

European Commission



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INDOXACARB

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Indoxacarb (DPX-KN128) is the insecticidally active, S-enantiomer of a compound belonging to the oxadiazine class of insecticides.

DPX-MP062 and DPX-JW062 are enantiomeric blends of DPX-KN128 and IN-KN127, where DPX-MP062 contains the respective isomers in a ratio of approximately 75:25, and DPX-JW062 is a racemic (50:50) mixture.

Development of the oxadiazine class of insecticides began with DPX-JW062. Processes were subsequently developed that allowed for commercial production of DPX-MP062, whose enhanced ratio of the insecticidally active enantiomer allowed for lower use rates of the end-use product and thus lower environmental and dietary exposures. Further process breakthroughs allowed for the production of >99% DPX-KN128 (indoxacarb) technical containing ≤1% IN-KN127.

Today, indoxacarb (DPX-KN128) is the primary technical material used as basis for formulation of end-user products.

Non-terrestrial vertebrate ecotoxicity studies were conducted with indoxacarb. Since indoxacarb was present at 50-75% in the ecotoxicity studies conducted on the racemic mixture of DPX-MP062, these studies can be used to support the ecotoxicity database for indoxacarb.

For clarity, the following development codes are used in the renewal dossier for indoxacarb:

- DPX-KN128: The pure insecticidal active isomer (S-isomer) with ISO name indoxacarb.
- DPX-MP062 is the development code for the technical material containing approximately 75% DPX-KN128 and 25% IN-KN127.
- DPX-JW062 is the development code for a racemic mixture of DPX-KN128 and IN-KN127.

Indoxacarb technical material is the basis for this active substance renewal dossier whereas DPX-MP062 was the technical material used as reference material in Indoxacarb monograph and review report (Indoxacarb SANCO/1408/2001 Rev.3) from 2005.

B.9. ECOTOXICOLOGY DATA

B.9.1. EFFECTS ON BIRDS AND OTHER TERRESTRIAL VERTEBRATES

B.9.1.1. Effects on birds

B.9.1.1.1. Acute oral toxicity to Birds

Report: [REDACTED] (1997); DPX-MP062 technical (approximately 75% DPX-KN128, 25% DPX-KN127): An acute oral toxicity study with the northern bobwhite

DuPont Report No.: AMR 3940-96, Revision No. 2

Guidelines: USEPA 71-1 **Deviations:** None

Testing Facility: [REDACTED]

Testing Facility Report No.: 112-432

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.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: DPX-MP062 technical
 Lot/Batch #: MP062-51A
 Purity: 94.5% DPX-MP062, by analysis
 CAS#: None for DPX-MP062 technical
 DPX-KN128: 173584-44-6
 Description: Off-white solid
 Stability of test compound: In the absence of evidence to the contrary, the test substance was assumed to be stable under the conditions of administration. Verification of test concentrations, stability, or homogeneity of the test substance in the diluent were not determined.
2. Control: Diluent control (corn oil)
 Test vehicle: Diluent (corn oil)
 Toxic reference: None
3. Test organism: Northern bobwhite
 Species: *Colinus virginianus*
 Age at dosing: 22 weeks
 Weight at dosing: 180 to 223 g
 Source: XX
 Acclimation period: Five weeks
 Diet: XX game bird ration (containing at least 27% protein and 2.5% fat, and no more than 5% crude fibre), *ad libitum*, except when fasted
 Water: Tap water, *ad libitum*
 Housing: Wire mesh pen with galvanised sheet sides, approximate 78 × 51 cm floor, approximate 20 to 25 cm height
4. Environmental conditions (in-life period):
 Temperature: Mean = 24.0 ± 1.2°C (SD)
 Relative humidity: Mean = 70 ± 12% (SD)
 Photoperiod: 8 hours light per day (approximately 193 lux)

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
 02-July-1996 to 23-July-1996
2. Test system
 A single-dose study was conducted with DPX-MP062 administered as an oral gavage at nominal dose levels of 0, 37.8, 63.0, 105, 175, 292, 486, 810, 1350, and 2250 mg DPX-MP062/kg body weight to fasted northern bobwhite quail (*Colinus virginianus*; 5 birds/sex/dose). The test substance was administered as a suspension in corn oil at a constant volume of 6 mL/kg body weight. Before and after treatment, birds were observed for abnormal behaviour, mortality, signs of toxicity, or physical injury. Body weight was measured on Days 0, 3, 7, 14, and 21. Feed consumption was determined for each group for Days 0-3, 4-7, 8-14, and 15-21.

