD	0.00/0.00	1.25/1.18	1.14/1.10	1.31/0.86	1.30/0.34	26.04

BFD0 Brood area fixing day

STD Standard deviation

* Significantly different compared to the control (no statistically significant results between the treatment groups occurred)

G. DEVELOPMENT OF OLD LARVAE IN THE MARKED CELLS:

The final brood indices were on a similar level in all treatment groups at the final relevant photographic assessment concerning the old larvae on BFD+16. It was 4.34 in the control, 3.37 in T1, 2.38 in T2 and 3.73 in R. There were no statistically significant differences between the treatment groups (pooled t-Test, Satterthwaite t-Test, Mann Whitney Exact Test or pooled t-Test, $p \leq 0.05$).

The compensation indices on BFD+16 were 4.4 in C, 3.64 in T1, 2.87 in T2 and 4.04 in R. There were no statistically significant differences between the treatment groups (pooled t-Test, Satterthwaite t-Test, Mann Whitney Exact Test or pooled t-Test, $p \leq 0.05$).

The termination rates on BFD+16 were 13.25% in C, 32.60% in T1, 52.52% in T2, and 25.51 in R. There were no statistically significant differences between the treatment groups (pooled t-Test, Satterthwaite t-Test, Mann Whitney Exact Test or pooled t-Test, $p \leq 0.05$).

Table 130
Honey bee old larvae brood/compensation indices and termination rate

Treatment Group/ Replicate	Brood/Compensation Indices at x days after brood area fixing day (BFD0) for marked cells containing old larvae at the first assessment				Termination rate (%)
	BFD0	BFD+5	BFD+11	BFD+16	BFD+16
Ca	3.00/3.00	3.84/3.84	3.80/3.80	4.75/4.78	5.08
Cb	3.00/3.00	2.32/2.32	2.22/2.22	2.78/2.96	44.40
Cc	3.00/3.00	3.93/3.93	3.88/3.88	4.85/4.86	3.04
Cd	3.00/3.00	3.98/3.98	3.98/3.98	4.98/4.98	0.47
Mean C	3.00/3.00	3.52/3.52	3.47/3.47	4.34/4.40	13.25
STD	0.00/0.00	0.80/0.80	0.84/0.84	1.04/0.96	20.85
T1a	3.00/3.00	2.58/2.58	2.53/2.53	2.83/3.10	43.33
T1b	3.00/3.00	3.95/3.95	3.85/3.86	4.82/4.84	3.65
T1c	3.00/3.00	3.91/3.91	3.90/3.90	4.87/4.91	2.58
T1d	3.00/3.00	0.94/1.45	0.77/0.99	0.96/1.72	80.85
Mean T	3.00/3.00	2.85/2.97	2.76/2.82	3.37/3.64	32.60
STD	0.00/0.00	1.42/1.20	1.47/1.38	1.87/1.53	37.34
T2a	3.00/3.00	2.07/2.15	1.20/1.25	1.50/1.97	70.00
T2b	3.00/3.00	2.72/3.25	2.68/3.22	3.35/4.25	33.03
T2c	3.00/3.00	2.83/2.84	2.43/2.43	3.04/3.39	39.15
T2d	3.00/3.00	2.52/2.54	1.28/1.31	1.61/1.88	67.89
Mean T2	3.00/3.00	2.54/2.70	1.90/2.05	2.38/2.87	52.52
STD	0.00/0.00	0.34/0.47	0.77/0.95	0.96/1.15	19.15
Ra	3.00/3.00	3.57/3.57	3.23/3.23	4.04/4.21	19.25
Rb	3.00/3.00	3.62/3.67	3.09/3.10	3.86/4.19	22.75
Rc	3.00/3.00	3.66/3.68	2.90/2.90	3.63/3.83	27.43
Rd	3.00/3.00	3.27/3.27	2.70/2.74	3.37/3.93	32.61
Mean R	3.00/3.00	3.53/3.55	2.98/2.99	3.73/4.04	25.51
STD	0.00/0.00	0.18/0.19	0.23/0.22	0.29/0.19	5.80

BFD0 Brood area fixing day

STD Standard deviation

Overall, the Indoxacarb 150 g/L EC application carried out after bee flight (T1) caused no harmful effects on the development of the marked eggs, young larvae, or old larvae (brood termination rate). The application of Indoxacarb 150 g/L EC carried out during bee flight (T2) had a slight effect on the development of the marked eggs, whereas no effect occurred on the development of young larvae and old larvae.

H. RESIDUES IN NECTAR AND POLLEN

No residues of indoxacarb could be found in the nectar samples prepared from forager bees or in nectar samples which were taken directly from the hive in the control group as well as in the treatment groups T1 and T2 taken two and four days after the application.

All pollen samples of the control group were free of residues of indoxacarb. In the pollen samples taken from the forager bees in the treatment group T1, 0.095 mg a.s./kg could be found two days after the application and 0.013 mg a.s./kg four days after the application. In the pollen samples which was taken directly from the hive, 0.047 mg a.s./kg could be found two days after the application and 0.020 mg a.s./kg could be found four days after the application.

In the sample taken from the forager bees in the treatment group T2, the residue level of indoxacarb was below the level of quantification (<0.010 mg a.s./kg) two days after the application. Four days after the application, residues of indoxacarb could also not be detected in the samples.

Table 131
Indoxacarb residue levels in nectar

Sample type	Treatment	Timing	Residue (mg/kg)
Nectar from forager bees	Ce	2 DAA	n.d.
Nectar from forager bees	Ce	4 DAA	n.d.
Nectar taken from the hive	Ce	2 DAA	n.d.
Nectar taken from the hive	Ce	4 DAA	n.d.
Nectar from forager bees	T1e	2 DAA	n.d.
Nectar from forager bees	T2e	2 DAA	n.d.
Nectar from forager bees	T1e	4 DAA	n.d.
Nectar from forager bees	T2e	4 DAA	n.d.
Nectar taken from the hive	T1e	2 DAA	n.d.
Nectar taken from the hive	T2e	2 DAA	n.d.
Nectar taken from the hive	T1e	4 DAA	n.d.
Nectar taken from the hive	T2e	4 DAA	n.d.

DAA: Days after application

LOQ: 0.010 mg/kg,

n.d. not detectable (<LOD = 0.003 mg/kg).

Ce: Control samples, T1e, T2e: Treated samples.

Table 132
Indoxacarb residue levels in pollen

Sample type	Treatment	Timing	Residue (mg/kg)
Pollen from forager bees	Ce	2 DAA	n.d.
Pollen from forager bees	Ce	4 DAA	n.d.
Pollen taken from the hive	Ce	2 DAA	n.d.
Pollen taken from the hive	Ce	4 DAA	n.d.
Pollen from forager bees	T1e	2 DAA	0.095
Pollen from forager bees	T2e	2 DAA	0.063
Pollen from forager bees	T1e	4 DAA	0.013
Pollen from forager bees	T2e	4 DAA	0.012
Pollen taken from the hive	T1e	2 DAA	0.047
Pollen taken from the hive	T2e	2 DAA	<LOQ
Pollen taken from the hive	T1e	4 DAA	0.020
Pollen taken from the hive	T2e	4 DAA	n.d.

DAA: Days after application

LOQ: 0.010 mg/kg

n.d. = Not detectable (<LOD = 0.003 mg/kg).

Ce: Control samples, T1e, T2e: Treated samples.

III. CONCLUSION

It can be concluded that Indoxacarb 150 g/L EC applied after bee flight had no effect on honeybee mortality of biological relevance. Since slightly increased numbers of dead bees/day occurred in the pre-application period of the treatment groups T1 and T2 and the values after the application were similar, these are not indicating any biologically relevant effect.

The application of Indoxacarb 150 g/L EC after bee flight or during bee flight had a slight effect on the flight intensity shortly after the applications in T1 and T2. One day after the applications, the flight activity in T1 and T2 was at the same level as in the control.

The application of Indoxacarb 150 g/L EC in T1 and T2 had a slight effect on the behaviour of the honey bees shortly after the applications. Since the numbers of honey bees showing unusual behaviour were generally low, this effect can be considered as not biologically relevant.

There was no effect on the colony size relating to the application of Indoxacarb 150 g/L EC in T1 and T2.

There was only a slight effect on the amount of brood cells with reduced numbers of larvae cells in the treatment group T2 shortly after the application of Indoxacarb 150 g/L EC. There was no test item related effect regarding the amount of brood cells in the treatment group T1.

Indoxacarb 150 g/L EC applications carried out after bee flight (T1) caused no harmful effects on the development of the marked eggs, young larvae or old larvae (brood termination rate). The application of Indoxacarb 150 g/L EC carried out during bee flight (T2) had a slight effect on the development of the marked eggs, whereas no effect occurred on the development of young larvae and old larvae.

Overall, the spray application of Indoxacarb 150 g/L EC carried out at 37.5 g a.s./ha during flowering and after bee flight (T1) caused no biologically relevant effect on honey bee colonies. The spray application of Indoxacarb 150 g/L EC carried out at 37.5 g a.s./ha during flowering and during bee flight (T2) caused slight effects on honey bee colonies.

No residues of indoxacarb could be found in the nectar samples prepared from forager bees or in nectar samples which were taken directly from the hive in the control group as well as in the treatment groups T1 and T2 taken two and four days after the application.

All pollen samples of the control group were free of residues of indoxacarb. In the pollen samples taken from the forager bees in the treatment group T1, the maximum value of 0.095 mg a.s./kg was found two days after the application. In the pollen samples which were taken directly from the hive, the maximum value of 0.047 mg a.s./kg was found two days after the application.

In the pollen samples taken from the forager bees in the treatment group T2, the maximum value of 0.063 mg a.s./kg was found two days after the application. In the pollen samples taken from the hive, no residues of indoxacarb above the level of quantification (<0.010 mg indoxacarb/kg) were detected.

(Berg, C., 2015)

RMS comment

Study submitted to the EU for the first time in this submission.

This study is considered valid. The mean brood termination rate for eggs, young larvae and old larvae in the control group was $\leq 50\%$ at the end of the study (as required by the study plan).

There were four bee colonies per treatment group (+ 1 tunnel tent for C, T1 and T2 for residue analysis).

RMS would not exclude an effect on mortality in T1 and T2 during 3 days after application in both cases. The increase, if any, is however very slight. It is agreed that mortality of adult honeybees were not of biological relevance in both T1 and T2. An increase of pupal mortality was however observed in T2 (application during bee flight).

The application of Indoxacarb 150 g/L EC after bee flight or during bee flight had a slight effect on the flight intensity shortly after the applications in T1 and T2. One day after the applications, the flight activity in T1 and T2 was at the same level as in the control.

The application of Indoxacarb 150 g/L EC in T1 and T2 had a slight effect on the behaviour of the honey bees shortly after the applications.

There was no effect on the colony size relating to the application of Indoxacarb 150 g/L EC in T1 and T2.

There was only a slight effect on the amount of brood cells with reduced numbers of larvae cells in the treatment group T2 shortly after the application of Indoxacarb 150 g/L EC. Besides RMS notes that two replicates in the treatment group T1, the original queens were lost due to unknown reasons (in one replicate, there was mainly male progeny left in the colony, in the other replicate, no eggs were found). This resulted in lower mean number of brood cells in this treatment group. The study author concludes that there was no test item related effect regarding the amount of brood cells in the treatment group T1. RMS considers that the lost of the queens is not explained and that an indirect effect of the treatment on the brood (via the lost of the queens) cannot be excluded.

According to the study author, Indoxacarb 150 g/L EC applications carried out after bee flight (T1) caused no harmful effects on the development of the marked eggs, young larvae or old larvae (brood termination rate). RMS disagrees as the brood and compensation indices are lower for eggs and old larvae (almost by factor 2 for the eggs) than in the control group (even if the difference was not significant). Besides these indices show high variability between replicates. The termination rates in T1 are higher than in control (except for young larvae but it has to be noted that the mean value in the control appears particularly high for young larvae). These termination also show high variability (for eggs, young larvae and old larvae). These results show a direct effect of the treatment T1 on the brood.

According to the study author, the application of Indoxacarb 150 g/L EC carried out during bee flight (T2) had a slight effect on the development of the marked eggs, whereas no effect occurred on the development of young larvae and old larvae. RMS disagrees as the brood and compensation indices are much lower for eggs, young larvae and old larvae than in the control group (even if the difference was not significant). Besides these indices show high variability between replicates. The termination rates in T2 are much higher than in control. These results show a direct effect of the treatment T2 on the brood.

No residues of indoxacarb could be found in the nectar samples prepared from forager bees or in nectar samples which were taken directly from the hive in the control group as well as in the treatment groups T1 and T2 taken two and four days after the application.

All pollen samples of the control group were free of residues of indoxacarb. In the pollen samples taken from the forager bees in the treatment group T1, the maximum value of 0.095 mg a.s./kg was found two days after the application. In the pollen samples which were taken directly from the hive, the maximum value of 0.047 mg a.s./kg was found two days after the application.

In the pollen samples taken from the forager bees in the treatment group T2, the maximum value of 0.063 mg a.s./kg was found two days after the application. In the pollen samples taken from the hive, no residues of indoxacarb above the level of quantification (<0.010 mg indoxacarb/kg) were detected. RMS notes that the content of residues was higher in T1 (application in evening). RMS however notes that the origin of the samples taken from the hive cannot be verified. It is not known if the samples are taken from newly deposited nectar and pollen or not (even if it is reported in the study report that the samples were

preferably taken from newly deposits. However the higher content of residue in the pollen of T1 seems confirmed by the samples directly taken from forager bees.

Report: Mamet, O. (2008a); DPX-KN128 150EC [150 g a.s./L (w/v)]: A semi-field study to evaluate effects on the honey bee (*Apis mellifera mellifera*; Hymenoptera, Apidae) on wheat treated with artificial honeydew in France 2007

DuPont Report No.: DuPont-19454

Guidelines: CEB Guideline No. 230 (November 2003) **Deviations:** None

Testing Facility: Testapi, Gennes, France

Testing Facility Report No.: 111-2007

GLP: Yes

Certifying Authority: Groupe Interministeriel des Produits Chimiques (GIPC) (Paris, France)

Executive summary:

A semi-field toxicity study on honeybees (*Apis mellifera mellifera* L.) in wheat treated with artificial honeydew was conducted with Indoxacarb 150 g/L EC in Gennes, in Southwestern France under CEB 230 (November 2003). The wheat was treated several times with a sugar solution to simulate honeydew. The study consisted of four treatment groups: the water-treated control applied during bee flight, 0.371 L formulated product/ha (equivalent to 55.6 g a.s./ha) applied before bee flight, 0.371 L formulated product/ha (equivalent to 55.6 g a.s./ha) applied during bee flight, and toxic standard, 1 L Diméthyl 40EC (400 g dimethoate/ha) applied during bee flight. All treatments were applied at 200 L/ha spray volume. One tent enclosing 140 m² was set up in each treatment and control area, and active honeybee colonies were established in the enclosures prior to application. A wheat crop area of 64 m² was available for foraging. Results of this study indicated that Indoxacarb 150 g/L EC applied during or before bee flight at a nominal rate of 0.371 L formulated product/ha (55.6 g a.s./ha) to winter wheat treated with sugar solution to simulate honeydew had an effect on mortality and foraging of honey bees.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Indoxacarb 150 g/L EC
Lot/Batch #: DPX-KN128-206
Purity: 150 g a.s./L
CAS#: None for the formulation
173584-44-6 for indoxacarb active substance
Description: Liquid/Amber
Stability of test compound: 100% of the indoxacarb remains in the delivery vehicle after one hour under agitation
2. Vehicle and/or positive control Tap water
Toxic reference Diméthyl 40EC (dimethoate a.s.)
3. Test organism
Species: *Apis mellifera* L.
Source: Supplied by a professional beekeeper, Meli-Bocage, in St. Michel (85)
Age at dosing: Direct exposure of adults; indirect exposure of all stages of development
Crop: *Triticum aestivum* (var. Aubusson)
Water: Water was supplied in a container filled with 0.3 L water
Artificial honeydew: Sugar solution (750-1000 g/L) applied daily at a rate of 250 L solution/ha in the morning
Tunnel tents (exposure): Tunnel tent: tents (7.0 m × 20.0 m and a height of 3.0 m) placed over plots of *Triticum aestivum* were made of iron and covered with an insect proof net; 1 replicate tunnel tent per treatment
Crop area: 4 crop plots per tent; crop plots (2 m × 8 m) furnished 64 m² to bees to forage on *Triticum aestivum*
Plastic sheet: The remaining surface was covered with plastic sheets for the assessment of dead bees in the tunnels.
4. Environmental conditions
Temperature: 5 to 29°C
Photoperiod (exposure): natural light conditions

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
19-May-2007 to 30-May-2007

2. Experimental treatments

A semi-field toxicity test on honeybees (*Apis mellifera* L.) in winter wheat, *Triticum aestivum* (var. Aubusson) was conducted with Indoxacarb 150 g/L EC in Gennes, in Southwestern France. The wheat was treated daily with a sugar solution to simulate honeydew. The study consisted of four treatments: the water-treated control, Indoxacarb 150 g/L EC applied during bee flight at 0.371 L formulated product/ha (equivalent to 55.6 g a.s./ha), Indoxacarb 150 g/L EC applied before bee flight at 0.371 L formulated product/ha (equivalent to 55.6 g a.s./ha), and the toxic reference treatment, Diméthyl 40EC applied at 1 L product/ha (equivalent to 400 g dimethoate/ha). All applications were carried out with a volume of 200 L/ha. The effects of the Indoxacarb 150 g/L EC treatment were examined on small honey bee colonies in tunnel tents placed over plots of *Triticum aestivum*. One replicate was used per treatment (small healthy colonies with 10 combs).

3. Observations

Mortality:	The numbers of dead honey bees within the tunnel were assessed daily on 6 plastic sheets (lanes) starting 3 days before application to 5 days after application.
Foraging activity	All forager honey bees were counted daily on the 4 crop plots of each tunnel.
Colony assessment:	Two apiarist visits were conducted: At the beginning and at the end of the experimental phase the bee colonies were inspected in order to assess the presence of a healthy queen and eggs, and the number of combs with brood and honey.
Behavioural observations:	Any abnormal behaviour was recorded.

4. Statistics

The data did not warrant statistical analysis, since only one replicate of each treatment and control was used.

II. RESULTS AND DISCUSSION

A. FINDINGS

1. Effects on Honey Bee Mortality

The daily honey bee mortalities were homogeneous from Day -3 to Day -1. The control mortality was quite stable over the entire study period after application with about 100 to 650 (last day) dead honey bees per day validating the test. The toxic reference (400 g dimethoate/ha) induced a high peak of mortality the day after spray application proving the sensitivity of the test system. The impact of the toxic reference treatment on the mortality was very high. The counts of dead honey bees performed the evening at Day 0aa (after application) and the following morning (Day +1) summed up to the total of 3979 individuals. Also at Day +2 a strong effect (867 dead honey bees) was determined in the toxic reference treatment.

When Indoxacarb 150 g/L EC was applied at 0.371 L formulated product/ha before daily bee flight, similar numbers of dead bees were found as in the toxic reference tunnel (4307 dead bees versus 3979 in the toxic reference at Day 0aa and Day+1). The increase in honey bee mortality occurred the day of application after the treatment (2745 dead honey bees at Day 0aa) and continue for the two following days (Day +1 to Day+2).

When Indoxacarb 150 g/L EC was applied at 0.371 L formulated product/ha during bee flight, an increase in honey bee mortality was recorded during the day of application after the treatment (2433 dead bees at Day 0aa versus 218 at Day 0ba). This increase continued for two days after spray treatment (Day +1, Day+2), slightly below the mortality level recorded in the toxic reference (3754 dead bees compared to around 3979 in the reference tunnel). Over the entire observation period the numbers of dead honey bees in toxic reference and the Indoxacarb 150 g/L EC treatment applied during bee flight showed a comparable pattern. From Day +3 to Day +6 after treatment the honey bee mortality in both Indoxacarb 150 g/L EC treatments was comparable to the control.

The calculated toxicity index for the day of application (before application) until Day +1 after application of 15.2 confirms the high toxicity of toxic reference, Dimézy 40EC, relative to the control (toxicity index = 1). The calculated toxicity indices for both Indoxacarb 150 g/L EC treatments (application during bee flight and application out of bee flight) were 15.3 and 13.3 respectively. The effect of Indoxacarb 150 g/L EC applications during or out of bee flight were close to the toxic reference demonstrating the similarity of effects observed in the Indoxacarb 150 g/L EC treatment and the toxic reference.

Table 133
Total number of dead honey bees/tunnel

Date	Day	Treatment groups			
		Indoxacarb 150 g/L EC treatment out of bee-flight	Indoxacarb 150 g/L EC treatment during bee- flight	Toxic standard, Dimézy 40EC	Control
21 May 2007	-3	363	292	144	351
22 May 2007	-2	624	375	387	506
23 May 2007	-1	222	176	284	237
24 May 2007	0ba	287	218	232	255
24 May 2007	0aa	2745	2433	2731	71
25 May 2007	+1	1562	1321	1248	216
26 May 2007	+2	694	1001	867	406
27 May 2007	+3	350	412	179	475
28 May 2007	+4	80	115	104	280
29 May 2007	+5	57	47	50	166
30 May 2007	+6	280	182	155	656
Toxicity index $i_{tox}(Day\ 0ba - Day\ 1aa)$		13.3	15.3	15.2	1.0

Day 0ba = Day of treatment before application

Day 1aa = Day 1 after application of test item

$i_{tox}(0\ ba-1aa)$ = toxicity index (Day 0ba – Day 1aa): evolution quotient of the daily mean mortality after/before treatment in the treated tunnel.

2. Effects on Honey Bee Flight Intensity

The daily mean honey bee foraging activity during the pre-application period varied between 5 to 10 honey bees/m² in the 4 different tents. The honey bee foraging activity was above the required level of >3 bees/m² in all treatments.

During the day of spray application (Day 0ba to Day 0aa) the honey bee foraging activity decreased in the control tunnel from 10.4 to 4.7 bees/m². In the Indoxacarb 150 g/L EC tunnel treated during bee flight, the foraging activity showed the same decrease pattern from 10.5 to 3.1 bees/m². The honey bee foraging activity in the Indoxacarb 150 g/L EC tunnel treated out of bee flight was 7.5 honey bees/m² in the afternoon after treatment. On the contrary in the reference tunnel the foraging activity dropped from 6.6 bees/m² to almost null in the afternoon after application (0.4 bees/m²) and almost no honey bees were foraging any longer.

On Day +1 and Day +2 the foraging activity in the control was 17.0 and 9.4 honey bees/m², respectively and on Day +5 a mean of 2.8 honey bees/m² were foraging. During the same time, the foraging activity in both Indoxacarb 150 g/L EC treatments was lower compared to the control and varied from 0.2 and 0.4 honey bees/m² (treated out of bee flight) and 0.7 and 0.1 honey bees/m² (treated during bee flight). No honey bee foraging activity was observed in the toxic reference at Day +1, Day +2, and Day+5.

The calculated forager mortality indices for the day of application until Day +1 after application for the water control according the two different calculation options – option 1 and 2 – were 1 and 0, respectively. The calculated forager mortality indices for the Indoxacarb 150 g/L EC treatment that was sprayed during bee flight were 109.3 and 5.3, respectively. In the same way the forager mortality indices for the toxic reference, Dimézy 40EC, were 183.5 and 8.9, respectively.

Table 134
Foraging activity (mean number of honeybees/m²)

Date	Day	Treatment groups			
		Indoxacarb 150 g/L EC treatment out of bee-flight	Indoxacarb 150 g/L EC treatment during bee- flight	Toxic standard, Dimézy 40EC	Control
21 May 2007	-3	1.8	3.4	3.3	3.1
22 May 2007	-2	7.3	5.3	3.8	3.9
23 May 2007	-1	5.4	6.3	5.5	6.7
24 May 2007	0ba	0.0*	10.5	6.6	10.4
24 May 2007	0aa	7.5	3.1	0.4	4.7
25 May 2007	+1	0.2	0.7	0.0	17.0
26 May 2007	+2	0.4	0.1	0.0	9.4
29 May 2007	+5	0.3	0.4	0.0	2.8
Forager mortality index					
Forager mortality index (i)		Indoxacarb 150 g/L EC treatment out of bee-flight	Indoxacarb 150 g/L EC treatment during bee- flight	Toxic standard, Dimézy 40EC	Control
i F option 1 (Day 0ba – Day 1aa)		NA	109.3	183.5	1.0
i F option 2 (Day 0ba – Day 1aa)		NA	8.9	8.9	0.0

Day 0ba = day of treatment before application

* no count at D0ba, closed hive

Day 0aa = in the evening after application of test item

NA: not applicable

3. Effects on Honey Bee Brood Development:

Some changes were detected between the two colony assessments at the beginning and end of the experimental phase. Due to the attractiveness of the artificial honeydew sprayed on the wheat crop, honey bees foraged actively and so the colonies had about the same level of food frame storage at the end of the test compared to the first assessment at the beginning of the field phase. For the control and the Indoxacarb 150 g/L EC treatment applied during bee flight, the proportion of brood slightly decreased between the two brood assessments from 5.5 and 5 brood combs to 4 and 3.5 brood combs respectively. For the Indoxacarb 150 g/L EC applied out of bee flight and the reference, the decrease was a little more important; from 5.5 and 4.5 brood combs to 2.5 and 2 brood combs, respectively.

The two colony assessments before and after application showed changes due to the test item, Indoxacarb 150 g/L EC applied during or after bee flight. The adult honey bee population (measured as a mark from 0 to 20 where 20 means that all combs are covered by honey bees and 0 means a empty hive) decreased in colonies which were under tents treated with Indoxacarb 150 g/L EC applied during bee flight, Indoxacarb 150 g/L EC applied after bee flight and the toxic reference (from 17 of 20, 17 of 20, and 16 of 20 (pre-applications) to 13 of 20, 12 of 20, and 10 of 20 (post-applications), respectively), due to the high level of mortality after the application. The adult honey bee population remained stable in the control colony (16 of 20 pre- and post applications).

4. Honey Bee Behaviour

First symptoms of poisoning, *e.g.* paralyzed honey bees, were recorded 2.5 hours after application in tunnel treated with Indoxacarb 150 g/L EC during bee flight and 4 hours after application in tunnel treated with Indoxacarb 150 g/L EC out of bee flight (one hour after opening the hive). The same kind of symptoms was recorded in the reference tunnel one hour after the application.

III. CONCLUSIONS

It was concluded that Indoxacarb 150 g/L EC applied during and before bee flight at 0.371 L formulated product/ha (equivalent to 55.6 g a.s./ha) on wheat treated with sugar solution to simulate honey dew had an effect on honey bees mortality and activity.

(Mamet, O., 2008a)

RMS comment

Study submitted to the EU for the first time in this submission.

The study is only informative. The mortality in control is very high making the results not reliable. However adverse effects in other tunnels were obvious.

Indoxacarb 150 g/L EC applied during and before bee flight (early morning) at 0.371 L formulated product/ha (equivalent to 55.6 g a.s./ha) on wheat treated with sugar solution to simulate honey dew had a strong effect on honey bees mortality (equivalent to toxic reference) and foraging activity.

RMS however notes that mortality in control seems high. The effects observed in the other treatment groups are nevertheless obvious. The (high) effects on mortality last at least until DAA+3 in both treatment groups. Foraging was affected until the end of the study. A decrease of the adult population and of the brood was also observed in all treated groups. Effects on behaviour were also observed in all treated groups.

Report: Mamet, O. (2008b); DPX-KN128 150EC [150 g a.s./L (w/v)]: A semi-field study to evaluate effects on the honey bee (*Apis mellifera mellifera*; Hymenoptera, Apidae) on wheat treated with artificial honeydew in France 2007

DuPont Report No.: DuPont-19455

Guidelines: CEB Guideline No. 230 (November 2003) **Deviations:** None

Testing Facility: Testapi, Gennes, France

Testing Facility Report No.: 113-2007

GLP: Yes

Certifying Authority: Groupe Interministeriel des Produits Chimiques (GIPC) (Paris, France)

Executive summary:

A semi-field toxicity study on honeybees (*Apis mellifera* L.) in wheat treated with artificial honeydew was conducted with Indoxacarb 150 g/L EC in Gennes, in Southwestern France under CEB 230 (November 2003). The wheat was treated several times with a sugar solution to simulate honeydew. The study consisted of four treatment groups: The water-treated control applied during bee flight, Indoxacarb 150 g/L EC at 0.25 L formulated product/ha (37.5 g a.s./ha) applied after bee flight, Indoxacarb 150 g/L EC at 0.25 L formulated product/ha (37.5 g a.s./ha) applied during bee flight, and toxic standard, 1 L Dimezyl 40EC (400 g dimethoate/ha) applied during bee flight. All treatments were applied at 200 L/ha spray volume. Toxic reference and control tunnels were common with another study conducted in the same conditions in the same time. One tent enclosing 140 m² was set up in each treatment and control area, and active honeybee colonies

were established in the enclosures prior to application. A wheat crop area of 64 m² was available for foraging. Results of this study indicated that Indoxacarb 150 g/L EC applied during or after bee flight at a nominal rate of 0.25 L formulated product/ha (37.5 g a.s./ha) to winter wheat treated with sugar solution to simulate honeydew did have an effect on honey bees.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Indoxacarb 150 g/L EC
 Lot/Batch #: DPX-KN128-206
 Purity: 150 g a.s./L
 CAS#: None for the formulation
 173584-44-6 for indoxacarb active substance
 Description: Deep brown
 Stability of test compound: 100% of the indoxacarb remains in the delivery vehicle after one hour under agitation
2. Vehicle and/or positive control: Tap water
 Toxic reference: Diméthyl 40EC (dimethoate a.s.)
3. Test organism
 Species: *Apis mellifera* L.
 Source: Supplied by a professional beekeeper, Meli-Bocage, in St. Michel (85)
 Age at dosing: Direct exposure of adults; indirect exposure of all stages of development
 Crop: *Triticum aestivum* (var. Aubusson)
 Water: Water was supplied in a container filled with 0.3 L water
 Artificial honeydew: Sugar solution (750-1000 g/L) applied daily at a rate of 250 L solution/ha in the morning
 Tunnel tents (exposure): Tunnel tent: tents (7.0 m × 20.0 m and a height of 3.0 m) placed over plots of *Triticum aestivum* were made of iron and covered with an insect proof net; 1 replicate tunnel tent per treatment
 Crop area: 4 crop plots per tent; crop plots (2 m × 8 m) furnished 64 m² to bees to forage on *Triticum aestivum*
 Plastic sheet: The remaining surface was covered with plastic sheets for the assessment of dead bees in the tunnels.
4. Environmental conditions
 Temperature: 5 to 29°C
 Photoperiod (exposure): natural light conditions

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
 06-June-2007 to 18-June-2007

2. Experimental treatments

A semi-field toxicity test on honeybees (*Apis mellifera* L.) in winter wheat, *Triticum aestivum* (var. Aubusson) was conducted with Indoxacarb 150 g/L EC in Gennes, in Southwestern France. The wheat was treated daily with a sugar solution to simulate honeydew. The study consisted of four treatments: The water-treated control, Indoxacarb 150 g/L EC applied during bee flight at 0.25 L formulated product/ha (equivalent to 37.5 g a.s./ha), Indoxacarb 150 g/L EC applied after bee flight at 0.25 L formulated product/ha (equivalent to 37.5 g a.s./ha), and the toxic reference treatment, Diméthyl 40EC applied at 1 L product/ha (equivalent to 400 g dimethoate/ha). All applications were carried out with a volume of 200 L/ha. The effects of the Indoxacarb 150 g/L EC treatment were

examined on small honey bee colonies in tunnel tents placed over plots of *Triticum aestivum*. One replicate was used per treatment (small healthy colonies with 10 combs).

3. Observations

Mortality:	The numbers of dead honey bees within the tunnel were assessed daily on 6 plastic sheets (lanes) starting 4 days before application to 6 days after application.
Foraging activity	All forager honey bees were counted daily on the 4 crop plots of each tunnel.
Colony assessment:	Two apiarist visits were conducted: At the beginning and at the end of the experimental phase the bee colonies were inspected in order to assess the presence of a healthy queen and eggs, and the number of combs with brood and honey.
Behavioural observations:	Any abnormal behaviour was recorded.

4. Statistics

The data did not warrant statistical analysis, since only one replicate of each treatment and control was used.

II. RESULTS AND DISCUSSION

A. FINDINGS

1. Effects on Honey Bee Mortality

Honey bee mortality was heterogeneous among the different tunnels during the pre-application period, *i.e.* during the whole observation period the number of dead honey bees found in the control tunnel was lower than in the other 3 test tunnels. Just before the spray application, the number of dead honey bees determined in the control tunnel was 123 individuals, while in the other 3 tunnels the honey bee mortality was 2.6-times higher or more: 653 (test item applied during bee flight), 317 (test item applied after bee flight), and 557 (reference compound).

The impact of the toxic reference treatment on the mortality was very high. The counts of dead honey bees performed the evening at Day 0aa (after application) and the following morning (Day +1) summed up to the total of 5139 individuals. Also at Day +2 a strong effect (2452 dead honey bees) was determined in the toxic reference treatment.

When Indoxacarb 150 g/L EC was applied at 0.25 L formulated product/ha during bee flight, an increase in mortality was recorded compared to the Day 0ba (317 dead honey bees) for two days after spray application (3018 dead honey bees at Day 0aa and Day +1, 1700 dead honey bees at Day+2), but lower than in the toxic reference, about 3000 daily dead bees at Day +1 compared to around 5000 in the reference tunnel. On Day +2, the mortality was decreasing in the Indoxacarb 150 g/L EC tunnel. Over the entire post-application period the numbers of dead honey bees in toxic reference and the Indoxacarb 150 g/L EC treatment applied during bee flight showed a comparable pattern.

When Indoxacarb 150 g/L EC was applied at 0.25 L formulated product/ha after daily bee flight, medium honey bee mortality (477 dead honey bees) was observed during the day after spray application. A high increase in honey bee mortality occurred in this Indoxacarb 150 g/L EC treatment two days after the application (2947 dead honey bees at Day +2) and continued the day after (1000 dead honey bees at Day +3). The impact in mortality followed the spray application of Indoxacarb 150 g/L EC after daily bee flight was about 50% less than the mortality level in the toxic reference with a delay of one day.

The calculated toxicity index for the day of application until Day +1 after application of 5.0 confirms the high toxicity of toxic reference, Dimézy 40EC, relative to the control (toxicity index = 1). The calculated toxicity indices for both Indoxacarb 150 g/L EC treatments (application during bee flight and application out of bee flight) were 5.2 and 0.9, respectively. The effect of the spray application

out of bee flight was not yet recordable at Day +1. If the mortality at Day +1 plus Day +2 is compared to Day 0ba, the toxicity index of Indoxacarb 150 g/L EC of 2.1 is half of the toxic reference.

Table 135
Total number of dead honey bees/tunnel

Date	Day	Treatment groups			
		Indoxacarb 150 g/L EC treatment after bee- flight	Indoxacarb 150 g/L EC treatment during bee- flight	Toxic standard, Dimézy 40EC	Control
08 June 2007	-4	1110	432	2249	310
09 June 2007	-3	552	414	1471	186
10 June 2007	-2	1141	425	1818	190
11 June 2007	-1	836	438	761	156
12 June 2007	0ba	653	317	557	123
12 June 2007	0aa	547	1380	2728	94
13 June 2007	+1	477	1638	2411	132
14 June 2007	+2	2947	1700	2452	136
15 June 2007	+3	1000	468	1316	159
16 June 2007	+4	415	238	695	145
17 June 2007	+5	257	163	494	179
18 June 2007	+6	118	96	331	104
Toxicity index $i_{tox}(Day\ 0ba - Day\ 1aa)$		0.9	5.2	5.0	1.0
Toxicity index $i_{tox}(Day\ 0ba - (Day\ 1aa + Day\ 2aa))$		2.1	-	-	1.0

Day 0ba = Day of treatment before application

Day 1aa = Day 1 after application of test item

$i_{tox}(0\ ba-1aa)$ = toxicity index (Day 0ba – Day 1aa): evolution quotient of the daily mean mortality after/before treatment in the treated tunnel.

2. Effects on Honey Bee Flight Intensity

The daily mean honey bee foraging activity during the pre-application period (Day-1 and Day0ba) varied from about 20 to 6.5 honey bees/m² in the 4 different tents. The honey bee foraging activity was above the required level of >3 bees/m² in all treatments just before the applications (from 6.5 to 18.8 bees/m²).

During the day of spray application (Day 0ba to Day 0aa) the honey bee foraging activity decreased in the control tunnel (from 6.5 to 2.1 bees/m²). In the Indoxacarb 150 g/L EC tunnel treated during bee flight, the foraging activity showed a noticeable decrease from 18.8 bees/m² before application to 4.2 bees/m² after application.

The foraging activity in the toxic reference tunnel dropped from 18.7 bees/m² (Day 0ba) to 0.6 bees/m² after application (Day 0aa) and almost no honey bees were foraging any longer.

On the contrary, in the Indoxacarb 150 g/L EC tunnel treated after the bee flight, the foraging activity stayed stable the day of application and at Day+1: 9.8 bees/m² at Day0ba, 8.9 bees/m² at Day0aa, and 9.0 bees/m² at Day+1.

On Day +1 and Day +2 the foraging activity in the control was 8.9 to 12.5 honey bees/m² respectively. During the same time period the foraging activity in the Indoxacarb 150 g/L EC treatment applied after bee flight was similar to the control and varied from 9.0 to 13.5 honey bees/m². In the Indoxacarb 150 g/L EC treatment applied during bee flight, the foraging activity reached almost nil (0.2 honey bees/m² at D+2), like in the toxic reference tunnel.

The calculated forager mortality indices for the day of application until Day +1 after application for the water control according the two different calculation options – option 1 and 2 – were 1 and 0.2, respectively. The calculated forager mortality indices for the Indoxacarb 150 g/L EC treatment that was sprayed during bee flight were 9.0 and 2.2, respectively. In contrast the forager mortality indices for the toxic reference, Dimezyl 40EC, were 15.4 and 3.8, respectively.

Table 136
Foraging activity (mean number of honeybees/m²)

Date	Day	Treatment groups			
		Indoxacarb 150 g/L EC treatment out of bee flight	Indoxacarb 150 g/L EC treatment during bee flight	Toxic standard, Dimézyl 40EC	Control
08 June 2007	-4	4.8	4.7	6.1	2.8
09 June 2007	-3	16.0	13.4	12.0	10.4
10 June 2007	-2	24.7	18.6	19.3	15.6
11 June 2007	-1	20.0	20.4	19.5	13.5
12 June 2007	0ba	9.8	18.8	18.7	6.5
12 June 2007	0aa	8.9	4.2	0.6	2.1
13 June 2007	+1	9.0	3.3	0.7	8.9
14 June 2007	+2	13.5	0.2	0.1	12.5
Forager mortality index					
Forager mortality index (i)		Indoxacarb 150 g/L EC treatment after bee flight	Indoxacarb 150 g/L EC treatment during bee flight	Toxic standard, Dimézyl 40EC	Control
i F option 1 1 (Day 0ba – Day 1aa)		NA	9.0	15.4	1.0
i F option 2 (Day 0ba – Day 1aa)		NA	2.2	3.8	0.2

Day 0ba = day of treatment before application

Day 0aa = in the evening after application of test item

NA: not applicable

3. Effects on Honey Bee Brood Development

Some changes were detected between the two colony assessments at the beginning and end of the experimental phase. Due to the attractiveness of the artificial honeydew sprayed on the wheat crop, honey bees foraged actively and so the colonies had about the same level of food frame storage at the end of the test compared to the first assessment at the beginning of the field phase. For the Indoxacarb 150 g/L EC treatment applied during or after the daily bee flight, the proportion of brood decreased between the two brood assessments from 5 and 6 brood combs to 2.5 and 3.5 brood combs, respectively. In the toxic reference colony, the decrease was from 6 to 2.5 brood combs and in the control colony, the evolution was from 5 and 4.5 brood combs between the two brood assessments.

The two colony assessments before and after application showed changes due to the test item, Indoxacarb 150 g/L EC applied during or after bee flight. The adult honey bee population (measured as a mark from 20, where all combs are covered by honey bees and 0 that means an empty hive) decreased in colonies that were under tents treated with Indoxacarb 150 g/L EC applied during bee flight, Indoxacarb 150 g/L EC applied after bee flight, and the toxic reference (from 16/20, 17/20 and 16/20 to 7/20, 10/20, and 11/20, respectively), due to the high level of mortality after the application.

The adult honey bee population remained stable in the control colony at 16/20 at beginning and at the end of the experimental phase.

4. Honey Bee Behaviour

No behaviour symptoms were recorded in the tunnel treated with Indoxacarb 150 g/L EC during the day after the application.

First symptoms of poisoning, e.g. paralyzed honey bees, were recorded 10 minutes and also 1 hour after application in tunnel treated with Dimezyl 40EC.

III. CONCLUSIONS

It was concluded Indoxacarb 150 g/L EC applied during bee flight at 0.25 L formulated product/ha (equivalent to 37.5 g a.s./ha) on wheat treated with sugar solution to simulate honeydew had an effect on honey mortality. Also, foraging activity was decreasing when Indoxacarb 150 g/L EC was applied during bee flight.

(Mamet, O., 2008b)

RMS comment

Study submitted to the EU for the first time in this submission.

Mortality was heterogeneous among the different tunnels during the pre-application period (Mortality in control group lower than in the other three test tunnels). Mortality was above 300 bees at day 0ba in the three treated groups. Just before the spray application, the number of dead honeybees determined in the control tunnel was 123 individuals, while in the other 3 tunnels the honey bee mortality was 2.6 times higher or more: 653, 317 and 557. The validity criteria are not fulfilled.

The mortalities before treatment are too high. Cumulative mortalities during this study are very high. As a consequence, the post-application mortalities could not be compared to the initial population as a lower mortality after treatment could be linked to a reduced population rather than to the absence of treatment effects. In the three treated groups however, an effect on mortality was nevertheless observed as an increase of mortality was observed after application (this increase might have been underestimated by the high mortality observed before the application).

A decrease of the adult population and of the brood (higher than in the control tunnel) was also observed in all treated groups.

This test is not reliable for a risk assessment but nevertheless confirms the strong effect of Indoxacarb 150 g/L EC applied during or after bee flight on wheat treated with sugar solution observed in other studies.

Report: Gonsior, G. (2008b); Indoxacarb (DPX-KN128) 150 g/L EC: A semi-field study to evaluate effects on the honey bee (*Apis mellifera carnica*; Hymenoptera, Apidae) on wheat treated with artificial honeydew in Northern France 2007

DuPont Report No.: DuPont-21944

Guidelines: CEB Draft Guideline No. 230 (November 2003), OEPP/EPPO Guideline No. 170 (3) (2001)

Deviations: None

Testing Facility: eurofins-GAB GmbH, Niefern-Oeschelbronn, Germany

Testing Facility Report No.: 20071084/F2-BZEU

GLP: Yes

Certifying Authority: Ministerium für Umwelt und Verkehr (Baden-Württemberg, Germany)

Executive summary:

The effects of the test item Indoxacarb 150 g/L EC were tested on the honey bee (*Apis mellifera carnica* L.) under semi-field conditions. This study was conducted in Drusenheim in Northern France (region: Alsace) in May 2007 and included four treatment groups (one replicate each = 1 tunnel tent). Indoxacarb 150 g/L EC was applied in the evening after bee-flight (treatment K1) and on the following day during bee-flight (treatment K2) in separated tunnel tents. The effects of the test item treatment were examined on small honey bee colonies in tunnel tents (5.0 m × 20.0 m and a height of 3.5 m) placed over plots of wheat. The semi-field test comprised of one replicate tunnel tent in each of the treatments (test item treatment after and during bee-flight, toxic standard, and control).

The influence of Indoxacarb 150 g/L EC was evaluated by comparing the results in the test item treatments K1 (applied after bee-flight) and K2 (applied during bee-flight) to the data in the control treatment as well as in the toxic standard treatment regarding the following observations:

- Number of dead honey bees at the edge of the treated area (linen sheets) and in the dead honey bee traps in front of the hives.
- Foraging activity on the crop (number of forager honey bees/m² wheat).
- Condition of the colonies and development of the honey bee brood.
- Behavior of the honey bees in the crop area and around the hives.

It can be concluded that Indoxacarb 150 g/L EC applied at 371 mL formulated product/ha (equivalent to 55.6 g a.s./ha) on honey dew treated winter wheat during and after bee-flight, does have an effect on honey bee mortality and honey bee flight intensity.

When applied after honey-bee flight slightly aggressive behavior was recorded on the day after application (DAA 0). When applied during honey bee flight abnormal behavior was observed on the day of application after treatment and on DAA+2.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Indoxacarb 150 g/L EC
Lot/Batch #: DPX-KN128-206
Purity: 150 g a.s./L
CAS#: None for the formulation
173584-44-6 for indoxacarb active substance
Description: dark, brown
Stability of test compound: 99.7% of the indoxacarb remains in the delivery vehicle after one hour under agitation
2. Vehicle and/or positive control: Tap water
Toxic reference: Perfekthion (dimethoate)
3. Test organism
Species: *Apis mellifera carnica* L.
Age at dosing: Direct exposure of adults; indirect exposure of all stages of development
Source: Beekeeper, Berthold Nengel (Dahlheim, Germany).
Diet: Artificial honeydew: Sucrose solution (50% API-Invert solution) at a rate of 500 L/ha.
Tunnel tents (exposure): Within the test field tents (5.0 m × 20.0 m and a height of 3.5 m) were installed soon before moving the hives to the experimental field. The tent frames were covered with light plastic gauze. Before start of the test, paths were created in each tunnel by removal of the plants and smoothing the ground. The crop area per tent was approx. 68 m². Subsequently, the paths (approx. 32 m²) were covered with linen sheets for the assessment of dead honey bees in the crop area.
Test age: Direct exposure of adults; indirect exposure of all stages of development
4. Environmental conditions
Temperature: 4.0 to 31.0°C
Relative humidity: Not reported
Photoperiod (exposure): Not reported

B. STUDY DESIGN AND METHODS

1. Experimental start./completion
18-May-2007 to 29-May-2007
2. Experimental treatments
The application rate of the test item was 371 mL formulated product/ha (equivalent to 55.6 g a.s./ha). A third group (tunnel) treated with tap water served as control. As toxic standard "Perfekthion" (dimethoate) was applied at a rate of 1 L product/ha. The applications in the control and toxic standard treatment were carried out during daily bee-flight. All applications were carried out with a rate of 300 L water/ha.
3. Observations
The influence of Indoxacarb 150 g/L EC was evaluated by comparing the results in the test item treatments K1 (applied after bee-flight) and K2 (applied during bee-flight) to the data in the control treatment as well as in the toxic standard treatment regarding the following observations:

- Number of dead honey bees at the edge of the treated area (linen sheets) and in the dead honey bee traps in front of the hives.
- Foraging activity on the crop (number of forager honey bees/m² wheat).
- Condition of the colonies and development of the honey bee brood.
- Behavior of the honey bees in the crop area and around the hives.

II. RESULTS AND DISCUSSION

A. FINDINGS:

1. Effects on Honey Bee Mortality

During the pre-application period (DAA-3 to DAA-1ba/0ba) a mean number of 40.7 dead honey bees/day was calculated in the Indoxacarb 150 g/L EC treatment group with application after bee-flight (K1) and 38.5 dead honey bees/day in the Indoxacarb 150 g/L EC treatment group applied during bee-flight (K2). During the same time period the control group showed a daily mean mortality of 38.0 dead honey bees/day and the reference item treatment group 32.3 dead honey bees/day.

In the evening of the day of application during bee-flight (DAA0aa) a total mortality of 490 dead honey bees/day was calculated in the Indoxacarb 150 g/L EC treatment K1, where application was performed in the preceding evening. For the treatment group K2 the total mortality on DAA 0 after application was 226 dead honey bees. In the control treatment a total number of 13.0 dead honey bees and in the reference item treatment a total number of 1743 dead honey bees were counted.

On the following assessment days (DAA+1 to DAA+5) the daily total mortality was in the range of 559 to 84 dead honey bees/day in the test item treatment K1 and of 1071 to 49 dead honey bees/day in test item treatment K2. Whereas the daily total number of dead honey bees in control treatment varied between 41 and 83 dead honey bees/day and between 104 and 222 dead honey bees/day in the reference item treatment.

Table 137
Total number of dead honey bees/tent (linen sheets 1-5 plus dead bee trap)

Date	DAA	Treatment groups			
		Indoxacarb 150 g/L EC Treatment after bee-flight (K1)	Indoxacarb 150 g/L EC Treatment during bee-flight (K2)	Reference item	Control
19 MAY 07	-3	53	83	49	75
20 MAY 07	-2	34	25	21	35
21 MAY 07	-1	18	21	16	11
21/22 MAY 07	-1ba/0ba	17	25	43	31
22 MAY 07	0aa	490	226	1743	13
23 MAY 07	+1	559	1071	222	45
24 MAY 07	+2	184	192	122	68
25 MAY 07	+3	155	204	193	83
26 MAY 07	+4	109	49	113	41
27 MAY 07	+5	84	53	104	78
mean number of dead honey bees (DAA-3 to DAA-1ba/0ba)		40.7	38.5	32.3	38.0
mean number of dead honey bees (DAA0aa to +5)		263.5	299.2	416.2	54.7
Toxicity index $i_{tox(0aa\ to\ +1)}$		33.0	27.7	24.4	1.0
Toxicity index $i_{tox(0aa\ to\ +5)}$		8.8	6.8	5.5	1.0

DAA = Days after application during bee-flight

ba = before application

aa = after application

The mean post-application mortality was determined to be 263.5 dead honey bees/day in the treatment group with test item application after bee-flight (K1), 299.2 dead honey bees/day with Indoxacarb 150 g/L EC application during bee-flight (K2), 54.7 dead honey bees/day in the control and 416.2 dead honey bees/day in the reference item treatment.

The toxicity index for day of application during bee-flight ($i_{tox(0aa\ to\ +1)}$) was calculated with 33.0 for the Indoxacarb 150 g/L EC applied after bee-flight (K1) and 27.7 for the treatment group with application during bee-flight (K2). The value in the reference item treatment was 24.4. The calculation of the toxicity index for the post-application period ($i_{tox(0aa\ to\ +5)}$) showed a value of 8.8 for the test item treatment K1 and of 6.8 for test item treatment K2. For the reference item the $i_{tox(0aa\ to\ +5)}$ value was 5.5.

2. Effects on Honey Bee Flight Intensity

The daily mean flight intensity during the pre-application period was 7.2 honey bees/m² in the Indoxacarb 150 g/L EC treatment with application after bee-flight (K1), 5.8 honey bees/m² in the test item treatment with application during bee-flight (K2), 10.1 honey bees/m² in the reference item treatment and 4.7 honey bees/m² in the control treatment.

Shortly before application during bee-flight a mean of 5.8 honey bees/m² was observed in the test item treatment K2, with honeydew treated wheat. After the application the flight intensity in both test item treatment groups slightly decreased to a mean of 4.9 honey bees/m² in the Indoxacarb 150 g/L EC treatment group K1 (application after bee flight) and 4.8 honey bees/m² in treatment K2 (application during bee-flight).

The mean flight intensity on the day of application during bee-flight before the application was 5.0 honey bees/m² in the control. In the reference item treatment a mean of 10.5 honey bees/m² was observed with honeydew treated wheat in the corresponding time. After the application the flight intensity in the reference item treatment decreased over the time of assessments on that day and resulted in a mean of 0.9 honey bees/m², whereas on the day of application during bee-flight after the application the control group showed a mean of 7.0 honey bees/m².

Table 138
Flight intensity (mean number of honey bees/m²)

Date	DAA	Treatment groups			
		Indoxacarb 150 g/L EC Treatment after bee-flight (K1)	Indoxacarb 150 g/L EC Treatment during bee-flight (K2)	Reference item	Control
19 MAY 07	-3	4.5	3.3	8.8	3.0
20 MAY 07	-2	6.0	4.3	9.0	3.5
21 MAY 07	-1	11.0	9.8	12.0	7.3
21/22 MAY 07	-1ba/0ba	0.0 ^a	5.8	10.5	5.0
22 MAY 07	0aa	4.9	4.8	0.9	7.0
23 MAY 07	+1	0.1	0.1	0.0	5.5
24 MAY 07	+2	0.1	0.1	0.0	6.3
25 MAY 07	+3	0.4	0.0	0.0	7.4
26 MAY 07	+4	0.7	0.0	0.0	9.9
27 MAY 07	+5	0.8	0.2	0.0	1.4
mean number of honey bees/m ² (DAA-3 to DAA-1ba/0ba)		7.2	5.8	10.1	4.7
mean number of honey bees/m ² (DAA0aa to +5)		1.2	0.9	0.2	6.3
i_{for1} (0aa to +1)		17.4	40.6	33.9	1.0
i_{for2} (0aa to +1)		93.8	219.3	183.0	5.4
i_{for1} (0aa to +5)		4.7	10.0	7.5	1.0
i_{for2} (0aa to +5)		22.4	47.3	35.5	4.7

DAA = Days after application during bee-flight

ba = before application

aa = after application

* = Calculation see chapter 3.5 Result Analysis

^a Assessment performed after bee-flight and excluded from further evaluation

From DAA+1 to DAA+5 the flight intensity in the test item treatment with application after bee-flight (K1) ranged between 0.1 and 0.8 and resulted in a mean flight intensity of 1.2 honey bees/m² which is on the same level as the test item treatment with application during bee flight (K2) (range: 0.0 to 0.2; mean: 0.9). In the control the mean flight intensity in the post-application period was calculated with 6.3 honey bees/m² and the reference item treatment with 0.2 honey bees/m² which is below the mean flight intensity of the test item treatment groups in the post application period.

The forager mortality index for day of application ($i_{for1(0aa +1)}$) was calculated with 17.4 for the Indoxacarb 150 g/L EC treatment applied after bee-flight (K1), with 40.6 for the Indoxacarb 150 g/L EC treatment applied during bee-flight (K2), 33.9 for the reference item treatment and 1.0 for the control.

Regarding the forager mortality index 1, comparing pre-application to post-application period, for Indoxacarb 150 g/L EC applied after bee-flight (K1), the $i_{for1(0aa\ to\ +5)}$ was calculated with 4.7 and for the test item treatment K2, with 10.0. For the reference item the $i_{for1(0aa\ to\ +5)}$ value was 7.5 and for the control 1.0.

The forager mortality index 2 for day of application ($i_{for2(0aa\ to\ +1)}$) was calculated with 93.8 for the Indoxacarb 150 g/L EC treatment applied after bee-flight (K1), with 219.3 for the treatment applied during bee-flight (K2) and 183.0 for the reference item group. For the control the corresponding value was determined with 5.4. Regarding the forager mortality index 2 beginning from day of application during bee-flight until Day +5 ($i_{for2(0aa\ to\ +5)}$) a value of 22.4 was calculated for test item treatment K1 and of 47.3 for the test item treatment K2. For the control treatment $i_{for2(0aa\ to\ +5)}$ was determined with 4.7 and for the reference item treatment the corresponding value is 35.5.

3. Effects on Honey Bee Behavior

Within the pre-application period (DAA -3 to DAA 0) all colonies of all treatment groups showed a normal behavior of the honey bees. For treatment group K1 with application after bee flight slightly aggressive behavior was recorded on DAA 0 in the afternoon. Depending test item treatment K2 the honey bees only showed aggressive behavior on DAA 0aa and +2 (honey bees cleaning themselves, hole up under the linen). After the application during bee flight the honey bees of the reference item treatment showed abnormal behavior up to the first assessment on DAA +1 (no collecting behavior, clustering and filtering at the bee hive entrance). The honey bees of the control treatment showed normal behavior throughout the five days of the post-application period.

4. Effects on Honey Bee Brood Development

The strength of the colonies (number of bee ways between combs filled with honey bees) in the colony of test item treatment K1 increased by 1.0 (7.0 to 8.0) from the brood assessment carried out before placing the colonies in the tunnel tents to the assessment after end of exposure of the honey bees. The strength of the colony of the Indoxacarb 150 g/L EC treatment K2 decreased over the time of exposure from 6.0 to 5.5. In the colony of the reference item treatment the number of combs covered with honey bees increased from 7.0 to 7.5 and in the control treatment from 7.0 to 8.0.

At the brood assessments carried out before application all brood stages (egg stage, larval and pupal stage) in the colonies of all treatment groups were available and the colonies contained 5-6 combs covered with brood. After the end of exposure in all treatment groups (K1, K2 and C) the number of combs covered with brood ranged between 4 and 6. In the colonies of both test item treatments, no larval stages were present whereby in the control colony no eggs were present at the brood assessment carried out at the end of exposure which was probably due to a lack of pollen towards the end of exposure. In the reference item group no eggs and no larvae were recorded at the second brood assessment.

III. CONCLUSIONS

It can be concluded that Indoxacarb 150 g/L EC applied during and after bee-flight at 371 mL formulated product/ha (equivalent to 55.6 g a.s./ha) on winter wheat treated daily with sugar solution to simulate honeydew, does have an effect on honey bee mortality and honey bee flight intensity.

When applied after honey bee flight slightly aggressive behaviour was recorded on the day after application (DAA 0). When applied during honey bee flight abnormal behaviour was observed on the day of application after treatment and on DAA+2.

(Gonsior, G., 2008b)

RMS comment

Study submitted to the EU for the first time in this submission.

The study is valid.

Indoxacarb 150 g/L EC applied during and after bee flight at 0.371 L formulated product/ha (equivalent to 55.6 g a.s./ha) on wheat treated with sugar solution to simulate honey dew had a strong effect on honey bees mortality and foraging activity. Mortality was comparable to control after 4 and 5 days respectively. Foraging activity remained close to 0 until the end of the test in both cases.

When applied after honey bee flight slightly aggressive behaviour was recorded on the day after application (DAA 0). When applied during honey bee flight abnormal behaviour was observed on the day of application after treatment and on DAA+2. RMS also notes that no larvae was found in the tunnels treated with test item at the end of the test.

Report: Kleinhenz, M. (2011a); Indoxacarb (DPX-KN128) 150 g/L EC: A study to evaluate effects on the honey bee (*Apis mellifera carnica*) in the field in *Brassica napus* L. in eastern Germany in 2009

DuPont Report No.: DuPont-26946

Guidelines: OEPP/EPPO 170 (3) 2001 **Deviations:** None

Testing Facility: Eurofins Agrosience Services GmbH, Niefern-Oeschelbronn, Germany

Testing Facility Report No.: S08-03156

GLP: Yes

Certifying Authority: Landesanstalt Fur Umwelt, Messungen Und Naturschutz Baden-Wurtemberg

Executive summary:

The effects of the test item Indoxacarb 150 g/L EC were tested on the honey bee (*Apis mellifera carnica* L.) under field conditions following the OEPP/EPPO guideline No. 170(3) (2001).

This study was conducted near Machern in eastern Germany dating April to May 2009 and included three treatment groups (fields of flowering winter oil-seed rape, *Brassica napus* L.) with one replicate each. Indoxacarb 150 g/L EC was applied at BBCH 55 (treatment T1; application date 09 April 2009) and at BBCH 57-59 (treatment T2; application date 14 April 2009) on separate fields. In each treatment, the application rate was 170 mL formulated product/ha (equivalent to 25.5 g a.s./ha). All applications were carried out with a spray volume of 300 L water/ha. A third group (field) was not treated during the experimental phase and served as the control.

The effects of the treatment with Indoxacarb 150 g/L EC were examined on 6 honey bee colonies placed at the edge of each field of winter oilseed rape (*Brassica napus* L.) on 23 April 2009 (BBCH 63-65), 14 days after the application in the treatment T1 and 9 days after the application in the treatment T2. Observations of mortality, flight intensity, and behaviour were carried out over a period of 7 days after set-up of the colonies at the field sites. Assessments of the colony conditions were carried out once before set-up and then in weekly intervals for up to 4 weeks after set-up of the honey bees. The influence of Indoxacarb 150 g/L EC was evaluated by comparing the results in the test item treatments T1 (treated at BBCH 55) and T2 (treated at BBCH 57-59) to the data in the control treatment regarding the following observations:

- Number of dead honey bees on the linen sheet in front of the hives and in the dead honey bee traps in front of the hives,
- Foraging activity on the crop (number of forager bees per m² of winter oilseed rape),

- Condition of the colonies and development of the brood,
- Behaviour of the honey bees in the crop area and around the hives.

Additionally, samples of sealed honey were taken from each hive on DAE+22. These samples were analysed for residues of indoxacarb with a level of quantification (LOQ) of 0.010 mg a.s./kg.

It can be concluded that Indoxacarb 150 g/L EC, applied once at a rate of 25.5 g a.s./ha (equivalent to 170 mL formulated product/ha) before flowering of *B. napus* L. (BBCH 55 in treatment T1 and BBCH 57-59 in treatment T2) had no negative effect on the mortality, flight activity, behaviour, colony size, and brood development of the honey bee colonies that were placed at the pre-treated fields during flowering, 14 days after the application in treatment T1 and 9 days after the application in treatment T2. No residues of indoxacarb were detectable in any of the samples of sealed honey taken from the experimental hives.

I. MATERIAL AND METHODS

A. MATERIALS:

- Test material: Indoxacarb 150 g/L EC
 Lot/Batch #: DPX-KN128-212
 Purity: 150 g a.s./L
 Description: Liquid
 CAS#: None for the formulation
 173584-44-6 for the active substance
 Stability of test compound: 99.2% of the indoxacarb remains in the delivery vehicle after one hour under agitation
- Vehicle and/or positive control: Tap water
- Test organism
 Species: *Apis mellifera carnica*. L.
 Age at dosing: Direct exposure of adults; indirect exposure of all stages of development
 Source: Beekeeper, Bienenfarm Kern GmbH, (Leipzig, Germany)
 Diet: Nectar and pollen of flowering oilseed rape (*Brassica napus* L.)
 Fields (exposure): Three fields of flowering oilseed rape (*Brassica napus* L.) were used, one for each treatment. The crop area per field was 4.0 ha (field T1), 3.6 ha (field T2) and 5.0 ha (field C). Within each field, 5 areas (1 m² each) were marked for assessment of flight activity and 3 linen sheets (0.5 × 10 m) were spread out for assessment of dead bees. Additionally, dead bees were counted on 1 linen sheet in front of the hives and in dead bee traps in front of each hive.
- Environmental conditions:
 Temperature: 1.7–26.6°C
 Relative Humidity: 17.2-91.9 %
 Photoperiod (exposure): natural light conditions

B. STUDY DESIGN AND METHODS:

- Experimental start/completion:
 09-April-2009 to 20-May-2010
- Experimental treatments
 T1: Application at BBCH 55
 T2: Application at BBCH 57-59
 C: control (untreated)

The application rate was 25.5 g a.s./ha (equivalent to 170 mL formulated product/ha). All applications were carried out at a rate of 300 L/ha. A third field remained untreated and served as the control.

The effects of the test item treatment were examined on honey bee colonies placed at the edge of fields (6 colonies per field) of flowering winter oilseed rape (*Brassica napus* L.), 14 days after the application in T1 and 9 days after the application in T2. Observations of mortality, flight intensity, and behaviour were carried out over a period of 7 days (DAE0 to +6). Assessments of the colony conditions were carried out once before setup of the colonies at the field sites (DAE-1) and then on DAE +6, +15, +21, and +29. The field test comprised one replicate (field) in each of the treatments (T1 (applied BBCH 55), T2 (applied BBCH 57-59), and control.

3. Observations

The influence of Indoxacarb 150 g/L EC was evaluated by comparing the results in treatments T1 (applied BBCH 55) and T2 (applied BBCH 57-59) to the data in the control treatment regarding the following observations:

- Number of dead honey bees on linen sheets in the crop area, on linen sheets in front of the hives and in the dead honey bee traps in front of the hives,
- Foraging activity on the crop (number of forager bees/m²),
- Condition of the colonies and development of the honey bee brood,
- Behaviour of the honey bees in the crop area and around the hives.

Additionally, samples of sealed honey were taken from each hive on DAE+22. These samples were analysed for residues of indoxacarb with a level of quantification (LOQ) of 0.010 mg a.s./kg.

II. RESULTS AND DISCUSSION

A. FINDINGS

1. Honey bee mortality

In the following, all mean mortality values are given as dead honey bees per hive (number of dead honey bees in the traps plus the number of dead honey bees on the linen sheet in front of the hive, divided by the number of hives).

Mortality of the honey bees of treatments T1 and T2 were comparable to the control on all days of the exposure period (DAE0 to DAE+6). Mortality values were low throughout the exposure period except for elevated values on the day after set-up of the colonies (DAE+1). On DAE0 and DAE+2 to DAE+6, daily mortality values were ranging from 3.5 to 7.3 dead honey bees (treatment T1), from 2.5 to 7.7 dead honey bees (treatment T2) and from 7.8 to 12.7 dead honey bees in the control. Elevated values on DAE+1 were clearly the result of the transport conditions before set-up of the hives and values on that day were similar in all treatments including the control (T1: 43.2 dead honey bees; T2: 38.8 dead honey bees; control: 45.0 dead honey bees).

The mean daily mortality during the whole exposure period (DAE0 to DAE+6) was 11.0 dead honey bees (treatment T1), 10.1 dead honey bees per day (treatment T2), and 15.2 dead honey bees per day in the control.

Table 139
Mean number of dead honey bees per day

Date	DAE ^c	Treatment groups ^{a,b}		
		Indoxacarb 150 g/L EC; Application of 25.5 g a.s./ha at BBCH 55 (T1)	Indoxacarb 150 g/L EC; Application of 25.5 g a.s./ha at BBCH 57-59 (T2)	Control
23 April 09	0	7.2	7.7	7.8
24 April 09	+1	43.2	38.8	45.0
25 April 09	+2	5.5	7.3	12.7
26 April 09	+3	7.3	4.3	9.3
27 April 09	+4	5.8	5.5	10.0
28 April 09	+5	4.7	4.7	10.5
29 April 09	+6	3.5	2.5	11.2
Mean number of dead honey bees (DAE0 to +6)		11.0	10.1	15.2
STD ^d		14.0	12.7	14.2

^a Mean mortality values for hives + linen in front of hives, divided by number of hives

^b Mean values and STD were calculated from the individual values per field

^c DAE = Days after start of exposure of the honey bees to the treated crop (DAE0 = first day of exposure)

^d STD = Standard deviation

2. Honey bee flight intensity

Flight activity during the exposure period (DAE0 to DAE+6) was similar in all treatment groups including the control, ranging from 0.6 to 1.6 forager bees/m² (treatment T1), from 0.6 to 1.8 forager bees/m² (treatment T2) and from 0.7 to 1.4 forager bees/m² in the control. The mean daily flight activity during the exposure period was 1.0 forager bees/m² (T1), 1.3 forager bees/m² (T2) and 1.1 forager bees/m² in the control.

Table 140
Mean number of honey bees/m² flying and foraging in the crop

Date	DAE ^b	Treatment groups ^a		
		Indoxacarb 150 g/L EC; Application of 25.5 g a.s./ha at BBCH 55 (T1)	Indoxacarb 150 g/L EC; Application of 25.5 g a.s./ha at BBCH 57-59 (T2)	Control
23 April 09	0	0.7	0.6	0.7
24 April 09	+1	1.0	1.1	1.1
25 April 09	+2	1.2	1.8	1.4
26 April 09	+3	0.8	1.4	0.8
27 April 09	+4	1.6	1.6	1.4
28 April 09	+5	0.6	1.2	1.0
29 April 09	+6	1.0	1.2	1.4
Mean number of forager bees/m² (DAE0 to +6)		1.0	1.3	1.1
STD ^c		0.6	0.7	0.6

^a Mean values and STD were calculated from the individual values per field

^b DAE = Days after start of exposure of the honey bees to the crop (DAE0 = first day of exposure)

^c STD = Standard deviation

3. Honey bee colony size and brood development

In all three treatment groups, the mean colony size increased during the observation period (DAE-1 to +29), namely from 8296 bees to 18424 bees (treatment T1), from 7301 to 16681 (treatment T2), and from 7397 to 15669 bees in the control.

Brood of all stages was present in all hives of the three treatment groups at the first assessment of colony condition (DAE-1) and on all later assessments until DAE+29, except for one hive in the control (hive 5C) where no eggs and larvae were noticed on the 2nd and later assessments. The queen of that hive (5C) was not noticed after the 2nd assessment (DAE+6) and on the last two assessments (DAE+21 and +29), there was no brood left in this hive. Queen cells were noticed in this hive at the 2nd to 4th assessment, indicating that queen replacement was taking place.

The mean number of brood cells increased in all treatment groups during the observation period (DAE-1 to DAE+29), namely from 20100 to 39900 brood cells (treatment T1), from 17340 to 38640 (treatment T2), and from 16020 to 35040 in the control. The amount of brood increased continuously in both test item treatments (T1 and T2) and in the control except for the last assessment where no further increase was observed in the control.

All hives of all treatment groups had sufficient amounts of nectar and pollen throughout the observation period (DAE-1 to +29).

4. Honey bee behaviour

Normal behaviour was recorded throughout the whole exposure period (DAE0 to DAE+6) in both test item treatments (T1 and T2) and in the control.

5. Residues in sealed honey

There were no residues of indoxacarb above the limit of detection in any of the honey samples from hives of the treatments T1 and T2 and the control.

III. CONCLUSIONS

It can be concluded that Indoxacarb 150 g/L EC, applied once at a rate of 25.5 g a.s./ha (equivalent to 170 mL formulated product/ha) before flowering of *B. napus* L. (BBCH 55 in treatment T1 and BBCH 57-59 in treatment T2) had no negative effect on the mortality, flight activity, behaviour, colony size, and brood development of the honey bee colonies that were placed at the pre-treated fields during flowering, 14 days after the application in treatment T1 and 9 days after the application in treatment T2. No residues of indoxacarb were detectable in any of the samples of sealed honey taken from the experimental hives.

(Kleinhenz, M., 2011a)

RMS comment

Study submitted to the EU for the first time in this submission.

This study is valid. The mean number of honey bees/m² flying and foraging in the crop seem quite low but this is not a validity criteria.

The test item was applied before start of flowering with no open flowers present in the field for T1 and T2 (BBCH 55 and 57-59 respectively). The test item was applied 14 days (for T1) and 9 days (for T2) before the set-up of the beehives at the experimental fields (BBCH 63-65). One field per treatment was used.

Indoxacarb 150 g/L EC, applied once at a rate of 25.5 g a.s./ha (equivalent to 170 mL formulated product/ha) before flowering of *B. napus* L. (BBCH 55 in treatment T1 and BBCH 57-59 in treatment T2) had no negative effect on the mortality, flight activity, behaviour, colony size, and brood development of the honey bee colonies that were placed at the pre-treated fields during flowering, 14 days after the application in treatment T1 and 9 days after the application in treatment T2. No residues of indoxacarb were detectable in any of the samples of sealed honey taken from the experimental hives.

This study is not used in the risk assessment as it is not representative of an application during flowering (application occurred before flowering) and because honey bee colonies were placed at the pre-treated fields only 9 and 14 days after application.

Report: Kleinhenz, M. (2011b); Indoxacarb (DPX-KN128) 150 g/L EC: A study to evaluate effects on the honey bee (*Apis mellifera carnica*) in the field in *Brassica napus* L. in northern Germany in 2009

DuPont Report No.: DuPont-26947

Guidelines: OEPP/EPPO 170 (3) 2001 **Deviations:** None

Testing Facility: Eurofins Agrosience Services GmbH, Niefern-Oeschelbronn, Germany

Testing Facility Report No.: S08-03366

GLP: Yes

Certifying Authority: Landesanstalt Fur Umwelt, Messungen Und Naturschutz Baden-Wurttemberg

Executive summary:

The effects of Indoxacarb 150 g/L EC were tested on the honey bee (*Apis mellifera carnica* L.) under field conditions following the OEPP/EPPO guideline No. 170(3) (2001).

This study was conducted near Celle in northern Germany in April to May 2009 and included three treatment groups (fields of flowering winter oil-seed rape, *Brassica napus* L.) with one replicate each. Indoxacarb 150 g/L EC was applied at BBCH 59–60 (T1; application date 18 April 2009) and at BBCH 57-59 (T2; application date

16 April 2009) on separate fields. In both treatments, the application rate was 25.5 g a.s./ha (equivalent to 170 mL formulated product/ha). All applications were carried out with a spray volume of 300 L water/ha. A third group (field) was not treated during the experimental phase and served as control.

The effects of the treatment with Indoxacarb 150 g/L EC were examined on 6 honey bee colonies placed at the edge of each field of winter oilseed rape (*Brassica napus* L.) on 22 April 2009 (BBCH 63), 4 days after the application in T1 and 6 days after the application in T2. Observations of mortality, flight intensity, and behaviour were carried out over a period of 7 days after set-up of the colonies at the field sites. Assessments of the colony conditions were carried out once before set-up and then in weekly intervals for up to 4 weeks after set-up of the honey bees. The effects of the treatment with Indoxacarb 150 g/L EC was evaluated by comparing the results in treatments T1 (treated at BBCH 59-60) and T2 (treated at BBCH 57-59) to the data in the control treatment regarding the following observations:

- Number of dead honey bees on the linen sheet in front of the hives and in the dead honey bee traps in front of the hives,
- Foraging activity on the crop (number of forager bees per m² of winter oilseed rape),
- Condition of the colonies and development of the brood,
- Behaviour of the honey bees in the crop area and around the hives.

Additionally, samples of sealed honey were taken from each hive on DAE+27. These samples were analysed for residues of indoxacarb with a level of quantification (LOQ) of 0.010 mg a.s./kg.

It can be concluded that Indoxacarb 150 g/L EC, applied once at a rate of 25.5 g a.s./ha (equivalent to 170 mL formulated product per ha) to winter oilseed rape (*Brassica napus* L.) at BBCH 59-60 (treatment T1) and at BBCH 57-59 (treatment T2), had no negative effect on honey bee mortality, flight activity, colony condition, brood development, and behaviour.

No residues of indoxacarb were detectable in any of the samples of sealed honey taken from the hives.

I. MATERIAL AND METHODS

A. MATERIALS:

1. Test material: Indoxacarb 150 g/L EC
Lot/Batch #: DPX-KN128-212
Purity: 150 g a.s./L
Description: Liquid
CAS#: None for the formulation
173584-44-6 for the active substance
Stability of test compound: 99.2% of the indoxacarb remains in the delivery vehicle after one hour under agitation
2. Vehicle and/or positive control: Tap water
3. Test organism
Species: *Apis mellifera carnica* L.
Age at dosing: Direct exposure of adults; indirect exposure of all stages of development
Source: LAVES Institut für Bienenkunde (Celle, Germany)
Diet: Nectar and pollen of flowering winter oilseed rape (*Brassica napus*)
Fields (exposure): Three fields of flowering oilseed rape (*Brassica napus*) were used, one for each treatment. The crop area per field was 2.4 ha (field T1), 2.8 ha (field T2) and 1.9 ha (field C). Within each field, 5 areas (1 m² each) were marked for assessment of flight activity and 3 linen sheets (0.5 x 10 m) were spread out for assessment of dead bees. Additionally, dead bees were counted on 1 linen sheet in front of the hives and in dead bee traps in front of each hive.
4. Environmental conditions during the experimental phase:
Temperature: 2.1 (min)–24.6°C (max)
Relative Humidity: 25–100%
Photoperiod (exposure): natural light conditions

B. STUDY DESIGN AND METHODS:

1. Experimental start/completion:
16-April-2009 to 18-May-2010
2. Experimental treatments
T1: Application at BBCH 59-60
T2: Application at BBCH 57-59
C: control (untreated)

The application rate was 25.5 g a.s./ha (equivalent to 170 mL formulated product/ha). All applications were carried out at a rate of 300 L water/ha. A third field remained untreated and served as a control.

The effects of the treatments were examined on honey bee colonies placed at the edge of fields (6 colonies per field) of flowering winter oilseed rape (*Brassica napus* L.) six days after the application in T2 and four days after the application in T1. Observations of mortality, flight intensity, and behaviour were carried out over a period of 7 days (DAE0 to +6). Assessments of the colony conditions were carried out once before setup of the colonies at the field sites and then on DAE -1, +6, +14/15, +20, and +27. The field test comprised one replicate (field) in each of the treatments (treatment T1 (applied at BBCH 59-60), treatment T2 (applied at BBCH 57-59), and the control).

3. Observations

The influence of Indoxacarb 150 g/L EC was evaluated by comparing the results in treatments T1 (applied BBCH 59-60) and T2 (applied BBCH 57-59) to the data in the control treatment regarding the following observations:

- Number of dead honey bees on linen sheets in the crop area, on linen sheets in front of the hives and in the dead honey bee traps in front of the hives,
- Foraging activity on the crop (number of forager bees/m²),
- Condition of the colonies and development of the honey bee brood,
- Behaviour of the honey bees in the crop area and around the hives.

Additionally, samples of sealed honey were taken from each hive on DAE+27. These samples were analysed for residues of indoxacarb with a level of quantification (LOQ) of 0.010 mg a.s./kg.

II. RESULTS AND DISCUSSION

A. FINDINGS

1. Honey bee mortality

Except for one record on DAE+6 in treatment T2, the daily mortality in treatments T1 and T2 was on a low level and comparable to the values in the control. Daily mortality was in a range from 3.7 to 20.0 dead honey bees/day in T1, 6.7 to 17.2 dead honey bees/day in T2 (except for the slightly higher value of 27.2 dead honey bees that was recorded on DAE+6), and 2.8 to 16.8 dead honey bees/day in the control. Since the higher mortality in treatment T2 on DAE+6 was observed only in three of the six hives and mortality in this treatment and hives was on a much lower level during the previous exposure period (DAE0 to DAE+5), it is unlikely that this is a test item effect but may be due to other, though unknown factors in the vicinity of the field T1.

The mean mortality during the whole exposure period was 11.4 dead honey bees/day in treatment T1, 14.2 dead honey bees/day in T2, and 8.5 dead honey bees/day in the control. Considering that the slightly elevated mean value in T2 was mainly due to the unusual observation on DAE+6, it can be stated that the mortality in the two treatments is on a low level that is not unusual for this kind of field study and is comparable to the control and within normal mortality variability observed for such honey bee colonies.

Table 141
Mean number of dead honey bees per day

Date	DAE ^b	Treatment groups ^a		
		Indoxacarb 150 g/L EC; Application of 25.5 g a.s./ha at BBCH 59-60 (T1)	Indoxacarb 150 g/L EC; Application of 25.5 g a.s./ha at BBCH 57-59 (T2)	Control
22 April 2009	0	9.8	12.0	7.3
23 April 2009 ^d	+1	10.7	15.0	11.5
24 April 2009 ^e	+2	20.0	17.2	16.8
25 April 2009	+3	15.7	13.8	11.8
26 April 2009	+4	12.5	7.5	5.5
27 April 2009	+5	7.2	6.7	3.8
28 April 2009	+6	3.7	27.2	2.8
Mean number of dead honey bees/day (DAE0 to +6)		11.4	14.2	8.5
STD ^c		6.4	11.6	6.3

^a Mortality values for hives plus linen in front of hives, divided by number of hives

^b DAE = Days after start of exposure of the honey bees to the treated crop (DAE0 = first day of exposure)

^c STD = Standard deviation

^d First assessment on that day (DAE+1), in the morning

^e Values of the two additional assessments in the afternoon of DAE+1 were added to the value in the next morning (DAE+2) and counted as one value.

2. Honey bee flight intensity

In general, flight activity on all three fields was rather low for this kind of crop and time of the year. Moreover, differences among the three fields may account for differences of flight activity on the three fields and, therefore, must be taken into consideration. For example, on each of the three fields there was a different variety of *B. napus*, which may have different attractiveness for the bees. Additionally, the size of the control field was *ca* $\frac{1}{5}$ to $\frac{1}{3}$ smaller than the fields that were used for the test item treatments, which may have caused a higher foraging density (number of forager bees/m²) in the control field. The daily flight intensity values recorded for treatment T1 ranged between 0.0 (DAE+6) and 0.6 honey bees/m² during the assessment period. For treatment T2, the equivalent values were calculated with 0.2 to 1.4 honey bees/m². In the control daily flight intensity ranged between 0.3 and 2.2 within the same time.

Throughout the exposure period, flight intensity recorded for treatment T1 was below the values recorded for the control and treatment T2 (except for DAE+4 where flight intensity in T1 and T2 was at the same level). Differences between T1 and the control on DAE0 to +2 and DAE+6 were rather small and within the natural range of variation that has to be expected in this kind of field study. Differences were more prominent on DAE+3 to +5 when 0.2 to 0.6 honey bees/m² were recorded in T1 compared to 1.2 to 2.2 honey bees/m² in the control where the highest values in this group during the whole exposure period were observed. In treatment T1, it was noted by the observers that a higher number of honey bees/m² was observed outside the observation squares that were marked at fix locations within the field. Therefore, the low flight activity in T1 may also be due to differences in the crop between the two fields.

Flight intensity in treatment T2 was comparable to the values in the control on most days of the exposure period, except for two slightly lower values on DAE+4 and +5 (T1: 0.6 and 1.0 honey bees/m²; control: 1.6 and 2.2 honey bees/m²). However, these values are within the natural range of variation. Considering that flight activity on the previous days (DAE+1 to +3) was higher in T2 than in the control, the data do not suggest a negative effect of the test item on flight activity.

Over the whole exposure period mean daily flight intensity of 0.3 and 0.7 honey bees/m² was determined for treatments T1 and T2, respectively, compared to 1.0 honey bees/m² in the control.

Overall, foraging activity was more on the lower side of the expected range, but the foraging data does not indicate any test item related effect.

Table 142
Mean number of honey bees/m² flying and foraging in the crop

Date	DAE ^b	Treatment groups ^a		
		Indoxacarb 150 g/L EC; Application of 25.5 g a.s./ha at BBCH 59-60 (T1)	Indoxacarb 150 g/L EC; Application of 25.5 g a.s./ha at BBCH 57-59 (T2)	Control
22 April 2009	0	0.1	0.2	0.3
23 April 2009	+1	0.3	0.7	0.6
24 April 2009	+2	0.4	0.8	0.6
25 April 2009	+3	0.2	1.4	1.2
26 April 2009	+4	0.6	0.6	1.6
27 April 2009	+5	0.4	1.0	2.2
28 April 2009	+6	0.0	0.2	0.4
Mean number of forager bees/m² (DAE0 to +6)		0.3	0.7	1.0
STD ^c		0.4	0.8	0.9

^a Mean values and STD were calculated from the individual values per field

^b DAE = Days after start of exposure of the honey bees to the treated crop (DAE0 = first day of exposure)

^c STD = Standard deviation

3. Honey bee colony size and brood development:

The strength of the colonies (number of honey bees according to Liebefeld method) of treatments T1 and T2 and the control colonies increased throughout the observation period, namely from a mean of 9659 to 25981 honey bees (T1), 9509 to 24910 honey bees (T2), and 9547 to 25301 honey bees in the control.

Regarding the brood development, it can be stated that all colonies of all three treatment groups (T1, T2, and control) had brood of all stages (eggs, larvae, sealed brood) at any time during the assessments, except for the lack of eggs in hive 1T2 at the assessment on DAE+6. However, in this hive (1T2) eggs and all other brood stages were present at the three following assessments until the end of the observation period.