II. RESULTS AND DISCUSSION

A. FINDINGS

Mortality, body mass, and food intake are summarised below (Table 1). Mortalities occurred up to 4 days after dosing. Clinical signs of toxicity were noted on the first day in the treatment groups above 37.8 mg DPX-MP062/kg, and all survivors recovered by end of the 19th day after dosing. The LD₅₀ and 95% confidence intervals were determined by probit analysis.

Table 1
Summary of mortality, body weight, and food intake of bobwhite quail following a single oral dose of DPX-MP062

Dose (mg/kg bw)	Sex	Cumulative mortality (%)	Mean body mass (g/bird)					Mean food intake (g/bird/d)			
			Days					Days			
			0	3	7	14	21	0-3	4-7	8-14	15-21
0	M	0	191	196	199	197	204	16	20	15	18
	F	0	203	207	210	211	218	14	18	15	15
37.8	M	0	198	202	208	207	214	16	18	14	14
	F	0	189	197	198	197	206	13	16	14	15
63.0	M	20	191	192	197	197	204	20	29	20	19
	F	20	192	196	199	199	208	9	18	16	15
105	M	40	200	199	215	212	225	25	33	19	22
	F	40	199	168	182	193	205	5	15	15	16
175	M	100	195	— ^a	—	—	—	—	—	—	—
	F	100	191	149	—	—	—	4	—	—	—
292	M	100	194	—	—	—	—	—	—	—	—
	F	100	194	—	—	—	—	—	—	—	—
486	M	100	191	—	—	—	—	—	—	—	—
	F	100	197	—	—	—	—	1	—	—	—
810	M	100	196	—	—	—	—	—	—	—	—
	F	100	202	—	—	—	—	—	—	—	—
1350	M	100	201	—	—	—	—	—	—	—	—
	F	100	206	—	—	—	—	—	—	—	—
2250	M	100	189	—	—	—	—	—	—	—	—
	F	100	195	—	—	—	—	—	—	—	—

^a indicates no data due to deaths of birds

III. CONCLUSIONS

The acute oral LD₅₀ value for northern bobwhite quail exposed to DPX-MP062 by single oral dose in corn oil was 98 mg DPX-MP062/kg body weight. The lowest concentration to have any mortality (20%) was in the 63.0 mg DPX-MP062/kg treatment group. The no-mortality dosage and no effects level were 37.8 mg DPX-MP062/kg body weight.

([REDACTED] 1997)

RMS comment

The acute oral toxicity to birds study AMR 3940-96, Revision No. 2, conducted with test material DPX-MP062 technical, was conducted under guideline U.S. EPA 71-1.

This study was assessed in original DAR. GLP compliance was checked by RMS. This study is valid according to validity criteria of OECD 223. The result for indoxacarb (79:21): LD50 = 98 mg DPX-MP062 /kg bw is still considered acceptable for the risk assessment. This is equivalent to 73.5 mg DPX-KN128/kg bw.

Report: [REDACTED] (1996); IN-JT333-20: An acute oral toxicity study with the northern bobwhite

DuPont Report No.: AMR 3890-96

Guidelines: USEPA 71-1 **Deviations:** None

Testing Facility: [REDACTED]

Testing Facility Report No.: 112-431

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.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: IN-JT333 technical metabolite
 Lot/Batch #: JT333-20
 Purity: 98.7%
 CAS#: 144171-39-1
 Description: Solid
 Stability of test compound: In the absence of evidence to the contrary, the test substance was assumed to be stable under the conditions of administration. Verification of test concentrations, stability, or homogeneity of the test substance in the diluent were not determined.
2. Control: Diluent control (corn oil)
 Test vehicle: Diluent (corn oil)
 Toxic reference: None
3. Test organism: Northern bobwhite quail
 Species: *Colinus virginianus*
 Age at dosing: 18 weeks
 Weight at dosing: 183 to 217 g
 Source: [REDACTED]
 Acclimation period: 18 days
 Diet: [REDACTED] game bird ration (containing at least 27% protein and 2.5% fat, and no more than 5% crude fibre), *ad libitum*
 Water: Tap water, *ad libitum*
 Housing: Wire mesh pen with galvanised sheet sides, approximate 78 × 51 cm floor, approximate 20 to 25 cm height
4. Environmental conditions (in-life period):
 Temperature: Mean = 21.0 ± 1.7°C (SD)
 Relative humidity: Mean = 54 ± 12% (SD)
 Photoperiod: 8 hours light per day (approximately 251 lux)