During the whole observation period, the mean size of the brood areas (number of cells containing eggs, larvae, and capped brood) increased in all the three treatment groups. Differences of the brood nest size between the first and second assessment were rather small, but generally an increase occurred during the observation period, namely from 29820 to 43080 brood cells in T1, 28890 to 43350 brood cells in T2, and 28860 to 42420 brood cells in the control.

In the five assessments carried out, all colonies of the treatment groups T1, T2, and the control had plenty of pollen and nectar resources. The amount and growth of the food stores was comparable in treatments T1 and T2 and in the control. In treatment T1, the number of cells containing nectar increased from 26190 to 87750 and the number of cells containing pollen increased from 12030 to 24870; in treatment T2, the food stores increased from 25470 to 93780 cells (nectar) and from 12660 to 23580 cells (pollen); and in the control, the food stores increased from 30540 to 93960 cells (nectar) and 9840 to 24150 cells (pollen).

4. Behaviour of the honey bees

All bees of all treatment groups showed normal behaviour throughout the observation period (DAE 0 to +6).

5. Residues in sealed honey

There were no residues of indoxacarb above the limit of detection (LOD = 0.003 mg a.s./kg) in any of the honey samples from hives of treatments T1, T2, and the control taken +27 days after exposure (about one month after spray application).

III. CONCLUSIONS

It can be concluded that Indoxacarb 150 g/L EC, applied once at a rate of 25.5 g a.s./ha (equivalent to 170 mL formulated product per ha) to winter oilseed rape (*Brassica napus* L.) at BBCH 59-60 (treatment T1) and at BBCH 57-59 (treatment T2), had no negative effect on honey bee mortality, flight activity, colony condition, brood development, and behaviour.

No residues of indoxacarb were detectable in any of the samples of sealed honey taken from the hives.

(Kleinhenz, M., 2011b)

RMS comment

Study submitted to the EU for the first time in this submission.

This study is valid. The mean number of honey bees/m² flying and foraging in the crop seem quite low but this is not a validity criteria.

The test item was applied at the beginning of flowering (BBCH 59-60 and 57-59 for T1 and T2 respectively). The number of flowering plants was :

- For T1, 46.2% plants without any open flower, 37.7% plants with 1-10 open flowers and 16.1% plants with >10 open flowers.

- For T2, 86.5% plants without any open flower, 13.5% plants with 1-10 open flowers and 0.0% plants with >10 open flowers.

The test item was applied 4 days (for T1) and 6 days (for T2) before the set-up of the beehives at the experimental fields (BBCH 63-65). One field per treatment was used.

A higher mortality in treatment T2 on DAE+6 was observed in three of the six hives. As the increase of mortality was observed in three hives (of the six hives) an effect of the treatment could be suspected however as this increase occurred at the same time (DAE+6 for the tree hives) and so far from the day of start of exposure (mortality was very low between DAE0 and DAE+5) RMS considers that the mortality observed at DAE+6 is not treatment related. The reason of the increase of mortality is however unknown.

Flight activity on all three fields was rather low for this kind of crop and time of the year. The mean number of honey bees/m² flying and foraging in the crop seems lower in the treated field fields than in control. However, differences among the three fields may account for differences of flight activity on the three fields. For example, on each of the three fields there was a different variety of *B. napus*, which may have different attractiveness for the bees. Additionally, the size of the control field (1.87 ha) was *ca* $\frac{1}{5}$ to $\frac{1}{3}$ smaller than the fields that were used for the test item treatments (2.4 and 2.8 ha for T1 and T2 respectively), which may have caused a higher foraging density (number of forager bees/m²) in the control field. RMS considers that the results on flight activity are inconclusive and cannot be used for the risk assessment.

Regarding the brood development, all colonies of all three treatments groups (T1, T2, and control) had brood of all stages (eggs, larvae, sealed brood) at any time during the assessments, except for the lack of eggs in hive 1T2 at the assessment on DAE+6. However, in this hive (1T2) eggs and all other brood stages were present at the three following assessments until the end of the observation period. RMS also notes that at DAE+6 the number of cells containing larvae is lower than in other hives. No differences was noted

before the set-up of the hives at DAE-1. This means that the egg-laying temporarily stopped from the time of set-up of the beehive on the experimental site. The reason of that is not known but might not be treatment related.

Indoxacarb 150 g/L EC applied once at a rate of 25.5 g a.s./ha (equivalent to 170 mL formulated product per ha) to winter oilseed rape (*Brassica napus* L.) at BBCH 59-60 (treatment T1) and at BBCH 57-59 (treatment T2), had no negative effect on honey bee mortality, colony condition, brood development, and behaviour of the honey bee colonies that were placed at the pre-treated fields during flowering, 4 days after the application in treatment T1 and 6 days after the application in treatment T2.

No residues of indoxacarb were detectable in any of the samples of sealed honey taken from the hives.

This study is not used in the risk assessment as it is not representative of an application during flowering (application occurred before full flowering) and because honey bee colonies were placed at the pre-treated fields only 4 and 6 days after application.

Report: Bocksch. S. (2011); Indoxacarb (DPX-KN128) 150 g/L EC: A study to evaluate effects on the honeybee (*Apis mellifera*) in the field in maize in Germany in 2010

DuPont Report No.: DuPont-30106

Guidelines: OEPP/EPPO 170 (3) 2001, Proposed revision to EPPO Standard PP1/170 (Lewis et al. 2009)
Deviations: None

Testing Facility: Eurofins Agrosience Services GmbH, Niefern-Oeschelbronn, Germany

Testing Facility Report No.: S10-02510

GLP: Yes

Certifying Authority: Landesanstalt Für Umwelt, Messungen Und Naturschutz Baden-Württemberg

Executive summary:

The effects of Indoxacarb 150 g/L EC were tested on the honey bee (*Apis mellifera carnica* L.) under field conditions following the guideline of the European and Mediterranean Plant Protection Organization No. 170 (3) (OEPP/EPPO, 2001) and the recommendations by Lewis *et al.* (2009).

The field part of this study was conducted near Tübingen and Hechingen in southern Germany (region: Baden-Württemberg) in July and August 2010 and included two treatment groups:

- Indoxacarb 150 g/L EC (treatment group T) with one application performed during flowering of maize (*Zea mays*) and after set-up of the honey bee colonies at the experimental fields, in the evening after daily honey bee-flight. The application was carried out at a rate of 37.5 g a.s./ha.
- Control group C with no application.

The honey bee colonies were set up at the experimental fields in the evening of the 19 July 2010 at the beginning of flowering of maize (BBCH 53-63) in treated field T and BBCH 61-63 in the control field (C). The application in treated field was performed on flowering maize on 25 July 2010 (BBCH 55-65) in the evening, after daily honey bee-flight. The application was carried out with a spray volume of 300 L/ha.

The effects of the test item treatment were examined on six commercial honey bee colonies placed at each experimental field. The study comprised one replicate field in each of the treatments.

The influence of Indoxacarb 150 g/L EC was evaluated by comparing the results to the data in the control treatment regarding the following observations:

- Number of dead honey bees on the linen sheet and in the dead honey bee traps in front of the hives,
- Foraging activity on the crop (number of forager honey bees/30 plants/2 minutes),
- Condition of the colonies and development of the brood,
- Behavior of the honey bees in the crop area and around the hives.

Samples of pollen for pollen source determination were taken with pollen traps at two dates from two hives per treatment group on 27 Jul 2010 (DAE+1) and 30 Jul 2010 (DAE+4).

It can be concluded that Indoxacarb 150 g/L EC, applied once at beginning of flowering and in the evening after daily bee-flight, with an application rate of 37.5 g a.s./ha, showed no obvious test item related impact on the honey bee mortality, flight intensity, behaviour, and brood development.

I. MATERIAL AND METHODS

A. MATERIALS:

1. Test material: Indoxacarb 150 g/L EC
Lot/Batch #: DPX-KN128-233
Purity: 150 g a.s./L
Description: Liquid
CAS#: None for the formulation
173584-44-6 for the active substance
Stability/Homogeneity: 100 % of the indoxacarb remains in the delivery vehicle after one hour under agitation.
2. Vehicle: Tap water
3. Control: untreated
4. Test organism
Species: *Apis mellifera carnica* L.
Age at dosing: Direct exposure of adult honey bees; indirect exposure of all stages of development
Source: Berthold Nengel, Beekeeper
Diet: Pollen of flowering *Zea mays*
5. Environmental conditions during the exposure period
Temperature: 8.1–31.7°C
Relative humidity: 18.6–90.4 %
Photoperiod (exposure): natural light conditions

B. STUDY DESIGN AND METHODS

1. Experimental start/completion
19-July-2009 to 20-October-2010
2. Experimental treatments
Indoxacarb 150 g/L EC (treatment group T) with one application performed during flowering of maize (*Zea mays*) and after set-up of the honey bee colonies at the experimental fields, in the evening after daily honey bee-flight. The application was carried out at a rate of 37.5 g a.s./ha and with a spray volume of 300 L/ha.

Control group C with no application.

3. Observations

The influence of Indoxacarb 150 g/L EC was evaluated by comparing the results to the data in the control treatment regarding the following observations:

- Number of dead honey bees on the linen sheets and in the dead bee traps in front of the hives,
- Foraging activity on the crop (number of forager honey bees/30 plants/2 minutes),
- Condition of the colonies and development of the brood,
- Behavior of the honey bees in the crop area and around the hives.

II. RESULTS AND DISCUSSION

A. FINDINGS

1. Honey bee mortality

During the pre-application period before the application (DAE-6 to -1ba) means of 16.8 dead honey bees/day in the control, and 10.7 dead honey bees/day in the treatment group were observed. Daily mean mortality varied between 4.8 and 29.5 dead honey bees/day in the control and 6.8 and 13.8 dead honey bees/day in the treatment group. On most of the days before the application the mortality in the control hives was slightly higher than in the hives at the treated field.

On the day of the application in T, before application (DAE-1ba) the mortality in the treatment group was slightly increased in comparison to the control with a mean of 4.8 dead honey bees/day in the control and 11.5 dead honey bees/day in the treatment.

Table 143
Number of dead honey bees per day (mean values of 6 hives and linen sheet in front of the hives)

Date	DAE ^b	Treatment groups ^a	
		Control	Indoxacarb 150 g/L EC; application after bee-flight (T)
20 Jul 2010	-6	9.8	13.8
21 Jul 2010	-5	29.5	12.8
22 Jul 2010	-4	17.5	9.8
23 Jul 2010	-3	15.7	11.3
24 Jul 2010	-2	22.3	8.5
25 Jul 2010	-1	18.0	6.8
25 Jul 2010	-1ba	4.8	11.5
Mean number of dead honey bees per day (DAE-6 to -1ba)		16.8	10.7
26 Jul 2010	0	11.5	18.0
27 Jul 2010	+1	6.3	8.2
28 Jul 2010	+2	11.8	24.3
29 Jul 2010	+3	12.0	5.8
20 Jul 2010	+4	14.7	7.5
31 Jul 2010	+5	9.3	15.7
01 Aug 2010	+6	10.2	23.3
02 Aug 2010	+7	9.5	11.3
Mean number of dead honey bees per day (DAE0 to DAE+7)		10.7	14.3
Mean number of dead honey bees per day (DAE-6 to DAE+7)		13.5	12.6

^a Mean values were calculated from the individual values per hive

^b DAE = Days after start of exposure

On the day after the application (first day of exposure) in T, the mortality in the treatment group was slightly increased in comparison to the control with a mean of 18.0 dead honey bees/day in the control and 11.5 dead honey bees/day in the treatment.

During the post-application period after the application (DAE0 to +7), means of 10.7 dead honey bees/day in the control and 14.3 dead honey bees/day in the treatment group were observed. Daily mean mortality varied between 6.3 and 14.7 dead honey bees/day in the control and 5.8 and 24.3 dead honey bees/day in the treatment group.

The mean mortality during the whole test period (DAE-6 to DAE+7) was 13.5 dead honey bees/day in the control and 12.6 dead honey bees/day in the treatment group.

2. Flight activity

The mean flight activity during the pre-application period (DAE-6 to DAE-1) was 0.28 honey bees/30 plants/2 minutes in the control and 1.15 honey bees/30 plants/2 minutes in the treatment group.

On the day of start of exposure (DAE0), the mean flight activity was 0.17 honey bees/30 plants/2 minutes in the control and 0.43 honey bees/30 plants/2 minutes in the treatment group.

The mean flight activity over the whole post-application period was 0.86 honey bees/30 plants/2 minutes in the control and 1.71 honey bees/30 plants/2 minutes in the treatment.

The mean flight activity over the whole test period (DAE-6 to DAE+7) was 0.61 honey bees/30 plants/2 minutes in the control and 1.47 honey bees/30 plants/2 minutes in the treatment.

Table 144
Number of forager honey bees/30 plants/2 minutes

Date	DAE ^b	Treatment groups ^a	
		Control	Indoxacarb 150 g/L EC; application after bee-flight) (T)
20 Jul 2010	-6	0.33	0.72
21 Jul 2010	-5	0.20	3.20
22 Jul 2010	-4	0.46	1.20
23 Jul 2010	-3	0.26	0.20
24 Jul 2010	-2	0.00	0.00
25 Jul 2010	-1	0.40	1.60
25 Jul 2010	-1ba ^c	0.00	0.00
Mean number of forager honey bees/30 plants/2 minutes per day (DAE-6 to -1)		0.28	1.15
26 Jul 2010	0	0.17	0.43
27 Jul 2010	+1	1.33	1.67
28 Jul 2010	+2	0.40	0.00
29 Jul 2010	+3	0.80	1.60
30 Jul 2010	+4	1.20	1.60
31 Jul 2010	+5	0.80	3.80
01 Aug 2010	+6	0.80	3.60
02 Aug 2010	+7	1.40	1.00
Mean number of forager honey bees/30 plants/2 minutes per day (DAE0 to DAE+7)		0.86	1.71
Mean number of forager honey bees/30 plants/2 minutes per day (DAE-6 to DAE+7)		0.61	1.47

^a Mean values were calculated from the individual values per hive

^b DAE = Days after start of exposure

^c Values of DAE-1ba were not considered for calculation of the mean pre-application flight activity.

3. Behaviour of the honey bees

The honey bees in the control and treatment groups showed normal behaviour throughout the observation period. No behavioural abnormalities of any of the colonies could be observed during exposure of the honey bees to the treated crop.

4. Brood development and colony condition

In both treatment groups, the colony size (number of honey bees) increased slightly during the study until DAE+21 and decreased afterwards on DAE+28.

The mean colony size was 13948 honey bees per hive in the control and 13791 in the treatment group at start of the test on DAE-7. On DAE+28, the mean number of honey bees per hive was on a similar level with 14675 and 14842 honey bees per hive in the control and treatment group, respectively.

The colonies of the control and the treatment group had a mean of 7.83 and 8.00 combs containing brood at beginning of the test, respectively. At the end of exposure on DAE+7 the mean number of brood combs per hive was 7.67 in the control and 6.33 in the treatment group. At the end of the study on DAE+28, the mean number of brood combs per hive was 6.67 and 6.17 in the control and treatment group, respectively.

All hives of both groups had brood of all stages (eggs, larvae, capped brood) during the first assessment (before set-up at the experimental fields) and throughout the rest of the observation period, except for hive C4 which had no eggs and larvae on DAE+15 due to lack of a fertile queen. This colony changed its queen between the assessments on DAE+7 and DAE+21.

With regard to the total observation period (DAE-7 to DAE+28), the area covered with brood remained on the level of test start in both groups, and was 18.60 % (DAE-7) and 19.06 % (DAE+28) in the control compared to 18.26 % (DAE-7) and 18.39 % (DAE+28) in the treatment group.

During the whole observation period, all hives had sufficient food resources (nectar, honey, and pollen).

5. Pollen source determination

On DAE+1, the pollen sample collected in the pollen trap of two hives per group contained a mean of 9.5% of maize pollen in the control and 5.5% in the treatment group. On DAE+4, the mean proportion of maize pollen in the two hives per group was 4.5% in the control and 54.5% in the treatment group. Beside maize pollen, the bees collected pollen from several wild flowers, mainly *Plantago* sp., *Filipendula* sp., *Anemone* sp., *Heracleum* sp., and *Trifolium* sp. at both test fields.

III. CONCLUSION

It can be concluded that Indoxacarb 150 g/L EC, applied once at beginning of flowering and in the evening after daily bee-flight, with an application rate of 37.5 g a.s./ha, showed no obvious test item-related impact on the honey bee mortality, flight intensity, behaviour, and brood development.

(Bocksch, S., 2011)

RMS comment

Study submitted to the EU for the first time in this submission.

This study is valid. The number of forager honey bees/30 plants/2 minutes seem quite low but this is not a validity criteria. RMS notes that the fields are however particularly large (11.0 ha for C, 25.45 ha for T).

RMS notes that important rain occurred especially on the day of application (13.4 mm). RMS considers that this might affect the actual exposure of the bees (by washing residues or by reducing foraging intensity).

The test item was applied at the beginning of flowering (BBCH 55-65). RMS considers that the application took place too early as only a few maize flowers were available for bees at the time of treatment. As a consequence, the pollen collected after the application might not have been exposed to the spray (pollen still enclosed at the time of application). This is confirmed by the amount of maize pollen found in the samples collected at DAA1 and DAA4. At DAA1, the amount of maize pollen was minimal (5.5% in T) but increase afterwards to a level of 54.5% at DAA+4. This means that even if the bees collected the pollen from the treated maize field, this pollen was not available at the time of application and was not directly exposed to the spray. This leads to an underestimation of the effects.

In the control field, the amount of maize pollen remains low during the test. As the control field was smaller, bees probably might have been able to choose other sources of pollen. It is noted that there were no attractive crops in the surroundings but the pollen of wild flowers was found in the samples. Besides, despite the presence of maize flowers at the end of the study, the flight intensity did not increase in this field.

RMS considers that an increase of mortality from DAA0 to DAA2 cannot be excluded even if it is slight.

RMS considers that the results available on flight intensity can not be used in the risk assessment.

No treatment related effect was observed on behaviour and brood development (until DAA28).

Indoxacarb 150 g/L EC, applied once at beginning of flowering and in the evening after daily bee-flight, with an application rate of 37.5 g a.s./ha, showed no obvious test item-related impact on the honey bee behaviour and brood development. Slight effect on mortality cannot be excluded. This test is however not representative of an application on a flowering crop.

Report: van der Steen, J.J.M., Dinter, A. (2006); A monitoring study to assess the acute mortality effects of indoxacarb on honey bees (*Apis mellifera* L.) in flowering apple orchards

DuPont Report No.: Pest Manag Sci 63 1095-1099 (2007)

Testing Facility: PPO Bijen, 6700 AB Wageningen, The Netherlands, DuPont de Nemours (Deutschland) GmbH, Bad Homburg v.d.H., Germany

GLP: No

Certifying Authority: Not applicable

To evaluate the effect of the Indoxacarb 300 g/kg WG, Steward 30WDG™, on the honey bee (*Apis mellifera* L.) in apple orchards, a monitoring study was conducted in Dutch apple orchards in April/May 2004. Before apple flowering began, two honey bee colonies were placed in each orchard to investigate honey bee mortality. Each hive was provided with a Münster dead bee trap to collect dead honey bees. The numbers of dead bees found in these Münster dead traps were counted every 3-4 days for about 2 weeks before and after the period of the insecticide treatment. In nine flowering orchards no indoxacarb was applied during the flowering period, which served as control sites. In 30 flowering orchards indoxacarb was sprayed by the fruit growers according to local practice at 170-260 g formulated product/ha (51-78 g a.s./ha). In the control orchards the average mortality was 8 honey bees/colony/day. The average daily honey bee mortality before and after indoxacarb application was 8 and 10 honey bees/colony/day, respectively. At one test site, indoxacarb was mixed with other plant protection

products plus plant nutrients, and in this orchard a slight but biologically non-significant increase in acute honey bee mortality was recorded. It was concluded that the application of indoxacarb caused no effects on honey bee mortality, and that the number of dead honey bees counted in the Münster traps in the orchard treated with indoxacarb was comparable with those determined in control orchards.

(van der Steen, J.M.M., Dinter, A., 2006)

RMS comment

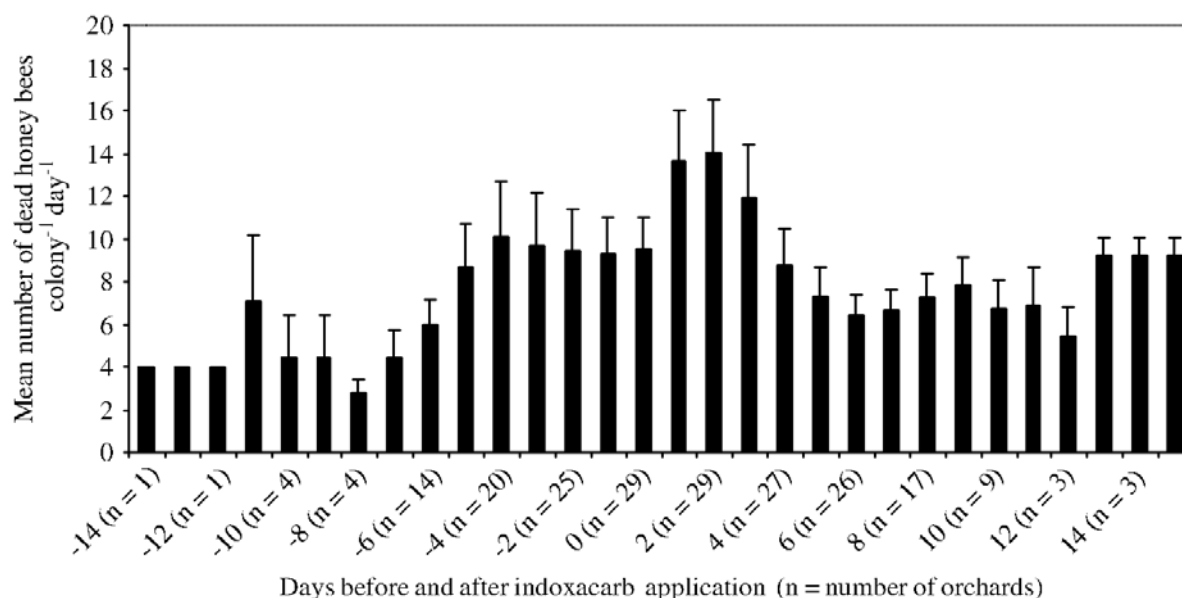
Study submitted to the EU for the first time in this submission.

It is noted in the publication that the selected orchards (1-3 ha) were separated from each other by a distance of at least 1 km. RMS considers that a distance of 1 km is quite short and thus it is not certain that bees did not forage in a field of an other modality (treated vs control). Considering the attractiveness and the short time of flowering of the apple trees, RMS considers the minimal distance of 1 km not sufficient (especially if flowering occurs not simultaneously in different orchards). Besides it is not known if other attractive crops or plants are available around the treated orchards. In such case where other attractive plants are available, it is difficult to force the bees to forage on the treated crop and thus the exposure might have been underestimated. It is thus not known if application on isolated attractive crops would result in significant effects or not. Besides the temperatures recorded were between 9.3 and 17°C. RMS considers the temperature quite low. No data on the foraging activity are available to ensure the foraging was sufficient at the time of the study.

It has also to be noted that the formulation tested is a WG formulation. The representative formulation to be assessed is an EC formulation. It is not certain if an extrapolation of effects can be made but the results of a semi-field study (Giffard, 2006, report n°92-2006) seem to show higher lethal effect of the EC formulation when the bees are exposed to fresh residues (application during bee flight).

It has to be noted that fungicide application occurred in the control orchards (not known to be hazardous according to the study authors). Mortality in control might then be overestimated.

RMS notes an increase of the mortality after the application that seems to last for 3 days. This increase was considered not significant by the study authors. It is however agreed by RMS that the increase is not of biological importance for the honey bee colonies.



It is noted in the report that an increase of mortality was observed in one site where indoxacarb was sprayed in mixture with four other substances. It is not known if the mortality is due to indoxacarb or not. It is also reported that all the bee colonies used in this study showed normal development and normal increase in honey bee population size during the test period. However no detail was provided in the

publication and the effects on brood could not have been assessed in this study aiming for the assessment of acute lethal effects.

RMS considers that the results of this monitoring study based on a WG formulation not reliable for the evaluation of the representative formulation (EC type). Uncertainty also remains on the presence or absence of other attractive plants in the surroundings of the treated orchards. Besides, the exposure of the bees during this monitoring might underestimate the exposure of other conditions of use (season, crops, regions,...).

B.9.5.2. Effects on non-target arthropods other than bees

Testing for effects on arthropod species other than bees was carried out using the formulated product Indoxacarb 150 g/L EC. Laboratory studies were conducted to assess effects on the sensitive indicator species, the phytoseiid mite, *Typhlodromus pyri*, and the parasitic wasp, *Aphidius rhopalosiphi*, and on the preferred species, the green lacewing *Chrysoperla carnea*, and the predatory hemipteran, *Orius laevigatus*. Summaries of these studies follow.

Report: Warmers, C. (2006a); Indoxacarb (DPX-KN128) 150 g/L EC: A laboratory rate response test to study the effects on the parasitoid *Aphidius rhopalosiphi* (Hymenoptera, Braconidae)

DuPont Report No.: DuPont-19443

Guidelines: Mead-Briggs *et al* (2000), Barrett *et al* (1994), Candolfi *et al* (2001) **Deviations:** None

Testing Facility: GAB Biotechnologie, GmbH, Niefern-Oeschelbronn, Germany

Testing Facility Report No.: 20061269/01-NLAp

GLP: Yes

Certifying Authority: Ministerium für Umwelt und Verkehr (Baden-Württemberg, Germany)

Executive summary:

An acute 48-hour toxicity study on the parasitic wasp *Aphidius rhopalosiphi* (Hymenoptera: Braconidae) was conducted according to the following methods: Mead-Briggs *et al.*, 2000; Barrett *et al.*, 1994; Candolfi *et al.*, 2001. The test organisms were exposed to an untreated control and dried residues of Indoxacarb 150 g/L EC at rates of 0.5, 1.2, 3.2, 8, and 20 g a.s./ha in a volume equivalent to 200 L water/ha. These rates are equivalent to 3.3, 8.0, 21.3, 53.3, and 133.3 mL product/ha. A toxic reference item (dimethoate) was also included in the test. The LR₅₀ of Indoxacarb 150 g/L EC was calculated to be 5.1 g a.s./ha (confidence limits: 3.8-6.9 g a.s./ha). This is equivalent to 34 mL product/ha (confidence limits: 25.3–46 mL product/ha).

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Indoxacarb 150 g/L EC
 Lot/Batch #: DPX-KN128-150
 Purity: 150 g a.s./L
 CAS#: None for the formulation
 173584-44-6 for indoxacarb active substance
 Description: EC formulation, liquid/amber
 Stability of test compound in solution: 98.8% of the indoxacarb remains in the delivery vehicle after one hour under agitation
2. Control: Deionised water
 Test Vehicle: Deionised water
 Toxic reference item: Perfekthion (dimethoate a.s.)
3. Test organism
 Species: *Aphidius rhopalosiphi*
 Age at dosing: <48-hour old adults
 Source: Pupal wasps from commercial supplier (Katz Biotech AG)
 Acclimation period: 48 hours
 Diet: Honey-agar-water solution
 Water: Tap water, *ad libitum*
 Test chambers (exposure): Glass plates (length of edges: 13 cm) were used to form the floor and the ceiling (treated surface inwards) of shallow arenas
4. Environmental conditions
 Temperature: 19.0–21.0°C
 Relative humidity: 76–83%
 Photoperiod (exposure): 16 hour photoperiod (800-1100 lux)

B. STUDY DESIGN AND METHODS

1. Experimental start/completed
 17-July-2006 to 19-July-2006
2. Experimental treatments
 In an acute toxicity study, parasitic wasps of the species *Aphidius rhopalosiphi* (Hymenoptera: Braconidae) were exposed to Indoxacarb 150 g/L EC. Adult mortality was evaluated in a rate response following a 48-hour exposure period to dried residues of the test item on treated glass plates. The test was comprised of 4 replicates of 10 individuals (5 male and 5 female) for each test item treatment rate, control, and toxic reference item (0.12 g dimethoate/ha). Indoxacarb 150 g/L EC was applied at 0.5, 1.2, 3.2, 8, and 20 g a.s./ha in a volume equivalent to 200 L water/ha. These rates are equivalent to 3.3, 8.0, 21.3, 53.3, and 133.3 mL product/ha.
3. Observations
 Assessments for adult wasp mortality were carried out approximately 1, 2, 24, and 48 hours after treatment.
4. Statistics
 Fisher's Exact Test ($\alpha = 0.05$): Analysis of mortality data for significance.

The LR₅₀ (lethal rate) was calculated by means of a moving average procedure computing over 3 values (Thompson, 1947).

II. RESULTS AND DISCUSSION

A. FINDINGS

Adult mortality in the control and toxic reference item groups were 0.0 and 100.0%, respectively. All validation criteria were met.

Results of the rate response testing are given below:

Table 145
The effects on mortality of the aphid parasitoid, *Aphidius rhopalosiphi*, exposed to fresh dried residue of Indoxacarb 150 g/L EC in the laboratory

Test Item	Rate (g a.s./ha)	Mortality (%) ^a	Corrected mortality (%) ^b
Untreated Control	-	0.0	-
Toxic reference item (dimethoate)	0.12	100.0	100.0
Indoxacarb 150 g/L EC	0.5	7.5	7.5
Indoxacarb 150 g/L EC	1.2	5.0	5.0
Indoxacarb 150 g/L EC	3.2	17.5 *	17.5
Indoxacarb 150 g/L EC	8.0	80.0 *	80.0
Indoxacarb 150 g/L EC	20	87.5 *	87.5

^a Mortality = moribund plus dead test organisms

^b Schneider-Orelli's Correction

* Statistically significant different to control (Fisher's Exact Test, one-tailed, $p \leq 0.05$)

III. CONCLUSIONS

Indoxacarb 150 g/L EC, at rates up to 1.2 g a.s./ha (equivalent to 8.0 mL product/ha), caused no statistically significant increase in mortality of *A. rhopalosiphi* when compared to controls. The LR₅₀ of Indoxacarb 150 g/L EC was calculated to be 5.1 g a.s./ha (confidence limits: 3.8–6.9 g a.s./ha). This is equivalent to 34.0 mL product/ha (confidence limits: 25.3–46.0 mL product/ha).

(Warmers, C., 2006a)

RMS comment

Study submitted to the EU for the first time in this dossier.

The study is valid.

The LR₅₀ of Indoxacarb 150 g/L EC was calculated to be 5.1 g a.s./ha equivalent to 34.0 mL product/ha.

Report: Warmers, C. (2006b); Indoxacarb (DPX-KN128) 150 g/L EC: A laboratory rate response test to study the effects on the predatory mite *Typhlodromus pyri* (Acari, Phytoseiidae)

DuPont Report No.: DuPont-19444

Guidelines: Blumel *et al* (2000), Barrett *et al* (1994), Candolfi *et al* (2001) **Deviations:** None

Testing Facility: GAB Biotechnologie, GmbH, Niefern-Oeschelbronn, Germany

Testing Facility Report No.: 20061269/01-NLTp

GLP: Yes

Certifying Authority: Ministerium für Umwelt und Verkehr (Baden-Württemberg, Germany)

Executive summary:

An acute 7-day toxicity study, on the predatory mite *Typhlodromus pyri* (Acari, Phytoseiidae) was conducted according to the following methods: Blümel *et al.*, 2000; Barrett *et al.*, 1994; Candolfi *et al.*, 2001. The test organisms were exposed to an untreated control and dried residues of Indoxacarb 150 g/L EC at rates of 50, 100, 200, 400, and 800 g a.s./ha in a volume of 200 L water/ha. These rates are equivalent to 333.3, 666.7, 1333.3, 2666.7, and 5333.3 mL product/ha. A toxic reference item (dimethoate 400 g/L) was also included in the test. The 7-day LR₅₀ was 220.5 g a.s./ha (confidence limits: 254.3–191.0 g a.s./ha). This is equivalent to a 7-day LR₅₀ of 1470 mL product/ha (confidence limits: 1273–1695 mL product/ha).

I. MATERIALS AND METHODS

A. MATERIALS

- | | |
|---|---|
| 1. Test material: | Indoxacarb 150 g/L EC |
| Lot/Batch #: | DPX-KN128-150 |
| Purity: | 150 g a.s./L |
| CAS#: | None for the formulation |
| | 173584-44-6 for indoxacarb active substance |
| Description: | EC formulation, liquid/amber |
| Stability of test compound in solution: | 98.8% of the indoxacarb remains in the delivery vehicle after one hour under agitation |
| 2. Control: | Deionised water |
| Test Vehicle: | Deionised water |
| Toxic reference item: | Perfekthion (dimethoate a.s.) |
| 3. Test organism: | Predatory mite |
| Species: | <i>Typhlodromus pyri</i> |
| Strain: | Eurofins-GAB GmbH; laboratory reared culture originating from 'Katz Biotech AG', An der Birkenpfuhlheide 10, D-15837 Baruth, Germany in 2003 |
| Age at dosing: | Protonymphs (<24 hours after molting) |
| Source: | The mites were obtained from a laboratory culture at the testing facility eurofins-GAB GmbH |
| Acclimation period: | 4 days |
| Diet: | Untreated broad bean and birch pollen |
| Water: | Mixture of tap water and deionized water (1:3), <i>ad libitum</i> |
| Test units: | Two glass cover slides (24 mm × 50 mm), placed side-by-side, and fixed together with two glass bars. A non-drying glue barrier resulted in an exposure area of approximately 10 to 13 cm ² . |
| 4. Environmental conditions | |
| Temperature: | 23.0 to 25.0°C |
| Relative humidity: | 62-82% |
| Photoperiod: | 16 hr light, 8 hr dark photoperiod (3500 lux) |

B. STUDY DESIGN AND METHODS

- Experimental start/completed
24-July-2006 to 31-July-2006

2. Experimental treatments

In an acute toxicity study, predatory mites, species *Typhlodromus pyri* Scheuten (Acarina: Phytoseiidae) were exposed to Indoxacarb 150 g/L EC. Adult mortality was evaluated in a rate response test during a 7-day exposure to dried residues of the test item on treated glass plates. The test was comprised of four replicates of 20 protonymphs for each treatment rate, control, and toxic reference (dimethoate). The test item was applied at 50, 100, 200, 400, and 800 g a.s./ha in a volume of 200 L water/ha. These rates are equivalent to 333.3, 666.7, 1333.3, 2666.7, and 5333.3 mL product/ha.

3. Observations

Assessments for mortalities (cumulative sum of dead and missing organisms) were carried out 3 and 7 days after treatment.

4. Statistics

Dunnett's Test (one-tailed, $\alpha = 0.05$): Analysis of mortality data for significance.

The LR₅₀ (lethal rate) was calculated by means of a normal logistic probit analysis with a goodness of fit of 0.163.

II. RESULTS AND DISCUSSION

A. FINDINGS

Juvenile mortality in the control and reference item group was 1.3 and 85.0%, respectively. All validation criteria were met or exceeded. Results of the rate response testing are given below:

Table 146
The effects on mortality of the predatory mite, *Typhlodromus pyri*, exposed to fresh-dried spray deposits of Indoxacarb 150 g/L EC in the laboratory

Test Item	Rate (g a.s./ha)	Mortality (%)	Corrected mortality (%) ^a
Untreated Control	-	1.3	-
Reference Item (dimethoate)	4.8	85.0	84.8
Indoxacarb 150 g/L EC	50	2.5	1.2
Indoxacarb 150 g/L EC	100	25.0 *	24.0
Indoxacarb 150 g/L EC	200	45.0 *	44.3
Indoxacarb 150 g/L EC	400	78.8 *	78.5
Indoxacarb 150 g/L EC	800	88.8 *	88.7

^a Corrected mortality according to Schneider-Orelli (1947)

* Statistically significantly different from the control (Dunnett's t-Test, one tailed, $p \leq 0.05$)

III. CONCLUSIONS

No statistically significant effects on mortality compared to the control were observed at the lowest rate of 50 g a.s./ha (333.3 mL product/ha).

The 7-day LR₅₀ of Indoxacarb 150 g/L EC was calculated to be 220.5 g a.s./ha (confidence limits: 254.3-191.0 g a.s./ha). This is equivalent to a 7-day LR₅₀ of 1470 mL product/ha (confidence limits: 1273-1695 mL product/ha).

(Warmers, C., 2006b)

RMS comment

Study submitted to the EU for the first time in this dossier.

The study is valid.

The 7-day LR₅₀ of Indoxacarb 150 g/L EC was calculated to be 220.5 g a.s./ha equivalent to a 7-day LR₅₀ of 1470 mL product/ha.

Report: Warmers, C. (2007a); Indoxacarb (DPX-KN128) 150 g/L EC: An extended laboratory rate response test to study the effects on the aphid parasitoid, *Aphidius rhopalosiphi* de Stefani Perez (Hymenoptera, Braconidae)

DuPont Report No.: DuPont-19445

Guidelines: Mead-Briggs et al (2000), Barrett et al (1994), Candolfi et al (2001) **Deviations:** None

Testing Facility: GAB Biotechnologie, GmbH, Niefern-Oeschelbronn, Germany

Testing Facility Report No.: 20061269/01-NEAp

GLP: Yes

Certifying Authority: Ministerium für Umwelt und Verkehr (Baden-Württemberg, Germany)

Executive summary:

Adult mortality and reproductive effects (parasitism rate) for *Aphidius rhopalosiphi* were evaluated in a dose-response test following a 48-hour exposure period to fresh-dried spray deposits of the test item, Indoxacarb 150g/L EC on detached treated apple leaves. The test comprised 4 replicates of 10 individuals (5 males and 5 females) for each test item rate, control and toxic reference item (10.0 mL dimethoate/ha). The test item was applied at 4.4, 10.0, 23.0, 52.5, and 120 g a.s./ha Indoxacarb 150 g/L EC in a volume equivalent to 200 L water/ha. These rates are equivalent to 29.3, 66.7, 153.3, 350, and 800 mL product/ha (corrected for content of active substance in the formulation). The LR₅₀ of Indoxacarb 150 g/L EC was calculated to be 74.2 g a.s./ha. This is equivalent to 494.7 mL product/ha. The reproduction ER₅₀ of Indoxacarb 150 g/L EC could not be calculated, because none of the rates tested caused a reduction of reproduction rate above 50%. The reproduction ER₅₀ of Indoxacarb 150 g/L EC can be assumed to be above 52.5 g a.s./ha (equivalent to 350 mL product/ha).

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Indoxacarb 150 g/L EC
 Lot/Batch #: DPX-KN128-150
 Purity: 150 g a.s./L
 CAS#: None for the formulation
 173584-44-6 for indoxacarb active substance
 Description: EC formulation, liquid/amber
 Stability of test compound in solution: 98.8% of the indoxacarb remains in the delivery vehicle after one hour under agitation
2. Control: Deionised water
 Test Vehicle: Deionised water
 Toxic reference item: Perfekthion (dimethoate a.s.)
3. Test organism
 Species: *Aphidius rhopalosiphi*
 Age at dosing: <48-hour old adults
 Source: Pupal wasps from commercial supplier (Katz Biotech AG)
 Acclimation period: 48 hours
 Diet: Honey-agar-water solution
 Water: Tap water, *ad libitum*
 Test chambers (exposure): Apple leaves were laid on a lower glass plates (length of edges: 13 cm). The lower glass plates with the apple leaves and an upper untreated glass plate served as upper and lower covers of the exposure cages.
 Test chambers (parasitation): Pots containing 5-10 day old barley seedlings enclosed with Plexiglas tubes (10 cm diameter; 25 cm height), covered with gauze
 Host Species: *Rhopalosiphum padi* (L.) Homoptera: Aphidae
 Test age: 2nd instar - adults
 Source: In-house laboratory culture
4. Environmental conditions
 Temperature: 9.0–22.5°C *
 Relative humidity: 60–88%
 Photoperiod (exposure): 16 hour photoperiod (1000-1300 lux)
 Photoperiod (parasitation period): 16 hour photoperiod (800-1100 lux)
 Photoperiod (after parasitation): 16 hour photoperiod (3000-5200 lux)

* Deviation: The temperature of the test chambers during the last few days of the reproductive phase of the test was outside the target range of $20 \pm 2^\circ\text{C}$. However, all validity criteria were met or exceeded and the controls behaved normally indicating that this deviation had no impact on the outcome of this study.

B. STUDY DESIGN AND METHODS

1. Experimental start/completed
 18-September-2006 to 03-October- 2006
2. Experimental treatments

In an extended laboratory study, parasitic wasps of the species *Aphidius rhopalosiphi* (Hymenoptera: Braconidae) were exposed to Indoxacarb 150 g/L EC. Adult mortality was evaluated in a rate response following a 48-hour exposure period to dried residues of the test item on detached treated apple leaves. The test was comprised of 4 replicates of 10 individuals. For the control, the toxic reference item (4.0 g dimethoate/ha) and each test item group, 5 males and 5 females were introduced. The test item was applied at 4.4, 10.0, 23.0, 52.5, and 120 g a.s./ha Indoxacarb 150 g/L EC in a

volume equivalent to 200 L water/ha. These rates are equivalent to 29.3, 66.7, 153.3, 350, and 800 mL product/ha (corrected for content of active substance in the formulation).

Reproduction (parasitisation rate) was evaluated by transferring 17 living female wasps from treatments with corrected mortality $\leq 50\%$ to individual test chambers containing 10 to 40 barley seedlings infested with >100 adult nymphal aphids (*Rhopalosiphum padi*). Each treatment level was comprised of 11 to 17 replicates of single female wasps. After a 24-hour parasitisation period the wasps were discarded and the plants were held for an additional eleven to twelve days. At this time the number of parasitised aphids (aphid mummies) per female wasp was determined.

3. Observations

Assessments for adult wasp mortality were carried out approximately 1, 2, 24, and 48 hours after treatment. Reproduction/fecundity was evaluated as the number of aphid mummies 11 to 12 days after the 24 hour parasitisation period.

4. Statistics

Dunnett's t-Test ($\alpha = 0.05$): Analysis of mortality data for significance.

Bonferroni U-Test ($\alpha = 0.05$): Analysis of reproduction data for significance.

For determining LR_{50} the moving averages procedure computing over 3 values (Thompson, 1947) was used. Due to the results of the test, no ER_{50} could be determined, as no reduction in reproduction $>50\%$ was observed.

II. RESULTS AND DISCUSSION

A. FINDINGS

Adult mortality in the control and the toxic reference item groups were 7.5 and 100.0%, respectively. All validation criteria were met or exceeded. Results of the dose response testing are given in Table 147.

Table 147
The effects on mortality and reproduction of the aphid parasitoid, *Aphidius rhopalosiphii*, exposed to fresh dried residue of Indoxacarb 150 g/L EC on apple leaves in the laboratory

Test Item	Rate (g a.s./ha)	Mortality (%)	Corrected Mortality (%) ^a	Parasitized aphid/female (mean \pm S.D.)	Reduction in Reproduction (%)
Untreated Control	-	-	-	11.7 \pm 9.6	-
Toxic reference item (dimethoate)	10.0 mL (product/ha)	100.0	100.0	n.a.	n.a.
Indoxacarb 150 g/L EC	4.4	0.0	-8.1	13.7 \pm 12.6	-17.1
Indoxacarb 150 g/L EC	10.0	12.5	5.4	13.5 \pm 8.9	-15.4
Indoxacarb 150 g/L EC	23.0	15.0	8.1	19.9 \pm 11.3	-70.1
Indoxacarb 150 g/L EC	52.5	47.5 *	43.2	6.0 \pm 7.3	48.7
Indoxacarb 150 g/L EC	120	72.5 *	70.3	n.a.	n.a.

^a Schneider-Orelli's Correction

* statistically significant difference to control (Dunnett's t-Test, one-tailed, $p \leq 0.05$)

SD standard deviation

n.a. not assessed

III. CONCLUSIONS

The LR₅₀ of Indoxacarb 150 g/L EC was calculated to be 74.2 g a.s./ha (95% confidence limits: 45.2-121.8 g a.s./ha). This is equivalent to 494.7 mL product/ha (95% confidence limits: 301.3- 812.0 mL product/ha).

Indoxacarb 150 g/L EC, at rates up to 52.5 g a.s./ha (equivalent to 350 mL product/ha), caused no statistically significant effect on the reproduction of *A. rhopalosiphi* when compared to the controls. The reproduction ER₅₀ of Indoxacarb 150 g/L EC could not be calculated because none of the rates tested caused a reduction of reproduction rate above 50%. The reproduction ER₅₀ of Indoxacarb 150 g/L EC can be assumed to be above 52.5 g a.s./ha (equivalent to 350 mL product/ha).

(Warmers, C., 2007a)

RMS comment

Study submitted to the EU for the first time in this dossier.

The study is valid.

The LR₅₀ of Indoxacarb 150 g/L EC was calculated to be 74.2 g a.s./ha equivalent to 494.7 mL product/ha.

Indoxacarb 150 g/L EC, at rates up to 52.5 g a.s./ha (equivalent to 350 mL product/ha), caused no statistically significant effect on the reproduction of *A. rhopalosiphi* when compared to the controls. The reproduction ER₅₀ of Indoxacarb 150 g/L EC could not be calculated because none of the rates tested caused a reduction of reproduction rate above 50%. RMS notes however that the real ER₅₀ must be near this dose rate as 48.7% effects were observed at 52.5 g a.s./ha (equivalent to 350 mL product/ha).

ER₅₀ of Indoxacarb 150 g/L EC was \geq 52.5 g a.s./ha equivalent to 350 mL product/ha.

Report: Warmers, C. (2007b); Indoxacarb (DPX-KN128) 150 g/L EC: An extended laboratory rate response test to study the effects on the green lacewing *Chrysoperla carnea* Steph. (Neuroptera, Chrysopidae)

DuPont Report No.: DuPont-19446

Guidelines: Vogt et al (2000), Barrett et al (1994), Candolfi et al (2001) **Deviations:** None

Testing Facility: GAB Biotechnologie, GmbH, Niefern-Oeschelbronn, Germany

Testing Facility Report No.: 20061269/01-NECc

GLP: Yes

Certifying Authority: Ministerium für Umwelt und Verkehr (Baden-Württemberg, Germany)

Executive summary:

The effects of Indoxacarb 150 g/L EC on mortality and reproduction (fecundity and fertility) on the green lacewing *Chrysoperla carnea*, were evaluated in a rate response test following at least a 6-11-day exposure period to fresh dried spray deposits of the test item on treated apple leaves. The test comprised 30 replicates of one individual for the control, for each treatment group and for the toxic reference (70 mL dimethoate/ha). The test item was applied at 6.9, 17.25, 43.2, 108, and 270.0 g a.s./ha Indoxacarb 150 g/L EC in a volume equivalent to 200 L water/ha. These rates are equivalent to 46, 115, 288, 720, and 1800 mL product/ha (corrected for the nominal content of active substance in the formulation). The LR₅₀ of Indoxacarb 150 g/L EC was calculated to be 29.8 g a.s./ha (95% confidence limits: 25.7 to 34.4 g a.s./ha). This is equivalent to 198.7 mL product/ha (95% confidence limits: 171.3 to 229.3 mL product/ha). Treatment-related effects were observed on fecundity of *Chrysoperla carnea* up to and including 17.25 g a.s./ha. The number of eggs/female/day in the 6.9 and 17.25 g a.s./ha Indoxacarb 150 g/L EC treatment groups were reduced compared to controls, 37.7 and 67.4%,

respectively. However, only slight effects were observed on fertility (hatching rate) of *Chrysoperla carnea* 1.3 and 11.4% reduction compared to controls for the 6.9 and 17.25 g a.s./ha treatments, respectively.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Indoxacarb 150 g/L EC
 Lot/Batch #: DPX-KN128-150
 Purity: 150 g a.s./L
 CAS#: None for the formulation
 173584-44-6 for indoxacarb active substance
 Description: EC formulation, liquid
 Stability of test compound in solution: Not determined in the test system
2. Control: Deionised water
 Test Vehicle: Deionised water
 Toxic reference item: Perfekthion (dimethoate a.s.)
3. Test organism
 Species: *Chrysoperla carnea*
 Age at dosing: 3 day old larvae
 Source: Larvae from commercial supplier (Sautter & Stepper)
 Acclimatisation period: 7 days (delivered as eggs; until hatching of larvae)
 Diet (during exposure): Eggs of *Sitotroga cerealella*
 Diet (during pre-reproduction and reproduction): Artificial diet (mixture of: 15 mL condensed milk, 1 egg, 1 egg yolk, 30 g honey, 20 g fructose, 30 g brewer's yeast, 50 g wheat germ and approximately 45 mL deionised water)
 Water: Tap water, *ad libitum*
 Exposure units: Treated apple leaves were placed on top of a wet cotton pad in a petri dish (diameter: 9 cm). A ring (acrylic glass: approx. 3.5 cm diameter, 2.5-3.5 cm height) with the inner surface covered by Dyneon® (formulation of Polytetrafluoroethylene), was attached to the surface of a leaf, providing a barrier to prevent *Chrysoperla carnea* from escaping. The ring was tightly fixed to the leaf with the help of rubber bands.
 Reproduction units: Untreated white plastic vessels (17 cm × 12.5 cm × 6 cm), containing artificial diet and water, covered by a lid of gauze
4. Environmental conditions
 Temperature: 23.0–26.5°C
 Relative humidity: 60–80%
 Photoperiod (exposure): 16 hour photoperiod; 1500-2000 lux

B. STUDY DESIGN AND METHODS

1. Experimental start/completed
 27-June-2006 to 04-October-2006
2. Experimental treatments
 The mortality and reproductive effects (fecundity and fertility) on *Chrysoperla carnea* were evaluated in an extended laboratory rate response test following at least an 11-day exposure period to fresh dried spray deposits of the test item on treated apple leaves. The test comprised 30 replicates of 1 individual for the control, for each treatment group and for the toxic reference (70 mL dimethoate/ha). The test item was applied at 6.9, 17.25, 43.2, 108.0, and 270.0 g a.s./ha Indoxacarb 150 g/L EC in a volume equivalent to 200 L water/ha. These rates are equivalent to 46, 115, 288,

720, and 1800 mL product/ha (corrected for the nominal content of active substance in the formulation).

3. Observations

The survival and development of the larvae was recorded at intervals of 1 to 3 days until pupation was completed and adults emerged.

After a 15 day period in pre-reproduction boxes, reproduction (fecundity and fertility) was evaluated 8 days after the first egg batch was observed.

4. Statistics

Fisher's Exact Test ($\alpha = 0.05$): Analysis of mortality data for significance.

The LR_{50} of Indoxacarb 150 g/L EC was calculated using the moving average procedure after Thompson (1947).

II. RESULTS AND DISCUSSION

A. FINDINGS

The mortality in the control and the toxic reference item groups was 13.3 and 100.0%, respectively. All validation criteria were met or exceeded. Results of the extended laboratory test are given below.

Table 148
The effects on mortality and reproduction (fecundity and fertility) of the green lacewing, *Chrysoperla carnea*, exposed to fresh dried spray deposits of Indoxacarb 150 g/L EC in the laboratory on apple leaves

Test Item	Rate (g a.s./ha)	Mortality (%) ^a	Corrected mortality (%) ^b	Fecundity [eggs/ female/day]	Reduction Fecundity (%) ^c	Fertility [hatching rate %]	Reduction Fertility (%) ^c
Untreated Control	-	13.3	-	21.5	-	94.1	-
Toxic reference item (dimethoate)	70.0 mL (product/ha)	100.0 *	100.0	n.a.	n.a.	n.a.	n.a.
Indoxacarb 150 g/L EC	6.9	13.3	0.0	13.4	37.7	92.9	1.3
Indoxacarb 150 g/L EC	17.25	13.3	0.0	7.0	67.4	83.4	11.4
Indoxacarb 150 g/L EC	43.2	93.3 *	92.3	n.a.	n.a.	n.a.	n.a.
Indoxacarb 150 g/L EC	108.0	96.7 *	96.2	n.a.	n.a.	n.a.	n.a.
Indoxacarb 150 g/L EC	270.0	100.0 *	100.0	n.a.	n.a.	n.a.	n.a.

^a Mortality based on the number of dead larvae and the no. of not emerged pupae and adults, which died during emergence.

^b Schneider-Orelli's Correction

^c Abbott's Correction

* Statistically significant difference to control (Fisher's Exact Test, one sided, $p \leq 0.05$)

SD standard deviation

n.a. not assessed

III. CONCLUSIONS

Indoxacarb 150 g/L EC, at rates up to 17.25 g a.s./ha (equivalent to 115 mL product/ha), caused no statistically significant increase in mortality of *Chrysoperla carnea* when compared to the control. The LR₅₀ of Indoxacarb 150 g/L EC was calculated to be 29.8 g a.s./ha (95% confidence limits: 25.7 to 34.4 g a.s./ha). This is equivalent to 198.7 mL product/ha (95% confidence limits: 171.3 to 229.3 mL product/ha). Treatment-related effects were observed on fecundity of *Chrysoperla carnea* as the number of eggs/female/day in the 6.9 and 17.25 g a.s./ha Indoxacarb 150 g/L EC treatment groups, were reduced compared to the controls by 37.7 and 67.4%, respectively. Slight treatment-related effects were observed on fertility (hatching) of *Chrysoperla carnea* (1.3 and 11.4% reduction compared to controls, respectively).

(Warmers, C., 2007b)

RMS comment

Study submitted to the EU for the first time in this dossier.

The study is valid.

The LR₅₀ of Indoxacarb 150 g/L EC was calculated to be 29.8 g a.s./ha equivalent to 198.7 mL product/ha. Treatment-related effects were observed on fecundity of *Chrysoperla carnea* as the number of eggs/female/day in the 6.9 and 17.25 g a.s./ha Indoxacarb 150 g/L EC treatment groups, were reduced compared to the controls by 37.7 and 67.4%, respectively. Slight treatment-related effects were observed on fertility (hatching) of *Chrysoperla carnea* (1.3 and 11.4% reduction compared to controls, respectively).

ER₅₀ of Indoxacarb 150 g/L EC was > 6.9 g a.s./ha equivalent to 46 mL product/ha.

Report: Adelberger, I. (2007); Indoxacarb (DPX-KN128) 150 g/L EC: An extended laboratory test with field-aged spray deposits to study the effects on the aphid parasitoid, *Aphidius rhopalosiphi* de Stefani Perez (Hymenoptera, Braconidae)

DuPont Report No.: DuPont-21947

Guidelines: Mead-Briggs *et al* (2000), Barrett *et al* (1994), Candolfi *et al* (2001) **Deviations:** None

Testing Facility: GAB Biotechnologie, GmbH, Niefern-Oeschelbronn, Germany

Testing Facility Report No.: 20071084/01-NEAp

GLP: Yes

Certifying Authority: Ministerium für Umwelt und Verkehr (Baden-Württemberg, Germany)

Executive summary:

Adult mortality and reproductive effects (parasitism rate) for *Aphidius rhopalosiphi* were evaluated in an aging test following a 48-hour exposure period to freshly applied and aged spray deposits of Indoxacarb 150 g/L EC on detached apple leaves. The study included 4 bioassays carried out with foliage collected at increasing intervals after the application to determine the rate of decline of any residual toxicity found. The test item was applied 4 times with a spray interval of 9-11 days at 100 g a.s./ha to potted apple trees under field conditions using a spray volume of 1500 L water/ha. The first two bioassays were conducted immediately after the 1st and the 4th application. Bioassay 3 and 4 were performed 28 and 56 days after the 4th application.

For the first 24 hours after each application the potted apple trees were protected from rain. Afterwards, the treated trees were allowed to weather under field conditions at the outdoor area of the testing facility. Tap water was applied as control. For the first bioassay Perfekthion (nominal content of dimethoate: 400 g/L) was applied at 80.0 g dimethoate/ha as a reference item treatment to potted apple trees. For the following bioassays the

reference item was applied to detached leaves with a track sprayer in the laboratory using 4.0 g a.s./ha. Each bioassay was comprised of 4 replicates of 5 female and 5 male wasps for each treatment and control.

Spray deposits of Indoxacarb 150 g/L EC, applied once at 100 g a.s./ha under field conditions, caused high mortality (97.5%) among *Aphidius rhopalosiphi* wasps when exposed on the day of application to fresh, dried spray deposits.

High effects on mortality (100%) were also observed following the 4th application of 100 g a.s./ha to fresh, dried spray deposits.

After ageing of the spray deposits for 28 days (outdoors without rain protection), a clear decline in the level of mortality was observed. The mortality conclusively passed the 50% threshold (less than 50% corrected mortality) with a corrected mortality of 13.2%, whereas the reduction of reproduction was slightly above the threshold of 50% (53.8%).

The 50% threshold for reproduction was passed in bioassay 4 (after 56 days of aging) with a reduction of reproduction of 37.6%.

I. MATERIALS AND METHODS

A. MATERIALS

- | | |
|---|--|
| 1. Test material: | Indoxacarb 150 g/L EC |
| Lot/Batch #: | DPX-KN128-206 |
| Purity: | 150 g a.s./L |
| CAS#: | None for the formulation
173584-44-6 for indoxacarb active substance |
| Description: | Liquid |
| Stability of test compound in solution: | Not measured in the test system |
| 2. Control: | Tap water |
| Test Vehicle: | Tap water |
| Toxic reference item: | Perfekthion (dimethoate 400 g a.s./L nominal) |
| 3. Test organism | |
| Species: | <i>Aphidius rhopalosiphi</i> |
| Age at dosing: | <48-hour old adults |
| Source: | Pupal wasps were obtained from a commercial supplier (Katz Biotech AG, An der Birkenpfuhlheide 10, D-15837 Baruth, Germany). |
| Acclimation period: | 48 hours |
| Diet: | Honey-agar-water solution |
| Water: | Tap water, <i>ad libitum</i> |
| Test chambers (exposure): | Apple leaves were laid on a lower glass plates (length of edges: 13 cm). The lower glass plates with the apple leaves and an upper untreated glass plate served as upper and lower covers of the exposure cages. |
| Test chambers (parasitation): | Pots containing 8 day old barley seedlings enclosed with Plexiglas tubes (10 cm diameter; 25 cm height), covered with gauze |
| Host Species: | <i>Rhopalosiphum padi</i> (L.) Homoptera: Aphidae |
| Test age: | 2 nd instar - adults |
| Source: | In-house laboratory culture |

4. Environmental conditions

Temperature:

Target: $20 \pm 2^\circ\text{C}$, actually recorded:

Bioassay 1: 19.0–20.5°C (min-max)

Bioassay 2: 19.0–22.0°C (min-max)

Bioassay 3: 17.5* –22.0°C (min-max)

Bioassay 4: 18.5–21.5°C (min-max)

Relative humidity:

Target: 60–90%, actually recorded:

Bioassay 1: 76–81% (min-max)

Bioassay 2: 66–75% (min-max)

Bioassay 3: 58** –74% (min-max)

Bioassay 4: 64–86% (min-max)

Light intensity:

Target: 400–3000 lux during exposure and 24 h parasitisation (>4000 lux after parasitisation); recorded:

Bioassay 1: Exposure: 950–1100 lux

Reproduction not assessed

Bioassay 2: Exposure: 1000–1300 lux

Reproduction not assessed

Bioassay 3: Exposure: 460–600 lux

24 h Parasitisation: 460–800 lux
(remaining period: 5200–6500 lux)

Bioassay 4: Exposure: 950 – 1100 lux

24 h Parasitisation: 900–1050 lux
(remaining period: 7000–8000 lux)* Deviation: The temperature during bioassay 3 was outside the target range of $20 \pm 2^\circ\text{C}$ for a few hours.

** Relative humidity < 60% .Deviations <2 hours.

However, all validity criteria were met or exceeded and the controls behaved normally indicating that this deviation had no impact on the outcome of this study.

B. STUDY DESIGN AND METHODS

1. Experimental start/completed

22-May-2007 to 28-August-2007

2. Experimental treatments

Adult mortality and reproductive effects (parasitism rate) for *Aphidius rhopalosiphi* were evaluated in an aging test following a 48-hour exposure period to freshly applied and aged spray deposits of Indoxacarb 150 g/L EC on detached apple leaves. The study included 4 bioassays carried out with foliage collected at increasing intervals after the application to determine the rate of decline of any residual toxicity found. The test item was applied 4 times with a spray interval of 9–11 days at 100 g a.s./ha to potted apple trees under field conditions using a spray volume of 1500 L water/ha. Tap water was applied as a control. For the 1st bioassay, Perfekthion (nominal content of dimethoate: 400 g/L) was applied at 80.0 g dimethoate/ha as a reference item treatment to potted apple trees (1500 L water/ha). For the following bioassays the reference item was applied to detached leaves with a track sprayer in the laboratory using 4.0 g a.s./ha (200 L water/ha). Bioassays were carried out directly after 1st application of the test item, and on the day of the 4th application and 28 days and 56 days after the 4th application. Each bioassay comprised 4 replicates with 5 female and 5 male wasps for each treatment and control. Reproduction (parasitisation rate) was evaluated at bioassay 3 and 4 by transferring 17 living female wasps from the control groups and 13–17 female survivors of the test item treatments individually to test chambers containing 10 to 13 barley seedlings infested with >100 adult and nymphal aphids (*Rhopalosiphum padi*). After a 24-hour parasitisation period the wasps were discarded and the plants were held for an additional 11 days. After this time the number of parasitised aphids (aphid mummies) per female wasp was determined.

3. Observations during each bioassay

Assessments for adult wasp mortality were carried out approximately 1, 2, 24, and 48 hours after treatment. Reproduction/fecundity was evaluated as the number of aphid mummies 12 days after the 24-hour parasitism period.

4. Statistics

Fisher's Exact Test ($\alpha = 0.05$): Analysis of mortality data for significance.

Dunnett's t-Test ($\alpha = 0.05$; one-tailed): Analysis of reproduction data for significance.

All statistical calculations were performed using the statistic program SAS release 9.1.3, service pack 4 (SAS Institute Inc., Ed. 2002-2003).

II. RESULTS AND DISCUSSION

A. FINDINGS

Adult mortality in the controls was between 0.0 and 5.0% for bioassay 1-4. The reference item caused a mortality of 100% in all four bioassays. All validation criteria were met or exceeded. Results of the aging study are given in Table 149.

Table 149

The effects on mortality and reproduction of the aphid parasitoid, *Aphidius rhopalosiphi*, exposed to fresh-dried spray deposits and aged spray deposits of Indoxacarb 150 g/L EC on detached apple leaves in the laboratory

Bioassays	Test Item	Rate (g a.s./ha)	Mortality (%)	Corrected Mortality (%) ^a	Parasitized aphids/female (n)	Reduction in Reproduction (%)
1 Exposure on the day of the 1 st application	Control	-	0.0	-	n.a.	-
	Reference item	80.0	100.0*	100.0	n.a.	-
	Indoxacarb 150 g/L EC	1 × 100	97.5*	97.5	n.a.	-
2 Exposure on the day of the 4 th application	Control	-	2.5	-	n.a.	-
	Reference item ^b	4.0	100.0*	100.0	n.a.	-
	Indoxacarb 150 g/L EC	4 × 100	100.0*	100.0	n.a.	-
3 Exposure following a period of 28 days of aging under field conditions	Control	-	5.0	-	6.5	-
	Reference item ^b	4.0	100.0*	100.0	n.a.	-
	Indoxacarb 150 g/L EC	4 × 100	17.5	13.2	3.0**	53.8
4 Exposure following a period of 56 days of aging under field conditions	Control	-	0.0	-	24.2	-
	Reference item ^b	4.0	100.0*	100.0	n.a.	-
	Indoxacarb 150 g/L EC	4 × 100	2.5	2.5	15.1**	37.6

^a Schneider-Orelli's Correction

^b Reference item applied with a laboratory track sprayer

*: Statistically significant different to control (Fisher's Exact Test, one-tailed, $\alpha = 0.05$)

** : Statistically significant different to control (t-Test, pooled, one-tailed, $\alpha = 0.05$)

n.a.: not assessed due to >50% mortality

III. CONCLUSIONS

Indoxacarb 150 g/L EC applied once at 100 g a.s./ha under field conditions caused high mortality (97.5%) among *Aphidius rhopalosiphi* wasps when exposed on the day of application to fresh, dried spray deposits.

High effects on mortality (100%) were also observed following the 4th application of 100 g a.s./ha to fresh, dried spray deposits.

After ageing of the spray deposits for 28 days (outdoors without rain protection), a clear decline in the level of mortality was observed. The mortality conclusively passed the 50% threshold (less than 50% corrected mortality) with a corrected mortality of 13.2%, whereas the reduction of reproduction was slightly above the threshold of 50% (53.8%).

The 50% threshold for reproduction was passed in bioassay 4 (after 56 days of aging) with a reduction of reproduction of 37.6%.

(Adelberger, I., 2007)

RMS comment

Study submitted to the EU for the first time in this dossier.

The study is valid.

Indoxacarb 150 g/L EC applied once at 100 g a.s./ha under field conditions caused high mortality (97.5%) among *Aphidius rhopalosiphi* wasps when exposed on the day of application to fresh, dried spray deposits. High effects on mortality (100%) were also observed following the 4th application of 100 g a.s./ha to fresh, dried spray deposits.

After ageing of the spray deposits for 28 days (outdoors without rain protection), a clear decline in the level of mortality was observed. The mortality conclusively passed the 50% threshold (less than 50% corrected mortality) with a corrected mortality of 13.2%, whereas the reduction of reproduction was slightly above the threshold of 50% (53.8%).

The 50% threshold for reproduction was passed in bioassay 4 (after 56 days of aging) with a reduction of reproduction of 37.6%.

Effects of Indoxacarb 150 g/L EC are < 50% at 4 x 100 g a.s./ha (equivalent to 46 mL product/ha) after 56 days of aging.

Report: Klug, T. (2007); Indoxacarb (DPX-KN128) 150 g/L EC: An extended laboratory test with field-aged spray deposits to study the effects on the green lacewing *Chrysoperla carnea* Steph. (Neuroptera, Chrysopidae)

DuPont Report No.: DuPont-21946

Guidelines: Vogt *et al* (2000), Barrett *et al* (1994), Candolfi *et al* (2001) **Deviations:** None

Testing Facility: GAB Biotechnologie, GmbH, Niefern-Oeschelbronn, Germany

Testing Facility Report No.: 20071084/01-NECc

GLP: Yes

Certifying Authority: Ministerium für Umwelt und Verkehr (Baden-Württemberg, Germany)

Executive summary:

The effects of Indoxacarb 150 g/L EC on mortality and reproduction (fecundity and fertility) on the green lacewing *Chrysoperla carnea* were evaluated in an ageing test with field sprayed deposits of the test item on apple leaves. The test comprised 50 replicates of one individual for the control, for the test item and for the toxic reference group. The test item was applied four times at a rate of 100 g a.s./ha in a volume equivalent to 1500 L water/ha. The reference item (dimethoate) was applied four times at a rate of 80 g a.s./ha at the first application in the field and with 28 g a.s./ha at the following applications in the laboratory. The corrected mortality of Indoxacarb 150 g/L EC on *Chrysoperla carnea* directly after first field application of 100 g a.s./ha was 94.7%. This bioassay did not meet the validity criteria because mortality in the control slightly exceeded the validity level of 20% but was nevertheless evaluated. The mortality in the toxic standard was 90%, indicating that the test organisms used in this study were sufficiently sensitive. Reproduction was not assessed at this rate because mortality in the test item group exceeded the trigger value of 50%. In bioassay 2 (started on the day of the fourth application), Indoxacarb 150 g/L EC caused significant mortality when compared to the control. Corrected mortality was 94.0%. No reproduction assessment was conducted because of the high mortality in the test item group. In the bioassay 3 (28 days after fourth application), no effects were observed on mortality, fecundity, or fertility compared to the control.

I MATERIALS AND METHODS

A. MATERIALS

1. Test material: Indoxacarb 150 g/L EC
 Lot/Batch #: DPX-KN128-206
 Purity: 150 g a.s./L
 CAS#: None for the formulation
 173584-44-6 for indoxacarb active substance
 Description: Liquid
 Stability of test compound in solution: Not measured in the test system
2. Control: Tap water
 Test Vehicle: Tap water
 Toxic reference item: Perfekthion (dimethoate a.s.)
3. Test organism
 Species: *Chrysoperla carnea*
 Age at dosing: 3 day old larvae
 Source: larvae from commercial supplier (Sauter & Stepper, Katz)
 Acclimatisation period: 7 days (delivered as eggs; until hatching of larvae)
 Diet (during exposure): Eggs of *Sitotroga cerealella*

 Diet (during pre-reproduction and reproduction): Artificial diet (mixture of: 15 mL condensed milk, 1 egg, 1 egg yolk, 30 g honey, 20 g fructose, 30 g brewer's yeast, 50 g wheat germ and approximately 45 mL deionized water)
 Water: Tap water, *ad libitum*
 Exposure units: Treated apple leaves were placed on top of a wet cotton pad in a petri dish (diameter: 9 cm). A ring (acrylic glass: approximately 3.5 cm diameter, 2.5 - 3.5 cm height) with the inner surface covered by Dyneon[®] (formulation of Polytetrafluoroethylene), was attached to the surface of a leaf, providing a barrier to prevent *Chrysoperla carnea* from escaping. The ring was tightly fixed to the leaf with the help of rubber bands.
 Reproduction units: Untreated white plastic vessels (17 cm × 12.5 cm × 6 cm), containing artificial diet and water, covered by a lid of gauze
4. Environmental conditions
 Temperature: 22.0–26.5°C (min. and max. temperature during all bioassays)
 Relative humidity: 56–87% (min. and max. humidity during all bioassays)
 Photoperiod (exposure): 16 hour photoperiod; 1850-2800 lux

B. STUDY DESIGN AND METHODS

1. Experimental start/completed
 22-May-2007 to 22-August-2007

2. Experimental treatments

The mortality and reproductive effects (fecundity and fertility) on *Chrysoperla carnea* were evaluated in an extended laboratory test with field-aged spray deposits of the test item on treated apple leaves. The test comprised 50 replicates of one individual for control, each treatment group, and toxic reference (70 mL dimethoate/ha). The test item was applied four times at 100g a.s./ha Indoxacarb 150 g/L EC in a volume equivalent to 1500 L water/ha. Bioassays were carried out directly after first application of the test item, on the day of the fourth application and 28 days after fourth application.

3. Observations

The survival and development of the larvae was recorded at intervals of 1 to 3 days until pupation was completed and adults emerged.

A reproduction assessment was carried out for the third bioassay (started 28 days after fourth application). After a 13 day period in pre-reproduction boxes, reproduction (fecundity and fertility) was evaluated 8 days after the first egg batch was observed.

4. Statistics

Fisher's Exact Test ($\alpha = 0.05$): Analysis of mortality data for significance.

II. RESULTS AND DISCUSSION

A. FINDINGS

Results of the extended laboratory test are given in Table 150.

Table 150
The effects on mortality and reproduction (fecundity and fertility) of the green lacewing, *Chrysoperla carnea*, exposed to fresh dried spray deposits of Indoxacarb 150 g/L EC on leaves of treated apple trees

Test Item	Bio-assay	Rate (g a.s./ha)	Mortality (%)	Corrected mortality (%) ^a	Fecundity (eggs/female/day)	Fertility [hatching rate] (%)
Untreated Control	1	-	22.4	-	n.a.	n.a.
Untreated Control	2	-	0.0	-	n.a.	n.a.
Untreated Control	3	-	4.1	-	20.33	97.17
Toxic reference item (dimethoate)	1	80.0	90.0	87.1	n.a.	n.a.
Toxic reference item (dimethoate)	2	28.0	96.0	96.0	n.a.	n.a.
Toxic reference item (dimethoate)	3	28.0	95.9	95.8	n.a.	n.a.
Indoxacarb 150 g/L EC	1	1 × 100	95.9	94.7	n.a.	n.a.
Indoxacarb 150 g/L EC	2	4 × 100	94.0	94.0	n.a.	n.a.
Indoxacarb 150 g/L EC	3	4 × 100	12.5	8.8	22.80	97.23

^a Schneider-Orelli's Correction

n.a.: not assessed

III. CONCLUSIONS

The corrected mortality of Indoxacarb 150 g/L EC on *Chrysoperla carnea* directly after first field application at 100 g a.s./ha on apple leaves was 94.7%. This bioassay did not meet the validity criteria, because mortality in the control slightly exceeded the validity level of 20%, but was nevertheless evaluated. The corrected mortality in the toxic standard was 87.1% indicating that the test organisms used in this study were sufficiently sensitive. Reproduction was not assessed at this rate, because mortality in the test item group exceeded the trigger value of 50%.

In bioassay 2 (started on the day of the fourth application) Indoxacarb 150 g/L EC caused significant mortality effects when compared to the control. A corrected mortality of 94.0% was recorded. No reproduction assessment was conducted, because of the high mortality in the test item group.

In the third bioassay (28 days after fourth application), no effects were observed neither on mortality nor on fecundity or fertility compared to the control. It may be concluded that Indoxacarb 150 g/L EC when applied up to 4-times at 100 g a.s./ha, has only a short-term effect on *Chrysoperla carnea*.

(Klug, T., 2007)

RMS comment

Study submitted to the EU for the first time in this dossier.

The bioassay 1 is not valid according to validity criteria (mortality > 20% in control) but this bioassay is not the most relevant in this test. Bioassays 2 and 3 are valid.

The test item was applied four times at a rate of 100 g a.s./ha.

In bioassay 2 (started on the day of the fourth application) Indoxacarb 150 g/L EC caused significant mortality effects when compared to the control. A corrected mortality of 94.0% was recorded. No reproduction assessment was conducted, because of the high mortality in the test item group.

In the third bioassay (28 days after fourth application), no effects were observed neither on mortality nor on fecundity or fertility compared to the control. It may be concluded that Indoxacarb 150 g/L EC when applied up to 4-times at 100 g a.s./ha, has only a short-term effect on *Chrysoperla carnea*.

Effects of Indoxacarb 150 g/L EC are < 50% at 4 x 100 g a.s./ha after 28 days of aging.

Report: Warmers, C. (2007c); Indoxacarb (DPX-KN128) 150 g/L EC: An extended laboratory test with field-aged spray deposits to study the effects on the predatory bug *Orius laevigatus* Fieber (Heteroptera, Anthocoridae)

DuPont Report No.: DuPont-22391

Guidelines: Bakker et al (2000), Barrett et al (1994), Candolfi et al (2001) **Deviations:** None

Testing Facility: GAB Biotechnologie, GmbH, Niefern-Oeschelbronn, Germany

Testing Facility Report No.: 20071084/01-NEOr

GLP: Yes

Certifying Authority: Ministerium für Umwelt und Verkehr (Baden-Württemberg, Germany)

Executive summary:

The effects of Indoxacarb 150 g/L EC on adult mortality and reproduction (parasitism rate) of the predatory bug *Orius laevigatus*, were evaluated in an aging test following a 9-day exposure period to freshly applied and aged spray deposits on detached apple leaves. The study included 2 bioassays carried out with foliage collected after the 1st and 4th application. The test item was applied 4 times with a spray interval of 9-11 days at 100 g a.s./ha to

potted apple trees under field conditions using a spray volume of 1500 L water/ha. The bioassays were conducted immediately after the 1st and the 4th application. For the first 24 hours after each application the potted apple trees were protected from rain. Afterwards, the treated trees were allowed to weather under field conditions at the outdoor area of the testing facility. Tap water was applied as a control. For the 1st bioassay, Perfekthion (nominal content of dimethoate: 400 g/L) was applied at 80.0 g dimethoate/ha as a reference item treatment to potted apple trees. For the 2nd bioassays, the reference item was applied to detached leaves with a track sprayer in the laboratory using 12.0 g dimethoate/ha, (nominal). The exposure was conducted under laboratory conditions with 2nd instar larvae of *Orius laevigatus* at both bioassays obtained as eggs from a commercial supplier. Fecundity assessments were carried out twice (on bean leaf discs) for both bioassays after the bugs had become adult and they had the possibility to mate in boxes with approximately 5 females and 3 males for 4 days. Single females (up to 20 per treatment group) had two laying opportunities for 2 days each on bean leaf discs. The hatching of the eggs from the first set of bean leaf discs was assessed.

I. MATERIALS AND METHODS

A. MATERIALS

- | | | |
|----|-------------------------------|---|
| 1. | Test material: | Indoxacarb 150 g/L EC |
| | Lot/Batch #: | DPX-KN128-206 |
| | Purity: | 150 g a.s./L |
| | CAS#: | None for the formulation |
| | | 173584-44-6 for indoxacarb the active substance |
| | Description: | Liquid |
| | Stability of test compound: | not measured under the test conditions |
| 2. | Control: | Tap water |
| | Test Vehicle: | Tap water |
| | Toxic reference item: | Perfekthion (dimethoate a.s.) |
| 3. | Test organism | |
| | Species: | <i>Orius laevigatus</i> Fieber |
| | Age at dosing: | 4 days |
| | Source: | Syngenta Bioline Production Ltd. (Telstar Nursery, Holland Road, GB-Little Clacton, Essex, England) |
| | Acclimation period: | 5 days prior to test start |
| | Feeding: | Eggs of <i>Ephestia</i> sp. |
| | Test chambers (exposure): | cell cultivate microtiter plates; a treated dwarf bean leave disc is fixed on the surface of an agar bacterial solution |
| | Test chambers (reproduction): | Same as exposure, but discs of untreated dwarf bean leaves were offered as oviposition substrate |
| | Test age: | 2 nd instar nymphs |
| 4. | Environmental conditions | |
| | Temperature: | 23.5–27.0°C (min. and max. temperature during all bioassays) |
| | Relative humidity: | 56–87% (min. and max. humidity during all bioassays) |
| | Photoperiod (exposure): | 16 hour photoperiod; 1000-1500 lux (during both bioassays) |

B. STUDY DESIGN AND METHODS

1. Experimental start/completed
22-May-2007 to 10-July-2007

2. Experimental treatments

The mortality and the reproduction (fecundity and fertility) of the predatory bug *Orius laevigatus* were evaluated in an aging test following a 9 day exposure period (mortality) to freshly applied and aged spray deposits of Indoxacarb 150 g/L EC on detached apple leaves. The study included 2 bioassays carried out with foliage collected after the 1st and 4th application. The test item was applied 4 times with a spray interval of 9-11 days at 100 g a.s./ha to potted apple trees under field conditions using a spray volume of 1500 L water/ha. The bioassays were conducted immediately

after the 1st and the 4th application. For the first 24 hours after each application the potted apple trees were protected from rain. Afterwards, the treated trees were allowed to weather under field conditions at the outdoor area of the testing facility. Tap water was applied as control. For the 1st bioassay, Perfekthion (nominal content of dimethoate: 400 g/L) was applied at 80.0 g dimethoate/ha as a reference item treatment to potted apple trees. For the 2nd bioassay, the reference item was applied to detached leaves with a track sprayer in the laboratory using 12.0 g dimethoate/ha, (nominal).

3. Observations

Adult mortality and reproductive effects (fecundity and hatching) were evaluated in an aging test following a 48-hour exposure period to freshly applied and field-aged spray deposits of Indoxacarb 150 g/L EC on detached apple leaves. Assessments for mortality were carried out 4, 7, and 9 days after treatment. Fecundity assessments were carried out twice (on bean leaf discs) for both bioassays after the bugs had become adult and they had the possibility to mate in boxes with approximately 5 females and 3 males for 4 days. Single females (up to 20 per treatment group) were transferred to bean leaf discs as oviposition substrate for 2 days. After this period the females were offered a fresh oviposition substrate again for 2 additional days. The hatching of the eggs from the first set of bean leaf discs was assessed.

4. Statistics

Mortality data were analysed for significance using Fisher's Exact test, which is a distribution-free test and does not require testing for normality or homoscedascity prior to analysis (ZAR, 1999). In the 1st and 2nd bioassay, reproduction data did meet normality criteria and therefore t-Test was used to analyse reproduction data for significance (ZAR, 1999). Hatching data did not meet normality criteria and therefore Mann-Whitney U-Test was used to analyse reproduction data for significance (ZAR, 1999).

II. RESULTS AND DISCUSSION

A. FINDINGS

Adult mortality in the control groups was 10.0 and 16.3% for the 1st and 2nd bioassay, respectively. Adult mortality in the toxic reference item groups was 98.8 and 95.0% for the 1st and 2nd bioassay, respectively. Indoxacarb 150 g/L EC resulted in statistically significant increase in mortality compared to controls in the 1st bioassay (after one application of 100 g a.s./ha), but not in the 2nd bioassay (after 4 applications of 100 g a.s./ha). All validation criteria were met or exceeded. Results of the rate-response testing are given below:

Table 151

The effects on mortality and reproduction of the predatory bug, *Orius laevigatus*, exposed to fresh-dried spray deposits and field aged spray deposits of Indoxacarb 150 g/L EC on detached apple leaves in the laboratory

Test Item	Mortality (%) ^a	Corrected mortality (%) ^b	Reproduction [eggs/female/day] (± SD)	Reduction in reproduction rate (%)	Hatching rate (%)	Reduction in hatching rate (%)
Untreated Control 1 st bioassay ^d	10.0	-	5.64	-	82.91	-
Toxic reference item (80 g a.s./ha dimethoate) 1 st bioassay ^d	98.8 *	96.6	n.a.	n.a.	n.a.	n.a.
Indoxacarb 150 g/L EC at 1 × 100 g a.s./ha 1 st bioassay ^d	30.0 *	22.2	7.34	-30.14 ^c	94.56 ^c	-14.05 ^c
Untreated Control 2 nd bioassay ^e	16.3	-	6.63	-	88.00	-
Toxic reference item (12 g a.s./ha dimethoate) 2 nd bioassay ^e	95.0 *	94.0	n.a.	n.a.	n.a.	n.a.
Indoxacarb 150 g/L EC at 4 × 100 g a.s./ha 2 nd bioassay ^e	23.8	9.0	5.72	13.46	79.04	10.18

^a Mortality based on the number of not recovered and dead bugs

^b Corrected mortality according to Schneider-Orelli (1947)

^c Negative values indicate better reproduction results than the control

^d Exposure on the day of the 1st application

^e Exposure on the day of the 4th application

* Statistically significant different compared to the control (Fisher's Exact Test, one-sided, $p \leq 0.05$) (mortality)

n.a.: not assessed

No statistically significant differences compared to the control were observed for reproduction and the hatching rate in both bioassays

(t-Test, one-sided $p \leq 0.05$ for the 1st bioassay; Mann-Whitney U-Test, one-sided $p \leq 0.05$ for the 2nd bioassay).

III. CONCLUSIONS

Exposure to fresh and field-aged residues of Indoxacarb 150 g/L EC on apple leaves following one or four applications of 100 g a.s./ha resulted in small (<50%) increases in mortality, but no effects on reproduction of *Orius laevigatus* Fieber compared to controls.

(Warmers, C., 2007c)

RMS comment

Study submitted to the EU for the first time in this dossier.

The study is valid.

Exposure to fresh and field-aged residues of Indoxacarb 150 g/L EC on apple leaves following one or four applications of 100 g a.s./ha resulted in less than 50% increase in mortality, but no effects on reproduction of *Orius laevigatus* Fieber compared to controls.