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
 26-April-1996 to 10-May-1996
2. Test system
 A single-dose study was conducted with IN-JT333 administered as an oral gavage at nominal dose levels of 0, 292, 486, 810, 1350, and 2250 mg IN-JT333/kg body weight to fasted northern bobwhite quail (*Colinus virginianus*; 5 birds/sex/dose). The test substance was administered as a suspension in corn oil at a constant volume of 6 mL/kg body weight. Before and after treatment, birds were observed for abnormal behaviour, mortality, signs of toxicity, or physical injury. Body weight was measured on Days 0, 3, 7, and 14. Feed consumption was determined for each group for Days 0-3, 4-7, and 8-14.

II. RESULTS AND DISCUSSION

A. FINDINGS:

Mortality, body mass, and food intake are summarised below (Table 2). Mortalities occurred up to 10 days after dosing. Clinical signs of toxicity were noted on the first day in all treatment groups, and all survivors recovered by end of the 11th day after dosing. The LD₅₀ and 95% confidence intervals were determined by probit analysis.

Table 2
Summary of mortality, body weight, and food intake of bobwhite quail following
a single oral dose of IN-JT333

Dose (mg/kg bw)	Sex	Cumulative mortality (%)	Mean body mass (g/bird)				Mean food intake (g/bird/d)		
			Days				Days		
			0	3	7	14	0-3	4-7	8-14
0	M	0	196	206	204	207	20	19	18
	F	0	200	215	211	212	20	17	17
292	M	40	196	213	210	216	15	26	26
	F	0	193	191	193	197	12	17	17
486	M	0	194	194	190	200	13	15	18
	F	0	206	210	205	213	11	16	19
810	M	40	193	190	170	201	11	8	16
	F	0	200	203	204	213	15	22	19
1350	M	80	195	189	192	203	12	8	41
	F	20	197	195	181	199	8	8	20
2250	M	80	193	181	181	197	7	14	21
	F	40	195	185	172	189	6	7	17

III. CONCLUSIONS

The acute oral LD₅₀ value for northern bobwhite quail exposed to IN-JT333 by single oral dose in corn oil was 1750 mg IN-JT333/kg body weight. The no mortality dosage was ≤292 mg IN-JT333/kg body weight. The no-observed effects level was <292 mg IN-JT333/kg, based on body weight effects at all doses.

([REDACTED] 1996)

RMS comment

The acute oral toxicity to birds study AMR 3890-96 was conducted under guideline U.S. EPA 71-1.

This study was assessed in original DAR. GLP compliance was checked by RMS. This study is valid according to validity criteria of OECD 223.

LD₅₀ = 1750 mg IN-JT333/kg bw.

B.9.1.1.2. Short-term dietary toxicity to birds

Report: [REDACTED] (1997a); DPX-MP062 technical (approximately 75% DPX-KN128, 25% DPX-KN127): A dietary LC₅₀ study with the northern bobwhite

DuPont Report No.: AMR 4094-96

Guidelines: USEPA 71-2, OECD 205 **Deviations:** None

Testing Facility: [REDACTED]

Testing Facility Report No.: 112-437

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.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: DPX-MP062 technical
 Lot/Batch #: MP062-51A
 Purity: 94.5%
 Description: Off-white solid
 CAS#: None for DPX-MP062
 DPX-KN128: 173584-44-6
 Stability of test compound: Shown to be stable for 5 days under the conditions of the test
2. Vehicle and/or positive control: Acetone
3. Test organism: Northern bobwhite
 Species: *Colinus virginianus*
 Age at dosing: 10 days
 Weight at dosing: 18–20 g
 Source: [REDACTED]
 Acclimation period: 10 days
 Diet: Testing laboratory ([REDACTED]) game bird ration, ($\geq 27\%$ protein, $\geq 2.5\%$ crude fat, $\leq 5\%$ crude fibre), *ad libitum*
 Water: Tap water, *ad libitum*
 Housing: Pen with 72 × 90 cm floor space and ceiling height 23 cm. External walls, ceilings and floors were constructed of wire mesh and/or galvanised sheeting
4. Environmental conditions
 Temperature: Average 38°C ($\pm 1^\circ\text{C}$) in brooding compartments
 Relative humidity: Average 65% ($\pm 8\%$) in brooding compartments
 Photoperiod: 16 hour photoperiod (average of approximately 195 lux)