B.9.6. RISK ASSESSMENT FOR ARTHROPODS

The use pattern for Indoxacarb 150 g/L EC includes two applications per season on maize and four applications on lettuce at a maximum single application rate of 37.5 g indoxacarb (DPX-KN128)/ha, equivalent to 250 g Indoxacarb 150 g/L EC.

Ecotoxicological studies used for the assessment of Indoxacarb 15 g/L EC were conducted using the formulated product and the active substance indoxacarb (DPX-KN128), and are summarised in Table 152.

Table 152
Ecotoxicological endpoints for honey bees and bumblebees

Species	Test substance	Type	Dose	Endpoint/observation	Reference
Laboratory testing					
Honey bee	Indoxacarb 150 g/L EC	48 h oral LD ₅₀	-	0.11 µg a.s./bee, (0.69 µg prod./bee)	DuPont- 18924 ^b
		48 h contact LD ₅₀	-	0.08 µg a.s./bee (0.50 µg prod./bee)	
Honey bee	Indoxacarb technical (DPX-KN128)	48 h oral LD ₅₀		0.232 µg a.s./bee	DuPont- 36500 ^a
		48 h contact LD ₅₀		0.0682 µg a.s./bee	
Bumble bee	Indoxacarb 150 g/L EC	96 h oral LD ₅₀	-	0.11 µg a.s./bee, (0.73 µg prod./bee)	DuPont- 38351 ^b
		96 h contact LD ₅₀	-	0.32 µg a.s./bee (2.13 µg prod./bee)	
Bumble bee	Indoxacarb technical (DPX-KN128)	96 h oral LD ₅₀	-	0.07 µg a.s./bee	DuPont- 38350 ^a
		96 h contact LD ₅₀	-	0.25 µg a.s./bee	
Honey bee	Indoxacarb technical (DPX-KN128)	10 d chronic LD ₅₀ oral	-	0.0649 µg a.s./bee/day	DuPont- 36490 ^a
Honey bee	Indoxacarb 150 g/L EC	10 d chronic LD ₅₀ oral	-	0.0399 µg a.s./bee/day (0.266 µg prod./bee)	DuPont- 36492 ^b
Honey bee	Indoxacarb 150 g/L EC	7 d lab larvae NOED	-	1.11 µg a.s./bee (7.4 µg prod./bee)	DuPont- 34817 ^b
Oomen Brood feeding testing					
Honey bee	Indoxacarb technical (DPX-KN128)	Bee brood feeding study	100 µg a.s./kg	Effects on mortality. Brood index and termination rate of eggs and young larvae were affected	DuPont- 36493 ^a
Honey bee	Indoxacarb technical (DPX-KN128)	Bee brood feeding study	100 µg a.s./kg	No adverse effects on any parameter tested.	DuPont- 43111 ^a
Honey bee	Indoxacarb 150 g/L EC	Bee brood feeding study	100 µg a.s./kg 667 µg prod./kg	No adverse effects on any parameter tested.	DuPont- 37488 ^b
Semi-field testing					
Honey bee colonies	Indoxacarb 150 g/L EC	Bee brood study on flowering <i>Phacelia tanacetifolia</i> in Germany	50 g a.s./ha 333 g prod./ha	Transient effect on flight activity, behaviour and mortality Strong effect on the brood development No effect on colony size, pupal mortality	DuPont- 34108 ^b

Honey bee colonies	Indoxacarb 150 g/L EC	Semi-field study on <i>Phacelia tanacetifolia</i> in Germany	55.5 g a.s./ha 370 mL prod./ha during bee flight	No harmful effects on honey bee mortality in 2 of the 3 tunnels, transient mortality in 1 tunnel. No harmful effects on flight intensity, brood development, and behaviour	DuPont-19449 ^b
Honey bee colonies	Indoxacarb 150 g/L EC	Semi-field study on <i>Phacelia tanacetifolia</i> in France	55.6 g a.s./ha 371 mL prod./ha during bee flight	No harmful effects on flight intensity and brood development, increased mortality at day of application and 1 day thereafter	DuPont-19450 ^b
Honey bee colonies	Indoxacarb 150 g/L EC	Semi-field study on <i>Phacelia tanacetifolia</i> in Germany	55.6 g a.s./ha 359 mL prod./ha during bee flight	No harmful effects on brood development, increased mortality at day of application and 1 day thereafter Transient effects on flight intensity and behaviour	DuPont-19451 ^b
Honey bee colonies	Indoxacarb 150 g/L EC	Semi-field study on flowering <i>Phacelia tanacetifolia</i> in Germany	37.5 g a.s./ha 250 mL prod./ha <u>during</u> bee flight	<u>During bee flight:</u> Transient increase in mortality (up to DAA2) when applied during daily bee flight. No effect on colony strength and brood development. Slight effect on flight intensity and behaviour when applied during daily bee flight.	DuPont-36482 ^b
			37.5 g a.s./ha 250 mL prod./ha <u>after</u> bee flight	<u>After bee flight:</u> No effect on mortality, flight intensity, colony strength and brood development when applied in evening after daily bee flight. Slight and transient effect on behaviour when applied in evening after daily bee flight.	

Honey bee colonies	Indoxacarb 150 g/L EC	Semi-field study on flowering <i>Phacelia tanacetifolia</i> in Germany	50 g a.s./ha 333 mL prod./ha <u>after</u> bee flight	Transient effect on mortality (up to DAA3), and on flight intensity (day after application) and behaviour. No effect on colony size but slight and transient effect on the amount of brood cells after treatment. Obvious effects on brood development, particularly on the eggs.	DuPont-38405 ^b
Honey bee colonies	Indoxacarb 150 g/L EC	Semi-field study on flowering <i>Phacelia tanacetifolia</i> in Germany	37.5 g a.s./ha 250 mL prod./ha <u>during</u> bee flight	<u>During bee flight:</u> Increase of pupal mortality when applied during daily bee flight. Slight and transient effect on flight intensity and behaviour. No adverse effect on mortality and colony size when applied during daily bee flight. Slight effect on the amount of brood. Obvious effects on the brood development (eggs and larvae).	DuPont-37489, Revision No. 1 ^b
			37.5 g a.s./ha 250 mL prod./ha <u>after</u> bee flight	<u>After bee flight:</u> Slight and transient effect on flight intensity and behaviour when applied after daily bee flight. No adverse effect on mortality and colony size when applied after daily bee flight.. Possible effect on the amount of brood. Obvious effects on the brood development (eggs and larvae).	
Honey bee colonies	Indoxacarb 150 g/L EC	Semi-field study on <i>Phacelia tanacetifolia</i> in France	50 g a.s./ha 333 mL prod./ha <u>during</u> bee flight	<u>During bee flight:</u> Transient effects on mortality and foraging activity were observed when applied during daily bee flight.	DuPont-19453 ^b

			50 g a.s./ha 333 mL prod./ha <u>after</u> bee flight	<u>After bee flight:</u> No harmful effects on honey bee mortality, foraging activity when applied after daily bee flight.	
Honey bee colonies	Indoxacarb 150 g/L EC	Semi-field study on <i>Phacelia tanacetifolia</i> in France	55.6 g a.s./ha 371 mL prod./ha <u>during</u> bee flight	Increase of mortality on day of application when applied during flight. Effects on foraging during 4 days.	DuPont- 21945 ^b
			55.6 g a.s./ha 371 mL prod./ha <u>after</u> bee flight	<u>After bee flight:</u> Results considered not fully reliable.	
Honey bee colonies	Indoxacarb 150 g/L EC	Semi-field study on flowering maize in Germany	37.5 g a.s./ha 250 mL prod./ha <u>during</u> bee flight	<u>During bee flight:</u> Increase in honey bee mortality when applied during flight (up to 3 DAA). No effect on honey bee flight activity, brood development and colony condition. Effects on behaviour.	DuPont- 37487 ^b
			37.5 g a.s./ha 250 mL prod./ha <u>after</u> bee flight	<u>After bee flight:</u> Slight increase in honey bee mortality when applied after flight (ODAA). No effect on honey bee flight activity, brood development and colony condition. Effects on behaviour.	
Honey bee colonies	Indoxacarb 150 g/L EC	Semi-field study on flowering <i>Phacelia tanacetifolia</i> in Germany	37.5 g a.s./ha 232.1 mL prod./ha <u>during</u> bee flight	Study considered valid but to be used with caution. <u>During bee flight:</u> No effect on mortality,	DuPont- 41668 ^b

			37.5 g a.s./ha 232.1 mL prod./ha <u>after</u> bee flight	flight intensity, colony strength when applied during flight. Slight and transient effect on behaviour. <u>After bee flight:</u> Slight effect on mortality and behaviour when applied after flight. No effect on flight intensity, colony strength.	
Honey bee colonies	Indoxacarb 150 g/L EC	Semi-field study on wheat treated with artificial honeydew in France	55.6 g a.s./ha 375 mL prod./ha <u>during</u> bee flight	Study not reliable. Effects on mortality were however obvious.	DuPont- 19454 ^b
			55.6 g a.s./ha 375 mL prod./ha 3 hours <u>before</u> bee flight		
Honey bee colonies	Indoxacarb 150 g/L EC	Semi-field study on wheat treated with artificial honeydew in France	37.5 g a.s./ha 250 mL prod./ha <u>during</u> bee flight	Not reliable for risk assessment.	DuPont- 19455 ^b
			37.5 g a.s./ha 250 mL prod./ha <u>after</u> bee flight		
Honey bee colonies	Indoxacarb 150 g/L EC	Semi-field study on wheat treated with artificial honeydew in France	55.6 g a.s./ha 375 mL prod./ha <u>during</u> bee flight	Increase in honey bee mortality and flight intensity up to 5 DAA in both treatments. Mortality was high in both cases. Changes in behaviour noted on the day of application when applied after bee flight and noted up to 2 DAA when applied during bee flight. No larvae was found at the end of the test in both cases.	DuPont- 21944 ^b
			55.6 g a.s./ha 375 mL prod./ha <u>after</u> bee flight		
Honey bee colonies	Indoxacarb 150 g/L EC	Semi-field study on oilseed rape in France	50 g a.s./ha during bee flight	<u>During bee flight:</u> Mortality was observed when applied during daily bee flight. No	Etude No. 92-2006 ^b

			50 g a.s./ha after bee flight	effect on flight activity. <u>After bee flight:</u> No effects on mortality and flight activity when applied after daily bee flight.	
Bumblebee colonies	Indoxacarb 150 g/L EC	Semi-field study on flowering <i>Phacelia tanacetifolia</i> in Germany	37.5 g a.s./ha 249.1 mL prod./ha in 400L water <u>after</u> bumblebee flight	Not considered valid for risk assessment.	DuPont- 38419 ^b
Field testing					
Honey bee colonies	Indoxacarb 150 g/L EC	Field study on oilseed rape before flowering (BBCH <59) in Germany, <u>hives exposed from d 9 and d 14 after application</u> <u>Application before flowering</u>	25.5 g a.s./ha 170 mL prod./ha in 300 L water/ha	No harmful effects on honey bee mortality, flight intensity, brood development, and behaviour 9 and 14 days after application; no residue of a.s. in honey	DuPont- 26946 ^b
Honey bee colonies	Indoxacarb 150 g/L EC	Field study on flowering oilseed rape (BBCH <61) in Germany, <u>hives exposed from d 4 and d 6 after application</u> <u>Application at the beginning of flowering</u>	25.5 g a.s./ha 170 mL prod./ha	No harmful effects on honey bee mortality, brood development, and behaviour 4 and 6 days after application; no residue of a.s. in honey	DuPont- 26947 ^b
Honey bee colonies	Indoxacarb 150 g/L EC	Field study on maize in Germany, colonies exposed in evening after flight <u>Application at the beginning of flowering</u>	37.5 g a.s./ha 250 mL prod./ha in 300 L water/ha	Increase of mortality cannot be excluded. Results on flight intensity inconclusive. No test item related impact on the behaviour, and brood development. Test not representative of an application on a flowering crop.	DuPont- 30106 ^b

^a Study summarised in Volume 3-CA^b Summarised in this document.

Hazard quotients for bees

The risk assessment used here follows the EC Guidance Document on Terrestrial Ecotoxicology (SANCO/10329, 17 October 2002).

The acute risk to honey bees from the use of Indoxacarb 150 g/L EC was assessed using the maximum single application rate and the LD₅₀ values to calculate hazard quotients (EPPO 2003) as follows:

$$Q_{HO} = \text{application rate/oral LD}_{50}.$$

$$Q_{HC} = \text{application rate/contact LD}_{50}.$$

Acute oral exposure Q_{HO}

The hazard quotient for oral exposure of honey bees based on the maximum recommended field use rate is presented in Table 153.

Table 153
Acute oral toxicity to honey bees (*Apis mellifera*)

Exposure route	Test compound	Application rate (g a.s./ha)	LD ₅₀ (µg a.s./bee)	Hazard quotient (Q _{HO})
Acute oral	Indoxacarb	37.5	0.232	161
Acute oral	Indoxacarb 150 g/L EC	37.5	0.11	341

Acute contact exposure Q_{HC}

The hazard quotient for contact exposure of honey bees based on the maximum recommended field use rate is presented in Table 154.

Table 154
Acute contact toxicity to honey bees (*Apis mellifera*)

Exposure route	Test compound	Application rate (g a.s./ha)	LD ₅₀ (µg a.s./bee)	Hazard quotient (Q _{HC})
Acute contact	Indoxacarb	37.5	0.0682	550
Acute contact	Indoxacarb 150 g/L EC	37.5	0.08	469

The acute oral and contact hazard quotients are exceeding the Regulation (EC) 546/2011 trigger value of 50. Therefore, further work, including semi-field and tunnel studies, was conducted with Indoxacarb 150 g/L EC to assess the potential risk to bees and bumblebees under actual conditions of use. The results of these tests are summarized in Table 152.

Risk to honey bees

Brood feeding studies

The results of acute oral and contact testing with honey bees indicate that Indoxacarb 150 g/L EC has intrinsic toxicity for bees and therefore has a potential to be acutely toxic to bees. To examine the potential effects of Indoxacarb 150 g/L EC on bees, six brood feeding studies were performed. Three studies were following Oomen *et al.* (1992)¹⁴, and three studies was conducted according to the OECD guidance document No. 75 (2007).

¹⁴ Oomen, P.A.; De Ruijter, A.; and Van der Steen, J. (1992): Method for honey bee brood feeding tests with insect growth-regulating insecticides. - EPPO Bulletin 22, 613 - 616

In the three studies conducted according to Oomen *et al.* (1992), 100 µg indoxacarb (DPX-KN128)/kg was applied as the technical substance (DuPont-36493, DuPont-43111) and in the formulated product Indoxacarb 150 g/L EC (DuPont-37488) in 200 mL 50% sucrose solution and administered over a period of 9 days to adult honey bees through feeding. The direct and indirect influence of the nine daily test item feedings were evaluated by comparing the results in the test item treatment to the control treatment as well as the reference item treatment regarding:

- Mortality
- Condition of colonies and amount of brood
- Detailed observation of the brood development in >600 selected cells (>200 eggs, >200 young larvae, and >200 old larvae),
- Behaviour of the honey bees in front of the hives.

In the opinion of the notifier, in the Oomen *et al.* (1992) study conducted with the technical substance (**DuPont-36493**), transient effects on adult mortality were observed, which were diminishing quickly and completely after feeding ended. No effects on colony size, amount of brood, brood index, compensation index and termination rate of young larvae and adult larvae were observed. The brood index for the development of eggs was lower and the termination rate was higher for the colonies exposed to the test item treatment when compared to the control, however, there was no effect on the compensation index. There was no effect of the indoxacarb technical treatments on the brood index, compensation index and termination rate of young larvae and old larvae.

RMS only partially agrees with the conclusions above. RMS considers that, for eggs and for young larvae, the termination rates on all assessments from BFD+5 to BFD+21 are higher than in control and reference item (even if not significant for young larvae according to the study report). The brood / compensation indices seem low even if not significantly different for the young larvae. Besides, RMS considers that the compensation index for the development of eggs is lower than in control (even if not considered significant in the study report).

In the opinion of the notifier, in the Oomen *et al.* (1992) studies, which were conducted with the technical substance (**DuPont-43111**) and product Indoxacarb 150 g/L EC (**DuPont-37488**), no effects on adult mortality, pupal mortality, malformations, behaviour, colony size, amount of brood, food consumption and colony condition were observed.

RMS agrees with the conclusions above for the study conducted with the technical substance (**DuPont-43111**) but not for the other study conducted with the product Indoxacarb 150 g/L EC (**DuPont-37488**). For this latter, RMS noted that, for eggs, young larvae and with a lesser extend old larvae, the termination rates on almost all assessments from BFD+5 to BFD+21 are higher than in control (even if not significant according to the study report). Even if these values are low, it is not known if this higher termination rates are treatment related or not.

In the study **DuPont-38405** performed according to OECD guidance document No. 75, (2007), Indoxacarb 150 g/L EC was applied on flowering *Phacelia* after daily bee flight at a dose of 50 g a.s./ha, equivalent to 333 g Indoxacarb 150 g/L EC/ha, while in **DuPont-34108**, Indoxacarb 150 g/L EC was applied on flowering *Phacelia* during bee flight at a dose of 50 g a.s./ha, equivalent to 333 g Indoxacarb 150 g/L EC/ha. In the study (**DuPont-37489, Revision No. 1**) Indoxacarb 150 g/L EC was applied on flowering *Phacelia* after daily bee flight in one treatment and during daily bee flight in a second treatment at a dose of 37.5 g a.s./ha, equivalent to 250 g Indoxacarb 150 g/L EC/ha. In **DuPont-38405**, pollen and nectar were analysed for indoxacarb residues. Mortality, foraging activity on the crop, condition of the colonies, brood development and behaviour of the honey bees in the crop area and around the hives were observed.

In the opinion of the notifier, in **DuPont-38405**, flight activity was reduced the day after application but returned to normal levels. No adverse effects on honey bee mortality, behaviour, colony size, or larval development were recorded when exposed to Indoxacarb 150 g/L EC. Indoxacarb residues were detected in pollen samples collected from foraging honey bees and cells in the hive during the study, but no residues were detected in nectar collected from foraging honey bees or cells in the hive.

RMS only partially agrees with the conclusions above. RMS considers that the mortality in the tunnels treated with Indoxacarb 150 g/L EC until DAA3 might be treatment related (considered of no biological relevance by the study author). It is also reported that a higher number of dead pupae and dead

malformed pupae was found in tunnels treated with Indoxacarb 150 g/L EC (considered of no biological relevance by the study author). RMS cannot ascertain if it is treatment related or not. This should be considered in conjunction with other studies.

The application of Indoxacarb 150 g/L EC after bee flight had a slight effect on the flight intensity on the day after the application. After this day, the flight intensity was at the same level as in the control. The application of Indoxacarb 150 g/L EC had a slight effect on the behaviour of the honey bees shortly after the applications. The numbers of honey bees showing unusual behaviour was low. There was no negative effect on the colony size relating to the application of Indoxacarb 150 g/L EC in treatment group S. There was only a slight and transient effect on the amount of brood cells in the treatment group S shortly after the application of Indoxacarb 150 g/L EC. RMS noted that the brood/compensation indices were much lower than in control at all assessment (even if not significant after BFD+5). The termination rates also appear higher than in the control (more than twice higher even if not significant according to the study report). RMS considers that it is treatment related. Lower brood/compensation indices and higher termination rates were also observed for young larvae and old larvae but the difference was lesser. RMS nevertheless considers that it is treatment related.

No residues of indoxacarb could be found in the nectar samples prepared from forager bees or in nectar samples which were taken directly from the hive in the control group as well as in the treatment group S. In the sample taken from the forager bees in the treatment group S, 0.059 mg/kg could be found two days after the application and 0.032 mg/kg four days after the application. In the pollen sample which was taken directly from the hive, 0.046 mg/kg could be found two days after the application and 0.017 mg/kg could be found four days after the application. RMS however notes that the origin of the samples taken from the hive cannot be verified. It is not known if the samples are taken from newly deposited nectar and pollen or not (even if it is reported in the study report that the samples were “preferably” taken from newly deposits).

In the opinion of the notifier, in **DuPont-34108**, a transient effect on mortality was observed at the day of application and one day thereafter. Likewise, on individual cell level an effect on honey bee brood was detected, which was shown to be only of transient nature on the whole bee colony and did not exhibit any effect on pupal mortality. No adverse effects on honey bee flight activity and on the size of the colonies were recorded when exposed to Indoxacarb 150 g/L EC.

RMS only partially agrees with the conclusions above. RMS considers that flight intensity decreased in the tunnels treated with Indoxacarb 150 g/L EC on the day of application and the day after (considered of no biological relevance by the study author). RMS also noted that the brood/compensation indices were lower than in control at all assessment (even if not significant at BFD+5). Indoxacarb 150 g/L EC at a rate of 50 g a.s./ha applied during bee flight has a transient effect on honey bee mortality on DAA0aa and DAA1 and on behaviour on DAA0aa. There was a transient decrease of flight activity on the day of application and the day after. No effect was observed on the size of the colonies in the Indoxacarb 150 g/L EC treatment. There was no effect on the amount of brood in the Indoxacarb 150 g/L EC treatment except a slight reduction of the number of larvae on DAA9 based on whole colony assessment. The termination rates of the individually marked cells were significantly higher in the Indoxacarb 150 g/L EC treatment than in the control. The brood index and compensation index were lower than in the control at all assessments. Overall, an effect on the brood was detected on the level of individual cells during the whole monitoring period but there was only a transient effect (DAA9 = BFD+11) detectable on the level of the whole bee colony and no effect on pupal mortality.

In the opinion of the notifier, in **DuPont-37489, Revision No. 1**, while there was no effect on forager mortality in either treatment a slight increase in pupal mortality was noted in the treatment during daily bee flight. This slight increase though did not result in any effect on colony size or number of brood cells, as recovery was seen once colonies were removed from the tunnels. Flight activity was reduced in both treatments the day after application but returned to normal levels after that. No adverse effects on honey bee behaviour or larval development were recorded when exposed to Indoxacarb 150 g/L EC.

RMS would not exclude an effect on mortality in T1 (application in evening after bee-flight) and T2 (application during bee-flight) during 3 days after application in both cases. The increase, if any, is however very slight. It is agreed that mortality of adult honeybees were not of biological relevance in both T1 and T2. An increase of pupal mortality was however observed in T2 (application during bee flight). The application of Indoxacarb 150 g/L EC after bee flight or during bee flight had a slight effect on the flight intensity shortly after the applications in T1 and T2. One day after the applications, the flight activity in T1 and T2 was at the same level as in the control. The application of Indoxacarb 150 g/L EC in T1 and T2 had a slight effect on the behaviour of the honey bees shortly after the applications. There was no effect on the colony size relating to the application of Indoxacarb 150 g/L EC in T1 and T2. There was only a slight effect on the amount of brood cells with reduced numbers of larvae cells in the treatment group T2 shortly after the application of Indoxacarb 150 g/L EC. Besides RMS notes that two replicates in the treatment group T1, the original queens were lost due to unknown reasons (in one replicate, there was mainly male progeny left in the colony, in the other replicate, no eggs were found). This resulted in lower mean number of brood cells in this treatment group. The study author concludes that there was no test item related effect regarding the amount of brood cells in the treatment group T1. RMS considers that the lost of the queens is not explained and that an indirect effect of the treatment on the brood (via the lost of the queens) cannot be excluded. According to the study author, Indoxacarb 150 g/L EC applications carried out after bee flight (T1) caused no harmful effects on the development of the marked eggs, young larvae or old larvae (brood termination rate). RMS disagrees as the brood and compensation indices are lower for eggs and old larvae (almost by factor 2 for the eggs) than in the control group (even if the difference was not significant). Besides these indices show high variability between replicates. The termination rates in T1 are higher than in control (except for young larvae but it has to be noted that the mean value in the control appears particularly high for young larvae). These termination also show high variability (for eggs, young larvae and old larvae). These results show a direct effect of the treatment T1 on the brood. According to the study author, the application of Indoxacarb 150 g/L EC carried out during bee flight (T2) had a slight effect on the development of the marked eggs, whereas no effect occurred on the development of young larvae and old larvae. RMS disagrees as the brood and compensation indices are much lower for eggs, young larvae and old larvae than in the control group (even if the difference was not significant). Besides these indices show high variability between replicates. The termination rates in T2 are much higher than in control. These results show a direct effect of the treatment T2 on the brood.

No residues of indoxacarb could be found in the nectar samples prepared from forager bees or in nectar samples which were taken directly from the hive in the control group as well as in the treatment groups T1 and T2 taken two and four days after the application.

In the pollen samples taken from the forager bees in the treatment group T1, the maximum value of 0.095 mg a.s./kg was found two days after the application. In the pollen samples which were taken directly from the hive, the maximum value of 0.047 mg a.s./kg was found two days after the application.

In the pollen samples taken from the forager bees in the treatment group T2, the maximum value of 0.063 mg a.s./kg was found two days after the application. In the pollen samples taken from the hive, no residues of indoxacarb above the level of quantification (<0.010 mg indoxacarb/kg) were detected. RMS notes that the content of residues was higher in T1 (application in evening). RMS however notes that the origin of the samples taken from the hive cannot be verified. It is not known if the samples are taken from newly deposited nectar and pollen or not (even if it is reported in the study report that the samples were “preferably” taken from newly deposits. However the higher content of residue in the pollen of T1 seems confirmed by the samples directly taken from forager bees.

Semi-field studies

A total of 15 semi-field tunnel studies were conducted in France and Germany to assess effects on honey bee mortality, behaviour, flight intensity, and brood development. In addition to that, a semi-field tunnel study was

conducted in Germany to assess effects of Indoxacarb 150 g/L EC applied on flowering *Phacelia* on bumble bees.

Six of the tests conducted with honey bees (**DuPont-19449**, **DuPont-19450**, **DuPont-19451**, **DuPont-37487**, **DuPont-36482**, and **DuPont-41668**) followed the EPPO 170 test guideline.

In **DuPont-19449**, **DuPont-19450**, and **DuPont-19451**, Indoxacarb 150 g/L EC was applied during bee flight to flowering *Phacelia tanacetifolia* at a rate of 55.5 g a.s./ha in a spray volume of 500 L/ha. Two of the EPPO 170 guideline tests were carried out in southern Germany in 2006 and 2007 and a third was conducted in France in 2007.

In **DuPont-37487**, **DuPont-36482**, and **DuPont-41668**, two different exposure scenarios were addressed. In one treatment, Indoxacarb 150 g/L EC was applied during bee flight at a rate of 37.5 g a.s./ha (equivalent to 250 g Indoxacarb 150 g/L EC/ha), and in a second treatment, the same dose was applied after bee flight in the evening. For **DuPont-41668** and **DuPont-36482**, the studies were conducted on *Phacelia* and for **DuPont-37487** the study was conducted on maize. In DuPont-36482 and DuPont-41668, additionally, residues in pollen and nectar were determined.

In the opinion of the notifier, in **DuPont-19449** which was conducted in southern Germany in 2006 demonstrated that Indoxacarb 150 g/L EC had no effects on honey bee mortality, flight activity, behaviour, or brood development; **DuPont-19450** and **DuPont-19451** which were semi-field tests conducted in 2007 did show some effects. The results of both tests in 2007 were similar, significant increases in mortality were observed on the day of application and to a lesser extent the following day. No significant mortality occurred on the second day after application in any of the 2007 tests. There was no significant effect on honey bee flight intensity, behaviour, or brood development in any of these semi-field tests.

RMS partially agrees with the conclusions above.

RMS considers that in **DuPont-19449** the mortality was higher in one replicate (one of the three tunnels treated with Indoxacarb 150 g/L EC). This explains the higher mortality observed after treatment on the basis of mean mortality values. According to the study author, this difference between replicates is possibly due to biological differences between hives. Excluding this colony the post-application mortality would be in the same range as the control. RMS considers that transient effects on mortality cannot be excluded as they appear precisely after the day of application. Indoxacarb 150 g/L EC when applied at 370 mL/ha (equivalent to 55.5 g a.s./ha) to *Phacelia tanacetifolia* during bee flight had no harmful effects (2 tunnels) or transient effects (1 tunnel) on honey bee mortality, and no effect on flight intensity, brood development, and behaviour.

RMS considers that in **DuPont-19450** abnormal behaviour on the day of application was observed. Indoxacarb 150 g/L EC when applied at 371 mL Indoxacarb 150 g/L EC/ha (equivalent to 55.6 g a.s./ha) to *Phacelia tanacetifolia* during bee flight had no effects on honey flight intensity and brood development. Concerning mortality, an increase occurred on the day of application and the day after application.

RMS considers that in **DuPont-19451**, effects on flight intensity and behaviour were seen on the day of application. Indoxacarb 150 g/L EC, when applied at 371 mL Indoxacarb 150 g/L EC/ha nominal (359 mL Indoxacarb 150 g/L EC/ha equivalent to 55.6 g a.s./ha, analysed) to *Phacelia tanacetifolia* during bee flight, had no effects on brood development. Concerning mortality, an increase could be noticed on the day of application after application and on the following day.

In the opinion of the notifier, in the test conducted on flowering maize **DuPont-37487**, no effect on honey bee flight activity, brood development and colony condition were recorded. In the treatment which was applied in the evening after honey bee flight, a slight increase in honey bee mortality was observed on the day of application. For the treatment which was applied during bee flight, an increased mortality up to three days after application was observed. For *Phacelia* (**DuPont-41668** and **DuPont-36482**), no difference was observed

between application during flight and thereafter in one study while a slight transient effect on behaviour was recorded in the second study. Different residue levels were recorded in pollen collected from forager bees and hives and in nectar from forager bees and hives (see study summary below). In general, no residues on pollen or nectar were detected in forager bees at Day 5 after application. Residues measured on Day 2 after application in forager bees (pollen and nectar) and on Day 2 and 5 in pollen in hives were slightly higher when the test item was applied during bee flight. No residues were detected in nectar from hives.

RMS partially agrees with the conclusions above. RMS considers that **DuPont-37487** is valid but to be used with caution. Indeed a deviation is reported: The distance to target during the application was partly not approx. 50 cm. Some of the plants in some of the tunnel were even higher than the boom height. RMS also noted that rain occurred the day after application (42.8mm) and the following days. The impact of such rainfall on the actual exposure of bees is not known. There was a slight increase in honey bee mortality in the Indoxacarb 150 g/L EC treatment T1 (application in evening) on the day of application and considering the post-application period from 0DAA to 7DAA. There was an increased daily mortality from 0DAA to 3DAA and considering the whole post-application period in the Indoxacarb 150 g/L EC treatment T2 (application during foraging). Indoxacarb 150 g/L EC, applied in the evening after honey bee flight or during daily honey bee flight had no effect on honey bee flight activity, brood development and colony condition in both test item treatments T1 and T2. Additionally, there was a effect on behaviour in the treatment group T1 and an effect on behaviour in the treatment group T2.

RMS considers that in **DuPont-41668** a slight effect on mortality cannot be discarded the day after treatment when Indoxacarb 150 g/L EC is applied in the evening after bee flight (not significant). Indoxacarb 150 g/L EC applied during bee flight had no effect on mortality. The Indoxacarb 150 g/L EC treatments had no effect on the flight intensity and colony strength. There was a slight and transient effect on the behaviour of the honey bees at the first two days after application of Indoxacarb 150 g/L EC in the evening after bee flight and also after application of Indoxacarb 150 g/L EC during bee flight at the application day. RMS noted that effects on brood were observed which might be due to the presence of Varroa and the use of a Varroa treatment. No larvae or capped brood was observed in several tunnels including one replicate of the control (no larvae in T2 in all tunnels at the last assessment). Besides, all treatments showed a decrease in the amount of brood cells. According to the study report the reduction of the brood cells in all groups can be related to the confined conditions and the changing weather. The decrease of brood cells was more important in tunnels where Indoxacarb 150 g/L EC was applied during honey bee flight. RMS considers that the observations on brood are inconclusive and an effect of the treatment cannot be discarded. This study is considered valid but results have to be used with caution (use of small colonies, presence of Varroa and use of a Varroa treatment).

RMS considers that in **DuPont-36482**, Indoxacarb 150 g/L EC, applied once at 37.5 g a.s./ha during flowering in the evening after honey bee-flight (treatment T1) or during daily honey bee-flight (treatment T2) had no effect on brood development and colony condition in both test item treatments T1 and T2. RMS noted that flight intensity slightly decreased in the tunnels treated with Indoxacarb 150 g/L EC during bee flight during all post-application period. The reason of this decrease is not known but RMS agrees that the difference is slight. No effect on flight intensity was observed when Indoxacarb 150 g/L EC is applied in the evening after honey bee-flight (treatment T1). There was no effect on honey bee mortality in T1. In T2, there was a transient increase in honey bee mortality (0DAA to 2DAA). A slight transient effect on behaviour was observed on 0DAA in T1 and T2.

Six additional semi-field studies were carried out following the CEB 230 test guideline in eastern, western and northern France in 2006 and 2007 (**DuPont-19453**, **DuPont-19454**, **DuPont-19455**, **DuPont-21944**, **Etude No. 92-2006** and **DuPont-21945**).

For **DuPont-19453** and **DuPont-21945**, applications of Indoxacarb 150 g/L EC were made either during bee flight or in the evening after bee flight to flowering *Phacelia tanacetifolia* at the equivalent of 50 g indoxacarb/ha. In the opinion of the notifier, in the test done in eastern France, there were no harmful effects on mortality, foraging activity, brood development or behaviour of the bees regardless of whether Indoxacarb 150 g/L EC was applied during bee flight or in the evening when the bees were inactive. However, in the test done

in western France, Indoxacarb 150 g/L EC resulted in greater honey bee mortality than the controls whether the applications were made during or following bee flight. The effects on mortality decreased rapidly in the days following the applications. There were no effects on flight intensity, behaviour or brood development for either application in this test. The lack of effects on brood development indicates that mid or long-term risks to honey bees from applications of Indoxacarb 150 g/L EC are low.

RMS considers that in **DuPont-19453**, the mortality in the tunnel treated during foraging activity was increased from the day of application to DAA+2. Foraging also appears lower on the day of application after the treatment (when the product is applied during the foraging activity). No effects were observed when Indoxacarb 150 g/L EC is applied after the bee flight (in evening).

RMS considers that in **DuPont-21945**, Indoxacarb 150 g/L EC has no effect on honey bee flight intensity and behaviour. Foraging decreased during 4 days after the treatment when the product was applied during the foraging activity. Only slight effect on behaviour was observed when Indoxacarb 150 g/L EC is applied during bee flight. When Indoxacarb 150 g/L EC was applied after bee-flight the number of dead honey bees increased on the following day (DAA 0). However RMS notes that mortality was higher before application than during the days after the application. Besides, mortality in this tunnel was also much higher than in the control tunnel. This tunnel is not considered reliable by RMS. The number of dead honey bees increased after application of Indoxacarb 150 g/L EC during bee-flight relative to the control treatment on the day of application (DAA 0aa).

Study No. 92-2006 had applications of Indoxacarb 150 g/L EC made either during bee flight or after daily bee flight to oilseed rape at the equivalent of 50 g indoxacarb/ha. The effects of another formulation, Steward (WG formulation) were also tested. In the opinion of the notifier, transient effects were seen on foraging bees when applications were made during bee flight, while no effects were seen on foraging bees when applications were made after daily bee flight.

RMS considers that in **Study No. 92-2006**, the mortality increased in the tunnels treated during foraging activity for both formulations. This increase lasted until DAA+2 and returned to a normal level afterwards. The peak of mortality seems higher in the tunnel treated with Indoxacarb 150 g/L EC. This resulted in higher cumulative mortality in this tunnel. It is not known if the difference in mortality values might reflect a difference of toxicity of the different types of formulations (EC and WG) when the bees are exposed to fresh residues. In the tunnels treated in the evening after foraging activity, no increase of mortality was observed for both formulations. No effect on foraging activity due to treatment was found in the tunnels treated with both test items.

In **DuPont-19454** and **DuPont-21944** applications of Indoxacarb 150 g/L EC were made either during or in the morning before or in the evening after bee flight to wheat that had been treated with artificial honeydew at the equivalent of 55.6 g indoxacarb/ha. In **DuPont-19455**, applications of Indoxacarb 150 g/L EC were made either during or in the evening after bee flight to wheat that had been treated with artificial honeydew at the equivalent of 37.5 g indoxacarb/ha. In the opinion of the notifier, in the two studies treated with 55.6 g indoxacarb/ha, honey bee mortality was increased for applications made both during and after daily bee flight for the first three days after the applications. Flight intensity was also decreased during the same time period and behavioural changes were noted. In the study treated with 37.5 g indoxacarb/ha, honey bee mortality was increased for applications made both during and after bee flight for the first two days after the applications. Flight intensity was decreased during the same time period in the tunnels with applications made during bee flight, but no change was noted in tunnels with applications made after bee flight. No behavioural changes were noted in either treatment.

RMS considers that in **DuPont-19454**, Indoxacarb 150 g/L EC applied during and before bee flight (early morning) at 0.371 L formulated product/ha (equivalent to 55.6 g a.s./ha) on wheat treated with sugar solution to simulate honey dew had a strong effect on honey bees mortality (equivalent to toxic reference) and foraging activity. However RMS considers the study only informative. The mortality in control is very high making the results not reliable. Adverse effects in other tunnels were nevertheless obvious.

RMS considers that in **DuPont-21944**, Indoxacarb 150 g/L EC applied during and after bee flight at 0.371 L formulated product/ha (equivalent to 55.6 g a.s./ha) on wheat treated with sugar solution to simulate honey dew had a strong effect on honey bees mortality and foraging activity. Mortality was comparable to control after 4 and 5 days respectively. Foraging activity remained close to 0 until the end of the test in both cases. When applied after honey bee flight slightly aggressive behaviour was recorded on the day after application (DAA 0). When applied during honey bee flight abnormal behaviour was observed on the day of application after treatment and on DAA+2. RMS also noted that no larvae was found in the tunnels treated with test item at the end of the test.

RMS considers that **DuPont-19455** is not reliable for a risk assessment but nevertheless confirms the strong effect of Indoxacarb 150 g/L EC applied during or after bee flight on wheat treated with sugar solution observed in other studies.

In addition to that, a bumble bee study was conducted under semi-field conditions (**DuPont-38419**). Indoxacarb 150 g/L EC was applied during and after bumble bee flight to flowering *Phacelia tanacetifolia* at a rate of 37.5 g a.s./ha in a spray volume of 400 L/ha. As currently no agreed guideline is available, the test was based on general SETAC/ESCORT recommendations (BARRETT *et al.*, 1994¹⁵) and OEPP/EPPO Guideline No 170 (4), 010. In the opinion of the notifier, Indoxacarb 150 g/L EC applied once *via* spray application did not have any effects on mortality, flight activity, consumption of sugar solution, hive weight, condition of colonies, development of bumble bee brood, production of young queen offspring and vigour relative to the water treated control. Residues of indoxacarb above the LOQ level were found for pollen samples after application in the test item treatment. Residues in nectar samples were below LOD.

RMS does not agree with the conclusions above. RMS noted that bumble bees were fed with sugar solution during the test. Bees were fed during the test to combat the effects of abnormally high temperatures on the colonies' feeding patterns. RMS is of the opinion that this feeding might have affected the results. Colonies would be strengthened and adverse effects due to the test item might have been underestimated as artificial feeding does not represent natural conditions. RMS also remarks that the sugar consumption seems higher in the tunnels treated with the test item (even if not significantly different with control). It is noted in the study report that higher sugar consumption in the test item hives is in accordance with the hives weight increase observed at the same timing. Besides the presence of syrup makes difficult to force the bumble bees to forage on the crop. The actual exposure of the bumble bees and the brood is uncertain. The applicant feels that it is a valid study as all colonies were fed, control as well as the treated colonies. RMS however, doubts the reliability of the study. RMS also noted that the max temperature recorded were particularly high in the tunnels (max 40.2°C). High temperatures was recorded for several days in the tunnels. The temperature was much lower outside the tunnels. It is not known if this high temperature might affect the behaviour of the substance on plants or the behaviour of the bumble bees but the representativeness of the study for normal climate conditions is wondered by RMS. RMS also noted an increase of larval mortality on DAA17 and DAA18 (not significant). A decrease of this larval mortality was observed as soon as the sugar solution supply was opened again. RMS considers the study inconclusive. RMS considers that the absence of effect of the test item Indoxacarb 150 g/L EC on bumble bees in more realistic conditions (natural conditions without feeding) cannot be proven on the basis of this study.

All applications made in these bee trials, using a downward directed boom sprayer to the top of the plants, represent a worst-case for the intended arable use scenarios with lower maximum application rates. These studies should also represent a worst-case for intended uses in maize with an application rate of 37.5 g a.s./ha. Beyond it should be considered that flowering *Phacelia tanacetifolia* is highly attractive for bees and therefore this testing scenario represents a worst-case versus the intended target crops that are not at all attractive to bees or much less attractive to bees than flowering *Phacelia tanacetifolia*.

¹⁵ BARRETT, K.L., GRANDY, N., HARRISON, E.G., HASSAN, S. AND OOMEN, P. (eds.) (1994): Guidance Document on Regulatory Testing Procedures for Pesticides with Non-Target Arthropods. From the SETAC/ESCORT Workshop (European Standard Characteristics of Beneficial Regulatory Testing); Wageningen, Holland, 28 – 30 March, 1994.

Semi-field tests with *Phacelia tanacetifolia*, by design (direct application to the flowers in a highly bee-attractive plant), result in significant direct contact and oral exposure to honey bees. Applications are made while bees are actively foraging on a single nectar and pollen source. The bees and hives are enclosed in the tunnels throughout the test and the bees have no alternative but to forage exclusively on treated flowering plants.