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
 22-August-1996 to 03-September-1996
2. Test system
 A 5-day feeding study was conducted with DPX-MP062 administered in the diet at nominal dose levels of 0, 178, 316, 562, 1000, 1780, 3160, and 5620 ppm to northern bobwhite quail chicks (*Colinus virginianus*; 10 birds/dose). Dose levels were confirmed by analysis. Chicks were acclimated from the day of hatch until test initiation at 10 days of age. All groups were given untreated feed for 3 days following the 5-day exposure period. Before, during, and after treatment, birds were observed for abnormal behaviour, mortality, or signs of toxicity. Body weight was measured at initiation of the test, on Days 5, 8, and 12. Average estimated feed consumption was determined for each test and control group for the exposure period, Days 0-5, and for the post-exposure observation period, Days 6-8 and 9-12. Dietary concentrations and the LC₅₀ value are reported as ppm of nominal.

II. RESULTS AND DISCUSSION

A. FINDINGS

Cumulative mortality, body weight changes, and food intake are given below (Table 3). Mortalities occurred up to 6 days after the first day of dosing. Clinical signs of toxicity were noted on the first day in

all treatment groups above 316 ppm DPX-MP062, and all survivors recovered by end of the fifth to seventh day after dosing. The LC_{50} and 95% confidence intervals were determined by probit analysis.

Table 3
Summary of mortality, body weight, and food intake of bobwhite quail chicks following administration of DPX-MP062 in the diet for 5 days

Nominal Dose (ppm)	Cumulative Mortality (%)	Mean Body Mass (g/bird)				Mean Food Intake (g/bird/day)		
		Days				Days		
		0	5	8	12	0-5	6-8	9-12
0 ^a	0	20	32	41	54	8	13	8
178	0	20	30	38	50	7	9	9
316	0	19	30	39	52	9	10	10
562	20	19	25	34	47	10	9	9
1000	80	18	20	29	39	8	20	16
1780	90	19	18	28	35	6	17	11
3160	100	19	-	-	-	-	-	-
5620	100	19	-	-	-	-	-	-

^a 4 control groups; 10 chicks/group

III. CONCLUSIONS

The short-term dietary LC_{50} value for northern bobwhite quail exposed to DPX-MP062 in the diet for 5 days was 808 ppm. The no-mortality and no-observed effect concentrations were 316 ppm based on mortality and clinical signs of toxicity. The LC_{50} is equivalent to 340 mg DPX-MP062/kg bw/d.

([REDACTED] 1997a)

RMS comment

The short-term dietary toxicity to birds study AMR 4094-96, conducted with test material DPX-MP062 technical, was conducted under guidelines U.S. EPA 71-2 and OECD 205.

This study was assessed for previous Annex I inclusion and a LC_{50} value of 808 ppm equivalent to 340 mg DPX-MP062/kg bw/d was used for risk assessment in the previous monograph. This study was conducted according to current guideline OECD 205. The values reported by the applicant in the tables of the summary were different than those in the study report. The reason of that was not understood by RMS so they were corrected by RMS. Dietary toxicity studies are no longer mandatory according to the new requirements and the dietary toxicity endpoint issued from this study (equivalent to 255 mg DPX-KN128/kg bw/d) is obviously higher than the acute endpoint, this study is not considered essential for the risk assessment. Validity criteria were nevertheless checked by RMS. The endpoint is still considered relevant and reliable.

Report: [REDACTED] (1997b); DPX-MP062 technical (approximately 75% DPX-KN128, 25% DPX-KN127): A dietary LC_{50} study with the mallard

DuPont Report No.: AMR 4093-96

Guidelines: USEPA 71-2, OECD 205 **Deviations:** None

Testing Facility: [REDACTED]

Testing Facility Report No.: 112-438

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.