Field studies

The effects of Indoxacarb 150 g/L EC on bees were also investigated in 3 field trials according to EPPO 170 test guideline. Two field trials investigated the use of Indoxacarb 150 g/L EC at 25.5 g a.s./ha in winter oil seed rape sprayed between BBCH 55 to 60 in 2009.

In the opinion of the notifier, in the field trials conducted in Eastern Germany (**DuPont-26946**) and in Northern Germany (**DuPont-26947**) no negative effect on honey bee mortality, flight activity, colony condition, brood development and behaviour were found due to Indoxacarb 150 g/L EC treatment. In 2010, the effects of Indoxacarb 150 g/L EC on bees were also investigated in a field trial according to EPPO 170 test guideline in flowering corn (**DuPont-30106**). The field trial investigated the use of Indoxacarb 150 g/L EC at 37.5 g a.s./ha sprayed at beginning of flowering and in the evening after daily bee-flight. In the opinion of the notifier, the field trial showed no negative effect on honey bee mortality, flight activity, colony condition, brood development and behaviour due to Indoxacarb 150 g/L EC treatment.

RMS reminds that in **DuPont-26946**, the test item was applied before start of flowering with no open flowers present in the field for T1 and T2 (BBCH 55 and 57-59 respectively). The test item was applied 14 days (for T1) and 9 days (for T2) before the set-up of the beehives at the experimental fields (BBCH 63-65). One field per treatment was used. Indoxacarb 150 g/L EC, applied once at a rate of 25.5 g a.s./ha (equivalent to 170 mL formulated product/ha) before flowering of *B. napus* L. (BBCH 55 in treatment T1 and BBCH 57-59 in treatment T2) had no negative effect on the mortality, flight activity, behaviour, colony size, and brood development of the honey bee colonies that were placed at the pre-treated fields during flowering, 14 days after the application in treatment T1 and 9 days after the application in treatment T2. No residues of indoxacarb were detectable in any of the samples of sealed honey taken from the experimental hives. This study is not used in the risk assessment as it is not representative of an application during flowering (application occurred before flowering) and because honey bee colonies were placed at the pre-treated fields only 9 and 14 days after application. RMS also reminds that the application rates intended for the intended uses of the representative formulation are not covered by this field study.

RMS reminds that in **DuPont-26947**, the test item was applied at the beginning of flowering (BBCH 59-60 and 57-59 for T1 and T2 respectively). The test item was applied 4 days (for T1) and 6 days (for T2) before the set-up of the beehives at the experimental fields (BBCH 63-65). Flight activity on all three fields was rather low for this kind of crop and time of the year. The mean number of honey bees/m² flying and foraging in the crop seems lower in the treated field fields than in control. However, differences among the three fields may account for differences of flight activity on the three fields. For example, on each of the three fields there was a different variety of *B. napus*, which may have different attractiveness for the bees. Additionally, the size of the control field (1.87 ha) was *ca* $\frac{1}{5}$ to $\frac{1}{3}$ smaller than the fields that were used for the test item treatments (2.4 and 2.8 ha for T1 and T2 respectively), which may have caused a higher foraging density (number of forager bees/m²) in the control field. RMS considers that the results on flight activity are inconclusive and cannot be used for the risk assessment. Regarding the brood development, all colonies of all three treatments groups (T1, T2, and control) had brood of all stages (eggs, larvae, sealed brood) at any time during the assessments, except for the lack of eggs in hive 1T2 at the assessment on DAE+6. However, in this hive (1T2) eggs and all other brood stages were present at the three following assessments until the end of the observation period. RMS also noted that at DAE+6 the number of cells containing larvae is lower than in other hives. No differences was noted before the set-up of the hives at DAE-1. This means that the egg-laying temporarily stopped from the time of set-up of the beehive on the experimental site. The reason of that is not known but might not be treatment related. Indoxacarb 150 g/L EC applied once at a rate of 25.5 g a.s./ha (equivalent to 170 mL formulated product per ha) to winter oilseed rape (*Brassica napus* L.) at BBCH 59-60 (treatment T1) and at BBCH 57-59 (treatment T2), had no negative effect on honey bee mortality, colony condition, brood development, and behaviour of the honey bee colonies that were placed at the pre-treated fields during flowering, 4 days after the application in treatment T1 and 6 days after the application in treatment T2. No residues of indoxacarb were detectable in any of the samples of sealed

honey taken from the hives. This study is not used in the risk assessment as it is not representative of an application during flowering (application occurred before full flowering) and because honey bee colonies were placed at the pre-treated fields only 4 and 6 days after application. RMS also reminds that the application rates intended for the intended uses of the representative formulation are not covered by this field study.

RMS noted that in **DuPont-30106**, important rain occurred especially on the day of application (13.4 mm). RMS considers that this might affect the actual exposure of the bees (by washing residues or by reducing foraging intensity). The test item was applied at the beginning of flowering (BBCH 55-65). RMS considers that the application took place too early as only a few maize flowers were available for bees at the time of treatment. As a consequence, the pollen collected after the application might not have been exposed to the spray (pollen still enclosed at the time of application). This is confirmed by the amount of maize pollen found in the samples collected at DAA1 and DAA4. At DAA1, the amount of maize pollen was minimal (5.5% in T) but increase afterwards to a level of 54.5% at DAA+4. This means that even if the bees collected the pollen from the treated maize field, this pollen was not available at the time of application and was not directly exposed to the spray. This leads to an underestimation of the effects. In the control field, the amount of maize pollen remains low during the test. As the control field was smaller, bees probably might have been able to choose other sources of pollen. It is noted that there were no attractive crops in the surroundings but the pollen of wild flowers was found in the samples. Besides, despite the presence of maize flowers at the end of the study, the flight intensity did not increase in this field. RMS considers that an increase of mortality from DAA0 to DAA2 cannot be excluded even if it is slight. No treatment related effect was observed on behaviour and brood development (until DAA28). Indoxacarb 150 g/L EC, applied once at beginning of flowering and in the evening after daily bee-flight, with an application rate of 37.5 g a.s./ha, showed no obvious test item-related impact on the honey bee behaviour and brood development. Slight effect on mortality cannot be excluded. This test is however not representative of an application on a flowering crop.

The effects of DPX-MP062 30WG applied commercially at 51 to 78 g indoxacarb/ha in flowering apple orchards on honey bees were investigated in 30 commercial apple orchards in The Netherlands during April/May 2004. DPX-MP062 30WG is a wettable granule formulation with 30% active substance and 70% inert ingredients. Indoxacarb 150 g/L EC is an emulsifiable concentrate formulation with 15% active substance and 85% inert ingredients. Both formulations only have one active ingredient, indoxacarb. In the opinion of the notifier, even though these are separate formulations, the results of studies conducted with the 30WG formulation are applicable as the higher proportion of indoxacarb in the 30WG formulation ensures greater exposure for bees to the active substance, and can be considered a worst case application. The numbers of dead honey bees found in “Münster” dead bee traps (2 honey bee colonies per orchard) were counted every 3 to 4 days for about 2 weeks before and after the period of insecticide treatment and compared to the numbers of dead honey bees found in nine orchards that did not receive a DPX-MP062 30WG application. The findings of the monitoring study were published in a peer-reviewed journal and can be summarized as follows:

*“It was concluded that the application of indoxacarb, **when applied at the equivalent rates of 51 and 78 g DPX-KN128/ha** caused no effects on honey bee mortality, and that the number of dead honey bees counted in the Münster traps in the orchard treated with indoxacarb was comparable with those determined in control orchards.”* (van der Steen & Dinter, 2007¹⁶).

Based on this monitoring study it can be concluded that negative effects on honey bees *i.e.* increased mortality following the commercial use of indoxacarb at up to 78 g indoxacarb/ha in flowering crops are unlikely on the basis of intensive monitoring of honey bee colonies in apple orchards.

RMS does not fully agree with the conclusions above. It is not known if other attractive crops or plants are available around the treated orchards. In such case where other attractive plants are available, it is difficult to force the bees to forage on the treated crop and thus the exposure might have been

¹⁶ van der Steen, J. J. and Dinter, A. (2007), A monitoring study to assess the acute mortality effects of indoxacarb on honey bees (*Apis mellifera* L.) in flowering apple orchards. Pest. Manag. Sci., 63: 1095–1099. doi: 10.1002/ps.1467

underestimated. It is thus not known if application on isolated attractive crops would result in significant effects or not. Besides the temperatures recorded were between 9.3 and 17°C. RMS considers the temperature quite low. No data on the foraging activity is available to ensure the foraging was sufficient at the time of the study. It has also to be noted that the formulation tested is a WG formulation. The representative formulation to be assessed is an EC formulation. It is not certain if an extrapolation of effects can be made but the results of a semi-field study (Giffard, 2006, report n°92-2006) seems to show higher lethal effect of the EC formulation when the bees are exposed to fresh residues (application during bee flight). RMS noted an increase of the mortality after the application that seems to last 3 days. This increase was considered not significant by the study authors. It is however agreed by RMS that the increase is not of biological importance for the honey bee colonies. It is also reported that all the bee colonies used in this study showed normal colony development and normal increase in honey bee population size during the test period. However no detail was provided in the publication and the effects on brood could not have been assessed in this study aiming for the assessment of acute lethal effects. RMS considers that the results of this monitoring study based on a WG formulation not reliable for the evaluation of the representative formulation (EC type). Uncertainty also remains on the presence or absence of other attractive plants in the surroundings of the treated orchards. Besides, the exposure of the bees during this monitoring might underestimate the exposure of other conditions of use (season, crops, regions,...).

Risk to honey bees under field conditions

The risk of Indoxacarb 150 g/L EC to honey bees under actual use conditions will depend on the level of exposure. Exposure of honey bees to residues of Indoxacarb 150 g/L EC will depend on the presence of flowers during the times in which applications of Indoxacarb 150 g/L EC are made (crop growth stage) and the attractiveness of these flowers as a source of nectar or pollen. The crops listed in the GAP for Indoxacarb 150 g/L EC can be divided into two categories:

- Category 1: Crops that are not intended to be sprayed during flowering with Indoxacarb 150 g/L EC: Lettuce

The risk to honey bees from applications of Indoxacarb 150 g/L EC to lettuce is considered to be negligible, because timing of applications in lettuce is before crop flowering (BBCH 13-59 for seed crops).

- Category 2: Crops that may produce flowers during the periods of spray applications of Indoxacarb 150 g/L EC, but the flowers do not strongly attract bees: Maize and sweet corn.

In the opinion of the notifier, the risk from applications to maize and sweet corn is considered to be low, and will depend on both the occurrence of mature flowers and the presence of bees actively foraging within the crop coincident with spray applications.

RMS, however, on the basis of available data, would recommend to avoid application on a flowering crop even for a crop like maize which does not strongly attract bees.

Conclusions:

The effects observed in semi-field tunnel studies with honey bees and *Phacelia tanacetifolia* suggest that a potential short-term and long-term risk to honey bees from applications of Indoxacarb 150 g/L EC may occur in flowering crops that are highly attractive to honey bees. These effects, and particularly effects on development, are expected even for an application in evening after bee-flight. The studies available with the less attractive crop maize are either not considered fully reliable (tunnel study) or not representative of an application during the flowering period (field study). It is then recommended to avoid application during flowering for these crops too. Strong effect of Indoxacarb 150 g/L EC were observed when applied on wheat treated with sugar solution (during and after bee flight). It is then recommended to avoid application during honeydew production periods.

Beneficial arthropod risk assessment

A summary of the relevant end points is given in Table 155.

Table 155
Summary of effects of Indoxacarb 150 g/L EC on non-target arthropods

Species	Test (test substance and test rate)	Measurement endpoint	Endpoint value	Reference ^a
<i>Aphidius rhopalosiphi</i>	Laboratory Tier 1 (Indoxacarb 150 g/L EC)	LR ₅₀	5.1 g a.s./ha (34 mL product/ha)	DuPont-19443
<i>Aphidius rhopalosiphi</i>	Extended laboratory Tier 2 (Indoxacarb 150 g/L EC)	LR ₅₀ (Mortality) ER ₅₀ (Reproduction)	74.2 g a.s./ha (494.7 mL product/ha) ≥52.5 g a.s./ha (≥350 mL product/ha)	DuPont-19445
<i>Aphidius rhopalosiphi</i>	Extended laboratory – aging test (1-4 × 100 g a.s./ha Indoxacarb 150 g/L EC)	Mortality and Reproduction after the 1 st , 4 th application (bioassay 1 and 2, respectively) and after 28 and 56 d field aging (bioassay 3 and 4, respectively)	Bioassay 1: 97.5% mortality Bioassay 2: 100% mortality Bioassay 3: 13.2% mortality and 57.8% reduction in reproduction Bioassay 4: 2.5% mortality and 37.6% reduction in reproduction	DuPont-21947
<i>Typhlodromus pyri</i>	Laboratory Tier 1 (Indoxacarb 150 g/L EC)	LR ₅₀	220.5 g a.s./ha (1470 mL product/ha)	DuPont-19444
<i>Chrysoperla carnea</i>	Extended laboratory Tier 2 (Indoxacarb 150 g/L EC)	LR ₅₀ Effect on reproduction	29.8 g a.s./ha (198.7 mL product/ha) 37.7% reduction at 6.9 g a.s./ha and 67.4% reduction at 17.25 g a.s./ha	DuPont-19446
<i>Chrysoperla carnea</i>	Extended laboratory – aging test (1-4 × 100 g a.s./ha Indoxacarb 150 g/L EC)	Mortality and Reproduction after the 1 st , 4 th application (bioassay 1 and 2, respectively) and after 28 field ageing (bioassay 3)	Bioassay 1: not valid Bioassay 2: 94% mortality Bioassay 3: 8.8% mortality and no adverse effect on fecundity and hatching (fertilization) compared to controls	DuPont-21946
<i>Orius laevigatus</i>	Extended laboratory – aging test (1-4 × 100 g a.s./ha Indoxacarb 150 g/L EC)	Mortality and Reproduction after the 1 st , 4 th application (bioassay 1 and 2, respectively)	Bioassay 1: 22% mortality, and no adverse effect on fecundity and hatching Bioassay 2: 9% mortality, 13.5% reduction in reproduction and 10.2% reduction in hatching	DuPont-22391

^a Summarised in this document.

The following equations were used to calculate the HQs:

$$\text{In-field HQ} = \frac{\text{Application rate} \times \text{MAF}}{\text{LR}_{50}}$$

$$\text{Off-field HQ} = \frac{\text{Application rate} \times \text{MAF} \times (\text{drift factor/veg. distr. factor}) \times \text{correction factor}}{\text{LR}_{50}}$$

Tier 1 risk assessment for maize and lettuce

Input parameters for the non-target arthropod worst-case Tier 1 risk assessment, for Indoxacarb 150 g/L EC use in maize (2 applications) and lettuce (4 applications), were the following:

- Application rate: 37.5 g DPX-KN128/ha
- MAF: 1.7 and 2.7 (default values for 2 and 4 applications; see ESCORT II Appendix III)
- Drift factor = 2.38% and 1.85% (basic drift value for two and four applications, 1 m distance, in field crops; see ESCORT II Appendix IV)
- Vegetation distribution factor = 10 (default value)
- Correction factor (uncertainty factor) = 10 (default value)

Table 156
Tier 1 summary of non-target arthropod in-field and off-field hazard quotients (HQ) for Indoxacarb 150 g/L EC use

Species	LR ₅₀ (g a.s./ha)	In-field HQ	Off-field HQ	Trigger
Leafy vegetables, 4 × 37.5 g a.s./ha				
<i>T. pyri</i>	220.5	0.46	0.01	2
<i>A. rhopalosiphi</i>	5.1	19.9	0.37	2
Maize, 2 × 37.5 g a.s./ha				
<i>T. pyri</i>	220.5	0.29	0.01	2
<i>A. rhopalosiphi</i>	5.1	12.5	0.30	2

Conclusions from the Tier 1 risk assessment for non-target arthropods

- In-field and off-field hazard quotients for *Typhlodromus pyri* are below the ESCORT II trigger value of 2 and therefore indicate that the risk to this standard sensitive non-target arthropod species is acceptable for all intended uses of Indoxacarb 150 g/L EC.
- In-field hazard quotients for *Aphidius rhopalosiphi* are above the ESCORT II trigger value of 2 for all uses of Indoxacarb 150 g/L EC and therefore trigger a higher-tiered assessment and the evaluation of potential effects on additional species.
- Off-field hazard quotients for *Aphidius rhopalosiphi* are below the ESCORT II trigger value of 2 for all proposed uses of Indoxacarb 150 g/L EC, therefore indicating that the off-field risk to this standard sensitive non-target arthropod species is acceptable for all intended uses of Indoxacarb 150 g/L EC.

Refined risk assessment for non-target arthropods

To address the need for a refined risk assessment for non-target arthropods, the following additional tests were conducted with Indoxacarb 150 g/L EC:

- Multi-rate extended laboratory studies with *Aphidius rhopalosiphi*, the most sensitive species identified in Tier 1 testing and an additional foliar species, *Chrysoperla carnea*.
- Extended-laboratory studies with residue ageing to assess the potential for recovery with *Aphidius rhopalosiphi*, *Chrysoperla carnea*, and *Orius laevigatus*. These studies were done to support a use on apples for Indoxacarb 150 g/L EC. However the results of these studies may be used to evaluate potential residual effects in the lower-use rate scenarios such as those also described in the GAP. Four applications of 100 g a.s./ha of Indoxacarb 150 g/L EC were made at 10-day intervals to apple trees. Bioassays to assess mortality and reproduction were conducted with leaf material taken immediately after the first and fourth applications and then again after ageing residues for 28 or 56 days.

Based on the results of the higher-tier studies with *Aphidius rhopalosiphi*, *Chrysoperla carnea*, and *Orius laevigatus* using a worst-case exposure scenario that exceeds all proposed uses described in the GAP (4×100 g a.s./ha was actually investigated in these studies) transient in-field effects may be expected for *Aphidius rhopalosiphi* and *Chrysoperla carnea*, but not for *Orius laevigatus* (Table 155). However, when residues of Indoxacarb 150 g/L EC were aged under natural conditions, the effects on both mortality and reproduction in these non-target arthropods were reduced. Aging residues for 28 days after the fourth application resulted in 8.8% mortality and no adverse effect on both fecundity and hatching of *Chrysoperla carnea* when compared to controls (Table 155). Aging residues for 28 days resulted in 13.2% mortality and 57.8% reduction in reproduction of *Aphidius rhopalosiphi*. A further 28-day aging period resulted in 2.5% mortality and 37.6% reduction in reproduction. The results demonstrate clearly that effects decline with time and any in-field effects observed are likely to be short-lived. This ensures that sensitive in-field non-target arthropod populations may recover rapidly for the intended uses with maximum use rates that are much lower than those tested in these studies.

Semi-field studies with non-target arthropods

Indoxacarb 150 g/L EC was found to pose an acceptable risk to non-target arthropods based on the proposed GAP and Tier 1 and Tier 2 testing. On this basis it is unlikely that the use of Indoxacarb 150 g/L EC will result in significant adverse effects on non-target terrestrial arthropods, and therefore semi-field testing was not carried out.

Field studies with non-target arthropods

Indoxacarb 150 g/L EC was found to pose an acceptable risk to non-target arthropods based on the proposed GAP and Tier 1 and Tier 2 testing. On this basis it is unlikely that the use of Indoxacarb 150 g/L EC will result in significant adverse effects on non-target terrestrial arthropods, and therefore field testing was not carried out.

B.9.7. EFFECTS ON NON-TARGET SOIL MESO- AND MACROFAUNA

B.9.7.1. Earthworms

Report: Lührs, U. (2006); Indoxacarb (DPX-KN128) 150 g/L EC: Acute toxicity to the earthworm, *Eisenia fetida* in artificial soil

DuPont Report No.: DuPont-18929

Guidelines: OECD 207 (1984), ISO 11268-1 (1993) **Deviations:** None

Testing Facility: IBACON, Rossdorf, Germany

Testing Facility Report No.: 29523021

GLP: Yes

Certifying Authority: Hessisches Ministerium für Umwelt, Landwirtschaft und Forsten

Executive summary:

The acute toxicity of Indoxacarb 150 g/L EC to earthworms, *Eisenia fetida* (Savigny), was determined in a 14-day soil exposure laboratory study according to OECD 207 (1984) and ISO 11268-1 (1993). Adult earthworms were exposed for 14 days to artificial soil (prepared according to OECD 207) treated with nominal concentrations of 28, 55, 110, 220, 440, and 880 mg of Indoxacarb 150 g/L EC/kg dry weight soil corresponding to 4.45, 8.73, 17.5, 34.9, 69.9, and 140 mg a.s./kg (based on analytical concentration of a.s.). The EC₅₀ for Indoxacarb 150 g/L EC was determined to be 770 mg formulated product/kg dry weight soil equivalent to 122 mg a.s./kg dry weight soil (95% confidence limits of 511 mg formulated product/kg (81.1 mg a.s./kg) and 1954 mg formulated product/kg (310 mg a.s./kg), Probit analysis). The Lowest-Observed-Effect Concentration (LOEC) was determined to be 440 mg formulated product/kg dry weight soil (69.9 mg a.s./kg) and the No-Observed-Effect Concentration (NOEC) was determined to be 220 mg formulated product/kg dry weight soil (34.9 mg a.s./kg).

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Indoxacarb 150 g/L EC
Lot/Batch #: DPX-KN128-150
Purity: 150 g a.s./L
CAS#: None for the formulation
173584-44-6 for indoxacarb active substance
Description: Liquid/yellow
Density: 0.953 g/mL
Stability of test compound: 98.8% of the indoxacarb remains in the delivery vehicle after one hour under agitation
2. Control: untreated (and moistened with deionised water)
Vehicle: Deionised water
Toxic reference: 2-Chloroacetamide (tested once per year)
3. Test System: Earthworm
Species: *Eisenia fetida*, Savigny
Age at test start: 9-10 months
Weight at test start: 300-600 mg
Source: In-house laboratory culture
Acclimation period: 1 day
Diet: None
Test medium: Artificial soil prepared according to OECD 207
Maximum water holding capacity of the OECD 207 artificial soil, as measured: 55%
Water content: Initiation: 30.6 to 32.7% (equivalent to 55.6 to 59.5% of the maximum water holding capacity)
Termination: 27.6 to 29.2% (equivalent to 50.2 to 53.1% of the maximum water holding capacity)
Test chamber: Glass containers (volume: 1 L), filled with *ca.* 500 g artificial soil dry weight
4. Environmental conditions
Temperature: 17 to 22°C
pH: 6.3 to 6.4 at test start and 6.1 at test termination
Photoperiod: continuous light, (480 to 780 lux)

B. STUDY DESIGN AND METHODS

1. Experimental start/completed
07-June-2006 to 28-June-2006
2. Experimental treatments
Acute toxicity of Indoxacarb 150 g/L EC to earthworms, *Eisenia fetida* (Savigny), was determined in a 14-day soil exposure laboratory study. Four replicates of ten earthworms each (total of 40 individuals per treatment group) were each exposed to nominal concentrations of 28, 55, 110, 220, 440, and 880 mg formulated product/kg soil dry weight (corresponding to 4.45, 8.73, 17.5, 34.9, 69.9, and 140 mg a.s./kg) and an untreated control (deionised water only). The toxic reference, 2-chloroacetamide, tested once per year was tested at five concentrations (the most recent test to this study was conducted in August 2005).
3. Observations
Earthworms were assessed for mortality and behavioural effects after 7 and 14 days of exposure and earthworm body weights were assessed at Day 0 and Day 14.

4. Statistics

Mortality data were analysed for significance by using Fisher-exact test (two-sided, $\alpha = 0.05$).

Data of weight changes were tested for normal distribution and homoscedascity using Kolmogoroff-Smirnov test and Cochran test ($\alpha = 0.05$). Further statistical evaluation of the NOEC for weight changes was performed using Bonferroni-Welch-t test (multiple comparison, $\alpha = 0.05$, two sided). The LC_{50} and its 95% confidence limits were determined by Probit analysis.

II. RESULTS AND DISCUSSION

A. FINDINGS

All validation criteria were met. The LC_{50} of the toxic reference standard in the most recent test was 26.0 mg 2-chloroacetamide/kg artificial soil dry weight.

Indoxacarb 150 g/L EC caused a significant increase in mortality of earthworms, *Eisenia fetida*, when exposed to concentration of 880 mg formulated product/kg artificial soil dry weight, the highest rate tested. No mortality was observed in the controls and the test item treated groups up to and including a concentration of 220 mg formulated product/kg artificial soil dry weight. At the concentration of 440 mg formulated product/kg artificial soil dry weight a non-significant mortality of 2.5% was observed.

Indoxacarb 150 g/L EC had significant effects on the weight development of earthworms, *Eisenia fetida*, when exposed to concentrations of 440 and 880 mg formulated product/kg artificial soil dry weight.

The Lowest-Observed-Effect Concentration (LOEC) was determined to be 440 mg formulated product/kg artificial soil dry weight. The overall No-Observed-Effect Concentration (NOEC) was determined to be 220 mg formulated product/kg artificial soil dry weight.

Table 157
The effects on mortality and weight change of earthworms, *Eisenia fetida*, exposed to Indoxacarb 150 g/L EC in artificial soil

Nominal Indoxacarb 150 g/L EC concentration (mg formulated product/kg soil dry weight)	Mean % mortality (\pm SD)	Mean % weight change (\pm SD)
Untreated control (0.0)	0 (\pm 0)	6.0 (\pm 3.5)
28 (4.45 mg a.s.)	0 (\pm 0)	5.0 (\pm 5.1)
55 (8.73 mg a.s.)	0 (\pm 0)	3.7 (\pm 6.2)
110 (17.5 mg a.s.)	0 (\pm 0)	-3.3 (\pm 12.9)
220 (34.9 mg a.s.)	0 (\pm 0)	-2.4 (\pm 7.2)
440 (69.9 mg a.s.)	2.5 (\pm 5.00)	-11.5 (\pm 5.3) ^b
880 (140 mg a.s.)	55.0 (\pm 42.0) ^a	-18.4 (\pm 3.0) ^b

^a There was a significant differences from the control (Fisher Exact test, two-sided, $\alpha = 0.05$)

^b There was a significant differences from the control (Bonferroni Welch-t test, two-sided, $\alpha = 0.05$)

III. CONCLUSIONS

The 14-day LC_{50} of Indoxacarb 150 g/L EC was determined to be 770 mg formulated product/kg dry weight soil equivalent to 122 mg a.s./kg dry weight soil, the Lowest-Observed-Effect Concentration (LOEC) was determined to be 440 mg formulated product/kg dry weight soil (69.9 mg a.s./kg) and the No-Observed-Effect Concentration (NOEC) was determined to be 220 mg formulated product/kg dry weight soil (34.9 mg a.s./kg).

(Lührs, U., 2006)

RMS comment

Study submitted to the EU for the first time in this dossier.

The study is valid.

The 14-day LC₅₀ of Indoxacarb 150 g/L EC was determined to be 770 mg formulated product/kg dry weight soil equivalent to 122 mg a.s./kg dry weight soil.

The No-Observed-Effect Concentration (NOEC) was determined to be 220 mg formulated product/kg dry weight soil (34.9 mg a.s./kg).

Report: Lührs, U. (2014); Indoxacarb (DPX-KN128) 150 g/L EC: Acute toxicity to the earthworm *Eisenia fetida* in artificial soil

DuPont Report No.: DuPont-38832

Guidelines: OECD 207 (1984), ISO 11268-1 (1993) **Deviations:** None

Testing Facility: IBACON, Rossdorf, Germany

Testing Facility Report No.: 83784021

GLP: Yes

Certifying Authority: Hessisches Ministerium für Umwelt, Energie, Landwirtschaft und Verbraucherschutz (Weisbaden, Germany)

Executive summary:

The acute toxicity of Indoxacarb 150 g/L EC to earthworms, *Eisenia fetida*, was determined in a 14-day soil exposure laboratory study according to OECD 207 (1984) and ISO 11268-1 (1993). Adult earthworms were exposed for 14 days to artificial soil (prepared according to OECD 207) treated with six nominal concentrations of 232.5, 465.0, 930.1, 1860, 3720 and 7441 mg formulated product/kg dry artificial soil equivalent to 35.0, 70.0, 140, 280, 560 and 1120 mg a.s./kg dry artificial soil and to an untreated control moistened with deionized water. The test item was applied *via* spreading different dilutions as homogeneously as possible onto the soil surface of each container using a Pasteur pipette.

The acute 14-day LC₅₀ for earthworms based on mortality and nominal concentrations was determined to be 921 mg a.s./kg dry weight soil. The 14-day NOEC (No-Observed-Effect Concentration) for earthworms based on body weight change and nominal concentrations was 70.0 mg a.s./kg soil dry weight.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Indoxacarb 150 g/L EC
 Lot/Batch#: DPX-KN128-311
 Purity: 150 g a.s./L
 CAS #: None for the formulation
 173584-44-6 for the active substance indoxacarb
- Stability of test compound: Not determined in the test system
2. Control: Untreated (the same amount of deionized water as in the test item groups was applied onto the surface of each test container)
- Test vehicle: Deionized water
- Reference item: 2-Chloroacetamide (tested once per year)
3. Test organism: Earthworm
 Species: *Eisenia fetida*
 Age at dosing: 11 to 12 months
 Weight at dosing: 300 to 600 mg
 Source: In-house laboratory culture (Laboratory: IBACON, Rossdorf, Germany)
- Acclimation period: 1 day
- Test chamber: Plastic boxes with perforated transparent lids (volume: 1 L), filled with *ca.* 500 g artificial soil dry weight
- Test medium: Artificial soil prepared according to OECD 207
- Diet: Unfed during test
- Water content of soil: Initiation: 24.4 to 25.0%, equivalent to 44.4 to 45.5% of the maximum water holding capacity or 30.4 to 31.0% equivalent to 55.3 to 56.4% of the maximum water holding capacity if considering the water added during the application
 Termination (only one value available): 26.8%, equivalent to 48.8% of the maximum water holding capacity
- Soil pH: 5.7 to 5.9 at test start and 5.6 at test termination (based on one value only)
4. Environmental conditions (in-life period)
- Temperature: Within the range of 18 to 22°C
- Photoperiod: Continuous light (within the range of 400 to 800 lux)

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
 25-October-2013 to 11-November-2013

2. Experimental treatments

Acute toxicity of Indoxacarb 150 g/L EC to earthworms *Eisenia fetida* was determined in a 14-day soil exposure laboratory study. Four replicates of ten earthworms (total 40 individuals per treatment group) were each exposed to six nominal concentrations of 232.5, 465.0, 930.1, 1860, 3720 and 7441 mg formulated product/kg dry artificial soil equivalent to 35.0, 70.0, 140, 280, 560 and 1120 mg a.s./kg dry artificial soil and to an untreated control moistened with deionized water. The test item was applied *via* spreading different dilutions as homogeneously as possible onto the soil surface of each container using a Pasteur pipette. The control was replicated four times, with ten earthworms in each replicate and was treated in the same way as the test item group but using deionized water. The reference item, 2-chloroacetamide, tested at least once per year was tested at five concentrations (the most recent test to this study was conducted in July 2013).

3. Observations

Earthworms were assessed for mortality and sublethal (behavioural) effects after 7 and 14 days of exposure. Earthworm body weight (adults) was assessed at test start (Day 0) and test end (Day 14).

4. Statistics

Mortality data were analysed for significance by using Fisher's Exact Test (one-sided greater, $\alpha = 0.05$).

Weight change data were tested for normal distribution and homoscedascity using Shapiro-Wilk's test and Levene's test ($\alpha = 0.05$). Further statistical evaluation of the NOEC for weight changes was performed using Williams t-test (multiple comparison, $\alpha = 0.05$, two-sided). The LC_{50} was determined by Probit Analysis.

II. RESULTS AND DISCUSSION

A. FINDINGS

The LC_{50} of the reference item in the most recent test was 29.9 mg 2-chloroacetamide/kg artificial soil dry weight. All validation criteria were within acceptable limits indicating the validity of this test.

At the highest concentration of 1120 mg a.s./kg soil dry weight worms were observed clustered on the bottom of the test container after 7 days exposure. After 14 days exposure this behaviour was also observed at the concentration of 560 mg a.s./kg soil dry weight. No additional adverse behavioural effects were observed at either 7 or 14 days exposure in any of the treatment groups. Cumulative mortality and body weight loss at 14 days are reported in the table that follows.

Table 158
Acute mortality and body weight change of earthworms, *Eisenia fetida*, exposed to Indoxacarb 150 g/L EC in artificial soil

Nominal Indoxacarb 150 g/L EC concentration (mg a.s./kg dry soil)	14-day Mean mortality (%)	14-day Mean weight change (%)
Untreated control (0.0)	0	+5.5
30.0	0 ^{n.s.}	+0.4 ^{n.s.}
70.0	0 ^{n.s.}	+0.1 ^{n.s.}
140	0 ^{n.s.}	-3.3*
280	0 ^{n.s.}	-12.1*
560	0 ^{n.s.}	-20.5*
1120	97.5*	-31.2 ^{n.e.}

n.s. No significant differences from the control

n.e. Not evaluated (only one surviving worm)

* Significantly different from the control

Mortality: Fisher's Exact Test, one-sided greater, $\alpha = 0.05$

Weight Changes: Williams t-test, two-sided, $\alpha = 0.05$)

III. CONCLUSIONS

The acute 14-day LC_{50} for earthworms based on mortality and nominal concentrations was 921 mg a.s./kg dry weight soil, the Lowest-Observed-Effect Concentration (LOEC) was determined to be 140 mg a.s./kg dry weight soil and the No-Observed-Effect Concentration (NOEC) was determined to be 70.0 mg a.s./kg dry weight soil.

(Lühns, U., 2014)

RMS comment

Study submitted to the EU for the first time in this dossier.

The study is valid.

A deviation is reported in the study report:

pH and water content at experimental end was not measured except for the highest concentration of 1120 mg a.s./kg soil dry weight (human error). Since all test containers were within the required range for water content and pH at experimental start and were all treated in the same way, it can be assumed that these values were comparable to those measured for the highest test concentration. Thus it is considered that the test conditions for all treatment groups were within the requirements for the complete test period so the deviation had no impact on the study.

The acute 14-day LC₅₀ for earthworms based on mortality and nominal concentrations was 921 mg a.s./kg dry weight soil.

The No-Observed-Effect Concentration (NOEC) was determined to be 70.0 mg a.s./kg dry weight soil.

Report: Pavic, B. (2014); Indoxacarb (DPX-KN128) 150 g/L EC: Effects on reproduction and growth of the earthworm, *Eisenia fetida*, in artificial soil

DuPont Report No.: DuPont-37891

Guidelines: OECD 222 (2004), ISO 11268-2 (2012) **Deviations:** None

Testing Facility: IBACON, Rossdorf, Germany

Testing Facility Report No.: 83783022

GLP: Yes

Certifying Authority: Hessisches Ministerium für Umwelt, Energie, Landwirtschaft und Verbraucherschutz (Weisbaden, Germany)

Executive summary:

The sublethal toxicity of Indoxacarb 150 g/L EC to earthworms, *Eisenia fetida*, were determined in a 56-day soil exposure laboratory study according to OECD 222 (2004) and ISO 11268-2 (2012). Adult earthworms were exposed to artificial soil (prepared according to OECD 222) treated with the test item to obtain the nominal concentrations of 5.9, 10.5, 19.0, 34.2, 61.5, 110.7, 199.3 and 358.7 mg formulated product/kg dry artificial soil (corresponding to 0.9, 1.6, 2.9, 5.1, 9.3, 16.7, 30.0 and 54.0 mg a.s./kg dry artificial soil, adjusted for purity) and to an untreated control (moistened with deionized water only). Mortality and growth (body weight) of the earthworms were assessed after 28 days and the effect on reproduction (number of juveniles produced) was assessed after 56 days. The LC₅₀ after 28 days was determined to be greater than 358.7 mg formulated product/kg dry artificial soil. The NOEC (No-Observable-Effect Concentration) for earthworms based on mortality, reproduction, growth and nominal concentrations was 199.3 mg formulated product/kg dry artificial soil.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Indoxacarb 150 g/L EC
Lot/Batch#: DPX-KN128-311
Purity: 150 g a.s./L
Description: Liquid
CAS#: None for the formulation
173584-44-6 for the active substance
Stability of test compound: Not analysed in the test system
2. Control: Untreated (moistened with deionized water)
Test vehicle: Deionized water
Toxic reference: Carbendazim
3. Test organism: Earthworm
Species: *Eisenia fetida*
Age at dosing: 9 to 10 months
Weight at dosing: 301 to 599 mg
Source: In-house laboratory culture (Laboratory: IBACON, Rossdorf, Germany)
Acclimation period: 1 day
Test chamber: Plastic boxes with perforated transparent lids (volume: 1 L), filled with *ca.* 500 g artificial soil dry weight
Test medium: Artificial soil prepared according to OECD 222, maximum water holding capacity of the artificial soil, as measured: 55%
Diet: Finely ground cattle manure
Water content of soil: Initiation: 29.6 to 31.2% (equivalent to 53.8 to 56.8% of the maximum water holding capacity)
Termination: 30.4 to 36.0% (equivalent to 55.2 to 65.4% of the maximum water holding capacity)
Soil pH: 5.8 to 5.9 at test start and 6.1 to 6.2 at test termination
4. Environmental conditions (in-life period)
Temperature: Within the range of 18 to 22°C
Photoperiod: 16 hour light, 8 hour dark, photoperiod within the range of 400 to 800 lux

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
19-November-2013 to 15-January-2014
2. Experimental treatments
The sublethal toxicity of Indoxacarb 150 g/L EC to earthworms, *Eisenia fetida*, was determined in a 56-day soil exposure laboratory study. Eight replicates for the control and four replicates per test item group, containing ten clitellated adult earthworms were each exposed to artificial soil (prepared according to OECD 222) treated with the test item to obtain the nominal concentrations of 5.9, 10.5, 19.0, 34.2, 61.5, 110.7, 199.3 and 358.7 mg formulated product/kg dry artificial soil (corresponding to 0.9, 1.6, 2.9, 5.1, 9.3, 16.7, 30.0 and 54.0 mg indoxacarb/kg dry artificial soil, adjusted for purity) and to an untreated control (moistened with deionized water only). The reference item, carbendazim, is tested at least once a year to ensure sensitivity of the test system at five concentrations. The most recent test relevant to this study was conducted from August 2013 to October 2013.
3. Observations
Worms were assessed for mortality and sublethal (behavioural) effects after 28 days of exposure. Body weight change (adults) was assessed between test start (Day 0) and 28 days after application. For reproduction, soil (without the adults) was replaced in the test container and juveniles were

allowed to grow for another 28 days (Day 56), at which time they were removed from soil, counted, and reproduction effects assessed.

4. Statistics

Data of weight changes and reproduction were tested for normal distribution and homogeneity of variance using Shapiro-Wilk's test and Levene's test ($\alpha = 0.05$). Further statistical evaluation of the NOEC for weight changes was performed using Welch t-test for weight changes (multiple comparison, $\alpha = 0.05$, two-sided) and Williams t-test for reproduction (multiple comparison, $\alpha = 0.05$, one-sided smaller).

The LC_{50} after 28 days was not determined by statistical analysis as no mortality was observed.

II. RESULTS AND DISCUSSION

A. FINDINGS

The EC_{50} for reproduction of the reference item in the most recent test was 1.32 mg carbendazim/kg dry artificial soil. All validation criteria were within acceptable limits indicating the validity of this test.

No mortality was observed in any treatment group. The LC_{50} after 28 days was determined to be greater than 358.7 mg formulated product/kg dry artificial soil. The food consumption of earthworms exposed to the test rates of the test item was comparable to the control. No adverse behavioural effects were observed after 28 days of exposure in any of the treatment groups. No statistically significant differences in weight change (28-day assessment) of earthworms compared to the control were observed. Reproduction (56-day assessment) was statistically significantly reduced at the concentration of 358.7 mg formulated product/kg dry artificial soil when compared to the control.

Cumulative mortality and weight change (of adults) at 28 days and reproduction at 56 days are reported in the table below.

Table 159
Sublethal toxicity of Indoxacarb 150 g/L EC to earthworms

Indoxacarb 150 g/L EC concentration (mg/kg soil dry weight)	28-day mortality (%) mean	28-day weight change (%)^a mean	56-day reproduction (# of juveniles)^b mean
Control (0.0)	0	24.8	266
5.9	0	29.6 n.s.	269 n.s.
10.5	0	26.9 n.s.	298 n.s.
19.0	0	30.2 n.s.	304 n.s.
34.2	0	28.3 n.s.	290 n.s.
61.5	0	31.2 n.s.	269 n.s.
110.7	0	28.6 n.s.	252 n.s.
199.3	0	28.5 n.s.	233 n.s.
358.7	0	26.9 n.s.	216 *

* significantly different from the control

n.s. not significantly different from the control

^a Welch t-test, two sided, $\alpha = 0.05$

^b Williams test, one-sided smaller, $\alpha = 0.05$

III. CONCLUSIONS

Indoxacarb 150 g/L EC had no significant lethal effects or effects on growth or feeding activity on the earthworm *Eisenia fetida* when exposed to concentrations up to and including 358.7 mg formulated product/kg dry artificial soil.

Indoxacarb 150 g/L EC had no statistically significant effects on reproduction of the earthworm *Eisenia fetida* when exposed to concentrations up to and including 199.3 mg/kg dry artificial soil. At the concentration of 358.7 mg formulated product/kg artificial soil dry weight reproduction was statistically significantly reduced when compared to the control

The overall NOEC (No-Observable-Effect Concentration) was determined to be 199.3 mg formulated product/kg dry artificial soil and the overall LOEC (Lowest-Observable-Effect Concentration) was determined to be 358.7 mg formulated product/kg dry artificial soil.