I. MATERIALS AND METHODS

A. MATERIALS

- | | |
|-------------------------------------|---|
| 1. Test material: | DPX-MP062 technical |
| Lot/Batch #: | MP062-51A |
| Purity: | 94.5% |
| Description: | Off-white solid |
| CAS#: | None for DPX-MP062 |
| | DPX-KN128: 173584-44-6 |
| Stability of test compound: | Shown to be stable for 5 days under the conditions of the test |
| 2. Vehicle and/or positive control: | Diet |
| 3. Test organism: | Mallard |
| Species: | <i>Anas platyrhynchos</i> |
| Age at dosing: | 10 days |
| Source: | [REDACTED] |
| Acclimation period: | 9 days |
| Diet: | Testing laboratory ([REDACTED]) game bird ration, ($\geq 27\%$ protein, $\geq 2.5\%$ crude fat, $\leq 5\%$ crude fibre), <i>ad libitum</i> |
| Water: | Tap water, <i>ad libitum</i> |
| Housing: | Pen with 62 × 92 cm floor space and ceiling height 25.5 cm. External walls, ceilings and floors were constructed of vinyl coated wire mesh |
| 4. Environmental conditions | |
| Temperature: | Average 30°C ($\pm 1^\circ\text{C}$) in brooding compartments |
| Relative humidity: | Average 74% ($\pm 11\%$) in brooding compartments |
| Photoperiod: | 16 hour photoperiod (average of 224 lux) |

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
22-August-1996 to 30-August-1996
2. Test system
A 5-day feeding study was conducted with DPX-MP062 administered in the diet at dose levels of 0, 178, 316, 562, 1000, 1780, 3160, and 5620 ppm to mallard ducklings (*Anas platyrhynchos*, 10 birds/dose). Dose levels were confirmed by analysis. Chicks were acclimated from the day of hatch until test initiation at 10 days of age. All groups were given untreated feed for 3 days following the 5-day exposure period. Before, during, and after treatment, birds were observed for abnormal behaviour, mortality, or signs of toxicity. Body weight was measured at initiation of the test, on Day 5, and at termination of the test on Day 8. Average estimated feed consumption was determined for each test and control group for the exposure period, Days 0–5, and for the post-exposure observation period, Days 6–8. Dietary concentrations and the LC₅₀ value are reported as ppm of nominal.

II. RESULTS AND DISCUSSION

A. FINDINGS

Cumulative mortality, body weight changes, and food intake are given below (Table 4). There were no mortalities in any dose group. Clinical signs of toxicity were noted on the first day in the 3160 and 5620 ppm DPX-MP062 treatment groups, and all survivors recovered by end of the second to sixth day after the first day of dosing.

Table 4
Summary of mortality, body weight, and food consumption of mallard duck chicks administered DPX-MP062 in the diet for 5 days

Nominal Dose (ppm)	Cumulative % Mortality	Mean Body Mass (g/bird)			Mean Intake (g/bird/day)	
		Days			Days	
		0	5	8	0-5	6-8
0 ^a	3	170	329	423	98	150
178	0	166	324	423	91	124
316	0	168	325	422	106	161
562	0	172	333	437	102	143
1000	0	164	293	386	91	106
1780	0	168	279	388	98	134
3160	0	163	237	343	63	107
5620	0	175	224	344	64	140

^a Three control groups; 10 birds per group

III. CONCLUSIONS

The short-term dietary LC₅₀ value for mallard ducks exposed to DPX-MP062 in the diet for 5 days was >5620 ppm, the highest concentration tested. The no-mortality level was 5620 ppm and NOEC was 562 ppm. The LC₅₀ is equivalent to >1803 mg DPX-MP062/kg bw/d.

([REDACTED]) 1997b)

RMS comment

This study was assessed for previous Annex I inclusion and a LC₅₀ > 5620 ppm was found. This study was conducted according to the current guideline OECD 205. Dietary toxicity studies are no longer mandatory according to the new requirements and the dietary toxicity endpoint issued from this study is obviously higher than the acute endpoint, this study is not considered essential for the risk assessment. Validity criteria were nevertheless checked by RMS. The endpoint is still considered relevant and reliable.