The LC₅₀ after 28 days was determined to be greater than 358.7 mg formulated product/kg dry artificial soil, the highest concentration tested.

(Pavic, B., 2014)

RMS comment

Study submitted to the EU for the first time in this dossier.

The study is valid.

Indoxacarb 150 g/L EC had no significant lethal effects or effects on growth or feeding activity on the earthworm *Eisenia fetida* when exposed to concentrations up to and including 358.7 mg formulated product/kg dry artificial soil.

Indoxacarb 150 g/L EC had no statistically significant effects on reproduction of the earthworm *Eisenia fetida* when exposed to concentrations up to and including 199.3 mg/kg dry artificial soil. At the concentration of 358.7 mg formulated product/kg artificial soil dry weight reproduction was statistically significantly reduced when compared to the control

The overall NOEC (No-Observable-Effect Concentration) was determined to be 199.3 mg formulated product/kg dry artificial soil and the overall LOEC (Lowest-Observable-Effect Concentration) was determined to be 358.7 mg formulated product/kg dry artificial soil.

The EC₁₀ was determined to be 142.88 mg formulated product/kg dry artificial soil (95% confidence intervals: 14.85-323.4) (RMS calculation).

B.9.7.2. Effects on non-target soil meso- and macrofauna (other than earthworms)

Report: Pavic, B. (2014a); Indoxacarb (DPX-KN128) 150 g/L EC: Effects on the collembola *Folsomia candida* in artificial soil with 5% peat

DuPont Report No.: DuPont-37892

Guidelines: OECD 232 (2009), ISO 11267 (1999) **Deviations:** None

Testing Facility: IBACON, Rossdorf, Germany

Testing Facility Report No.: 83782016

GLP: Yes

Certifying Authority: Hessisches Ministerium für Umwelt, Energie, Landwirtschaft und Verbraucherschutz (Weisbaden, Germany)

Executive summary:

The effects of Indoxacarb 150 g/L EC on the mortality and reproduction of Collembola (*Folsomia candida*) were determined in a 28-day soil exposure laboratory study according to OECD 232, 2009 and ISO 11267, 1999. Ten to twelve day-old Collembola were exposed for 28 days to artificial soil (prepared according to OECD 232) treated with eight nominal concentrations of Indoxacarb 150 g/L EC of 2.0, 3.7, 6.6, 11.9, 21.4, 38.6, 69.4, and 125 mg formulated product/kg artificial soil dry weight (corresponding to 0.3, 0.6, 1.0, 1.8, 3.2, 5.8, 10.4 and 18.8 mg a.s./kg dry artificial soil, adjusted for purity) and an untreated control moistened with deionized water only. Mortality and reproduction (number of juveniles produced) were assessed after 28 days. The overall 28-day NOEC (No-Observed-Effect Concentration) based on mortality and reproduction was determined to be 6.6 mg formulated product/kg artificial soil dry weight.

I. MATERIALS AND METHODS**A. MATERIALS**

- | | | |
|----|-----------------------------|---|
| 1. | Test material: | Indoxacarb 150 g/L EC |
| | Lot/Batch #: | DPX-KN128-311 |
| | Purity: | 150 g a.s./L |
| | Description: | Liquid |
| | CAS#: | None for the formulation
173584-44-6 for indoxacarb active substance |
| | Stability of test compound: | Not analysed in the test system |
| 2. | Control: | Untreated (moistened with deionized water) |
| | Test vehicle: | Deionized water |
| | Toxic reference: | Boric acid |
| 3. | Test System: | Collembola |
| | Species: | <i>Folsomia candida</i> , Willem (Collembola: Isotomidae) |
| | Age at dosing: | 10 to 12 days |
| | Weight at dosing: | Not determined |
| | Source: | In-house laboratory culture |
| | Acclimation period: | 10 to 12 days |
| | Test chamber: | Glass containers (volume: 100 mL; diameter: 5.0 cm), closed,
filled with 30 ± 1.0 g artificial soil fresh weight |
| | Test medium: | Artificial soil prepared according to OECD 232, maximum water
holding capacity of the artificial soil, as measured: 41% |
| | Diet: | Granulated dry yeast |
| | Water content of soil: | Initiation: 22.8 to 23.7%, equivalent to 55.7% to 57.7% of the
maximum water holding capacity
Termination: 16.4 to 22.6% equivalent to 40.0 to 55.1% of the
maximum water holding capacity |
| | Soil pH: | 5.6 to 5.9 at test start; 5.6 to 5.7 at test termination |
| 4. | Environmental conditions | |
| | Temperature: | Within the range of 18 to 22°C |
| | Photoperiod: | 16 h light, 8 h dark, photoperiod within the range of 400 to
800 lux |

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
30-October-2013 to 28-November-2013

2. Experimental treatments

A study was conducted to determine the effects of Indoxacarb 150 g/L EC on the mortality and reproduction of Collembola (*Folsomia candida*). Eight replicates for the control and four replicates per test item group, containing ten Collembola each (total 80 per control and 40 individuals per test item group) were each exposed for 28 days to the nominal concentrations of Indoxacarb 150 g/L EC

of 2.0, 3.7, 6.6, 11.9, 21.4, 38.6, 69.4 and 125 mg formulated product/kg dry artificial soil (corresponding to 0.3, 0.6, 1.0, 1.8, 3.2, 5.8, 10.4 and 18.8 mg a.s./kg dry artificial soil, adjusted for purity) and an untreated control moistened with deionized water only. A reference item (boric acid, at a concentration range of 33.6 to 220 mg/kg artificial soil dry weight) is tested at least once per year to ensure sensitivity of the test system. The most recent test to this study was conducted in August/September 2013.

3. Observations

After the 28-day exposure period, adult Collembola were counted and the mean number of adults in each treatment group was determined. The number of juveniles produced in each treatment group over 28 days exposure period was also determined and the percent reduction in juveniles produced relative to the untreated control group was calculated.

4. Statistics

Mortality data were analysed for significance by using Fisher's Exact Test (one-sided greater, $\alpha = 0.05$).

Reproduction data were tested for normal distribution and homoscedascity using Shapiro-Wilk's test and Levene's test ($\alpha = 0.05$). Further statistical evaluation of the NOEC for reproduction was performed using Williams t-test (multiple comparison, $\alpha = 0.05$, one-sided smaller). EC_{50} was not determined by statistical analysis as the reduction of reproduction was less than 50%.

II. RESULTS AND DISCUSSION

A. FINDINGS

All validity criteria were met. The EC_{50} for reproduction of the reference item (boric acid) in the most recent test was 99.6 mg boric acid/kg dry artificial soil. A summary of the results is provided in the table below:

Table 160
The effects on mortality and reproduction of Collembola, *Folsomia candida*, exposed to Indoxacarb 150 g/L EC in artificial soil for 28 days

Indoxacarb 150 g/L EC concentration (mg formulated product/kg soil dry weight)	Mean % mortality	Reproduction	
		Mean juveniles per replicate	% of control
Untreated control (0.0)	4	694	-
2.0	8	673	97
3.7	13	725	104
6.6	8	674	97
11.9	10	520	75*
21.4	5	555	80*
38.9	13	650	94*
69.4	15	571	82*
125	28*	408	59*

* Statistically significant (mortality: Fisher's Exact Test, $\alpha = 0.05$, one-sided greater; number of juveniles: Williams t-test, $\alpha = 0.05$, one-sided smaller)

- not applicable

III. CONCLUSIONS

Indoxacarb 150 g/L EC had no statistically significant lethal effects on the Collembola *Folsomia candida* when exposed to concentrations up to and including 69.4 mg formulated product/kg soil dry weight for 28 days. At the test concentration of 125 mg formulated product/kg soil dry weight mortality was statistically significantly different when compared to the control.

Indoxacarb 150 g/L EC had no statistically significant reproductive effects on the Collembola *Folsomia candida* when exposed to concentrations up to and including 6.6 mg formulated product/kg soil dry weight for 28 days. At the test concentration of 11.9 mg formulated product/kg soil dry weight and higher mortality was statistically significantly different when compared to the control.

The 28-day EC₅₀ based on reproduction was determined to be greater than 125 mg formulated product/kg soil dry weight and the overall Lowest-Observed-Effect Concentration (LOEC) for Indoxacarb 150 g/L EC was determined to be 11.9 mg formulated product/kg soil dry weight. The overall 28-day No-Observed-Effect Concentration (NOEC) was determined to be 6.6 mg formulated product/kg soil dry weight.

(Pavic, B., 2014a)

RMS comment

Study submitted to the EU for the first time in this dossier.

The study is valid.

Indoxacarb 150 g/L EC had no statistically significant lethal effects on the Collembola *Folsomia candida* when exposed to concentrations up to and including 69.4 mg formulated product/kg soil dry weight for 28 days. At the test concentration of 125 mg formulated product/kg soil dry weight mortality was statistically significantly different when compared to the control.

Indoxacarb 150 g/L EC had no statistically significant reproductive effects on the Collembola *Folsomia candida* when exposed to concentrations up to and including 6.6 mg formulated product/kg soil dry weight for 28 days. At the test concentration of 11.9 mg formulated product/kg soil dry weight and higher mortality was statistically significantly different when compared to the control.

The overall Lowest-Observed-Effect Concentration (LOEC) for Indoxacarb 150 g/L EC was determined to be 11.9 mg formulated product/kg soil dry weight. The overall 28-day No-Observed-Effect Concentration (NOEC) was determined to be 6.6 mg formulated product/kg soil dry weight. The EC10 was calculated by RMS: EC10 = 14.86 mg formulated product/kg soil dry weight (95% confidence intervals: 1.31-51.84).

Report: Pavic, B. (2014b); Indoxacarb (DPX-KN128) 150 g/L EC: Effects on reproduction of the predatory mite *Hypoaspis aculeifer* in artificial soil with 5% peat

DuPont Report No.: DuPont-37893

Guidelines: OECD 226 (2008) **Deviations:** None

Testing Facility: IBACON, Rossdorf, Germany

Testing Facility Report No.: 83781089

GLP: Yes

Certifying Authority: Hessisches Ministerium für Umwelt, Energie, Landwirtschaft und Verbraucherschutz (Weisbaden, Germany)

Executive summary:

A study was conducted to determine the effect of Indoxacarb 150 g/L EC on the mortality and reproduction of the soil mite (*Hypoaspis aculeifer*) according to OECD 226. The soil mites were exposed for 14 days to artificial soil (prepared according to OECD 226) treated with the test item to obtain the nominal concentrations of 16.3, 29.4, 52.9, 95.3, 171.5, 308.6, 555.6, and 1000 mg formulated product/kg artificial soil dry weight (corresponding to 2.5, 4.4, 8.0, 14.3, 25.8, 46.5, 83.6 and 150.5 mg a.s./kg artificial soil dry weight, adjusted for purity) and to an untreated control moistened with deionized water only. Indoxacarb 150 g/L EC had no statistically significant lethal effects on the predatory mite *Hypoaspis aculeifer* when exposed to concentrations up to and including 555.6 mg formulated product/kg artificial soil dry weight for 14 days and no reproductive effects when exposed to concentrations up to and including 95.3 mg formulated product/kg artificial soil dry weight for 14 days.

The 14-day EC₅₀ and the Lowest-Observed-Effect Concentration (LOEC) for Indoxacarb 150 g/L EC, based on reproduction were determined to be 593.55 mg formulated product/kg artificial soil dry weight and 171.5 mg formulated product/kg artificial soil dry weight, respectively. The Lowest-Observed-Effect Concentration (LOEC) for Indoxacarb 150 g/L EC based on mortality was determined to be 1000 mg formulated product/kg artificial soil dry weight. The overall 14-day No-Observed-Effect Concentration (NOEC) was determined to be 95.3 mg formulated product/kg artificial soil dry weight.

I. MATERIALS AND METHODS**A. MATERIALS**

- | | | |
|----|-----------------------------|--|
| 1. | Test material: | Indoxacarb 150 g/L EC |
| | Lot/Batch #: | DPX-KN128-311 |
| | Purity: | 150 g a.s./L |
| | Description: | Liquid |
| | CAS#: | None for the formulation |
| | | 173584-44-6 for indoxacarb the active substance |
| | Stability of test compound: | Not analysed in the test system |
| 2. | Control: | Untreated (moistened with deionized water) |
| | Test vehicle: | Deionized water |
| | Toxic reference: | Dimethoate |
| 3. | Test System: | Predatory soil mites (adult females) |
| | Species: | <i>Hypoaspis aculeifer</i> |
| | Age at dosing: | Adults, approximately 11 days after reaching the adult stage
(32 days after placing adult females in clean rearing vessels over a period of 3 days) |
| | Weight at dosing: | Not determined |
| | Source: | Cultured by IBACON |
| | Acclimation period: | 32 days |
| | Test chamber: | Glass containers (volume: 100 mL; diameter: 5 cm), closed,
filled with 20 ± 1.0 g artificial soil dry weight |
| | Test medium: | Artificial soil prepared according to OECD 226, maximum
water holding capacity of the artificial soil, as measured: 41% |
| | Diet: | Cheese mites (<i>Tyrophagus putrescentiae</i>) |
| | Water content of soil: | Initiation: 22.3 to 22.9%, equivalent to 54.3 to 56.0% of the
maximum water holding capacity
Termination: 20.5 to 22.1% equivalent to 49.9 to 54.0% of the
maximum water holding capacity |
| | Soil pH: | 5.5 to 6.0 at test start; 5.5 at test termination |
| 4. | Environmental conditions | |
| | Temperature: | Within a range of 18 to 22°C |
| | Photoperiod: | 16 hour light, 8 hour dark, photoperiod within a range of
400 to 800 lux |

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed

05-November-2013 to 21-November-2013

2. Experimental treatments

A study was conducted to determine the effect of Indoxacarb 150 g/L EC on the mortality and reproduction of the predatory soil mite (*Hypoaspis aculeifer*). Eight replicates for the control and four replicates per test item group, containing ten predatory mites each (total 80 individuals per control and 40 individuals per test item group) were each exposed for 14 days to nominal concentrations of 16.3, 29.4, 52.9, 95.3, 171.5, 308.6, 555.6 and 1000 mg formulated product/kg dry artificial soil (corresponding to 2.5, 4.4, 8.0, 14.3, 25.8, 46.5, 83.6 and 150.5 a.s./kg dry artificial soil, adjusted for purity) and to an untreated control (deionized water only). A reference item (dimethoate) is tested at least once a year to ensure sensitivity of the test system at five concentrations. The most recent test relevant to this study was conducted in June 2013.

3. Observations

After the 14-day exposure period, surviving soil mites were extracted by a heat gradient. The mean number of adults in each treatment group was determined. The mean number of juveniles produced in each treatment group over 14-day exposure period was also determined and the percent reduction in juveniles produced relative to the untreated control group was calculated.

4. Statistics

Mortality data were analysed for significance by using Fisher's Exact Test (one-sided greater, $\alpha = 0.05$). Reproduction data were tested for normal distribution and homoscedascity using Shapiro-Wilk's test, Levene's test and Cochran's test ($\alpha = 0.05$). Further statistical evaluation of the NOEC for reproduction was performed using Bonferroni-Welch t-test (multiple comparison, $\alpha = 0.05$, one-sided smaller). EC_{50} of reproduction and its 95% confidence limits were determined by applying Probit-Analysis.

II. RESULTS AND DISCUSSION

A. FINDINGS

All validity criteria were met. The reference item caused statistically significant effects on reproduction at a concentration of 3.0 mg dimethoate/kg dry artificial soil and above; the EC_{50} for reproduction was 4.2 mg dimethoate/kg dry artificial soil.

A summary of the results is provided in the table below:

Table 161
The effects on mortality and reproduction of *Hypoaspis aculeifer*, exposed to
Indoxacarb 150 g/L EC in artificial soil for 14 days

Indoxacarb 150 g/L EC concentration (mg formulated product/kg soil dry weight)	Mean % mortality	Reproduction	
		Mean juveniles per replicate	% of control
Untreated control (0.0)	14	167	-
16.3	5	173	104
29.4	10	164	98
52.9	10	144	86
95.3	20	156	93
171.5	15	133	80*
308.6	23	131	78
555.6	18	89	54*
1000	44*	48*	29*

* Statistically significant (mortality: Fisher's Exact Test, one-sided greater, $\alpha = 0.05$; number of juveniles: Bonferroni-Welch t-test, one-sided smaller, $\alpha = 0.05$)

- not applicable

III. CONCLUSIONS

Indoxacarb 150 g/L EC had no statistically significant lethal effects on the soil mite *Hypoaspis aculeifer* when exposed to concentrations up to and including 555.6 mg formulated product/kg artificial soil dry weight. At the test concentration of 1000 mg formulated product/kg soil dry weight mortality was statistically significantly different when compared to the control.

Indoxacarb 150 g/L EC had no statistically significant reproductive effects on the soil mite *Hypoaspis aculeifer* when exposed to concentrations up to and including 95.3 mg formulated product/kg artificial soil dry weight. At concentrations of 171.5 mg formulated product/kg artificial soil dry weight and higher reproduction was statistically significantly reduced when compared to the control. The statistically non-significant value of reproduction at the concentration of 308.6 mg formulated product/kg soil dry weight was nevertheless considered to be a test item-related effect since the mean reproduction is even lower than at the concentration of 171.5 mg formulated product/kg artificial soil dry weight which was defined as statistically significantly different compared to the control. The reason why this value could not be determined as a clear effect by the statistical evaluation lies in the variance of the reproduction values, which was very high, reaching from 93 to 167 individuals.

The 14-day EC_{50} based on reproduction was determined to be 593.55 mg formulated product/kg dry artificial soil dry weight and the overall Lowest-Observed-Effect Concentration (LOEC) for Indoxacarb 150 g/L EC was determined to be 171.5 mg formulated product/kg artificial soil dry weight. The overall 14-day No-Observed-Effect Concentration (NOEC) was determined to be 95.3 mg formulated product/kg dry artificial soil.

(Pavic, B., 2014b)

RMS comment

Study submitted to the EU for the first time in this dossier.

The study is valid.

Indoxacarb 150 g/L EC had no statistically significant lethal effects on the soil mite *Hypoaspis aculeifer* when exposed to concentrations up to and including 555.6 mg formulated product/kg artificial soil dry weight. At the test concentration of 1000 mg formulated product/kg soil dry weight mortality was statistically significantly different when compared to the control.

Indoxacarb 150 g/L EC had no statistically significant reproductive effects on the soil mite *Hypoaspis aculeifer* when exposed to concentrations up to and including 95.3 mg formulated product/kg artificial soil dry weight. At concentrations of 171.5 mg formulated product/kg artificial soil dry weight and higher reproduction was statistically significantly reduced when compared to the control.

The overall Lowest-Observed-Effect Concentration (LOEC) for Indoxacarb 150 g/L EC was determined to be 171.5 mg formulated product/kg artificial soil dry weight. The overall 14-day No-Observed-Effect Concentration (NOEC) was determined to be 95.3 mg formulated product/kg dry artificial soil.

The EC10 was calculated by RMS: EC10 = 137.2 mg formulated product/kg soil dry weight (95% confidence intervals: 59.64-246.85).

B.9.8. RISK ASSESSMENT FOR NON-TARGET SOIL MESO- AND MACROFAUNA

Studies on indoxacarb (DPX-KN128), Indoxacarb 150 g/L EC, and the major soil metabolites of indoxacarb were conducted in soil meso and macrofauna. All relevant data and assessments are provided here and are considered adequate for the assessment of the risk to meso and macrofauna.

Table 162
Ecotoxicological endpoints for soil macro organisms

Test organism	Test substance	Application method of test a.s./ OM ¹	Time scale	End point	Toxicity
Earthworms					
<i>Eisenia fetida</i>	DPX-KN128	5% peat in test soil, test item mixed into soil	Chronic 56 d	reproduction	56-d NOEC = 29.2 mg/kg dry soil EC10 = 23.95 mg a.s./kg dry soil
<i>Eisenia fetida</i>	Indoxacarb 150 g/L EC	10% peat in test soil, test item mixed into soil	Chronic 56 d	reproduction	56-d NOEC = 199.3 mg product/kg dry soil (29.9 mg a.s./kg dry soil) EC10 = 142.88 mg prep./kg dry soil (21.43 mg a.s./kg dry soil)
<i>Eisenia fetida</i>	IN-JT333	5% peat in test soil, test item mixed into soil	Chronic 56 d	Growth, reproduction, behaviour	56-d NOEC = 100 mg/kg dry soil EC10 = 54.86 mg/kg dry soil

<i>Eisenia fetida</i>	IN-JT333	10% peat in test soil, test item mixed into soil	Chronic 56 d	reproduction	56-d NOEC = 2.5 mg/kg dry soil
<i>Eisenia fetida</i>	IN-JU873	5% peat in test soil, test item mixed into soil	Chronic 56 d	reproduction	56-d NOEC = 50 mg/kg dry soil EC10 = 89.12 mg/kg dry soil
<i>Eisenia fetida</i>	IN-KG433	5% peat in test soil, test item mixed into soil	Chronic 56 d	reproduction	56-d NOEC = 50 mg/kg dry soil EC10 = 55.3 mg/kg dry soil
<i>Eisenia fetida</i>	IN-KT413	5% peat in test soil, test item mixed into soil	Chronic 56 d	Growth, reproduction, behaviour	56-d NOEC = 100 mg/kg dry soil
<i>Eisenia fetida</i>	IN-MK638	5% peat in test soil, test item mixed into soil	Chronic 56 d	Growth	56-d NOEC = 50 mg/kg dry soil
<i>Eisenia fetida</i>	IN-MK643	5% peat in test soil, test item mixed into soil	Chronic 56 d	Growth, reproduction, behaviour	56-d NOEC = 25 mg/kg dry soil EC10 = 22.25 mg/kg dry soil
<i>Eisenia fetida</i>	IN-KB687	5% peat in test soil, test item mixed into soil	Chronic 56 d	reproduction	56-d NOEC = 50 mg/kg dry soil
Other soil macroorganisms					
<i>Folsomia candida</i>	DPX-KN128	5% peat in test soil, test item mixed into soil	Chronic 28 d	reproduction	NOEC = 125 mg/kg dry soil EC10 = 106.8 mg/kg dry soil
<i>Folsomia candida</i>	Indoxacarb 150 g/L EC	5% peat in test soil, test item mixed into soil	Chronic 28 d	reproduction	28-d NOEC = 6.6 mg prep./kg dry soil (1 mg a.s./kg dry soil) EC10 = 14.86 mg prep./kg dry soil

<i>Folsomia candida</i>	IN-JT333	5% peat in test soil, test item mixed into soil	Chronic 28 d	reproduction	NOEC = 50 mg/kg dry soil EC10 = 64.17 mg/kg dry soil
<i>Folsomia candida</i>	IN-JU873	5% peat in test soil, test item mixed into soil	Chronic 28 d	reproduction	NOEC = 100 mg/kg dry soil
<i>Folsomia candida</i>	IN-KG433	5% peat in test soil, test item mixed into soil	Chronic 28 d	reproduction	NOEC = 6.25 mg/kg dry soil
<i>Folsomia candida</i>	IN-KT413	5% peat in test soil, test item mixed into soil	Chronic 28 d	reproduction	NOEC= 100 mg/kg dry soil
<i>Folsomia candida</i>	IN-MK638	5% peat in test soil, test item mixed into soil	Chronic 28 d	reproduction	NOEC= 25 mg/kg dry soil EC10 = 10.15 mg/kg dry soil
<i>Folsomia candida</i>	IN-MK643	10% peat in test soil, test item mixed into soil	Chronic 28 d	reproduction	NOEC= 10 mg/kg dry soil EC10 = 80.28 mg/kg dry soil
<i>Folsomia candida</i>	IN-KB687	5% peat in test soil, test item mixed into soil	Chronic 28 d	reproduction	NOEC= 6.25 mg/kg dry soil EC10 = 10.80 mg/kg dry soil
<i>Hypoaspis aculeifer</i>	DPX-KN128	5% peat in test soil, test item mixed into soil	Chronic 14 d	reproduction	NOEC = 1000 mg/kg dry soil
<i>Hypoaspis aculeifer</i>	Indoxacarb 150 g/L EC	5% peat in test soil, test item mixed into soil	Chronic 14 d	reproduction	NOEC = 95.3 mg prep./kg dry soil (14.3 mg a.s./kg dry soil) EC10 = 137.2 mg prep./kg dry soil

<i>Hypoaspis aculeifer</i>	IN-JT333	5% peat in test soil, test item mixed into soil	Chronic 14 d	reproduction	NOEC = 100 mg/kg dry soil
<i>Hypoaspis aculeifer</i>	IN-JU873	5% peat in test soil, test item mixed into soil	Chronic 14 d	reproduction	NOEC = 100 mg/kg dry soil
<i>Hypoaspis aculeifer</i>	IN-KG433	5% peat in test soil, test item mixed into soil	Chronic 14 d	reproduction	NOEC = 100 mg/kg dry soil
<i>Hypoaspis aculeifer</i>	IN-KT413	5% peat in test soil, test item mixed into soil	Chronic 14 d	reproduction	NOEC = 100 mg/kg dry soil EC10 = 62.7 mg/kg dry soil
<i>Hypoaspis aculeifer</i>	IN-MK638	5% peat in test soil, test item mixed into soil	Chronic 14 d	reproduction	NOEC = 100 mg/kg dry soil
<i>Hypoaspis aculeifer</i>	IN-MK643	5% peat in test soil, test item mixed into soil	Chronic 14 d	reproduction	NOEC = 100 mg/kg dry soil
<i>Hypoaspis aculeifer</i>	IN-KB687	5% peat in test soil, test item mixed into soil	Chronic 14 d	reproduction	NOEC = 3.125 mg/kg dry soil EC10 = 5.44 mg/kg dry soil

Toxicity exposure ratios for soil macroorganisms

The risk of Indoxacarb 150 g/L EC is assessed according to the Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002, October 2002). The TER values were determined for indoxacarb (DPX-KN128), Indoxacarb 150 g/L EC, and indoxacarb metabolites based on the ratio of the NOEC values to the maximum PEC_{soil} .

Use on maize at 37.5 g a.s./ha x 2applications

Table 163
TER for soil macroorganisms for the use of Indoxacarb 150 g/L EC in maize

Test organism	Test substance	Time scale	Endpoint	Soil PEC	TER	Trigger
Earthworms						
<i>Eisenia fetida</i>	DPX-KN128	Chronic	EC10 = 23.95 mg a.s./kg dry soil ^c	0.042 (plateau)	570	5
<i>Eisenia fetida</i>	Indoxacarb 150 g/L EC	Chronic	EC10corr = 10.71 mg a.s./kg dry soil ^a	0.042 (plateau)	255	5
<i>Eisenia fetida</i>	IN-JT333	Chronic	EC10 = 54.86 mg/kg dry soil ^c	0.007 (plateau)	7837	5
<i>Eisenia fetida</i>	IN-JT333	Chronic	NOECcorr = 1.25 mg/kg dry soil ^b	0.007 (plateau)	178	5
<i>Eisenia fetida</i>	IN-JU873	Chronic	NOEC = 50 mg/kg dry soil ^c	0.004 (plateau)	12500	5
<i>Eisenia fetida</i>	IN-KG433	Chronic	NOEC = 50 mg/kg dry soil ^c	0.015 (initial)	3333	5
<i>Eisenia fetida</i>	IN-KT413	Chronic	NOEC = 100 mg/kg dry soil ^c	0.007 (initial)	14285	5
<i>Eisenia fetida</i>	IN-MK638	Chronic	NOEC = 50 mg/kg dry soil ^c	0.004 (initial)	12500	5
<i>Eisenia fetida</i>	IN-MK643	Chronic	EC10 = 22.25 mg/kg dry soil ^c	0.002 (plateau)	11125	5
<i>Eisenia fetida</i>	IN-KB687	Chronic	NOEC = 50 mg/kg dry soil ^c	0.004 (initial)	12500	5
<i>Eisenia fetida</i>	IN-ML438	Chronic	EC10 = 2.395 mg a.s./kg dry soil ^d	0.003 (plateau)	798	5
<i>Eisenia fetida</i>	IN-U8E24	Chronic	EC10 = 2.395 mg a.s./kg dry soil ^d	0.022 (plateau)	109	5
<i>Folsomia candida</i>	DPX-KN128	Chronic	EC10 = 106.8 mg/kg dry soil ^c	0.042 (plateau)	2542	5
<i>Folsomia candida</i>	Indoxacarb 150 g/L EC	Chronic	NOEC = 1 mg a.s./kg dry soil ^c	0.042 (plateau)	23.8	5
<i>Folsomia candida</i>	IN-JT333	Chronic	NOEC = 50 mg/kg dry soil ^c	0.007 (plateau)	7142	5
<i>Folsomia candida</i>	IN-JU873	Chronic	NOEC = 100 mg/kg dry soil ^c	0.004 (plateau)	25000	5
<i>Folsomia candida</i>	IN-KG433	Chronic	NOEC = 6.25 mg/kg dry soil ^c	0.015 (initial)	416	5
<i>Folsomia candida</i>	IN-KT413	Chronic	NOEC= 100 mg/kg dry soil ^c	0.007 (initial)	14285	5

<i>Folsomia candida</i>	IN-MK638	Chronic	EC10 = 10.15 mg/kg dry soil ^c	0.004 (initial)	2537	5
<i>Folsomia candida</i>	IN-MK643	Chronic	NOECcorr= 5 mg/kg dry soil ^b	0.002 (plateau)	2500	5
<i>Folsomia candida</i>	IN-KB687	Chronic	NOEC= 6.25 mg/kg dry soil ^c	0.004 (initial)	1562	5
<i>Folsomia candida</i>	IN-ML438	Chronic	EC10 = 10.68 mg/kg dry soil ^d	0.003 (plateau)	3560	5
<i>Folsomia candida</i>	IN-U8E24	Chronic	EC10 = 10.68 mg/kg dry soil ^d	0.022 (plateau)	485	5
<i>Hypoaspis aculeifer</i>	DPX-KN128	Chronic	NOEC = 1000 mg/kg dry soil ^c	0.042 (plateau)	23809	5
<i>Hypoaspis aculeifer</i>	Indoxacarb 150 g/L EC	Chronic	NOEC = 14.3 mg a.s./kg dry soil ^c	0.042 (plateau)	340	5
<i>Hypoaspis aculeifer</i>	IN-JT333	Chronic	NOEC = 100 mg/kg dry soil ^c	0.007 (plateau)	14285	5
<i>Hypoaspis aculeifer</i>	IN-JU873	Chronic	NOEC = 100 mg/kg dry soil ^c	0.004 (plateau)	25000	5
<i>Hypoaspis aculeifer</i>	IN-KG433	Chronic	NOEC = 100 mg/kg dry soil ^c	0.015 (initial)	6666	5
<i>Hypoaspis aculeifer</i>	IN-KT413	Chronic	EC10 = 62.7 mg/kg dry soil ^c	0.007 (initial)	8957	5
<i>Hypoaspis aculeifer</i>	IN-MK638	Chronic	NOEC = 100 mg/kg dry soil ^c	0.004 (initial)	25000	5
<i>Hypoaspis aculeifer</i>	IN-MK643	Chronic	NOEC = 100 mg/kg dry soil ^c	0.002 (plateau)	50000	5
<i>Hypoaspis aculeifer</i>	IN-KB687	Chronic	NOEC = 3.125 mg/kg dry soil ^c	0.004 (initial)	781	5
<i>Hypoaspis aculeifer</i>	IN-ML438	Chronic	NOEC = 100 mg/kg dry soil ^d	0.003 (plateau)	33333	5
<i>Hypoaspis aculeifer</i>	IN-U8E24	Chronic	NOEC = 100 mg/kg dry soil ^d	0.022 (plateau)	4545	5

^a The toxicity endpoint has been divided by 2 since the log P_{ow} is >2 and peat content in artificial soil was of 10%.

^b No log Pow value was indicated. The toxicity endpoint has been conservatively divided by 2 since the peat content in artificial soil was of 10%.

^c No endpoint correction as substrate contained reduced amount of peat (5%).

^d As a worst-case assumption the endpoint of the parent compound divided by 10 was used.

Use on lettuce at 37.5 g a.s./ha x 4applications

Table 164
TER for soil macroorganisms for the use of Indoxacarb 150 g/L EC in lettuce

Test organism	Test substance	Time scale	Endpoint	Soil PEC	TER	Trigger
Earthworms						
<i>Eisenia fetida</i>	DPX-KN128	Chronic	EC10 = 23.95 mg a.s./kg dry soil ^c	0.169 (plateau)	142	5
<i>Eisenia fetida</i>	Indoxacarb 150 g/L EC	Chronic	EC10corr = 10.71 mg a.s./kg dry soil ^a	0.169 (plateau)	63	5
<i>Eisenia fetida</i>	IN-JT333	Chronic	EC10 = 54.86 mg/kg dry soil ^c	0.026 (plateau)	2110	5
<i>Eisenia fetida</i>	IN-JT333	Chronic	NOECcorr = 1.25 mg/kg dry soil ^b	0.026 (plateau)	48	5
<i>Eisenia fetida</i>	IN-JU873	Chronic	NOEC = 50 mg/kg dry soil ^c	0.017 (plateau)	2941	5
<i>Eisenia fetida</i>	IN-KG433	Chronic	NOEC = 50 mg/kg dry soil ^c	0.059 (initial)	847	5
<i>Eisenia fetida</i>	IN-KT413	Chronic	NOEC = 100 mg/kg dry soil ^c	0.027 (initial)	3703	5
<i>Eisenia fetida</i>	IN-MK638	Chronic	NOEC = 50 mg/kg dry soil ^c	0.018 (initial)	2777	5
<i>Eisenia fetida</i>	IN-MK643	Chronic	EC10 = 22.25 mg/kg dry soil ^c	0.009 (plateau)	2472	5
<i>Eisenia fetida</i>	IN-KB687	Chronic	NOEC = 50 mg/kg dry soil ^c	0.015 (initial)	3333	5
<i>Eisenia fetida</i>	IN-ML438	Chronic	EC10 = 2.395 mg a.s./kg dry soil ^d	0.011 (plateau)	217	5
<i>Eisenia fetida</i>	IN-U8E24	Chronic	EC10 = 2.395 mg a.s./kg dry soil ^d	0.035 (plateau)	109	5
<i>Folsomia candida</i>	DPX-KN128	Chronic	EC10 = 106.8 mg/kg dry soil ^c	0.169 (plateau)	632	5
<i>Folsomia candida</i>	Indoxacarb 150 g/L EC	Chronic	NOEC = 1 mg a.s./kg dry soil ^c	0.169 (plateau)	5.9	5
<i>Folsomia candida</i>	IN-JT333	Chronic	NOEC = 50 mg/kg dry soil ^c	0.026 (plateau)	1923	5
<i>Folsomia candida</i>	IN-JU873	Chronic	NOEC = 100 mg/kg dry soil ^c	0.017 (plateau)	5882	5
<i>Folsomia candida</i>	IN-KG433	Chronic	NOEC = 6.25 mg/kg dry soil ^c	0.059 (initial)	106	5

<i>Folsomia candida</i>	IN-KT413	Chronic	NOEC= 100 mg/kg dry soil ^c	0.027 (initial)	3703	5
<i>Folsomia candida</i>	IN-MK638	Chronic	EC10 = 10.15 mg/kg dry soil ^c	0.018 (initial)	564	5
<i>Folsomia candida</i>	IN-MK643	Chronic	NOECcorr= 5 mg/kg dry soil ^b	0.009 (plateau)	555	5
<i>Folsomia candida</i>	IN-KB687	Chronic	NOEC= 6.25 mg/kg dry soil ^c	0.015 (initial)	416	5
<i>Folsomia candida</i>	IN-ML438	Chronic	EC10 = 10.68 mg/kg dry soil ^d	0.011 (plateau)	971	5
<i>Folsomia candida</i>	IN-U8E24	Chronic	EC10 = 10.68 mg/kg dry soil ^d	0.035 (plateau)	305	5
<i>Hypoaspis aculeifer</i>	DPX-KN128	Chronic	NOEC = 1000 mg/kg dry soil ^c	0.169 (plateau)	5917	5
<i>Hypoaspis aculeifer</i>	Indoxacarb 150 g/L EC	Chronic	NOEC = 14.3 mg a.s./kg dry soil ^c	0.169 (plateau)	84	5
<i>Hypoaspis aculeifer</i>	IN-JT333	Chronic	NOEC = 100 mg/kg dry soil ^c	0.026 (plateau)	3846	5
<i>Hypoaspis aculeifer</i>	IN-JU873	Chronic	NOEC = 100 mg/kg dry soil ^c	0.017 (plateau)	5882	5
<i>Hypoaspis aculeifer</i>	IN-KG433	Chronic	NOEC = 100 mg/kg dry soil ^c	0.059 (initial)	1694	5
<i>Hypoaspis aculeifer</i>	IN-KT413	Chronic	EC10 = 62.7 mg/kg dry soil ^c	0.027 (initial)	2322	5
<i>Hypoaspis aculeifer</i>	IN-MK638	Chronic	NOEC = 100 mg/kg dry soil ^c	0.018 (initial)	5555	5
<i>Hypoaspis aculeifer</i>	IN-MK643	Chronic	NOEC = 100 mg/kg dry soil ^c	0.009 (plateau)	11111	5
<i>Hypoaspis aculeifer</i>	IN-KB687	Chronic	NOEC = 3.125 mg/kg dry soil ^c	0.015 (initial)	208	5
<i>Hypoaspis aculeifer</i>	IN-ML438	Chronic	NOEC = 100 mg/kg dry soil ^d	0.011 (plateau)	9091	5
<i>Hypoaspis aculeifer</i>	IN-U8E24	Chronic	NOEC = 100 mg/kg dry soil ^d	0.035 (plateau)	2857	5

^a The toxicity endpoint has been divided by 2 since the log P_{ow} is >2 and peat content in artificial soil was of 10%.

^b No log P_{ow} value was indicated. The toxicity endpoint has been conservatively divided by 2 since the peat content in artificial soil was of 10%.

^c No endpoint correction as substrate contained reduced amount of peat (5%).

^d As a worst-case assumption the endpoint of the parent compound divided by 10 was used.

The TER_{It} values exceed the relevant Regulation (EC) 546/2011 decision-making criteria of 5 for earthworms and other soil macroorganisms. Therefore, it can be concluded that chronic risk from the use of Indoxacarb 150 g/L EC in maize and lettuce is acceptable.

B.9.9. EFFECTS ON SOIL NITROGEN TRANSFORMATION

Report: Reis, K.-H. (2006); Indoxacarb (DPX-KN128) 150 g/L EC: Assessment of the effects on soil microflora

DuPont Report No.: DuPont-18925

Guidelines: OECD 216 (2000), OECD 217 (2000) **Deviations:** None

Testing Facility: IBACON, Rossdorf, Germany

Testing Facility Report No.: 29522080

GLP: Yes

Certifying Authority: Hessisches Ministerium für Umwelt, Landwirtschaft und Forsten

Executive summary:

A laboratory soil microflora study was conducted in a loamy sand soil to determine the effects of Indoxacarb 150 g/L EC on nitrogen transformation and soil respiration. This study was conducted according to OECD 216 (2000) and OECD 217 (2000). Indoxacarb 150 g/L EC was applied to the soil at nominal application rates of 0.84, 4.17, and 8.35 mg formulated product/kg of soil (dry weight equivalent). These rates were equivalent to 1×, 5×, and 10× the maximum recommended field application rate of 0.67 L formulated product/ha (equivalent to 100 g a.s./ha, based on nominal content). An untreated control soil was also tested. At the end of 28 days, deviation in respiration rate at the highest concentration tested 8.35 mg formulated product/kg of soil (dry weight equivalent) compared to the control was <25% (actual, 10.22%), the effect threshold specified by the OECD guidelines. At the end of 28 days, deviation in nitrate formation rate at the highest concentration tested, 8.35 mg formulated product/kg of soil (dry weight equivalent) compared to the control was <25% (actual, 6.58%), the effect threshold specified by the OECD test guidelines.

I. MATERIALS AND METHODS

A. MATERIALS

- | | |
|---------------------------------|--|
| 1. Test material: | Indoxacarb 150 g/L EC |
| Lot/Batch #: | DPX-KN128-150 |
| Purity: | 150 g a.s./L |
| CAS#: | None for the formulation
173584-44-6 for indoxacarb the active substance |
| Description: | Yellow liquid |
| Stability of test compound: | Considered to be stable under the conditions of the test |
| 2. Control: | Untreated soil/deionised water |
| Test vehicle: | Deionised water |
| Toxic standard: | Sodium chloride (tested once per year) |
| 3. Test organism: | Soil microflora |
| Source: | Fallow land near Rossdorf, Germany |
| Test Chambers: | Nitrogen transformation test: 500 mL plastic boxes with perforated plastic lids containing approximately 400 g soil dw
Respiration test: 1000 mL plastic boxes, with perforated plastic lids containing approximately 800 grams of soil dw
Lucerne meal: 5g/kg soil dry weight (nitrogen determination),
Glucose: 4 g/kg soil dry weight (short-term respiration study) |
| Substrates: | |
| Acclimation period: | 20 days |
| 4. Environmental conditions | |
| Temperature: | 20 to 22°C |
| Photoperiod: | Continuous dark |
| Soil: | Natural soil |
| Soil type: | Loamy sand |
| Soil pH: | 6.8 |
| % total organic carbon: | 1.06 |
| CEC (meg)/100 g: | 66.5 |
| Water holding capacity (%): | 50.5 |
| Soil moisture range during test | |
| % of water holding capacity: | 46-51% |

B. STUDY DESIGN AND METHODS

1. Experimental start/completed
30-May-2006 to 29-June-2006

2. Experimental treatments

A laboratory study was conducted in a loamy sand soil to determine the effects of Indoxacarb 150 g/L EC on nitrogen transformation and soil respiration. Indoxacarb 150 g/L EC was emulsified in deionised water and mixed with the soil at nominal application rates of 0.84, 4.17, and 8.35 mg formulated product/kg of soil (dry weight equivalent). Assuming the test material is uniformly distributed in the top 5 cm of the soil and has a soil bulk density of 1.5 g dry weight, these rates were equivalent to 1×, 5×, and 10× the maximum recommended field application rate of 100 g a.s./ha. The control consisted of the soil treated with deionised water. The reference item, sodium chloride, is tested once a year at a concentration of 16 g/kg dry weight. Samples for nitrogen determination and soil respiration were incubated for 28 days.