B.9.1.1.3. Sub-chronic toxicity and reproduction to birds

Report: [REDACTED] (1997a); DPX-MP062 technical (approximately 75% DPX-KN128, 25% DPX-KN127); A reproduction study with the mallard (*Anas platyrhynchos*)

DuPont Report No.: AMR 4095-96

Guidelines: USEPA 71-4, OECD 206 **Deviations:** None

Testing Facility: [REDACTED]

Testing Facility Report No.: 112-442

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.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: DPX-MP062 technical
 Lot/Batch #: MP062-51A
 Purity: 94.5%
 Description: Off-white solid
 CAS#: None for DPX-MP062 technical
 DPX-KN128: 173584-44-6
 Stability of test compound: Stability was demonstrated by chemical analyses of diet samples.
2. Control: Untreated diet with acetone equivalent to the highest amount used in the treated diets
 Test vehicle: Laboratory diet mixed with test substance dissolved in acetone
 Toxic reference: None
3. Test organism: Mallard duck
 Species: *Anas platyrhynchos*
 Age at dosing: 34 weeks
 Weight at dosing: 940 to 1330 g (males); 1226 to 846 g (females)
 Source: [REDACTED]
 Acclimation period: 14 weeks
 Diet: [REDACTED] . game bird ration (containing at least 27% protein and 2.5% fat, and no more than 5% crude fibre), *ad libitum* (addition of 5% w/w limestone for adults)
 Water: Tap water, *ad libitum*
 Housing: Vinyl-coated wire mesh pen, approximate 72 × 90 cm floor, approximate 45 cm height
4. Environmental conditions
 Temperature: Mean = 19.8 ± 1.3°C (SD)
 Relative humidity: Mean = 42 ± 13% (SD)
 Photoperiod: Acclimation through Week 10 of study: 8 hours or less light per day (approximately 240 lux)
 Week 11 through termination: 17 hours light per day (approximately 240 lux)

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
 15-January-1997 to 17-July-1997
2. Test system

The reproductive toxicity of DPX-MP062 to mallard ducks (*Anas platyrhynchos*) was assessed. DPX-MP062 was administered in the diet to 128 young adult (34-week-old) birds (16 pairs of adults/replicate; 1 replicate/control; 1 replicate/treatment) for 21 weeks). Treatment levels were 0, 28.8, 86.4, 144, and 720 ppm. Homogeneity, stability, and dose verification were confirmed by analysis of diet. Observations of adult mortality and overt signs of toxicity were made daily. Adult food consumption was measured weekly and body weight was taken on Weeks 0, 2, 4, 6, 8, and at

adult termination. Reproductive parameters were measured beginning at the onset of egg laying. Observations on chicks were made for 14 days following hatching.

II. RESULTS AND DISCUSSION

A. FINDINGS

There was one incidental adult mortality at 28.8 ppm, and one at 86.4 ppm. There were no treatment-related mortalities, overt signs of toxicity, or treatment-related effects on feed consumption or body weight at any of the concentrations tested (Dunnett's test, $p > 0.05$, Table 5).

There were no apparent treatment-related effects on any reproductive parameter measured at 28.8, 86.4, 144, or 720 ppm (Dunnett's test, $p > 0.05$, Table 6 and Table 7).

Table 5
Summary of mean body weight of adult mallard ducks exposed to DPX-MP062 in the diet

Nominal dose (ppm)	Sex	Week of the test						Total weight change (g)
		0	2	4	6	8	Adult termination	
Control	M	1143	1126	1149	1142	1146	1236	93
	F	1012	988	1007	1004	1021	1130	118
28.8	M	1125	1111	1151	1147	1145	1187	65
	F	992	975	988	1003	1008	1115	124
86.4	M	1144	1116	1123	1117	1132	1200	51
	F	991	961	995	987	1008	1155	157
144	M	1130	1118	1144	1128	1129	1193	62
	F	1016	1011	1037	1055	1109	1146	130
720	M	1118	1098	1123	1116	1124	1200	82
	F	1007	979	1005	1009	1014	1126	118

Table 6
Summary of reproductive effects of DPX-MP062 on mallard ducks

Reproductive parameter	Test group (nominal dietary concentration in mg a.s./kg bw)				
	0	28.8	86.4	144	720
Number of replicates	16	15	15	16	16
Total eggs laid/group	677	709	577	783	639
Eggs cracked	5	14	12	8	7
Eggs set	600	616	500	704	569
Viable embryos	573	532	467	630	527
Live three-week embryos	566	523	463	620	517
Hatchlings	415	341	402	463	388
14-day-old survivors	396	326	393	461	374
Eggs laid/hen	42	47	38	49	40
Eggs laid/hen/day ^a	0.59	0.66	0.53	0.68	0.55
14-day-old survivors/hen	25	22	26	29	23