3. Observations

Samples were collected for determination of nitrogen transformation and soil respiration at Days 0, 7, 14, and 28 days following application of the test item.

4. Statistics

R/S-Test and Bartlett's-Test ($\alpha = 0.05$): normality and homogeneity of variance

Student-t-test, two sided, $\alpha = 0.05$: test for significant differences between the treatment groups and the control group.

Calculations:

$$\% \text{ deviation from the control} = [(C - T)/C] \times 100;$$

Nitrate formation rate (mg/day) = the difference between the $\text{NO}_3\text{-N}$ (mg/kg soil dry weight) content between the sampling day and Day 0, divided by the number of sampling days.

II. RESULTS AND DISCUSSION

A. FINDINGS

Indoxacarb 150 g/L EC, at 0.84, 4.17, and 8.35 mg formulated product/kg of soil (equivalent to 100, 500, and 1000 g a.s./ha), had no significant effect on the nitrate content in soil. At the end of the 28-day study, the deviations in nitrate content compared to the control soil were below the 25% trigger value in accordance with OECD guideline 216.

The rate of nitrate formation per day was below the 25% trigger value according to the OECD guideline 216. On Day 28, the nitrate formation rate deviated 22.6, -9.4, and 11.3% when compared to the control for the test concentrations of 0.84, 4.17, and 8.35 mg formulated product/kg soil dry weight, respectively. The differences from the control were statistically significant in the lower and upper test concentration.

At the end of the study on Day 28, deviations in respiration rates at concentrations up to and including 8.35 mg formulated product/kg of soil (dry weight equivalent) compared to the control were <25%, the effect threshold specified by the OECD guidelines. The short-term respiration rate in soil treated with Indoxacarb 150 g/L EC was not statistically significantly different from the control at 0.84, 4.17, and 8.35 mg formulated product/kg soil dry weight at the end of the study (Day 28).

Table 165
Summary of effects of Indoxacarb 150 g/L EC on nitrate formation and short-term respiration in soil

Indoxacarb 150 g/L EC mg formulated product/kg dry soil ^a	NO ₃ -N Levels (Day 28)		Nitrate formation rate (Day 0 to 28)		Respiration rate (Day 28)	
	mg formulated product/kg dry soil	% Deviation from control ^{b,c}	mg formulated product/kg dry soil/day	% Deviation from control ^{b,c}	mg CO ₂ /hr/kg dry soil	% Deviation from control ^{b,c}
Control (0.0)	28.383	---	0.53	---	12.978	---
0.84	31.949	12.56*	0.65	22.6*	13.369	3.01
4.17	27.758	-2.20	0.48	-9.4	13.281	2.33
8.35	30.252	6.58*	0.59	11.3	14.305	10.22

^a Test item concentrations correspond to 0.66, 3.30 and 6.61 L product/ha, 0.63, 3.13 and 6.26 kg product/ha, and 1-, 5- and 10-times the field application rate

^b Negative value = % inhibition, positive value = % stimulation

^c Statistical evaluation (Student t-test, two sided, $\alpha = 0.05$):

* Significant differences from the control

III. CONCLUSION

At the end of 28 days, deviations in respiration rates and nitrate formation rates compared to the control were <25% at all tested concentrations of Indoxacarb 150 g/L EC, the effect threshold specified by the OECD guidelines. It can be concluded that Indoxacarb 150 g/L EC, at concentrations up to and including 8.35 mg formulated product/kg soil dry weight, corresponding to 6.61 L formulation per hectare (equivalent to 1000 g a.s./ha) pose a minimal risk to soil microflora.

(Reis, K.-H., 2006)

RMS comment

Study submitted to the EU for the first time in this dossier.

The study is valid.

At the end of 28 days, deviations in respiration rates and nitrate formation rates compared to the control were <25% at concentrations up to and including 8.35 mg Indoxacarb 150 g/L EC /kg soil dry weight.

B.9.10. RISK ASSESSMENT FOR SOIL NITROGEN TRANSFORMATION

The risk of Indoxacarb 150 g/L EC is assessed according to the Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002, October 2002).

Laboratory testing was conducted to evaluate the effects of Indoxacarb 150 g/L EC and its soil metabolites on non-target soil micro-organisms.

Table 166
Ecotoxicological endpoints for soil nitrogen transformation

Nitrogen transformation	Indoxacarb 150 g/L EC	0.84, 4.17, and 8.35 mg formulated product/kg soil dry weight	<25% effect at day 28 at 8.35 mg formulated product/kg soil dry weight
Nitrogen transformation	DPX-MP062 (79:21 mixture DPX-KN128/IN-KN127)	250 g DPX-MP062/ha	<25% effect at day 28 at 250 g a.s./ha (0.333 mg DPX-MP062/kg soil dry weight).
Nitrogen transformation	IN-JT333	60 g IN-JT333/ha	<25% effect at day 28 at 60 g/ha (0.08 mg IN-JT333/kg soil dry weight).
Nitrogen transformation	IN-JU873	0.087 and 0.87 mg IN-JU873/kg soil dry weight	<25% effect at day 28 at 0.087 and 0.87 mg IN-JU873/kg soil dry weight
Nitrogen transformation	IN-KG433	0.076 mg IN-KG433/kg soil dry weight	<25% effect at day 28 at 0.076 mg IN-KG433/kg soil dry weight (14.87%) based on nitrogen levels 28% effect at day 28 at 0.076 mg IN-KG433/kg soil dry weight based on nitrogen transformation

Nitrogen transformation	IN-KT413	0.102 and 1.02 mg IN-KT413/kg soil dry weight	<25% effect at day 28 at 0.102 and 1.02 mg IN-KT413/kg soil dry weight
Nitrogen transformation	IN-MK638	0.042 and 0.42 mg IN-MK638/kg soil dry weight	<25% effect at day 28 at 0.042 and 0.42 mg IN-MK638/kg soil dry weight
Nitrogen transformation	IN-MK643	0.041 and 0.41 mg IN-MK643/kg soil dry weight	<25% effect at day 28 at 0.041 and 0.41 mg IN-MK643/kg soil dry weight
Nitrogen transformation	IN-KB687	0.13, 0.67, and 1.33 mg/kg soil dry weight	<25% effect at day 28 at up to 1.33 mg IN-KB687/kg soil dry weight

A risk assessment was conducted using the worst-case PEC values for the use on lettuce. This is given in the table below:

Table 167

Risk assessment for effects on nitrogen transformation of non-target micro-organisms

Test item	Endpoint	PEC soil (mg/kg soil)	Risk acceptable?
Indoxacarb 150 g/L EC	<25% effect at day 28 at 8.35 mg formulated product/kg soil dry weight (1.25 mg DPX-KN128/kg soil)	0.169	yes
IN-JT333	<25% effect at day 28 at 60 g/ha (0.08 mg IN-JT333/kg soil dry weight).	0.026	yes
IN-JU873	<25% effect at day 28 at 0.087 and 0.87 mg IN-JU873/kg soil dry weight	0.017	yes
IN-KG433	<25% effect at day 28 at 0.076 mg IN-KG433/kg soil dry weight (14.87%) based on nitrogen levels 28% effect at day 28 at 0.076 mg IN-KG433/kg soil dry weight based on nitrogen transformation	0.059	yes
IN-KT413	<25% effect at day 28 at 0.102 and 1.02 mg IN-KT413/kg soil dry weight	0.027	yes
IN-MK638	<25% effect at day 28 at 0.042 and 0.42 mg IN-MK638/kg soil dry weight	0.018	yes
IN-MK643	<25% effect at day 28 at 0.041 and 0.41 mg IN-MK643/kg soil dry weight	0.009	yes
IN-KB687	<25% effect at day 28 at up to 1.33 mg IN-KB687/kg soil dry weight	0.015	yes
IN-ML438	0.125 mg DPX-KN128/kg soil ^a	0.011	yes
IN-U8E24	0.125 mg DPX-KN128/kg soil ^a	0.035	yes

^a As a worst-case assumption the endpoint of the parent compound divided by 10 was used.

For metabolite IN-KG433, nitrogen transformation was slightly higher than 25 % (28%) at day 28. It is RMS opinion to consider the risk acceptable for this metabolite as the concentration tested (0.076 mg/kg soil) was above the maximum expected concentration of 0.059 mg/kg soil for the use on lettuce and because the comparison based on nitrogen levels results in effects < 25%.

For metabolites IN-ML438 and IN-U8E24, no study was conducted. Considering the endpoint of the parent compound divided by 10 as a worst-case assumption, the risk is acceptable for these metabolites.

Therefore, it is concluded that Indoxacarb 150 g/L EC and its metabolites pose acceptable risk to soil non-target micro-organisms for all proposed uses of Indoxacarb 150 g/L EC.

B.9.11. EFFECTS ON TERRESTRIAL NON-TARGET HIGHER PLANTS

B.9.11.1. Summary of screening data

Screening data to establish whether indoxacarb exhibits herbicidal or plant growth regulatory activity are presented in Indoxacarb Volume 3CA. Screening for pre-and post-emergent herbicidal activity was conducted with DPX-JW062 (50% DPX-KN128:50% IN-KN127) at two rates (0.5 and 1 kg a.s./ha) on nine species of plants. No herbicidal activity was indicated however the origin of these data was not found by RMS. No study was provided. These data are not used in the risk assessment.

B.9.11.2. Testing on non-target plants

The effects of Indoxacarb 150 g/L EC on vegetative vigour of a range of terrestrial non-target plants was assessed in a laboratory study.

Report: Porch, J.R., Martin, K.H. (2006); Indoxacarb (DPX-KN128) 150 g/L EC: A greenhouse study to investigate the effects on vegetative vigor of ten sensitive terrestrial plants following foliar exposure

DuPont Report No.: DuPont-19456

Guidelines: U.S. EPA 122-1 (1986), OPPTS 850.4250 (1996) **Deviations:** None

Testing Facility: Wildlife International Ltd. (USA), Easton, Maryland, USA

Testing Facility Report No.: 112-582

GLP: Yes

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

Executive summary:

Non-target terrestrial plant response to Indoxacarb 150 g/L EC was evaluated on ten common plant species (corn, oat, onion, ryegrass, cucumber, pea, rape, soybean, sugar beet, and tomato). Effects on vegetative vigour following foliar application to seedlings were assessed based on OECD Draft Guideline 227, U.S. EPA OPPTS 850.4150, and U.S. EPA, Subdivision J, Series 122-1 after 21 days. Tests were conducted as a limit test at an application rate of 100 g a.s./ha (0.67 L product/ha) using a sandy loam artificial soil (pH 7.3, O.M. 2.2%) under greenhouse conditions. The test was conducted to determine if a 25% inhibition relative to the control was observed. For the ten test species, none of the mean shoot height, shoot dry weight, survival or visual response in the 100 g a.s./ha test level were reduced by 25% or more relative to the respective control (no effect greater than 7% was observed).

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Indoxacarb 150 g/L EC
 Lot/Batch #: DPX-KN128-150
 Purity: 150 g a.s./L
 CAS#: None for the formulation
 173584-44-6 for indoxacarb the active substance
 Description: Liquid
 Stability of test compound: 98.8% of the indoxacarb remains in the delivery vehicle after one hour under agitation
2. Control: Well water purified by reverse osmosis
 Test vehicle: Well water purified by reverse osmosis
3. Test organisms: Terrestrial plants
 Species: *Zea mays* (corn)
Avena sativa (oat)
Allium cepa (common onion)
Lolium perenne, (perennial ryegrass)
Cucumis sativa (cucumber)
Brassica napus, (oilseed rape)
Pisum sativum (pea)
Glycine max (soybean)
Beta vulgaris (sugar beet)
Lycopersicon esculentum (tomato)
 Age at dosing: 2-6 true leaves (7-38 cm height) depending on species
 Initial population: 5 replicates of 4 plants (20 plants total) each per treatment and control
 Test vessels: Plastic pots, 11 cm diameter × 10 cm depth for onion and ryegrass and 16 cm diameter × 12 cm depth for other species
 Growth medium: Sandy loam soil (pH 7.3, Organic Matter 2.2%, Organic Carbon 1.3%)
 Water: Laboratory well water; Initial top watering (foliage avoided) followed by sub-irrigation
4. Environmental conditions
 Temperature: 17 to 38°C
 Relative humidity: 32 to 95%
 Photoperiod: 16 hour photoperiod
 Photosynthetically active radiation: 13.1 to 16.2 moles/M²/day (Einsteins)

B. STUDY DESIGN AND METHODS

1. Experimental start/completed
 22-August-2006 to 12-September-2006
2. Experimental treatments
 This study was carried out to assess the effects of a 100 g a.s./ha application of Indoxacarb 150 g/L EC (0.67 L product/ha) on the vegetative vigour of non-target plants following application to the foliage of young seedling plants (post-emergent application).

Seeds were planted at uniform depth (depth depending on species). Indoxacarb 150 g/L EC was applied to corn, oat, onion, ryegrass, cucumber, oilseed rape, pea, soybean, sugar beet, and tomato using a compressed nitrogen laboratory sprayer calibrated to apply approximately 400 L water/ha. After application the pots were maintained in a greenhouse under controlled conditions in a randomised complete block arrangement by species.

3. Observations

The visual response and survival of the seedlings were recorded on Days 7, 14, and 21 after application. Additionally, shoot height and shoot dry weight were determined on Day 21.

4. Statistics

Treatment group means were compared to control means in order to determine if a 25% or greater reduction in any endpoint had occurred. No statistical analysis was warranted due to the lack of negative response relative to the control.

II. RESULTS AND DISCUSSION

A. FINDINGS

The results of a post-emergent application of 100 g a.s./ha expressed in percent reductions from respective control means are summarised in Table 168.

Table 168
Effects of Indoxacarb 150 g/L EC on ten non-target plants following post-emergent application (foliar application) at 100 g a.s./ha (Tier 1 limit testing)

Plant	Family	Genus/species	Reduction from control (%)	Most sensitive parameter
Monocotyledons				
Corn	Poaceae	<i>Zea mays</i>	0	Shoot Height, Visual Response, Survival
Oat	Poaceae	<i>Avena sativa</i>	5	Shoot Dry Weight
Onion	Liliaceae	<i>Allium cepa</i>	5	Shoot Height
Ryegrass	Poaceae	<i>Lolium perenne</i>	0	Visual Response, Survival
Dicotyledons				
Cucumber	Cucurbitaceae	<i>Cucumis sativa</i>	7	Shoot Dry Weight
Oilseed rape	Brassicaceae	<i>Brassica napus</i>	1	Shoot Height
Pea	Fabaceae	<i>Pisum sativum</i>	2	Visual Response
Soybean	Fabaceae	<i>Glycine max</i>	0	Survival
Sugar beet	Chenopodiaceae	<i>Beta vulgaris</i>	7	Shoot Dry Weight
Tomato	Solanaceae	<i>Lycopersicon esculentum</i>	0	Shoot Height, Visual Response, Survival

III. CONCLUSIONS

For the ten species tested, no effect greater than 7% was observed, therefore the ER₂₅ for all species was >100 g a.s./ha (0.67 L formulated product/ha).

(Porch, J.R., Martin, K.H., 2006)

RMS comment

Study submitted to the EU for the first time in this dossier.

The study is valid.

For the ten species tested, the ER₂₅ was >100 g a.s./ha (0.67 L formulated product/ha).

Report: Porch, J.R., Keller, K. (2015); Indoxacarb (DPX-KN128) 150 g/L EC: A greenhouse study to investigate the effects on vegetative vigor of ten terrestrial plants following foliar exposure

DuPont Report No.: DuPont-42153

Guidelines: OECD 227, OCSPP 850.4150 **Deviations:** None

Testing Facility: Wildlife International Ltd. (USA), Easton, Maryland, USA

Testing Facility Report No.: 112P-225

GLP: Yes

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

Executive summary:

Non-target terrestrial plant response to Indoxacarb 150 g/L EC was evaluated on ten common plant species representing seven plant families. Effects on vegetative vigour following foliar application to seedlings were assessed based on OECD Guideline 227 and U.S. EPA OCSPP 850.4150 after 21 days. The test was conducted using multiple rates up to a maximum of 0.45 lb a.s. per acre (equivalent to 2.78 lb Indoxacarb 150 g/L EC/Ac and 504 g a.s./ha). Plants were grown in an artificial greenhouse soil mixture (loamy sand, pH 6.5, O.M. 1.01%) and maintained under greenhouse conditions for the duration of the study (21 days following application). The ER₂₅ and ER₅₀ for all species were greater than 0.45 lb a.s./Ac (equivalent to 2.78 lb Indoxacarb 150 g/L EC/Ac and 504 g a.s./ha), and the NOER for all species was 0.45 lb a.s./Ac (504 g a.s./ha).

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Indoxacarb 150 g/L EC
 Lot/Batch #: KN128-434
 Purity: 16.2% (w/w) indoxacarb (150 g a.s./L nominal)
 Description: Liquid
 CAS#: None for test formulation
 173584-44-6 for indoxacarb active substance
2. Control: Domestic well water purified by reverse osmosis
 Test vehicle: Well water purified by reverse osmosis.
 Toxic reference: None
3. Test organisms: Terrestrial plant
 Species: *Allium cepa* (common onion)
Triticum aestivum (wheat)
Sorghum bicolor (sorghum)
Zea mays (corn)
Beta vulgaris (sugarbeet)
Brassica napus (oilseed rape)
Cucumis sativa (cucumber)
Glycine max (soybean)
Lycopersicon esculentum (tomato)
Pisum sativum (pea)
 Age at dosing: 2 to 5 true leaves, 4 to 30 cm height depending on species
 Initial population: 5 replicates of 4 plants (20 plants total) each per treatment and control
 Test chamber: Plastic pots, 16 cm diameter × 12 cm depth for corn, wheat, sorghum, cucumber, oilseed rape, pea, soybean, sugarbeet and tomato, and 11 cm diameter × 10 cm depth for onion
 Growth medium: Artificial loamy sand soil (pH: 6.3 [CaCl₂], 6.5 [water], Organic Matter: 1.01%, Organic Carbon: 0.59%)
 Water: Laboratory well water; Initial top watering followed by sub-irrigation
4. Environmental conditions (in-life period)
 Temperature: Range: 12°C to 33°C, Mean: 23°C (for onion, sorghum, wheat, pea and tomato); 23°C (for corn, cucumber, oilseed rape soybean and sugarbeet)
 Relative humidity: Range: 16% to 78%, Mean: 46% (for onion, sorghum, wheat, pea and tomato), Mean: 46% (for corn, cucumber, oilseed rape soybean and sugarbeet)
 Photoperiod: 16 hour photoperiod
 Photosynthetically active radiation:
 Range: 12 to 16 moles/M²/day (Einsteins/M²/day)
 Mean: 14 Einsteins/M²/day (for onion, sorghum, wheat, pea and tomato), 14 Einsteins/M²/day (for corn, cucumber, oilseed rape soybean and sugarbeet)

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed:
 26-November-2014 to 26-December-2014
2. Experimental treatments
 The effects of Indoxacarb 150 g/L EC were evaluated on the vegetative vigour of non-target plants

following application to the foliage of young seedling plants (post-emergent application). Corn, wheat, onion, sorghum, cucumber, oilseed rape, pea, soybean, sugarbeet, and tomato were selected for testing based upon the range of response these species are likely to represent. The rates applied ranged from 0.0035 to 0.45 lb a.s./Ac. Application was conducted using a laboratory sprayer calibrated to apply approximately 400 L water/ha with compressed nitrogen gas. After application, plants were maintained in a greenhouse under controlled conditions in a randomised block arrangement by species.

3. Observations

The shoot height and visual response of the seedlings were recorded on Days 7, 14, and 21 after application. Additionally, shoot dry weight and survival were determined on Day 21. No endpoint values were calculated on the basis of visual evaluations due to the subjective nature of this type of evaluation.

4. Statistics

The NOER was determined from a Jonckheere-Terpstra trend test ($\alpha = 0.05$). Non-linear regression analysis was used to estimate the ER_{25} and ER_{50} values when possible.

II. RESULTS AND DISCUSSION

A. FINDINGS

The test results based on the ER_{50} as determined for the most sensitive parameter(s) following foliar application of Indoxacarb 150 g/L EC are summarised in Table 169.

Table 169
Effects of Indoxacarb 150 g/L EC on ten non-target plants following foliar application

Plant	Family	Genus/species	ER_{50} (lb a.s./Ac)	ER_{50} (g a.s./ha)	Parameter
Monocots					
Corn	Poaceae	<i>Zea mays</i>	>0.45	>504	all parameters
Onion	Liliaceae	<i>Allium cepa</i>	>0.45	>504	all parameters
Sorghum	Poaceae	<i>Sorghum bicolor</i>	>0.45	>504	all parameters
Wheat	Poaceae	<i>Triticum aestivum</i>	>0.45	>504	all parameters
Dicots					
Cucumber	Cucurbitaceae	<i>Cucumis sativa</i>	>0.45	>504	all parameters
Oilseed Rape	Brassicaceae	<i>Brassica napus</i>	>0.45	>504	all parameters
Pea	Fabaceae	<i>Pisum sativum</i>	>0.45	>504	all parameters
Soybean	Fabaceae	<i>Glycine max</i>	>0.45	>504	all parameters
Sugarbeet	Chenopodiaceae	<i>Beta vulgaris</i>	>0.45	>504	all parameters
Tomato	Solanaceae	<i>Lycopersicon esculentum</i>	>0.45	>504	all parameters

III. CONCLUSIONS

The ER₂₅ and ER₅₀ for all monocot species were greater than 0.45 lb a.s./Ac and the NOER was 0.45 lb a.s./Ac (equivalent to 2.78 lb Indoxacarb 150 g/L EC/Ac, and equivalent to 504 g a.s./ha) based on all parameters.

The ER₂₅ and ER₅₀ for all dicot species were greater than 0.45 lb a.s./Ac and the NOER was 0.45 lb a.s./Ac (equivalent to 2.78 lb Indoxacarb 150 g/L EC/Ac and equivalent to 504 g a.s./ha) based on all parameters.

(Porch, J.R., Keller, K., 2015)

RMS comment

Study submitted to the EU for the first time in this dossier.

The study is valid.

The ER₂₅ and ER₅₀ for all monocot species were greater than 504 g a.s./ha and the NOER was 504 g a.s./ha based on all parameters.

The ER₂₅ and ER₅₀ for all dicot species were greater than 504 g a.s./ha and the NOER was 504 g a.s./ha based on all parameters.

B.9.11.3. Extended laboratory studies on non-target plants

On the basis of the non-target plant risk assessment, no extended laboratory testing is needed.

B.9.11.4. Semi-field and field tests on non-target plants

On the basis of the non-target plant risk assessment, no semi-field or field testing is needed.

B.9.12. RISK ASSESSMENT FOR TERRESTRIAL NON-TARGET HIGHER PLANTS

Non-target plant testing with Indoxacarb 150 g/L EC to evaluate potential effects following post-emergent (foliar) exposure indicated that the product exhibited virtually no toxicity to terrestrial plants at the maximum recommended field application rate. The ER₅₀ for all 10 tested plants based on the most sensitive assessment parameter was >504 g a.s./ha. PEC_{drift} values were calculated based on the maximum EU application rates for both crops. The resulting TER values are given in Table 170.

Table 170
Indoxacarb 150 g/L EC: TERs for terrestrial non-target plants based on an application to maize and lettuce (vegetative vigour at 21 days)

Crop	Application rate (g a.s./ha)	Drift value ^a (%)	PEC _{drift} (off-field drift rate in g a.s./ha)	Endpoint used in risk assessment	TER
Maize	37.5	2.77	1.04	ER ₅₀ >504 g a.s./ha	>485
Lettuce	37.5	2.77	1.04		>485

^a Drift estimates are based on 90th percentile values for field crops (BBA 2000).

Indoxacarb 150 g/L EC is applied post-emergence to the foliage of crops and the most likely route of exposure to non-target plants is through contact with the foliage. The notifier considers that the vegetative vigour test described above represents a worst-case exposure scenario and therefore a seedling emergence test was not conducted.

Conclusion:

Indoxacarb 150 g/L EC will not pose an off-field risk to non-target terrestrial plants because the EC₅₀ (vegetative vigour) (>504 g a.s./ha) exceeds all uses proposed. Furthermore, Indoxacarb 150 g/L EC will not pose an off-field risk to non-target terrestrial plants because TERs based on maximum drift exceed the trigger of 5. It may be concluded that Indoxacarb 150 g/L EC poses no unacceptable risk to plants in the off-crop areas for all uses proposed in the GAP.

B.9.13. EFFECTS ON OTHER TERRESTRIAL ORGANISMS (FLORA AND FAUNA)

No further data on terrestrial organisms are considered required.

B.9.14. RISK ASSESSMENT FOR OTHER TERRESTRIAL ORGANISMS (FLORA AND FAUNA)

No further risk assessment for terrestrial organisms are considered required.

B.9.15. 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
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CP, 10.1.1.1/ 01	F	2006	Indoxacarb (DPX-KN128) 150 g/L EC: An acute oral toxicity study with the northern bobwhite [REDACTED] DuPont-18923 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	<i>Submitted for the purpose of renewal</i>
CP, 10.2.1/01	[REDACTED]	2006	Indoxacarb (DPX-KN128) 150 g/L EC: Static, acute, 96-hour toxicity test to rainbow trout, <i>Oncorhynchus mykiss</i> [REDACTED] DuPont-18927 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	<i>Submitted for the purpose of renewal</i>
CP, 10.2.1/02	Dinehart, S.	2014	Indoxacarb (DPX-KN128) 150 g/L EC: Acute toxicity with the Mysid shrimp, <i>Americamysis bahia</i> , determined under static test conditions ABC Laboratories, Inc. (Missouri) DuPont-38348 GLP: Yes Published: No	N	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	<i>Submitted for the purpose of renewal</i>
CP, 10.2.1/03	Sloman, T.L.	2006	Indoxacarb (DPX-KN128) 150 g/L EC:	N	Y	The study is necessary for the regulatory	DuPont	<i>Submitted for the purpose of renewal</i>

			Static, 72-hour growth inhibition toxicity test to the green alga, <i>Pseudokirchneriella subcapitata</i> DuPont Haskell Laboratory DuPont-18926 GLP: Yes Published: No			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, 10.2.1/04	Turner, J.T.	2006	Indoxacarb (DPX-KN128) 150 g/L EC: Static, acute, 48-hour toxicity test with <i>Daphnia magna</i> DuPont Haskell Laboratory DuPont-18928 GLP: Yes Published: No	N	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	<i>Submitted for the purpose of renewal</i>
CP, 10.3.1.1.1/01	Haupt, S.	2014	Indoxacarb (DPX-KN128) 150 g/L EC: Acute oral and contact toxicity to the bumblebee, <i>Bombus terrestris</i> L. (Hymenoptera) IBACON DuPont-38351 GLP: Yes Published: No	N	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	<i>Submitted for the purpose of renewal</i>
CP, 10.3.1.1.1/02	Schmitzer, S.	2006	Indoxacarb (DPX-KN128) 150 g/L EC: Acute oral and contact toxicity to the honey	N	Y	The study is necessary for the regulatory decision, conducted according to	DuPont	<i>Submitted for the purpose of renewal</i>

			bee, <i>Apis mellifera</i> L. IBACON DuPont-18924 GLP: Yes Published: No			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, 10.3.1.2/ 01	Kling, A.	2014	Indoxacarb 150 g/L EC: Assessment of chronic effects to the honeybee, <i>Apis mellifera</i> L., in a 10 days continuous laboratory feeding test Eurofins Agrosience Services EcoChem GmbH DuPont-36492 GLP: Yes Published: No	N	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	<i>Submitted for the purpose of renewal</i>
CP, 10.3.1.3/ 01	Klank, C.	2014	Indoxacarb (DPX-KN128) 150 g/L EC: Honey bee (<i>Apis mellifera</i> L.) larval toxicity test (single feeding exposure) Eurofins Agrosience Services EcoChem GmbH DuPont-34817 GLP: Yes Published: No	N	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	<i>Submitted for the purpose of renewal</i>
CP, 10.3.1.3/ 02	Kleinhenz, M.	2014	Indoxacarb (DPX-KN128) 150 g/L EC: A feeding study to evaluate effects on the brood of honey bees (<i>Apis</i>	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously	DuPont	<i>Submitted for the purpose of renewal</i>

			<i>mellifera</i> ; Hymenoptera, apidae) in Germany 2013 Eurofins Agrosience Services EcoChem GmbH DuPont-37488 GLP: Yes Published: No			been protected or if previously protected the period of data protection has not expired at the time of submission of this dossier.		
CP, 10.3.1.3/03	Giffard, H.	2006	Evaluation des effets sur l'abeille domestique d'une application de Steward et de DPX KN128 150 EC (indoxacarbe) sur culture de phacélie. (Evaluation of the effects on domestic bees from one application of Steward and of DPX-KN128 150 EC (indoxacarb) on a blue tansy (<i>Phacelia</i>) crop) Testapi à Sarré, Etude No. 92-2006 GLP: Yes Published: No	N	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	<i>Submitted for the purpose of renewal</i>
CP, 10.3.1.6/01	Berg, C.	2013	Indoxacarb (DPX-KN128) 150 g/L EC: A semi-field study to evaluate effects on the honey bee (<i>Apis mellifera</i> ; Hymenoptera, Apidae) in maize (<i>Zea mays</i>) in Germany 2013 Eurofins Agrosience	N	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	<i>Submitted for the purpose of renewal</i>

			Services GmbH DuPont-37487 GLP: Yes Published: No			submission of this dossier.		
CP, 10.3.1.6/02	Berg, C.	2014	Indoxacarb (DPX-KN128) 150 g/L EC: A semi-field study to evaluate effects on the brood of honey bees (<i>Apis mellifera</i> ; Hymenoptera, Apidae) in <i>Phacelia tanacetifolia</i> in Germany 2014 Eurofins Agrosience Services GmbH DuPont-38405 GLP: Yes Published: No	N	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	<i>Submitted for the purpose of renewal</i>
CP, 10.3.1.6/03	Berg, C.	2015	Indoxacarb (DPX-KN128) 150 g/L EC: A semi-field study to evaluate effects on the brood of honey bees (<i>Apis mellifera</i> ; Hymenoptera, Apidae) in <i>Phacelia tanacetifolia</i> in Germany 2014 Eurofins Agrosience Services GmbH DuPont-37489, Revision No. 1 GLP: Yes Published: No	N	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	<i>Submitted for the purpose of renewal</i>
CP, 10.3.1.6/04	Bocksch. S.	2011	Indoxacarb (DPX-KN128) 150 g/L EC: A study to evaluate effects on the honeybee (<i>Apis mellifera</i>) in the field in	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been	DuPont	<i>Submitted for the purpose of renewal</i>

			maize in Germany in 2010 Eurofins Agrosience Services GmbH DuPont-30106 GLP: Yes Published: No			protected or if previously protected the period of data protection has not expired at the time of submission of this dossier.		
CP, 10.3.1.6/05	Giffard, H.	2008	DPX-KN128 150EC [150 g a.s./L (w/v)]: A semi-field study to evaluate effects on the honey bee (<i>Apis mellifera mellifera</i> ; Hymenoptera, Apidae) on phacelia in France 2007 Testapi DuPont-19453 GLP: Yes Published: No	N	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	<i>Submitted for the purpose of renewal</i>
CP, 10.3.1.6/06	Gonsior, G.	2006	Indoxacarb (DPX-KN128) 150 g/L EC: A semi-field study to evaluate effects on the honey bee (<i>Apis mellifera carnica</i> ; Hymenoptera, Apidae) in Phacelia in Germany 2006 GAB Biotechnologie, GmbH DuPont-19449 GLP: Yes Published: No	N	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	<i>Submitted for the purpose of renewal</i>
CP, 10.3.1.6/07	Gonsior, G.	2007a	Indoxacarb (DPX-KN128) 150 g/L EC: A semi-field study to evaluate effects on the honey bee (<i>Apis mellifera</i>	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been	DuPont	<i>Submitted for the purpose of renewal</i>

			<i>carnica</i> ; Hymenoptera, Apidae) in Phacelia tanacetifolia in France 2007 GAB Biotechnologie , GmbH DuPont-19450 GLP: Yes Published: No			protected or if previously protected the period of data protection has not expired at the time of submission of this dossier.		
CP, 10.3.1.6/ 08	Gonsior, G.	2007b	Indoxacarb (DPX-KN128) 150 g/L EC: A semi-field study to evaluate effects on the honey bee (<i>Apis mellifera carnica</i> ; Hymenoptera, Apidae) in Phacelia tanacetifolia in Germany 2007 GAB Biotechnologie , GmbH DuPont-19451 GLP: Yes Published: No	N	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	<i>Submitted for the purpose of renewal</i>
CP, 10.3.1.6/ 09	Gonsior, G.	2008a	Indoxacarb (DPX-KN128) 150 g/L EC: A semi-field study to evaluate effects on the honey bee (<i>Apis mellifera carnica</i> ; Hymenoptera, Apidae) in <i>Phacelia tanacetifolia</i> in Alsace, France 2007 eurofins-GAB GmbH DuPont-21945 GLP: Yes Published: No	N	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	<i>Submitted for the purpose of renewal</i>
CP, 10.3.1.6/ 10	Gonsior, G.	2008b	Indoxacarb (DPX-KN128) 150 g/L EC: A	N	Y	The study is necessary for the regulatory	DuPont	<i>Submitted for the purpose of renewal</i>

			semi-field study to evaluate effects on the honey bee (<i>Apis mellifera carnica</i> ; Hymenoptera, Apidae) on wheat treated with artificial honeydew in Northern France 2007 eurofins-GAB GmbH DuPont-21944 GLP: Yes Published: No			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, 10.3.1.6/12	Kleinhenz, M.	2011a	Indoxacarb (DPX-KN128) 150 g/L EC: A study to evaluate effects on the honey bee (<i>Apis mellifera carnica</i>) in the field in <i>Brassica napus</i> L. in eastern Germany in 2009 Eurofins Agrosience Services GmbH DuPont-26946 GLP: Yes Published: No	N	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	Submitted for the purpose of renewal
CP, 10.3.1.6/13	Kleinhenz, M.	2011b	Indoxacarb (DPX-KN128) 150 g/L EC: A study to evaluate effects on the honey bee (<i>Apis mellifera carnica</i>) in the field in <i>Brassica napus</i> L. in northern Germany in 2009 Eurofins Agrosience Services	N	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	DuPont	Submitted for the purpose of renewal

			GmbH DuPont-26947 GLP: Yes Published: No			this dossier.		
CP, 10.3.1.6/ 14	Kleinhenz, M.	2014a	Indoxacarb (DPX-KN128) 150 g/L EC: A semi-field study to evaluate effects on the brood of honey bees (<i>Apis mellifera</i> ; Hymenoptera, Apidae) in <i>Phacelia tanacetifolia</i> in Germany 2012 Eurofins Agroscience Services GmbH DuPont-34108 GLP: Yes Published: No	N	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	<i>Submitted for the purpose of renewal</i>
CP, 10.3.1.6/ 15	Kleinhenz, M.	2014b	Indoxacarb (DPX-KN128) 150 g/L EC: A semi-field study to evaluate effects on the honey bee (<i>Apis mellifera</i> ; Hymenoptera, apidae) in <i>Phacelia tanacetifolia</i> in Germany 2013 Eurofins Agroscience Services EcoChem GmbH DuPont-36482 GLP: Yes Published: No	N	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	<i>Submitted for the purpose of renewal</i>
CP, 10.3.1.6/ 18	Rentschler, S.	2014	Indoxacarb (DPX-KN128) 150 g/L EC: A semi-field study to evaluate effects on the honey bee (<i>Apis mellifera</i> ; Hymenoptera,	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or if	DuPont	<i>Submitted for the purpose of renewal</i>

			Apidae) in <i>Phacelia tanacetifolia</i> in Germany 2014 Eurofins Agrosience Services EcoChem GmbH DuPont-41668 GLP: Yes Published: No			previously protected the period of data protection has not expired at the time of submission of this dossier.		
CP, 10.3.2.1/01	Warmers, C.	2006a	Indoxacarb (DPX-KN128) 150 g/L EC: A laboratory rate response test to study the effects on the parasitoid <i>Aphidius rhopalosiphi</i> (Hymenoptera, Braconidae) GAB Biotechnologie, GmbH DuPont-19443 GLP: Yes Published: No	N	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	<i>Submitted for the purpose of renewal</i>
CP, 10.3.2.1/02	Warmers, C.	2006b	Indoxacarb (DPX-KN128) 150 g/L EC: A laboratory rate response test to study the effects on the predatory mite <i>Typhlodromus pyri</i> (Acari, Phytoseiidae) GAB Biotechnologie, GmbH DuPont-19444 GLP: Yes Published: No	N	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	<i>Submitted for the purpose of renewal</i>
CP, 10.3.2.2/01	Adelberger, I.	2007	Indoxacarb (DPX-KN128) 150 g/L EC: An extended laboratory test with field-aged spray deposits to study the effects on the	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been	DuPont	<i>Submitted for the purpose of renewal</i>

			aphid parasitoid, <i>Aphidius rhopalosiphii</i> de Stefani Perez (Hymenoptera, Braconidae) GAB Biotechnologie, GmbH DuPont-21947 GLP: Yes Published: No			protected or if previously protected the period of data protection has not expired at the time of submission of this dossier.		
CP, 10.3.2.2/02	Klug, T	2007	Indoxacarb (DPX-KN128) 150 g/L EC: An extended laboratory test with field-aged spray deposits to study the effects on the green lacewing <i>Chrysoperla carnea</i> Steph. (Neuroptera, Chrysopidae) GAB Biotechnologie, GmbH DuPont-21946 GLP: Yes Published: No	N	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	<i>Submitted for the purpose of renewal</i>
CP, 10.3.2.2/03	Warmers, C.	2007a	Indoxacarb (DPX-KN128) 150 g/L EC: An extended laboratory rate response test to study the effects on the aphid parasitoid, <i>Aphidius rhopalosiphii</i> de Stefani Perez (Hymenoptera, Braconidae) GAB Biotechnologie, GmbH DuPont-19445 GLP: Yes Published: No	N	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	<i>Submitted for the purpose of renewal</i>
CP,	Warmers,	2007b	Indoxacarb	N	Y	The study is	DuPont	<i>Submitted for</i>

10.3.2.2/ 04	C.		(DPX-KN128) 150 g/L EC: An extended laboratory rate response test to study the effects on the green lacewing <i>Chrysoperla carnea</i> Steph. (Neuroptera, Chrysopidae) GAB Biotechnologie , GmbH DuPont-19446 GLP: Yes Published: No			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.	t	<i>the purpose of renewal</i>
CP, 10.3.2.2/ 05	Warmers, C.	2007c	Indoxacarb (DPX-KN128) 150 g/L EC: An extended laboratory test with field-aged spray deposits to study the effects on the predatory bug <i>Orius laevigatus</i> Fieber (Heteroptera, Anthocoridae) GAB Biotechnologie , GmbH DuPont-22391 GLP: Yes Published: No	N	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	<i>Submitted for the purpose of renewal</i>
CP, 10.4.1.1.1 /01	Pavic, B.	2014	Indoxacarb (DPX-KN128) 150 g/L EC: Effects on reproduction and growth of the earthworm, <i>Eisenia fetida</i> , in artificial soil IBACON DuPont-37891 GLP: Yes Published: No	N	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	<i>Submitted for the purpose of renewal</i>

CP, 10.4.2.1/ 01	Pavic, B.	2014a	Indoxacarb (DPX-KN128) 150 g/L EC: Effects on the collembola <i>Folsomia candida</i> in artificial soil with 5% peat IBACON DuPont-37892 GLP: Yes Published: No	N	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	<i>Submitted for the purpose of renewal</i>
CP, 10.4.2.1/ 02	Pavic, B.	2014b	Indoxacarb (DPX-KN128) 150 g/L EC: Effects on reproduction of the predatory mite <i>Hypoaspis aculeifer</i> in artificial soil with 5% peat IBACON DuPont-37893 GLP: Yes Published: No	N	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	<i>Submitted for the purpose of renewal</i>
CP, 10.5/01	Reis, K.-H.	2006	Indoxacarb (DPX-KN128) 150 g/L EC: Assessment of the effects on soil microflora IBACON DuPont-18925 GLP: Yes Published: No	N	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	<i>Submitted for the purpose of renewal</i>
CP, 10.6.2/01	Porch, J.R., Martin, K.H.	2006	Indoxacarb (DPX-KN128) 150 g/L EC: A	N	Y	The study is necessary for the regulatory	DuPont	<i>Submitted for the purpose of renewal</i>

			greenhouse study to investigate the effects on vegetative vigor of ten sensitive terrestrial plants following foliar exposure Wildlife International Ltd. (USA) DuPont-19456 GLP: Yes Published: No			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, 10.6.2/02	Porch, J.R., Keller, K.	2015	Indoxacarb (DPX-KN128) 150 g/L EC: A greenhouse study to investigate the effects on vegetative vigor of ten terrestrial plants following foliar exposure Wildlife International Ltd (USA) DuPont-42153 GLP: Yes Published: No	N	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	<i>Submitted for the purpose of renewal</i>