^a Based on 80 days of egg production

Table 7

Reproduction parameters	Test group (Nominal dietary concentration in mg a.s./kg diet)				
	0	28.8	86.4	144	720
Number of replicates ^a	16	15	15	16	16
Total eggs laid/group ^a	677	709	577	783	639
Eggs laid/maximum laid	57	64	52	66	54
Eggs cracked/eggs laid	1	2	2	1	1
Viable embryos/eggs set	96	85	90	91	93
Live three-week embryos/viable embryos	99	97	99	98	98
Hatchlings/live 3-week embryos	74	62	86	75	74
14-day-old survivors/hatchlings	95	95	98	100 ^b	97
Hatchlings/eggs set	70	52	76	66	68
14-day old survivors/eggs set	66	49	74	66	66
Hatchlings/maximum set	38	33	39	43	36
14-day-old survivors/maximum set	36	32	39	42	34

^b Significantly different from the control at $p < 0.05$.

III. CONCLUSIONS

([REDACTED] 1997a)

This study was assessed for previous Annex I inclusion and a NOEC of 720 mg DPX-MP062/kg feed equivalent to 105 mg DPX-MP062/kg bw/d was found. This is equivalent to 78.75 mg DPX-KN128/kg bw/d. This study was conducted according to the current guideline OECD 206. Validity criteria were checked by RMS. The values reported by the applicant in the tables of the summary were different than those in the study report. The reason of that was not understood by RMS so they were corrected by RMS. RMS notes that effects were observed at the lowest rate tested of 28.8 mg a.s./kg diet. As no dose-response relationship was found, these effects are not considered treatment related. The endpoint is still considered relevant and reliable.

DuPont Report No.: AMR 4096-96

Guidelines: USEPA 71-4, OECD 206 **Deviations:** None

Testing Facility:

Testing Facility Report No.: 112-441

GLP: Yes

I. MATERIALS AND METHODS

1. Test material:	DPX-MP062 technical
Lot/Batch #:	MP062-51A-
Purity:	94.5%
Description:	Off-white solid
CAS#:	None for DPX-MP062 DPX-KN128:173584-44-6
Stability of test compound:	Stability was demonstrated by chemical analyses of diet samples.
2. Control:	Untreated diet
Test vehicle:	Diet
Toxic reference:	None
3. Test organism:	Northern bobwhite
Species:	<i>Colinus virginianus</i>
Age at dosing:	28 weeks
Weight at dosing:	179 to 239 g
Source:	
Acclimation period:	14 weeks
Diet:	Basal game bird ration (containing at least 27% protein and 2.5% fat, and no more than 5% crude fibre), <i>ad libitum</i> (addition of 5% w/w limestone)
Water:	Tap water, <i>ad libitum</i>
Housing:	Adults: wire mesh pen with galvanised sheet sides, approximate 25 x 51 cm floor, approximate 20 to 26 cm height Hatchlings: wire mesh pen with galvanised sheet sides, approximate 72 x 90 cm floor, approximate 23 cm height
4. Environmental conditions	
Temperature:	Adults' room: mean = 21.1°C (± 1.3°C SD) Hatchlings' room: mean = 27.5°C (± 0.9°C SD)
Relative humidity:	Adults: mean = 34% (± 15% SD) Hatchlings: mean = 44% (± 14% SD)
Photoperiod:	Adults: Acclimation period and first seven weeks of the test period: 8 hours light per day (approximately 210 lux) From start of Week 8 through the end of the test period: 17 hours light per day (approximately 210 lux) Hatchlings: 16 hours light per day

1. In-life initiated/completed
15-January-1997 to 14-July-1997

2. Test system

The reproductive toxicity of DPX-MP062 to bobwhite quail (*Colinus virginianus*) was assessed. DPX-MP062 was administered in the diet to 128 young adult (28-week-old) birds (16 pairs of adults/replicate; 1 replicate/control; 1 replicate/treatment) for 20 weeks (145 days). Treatment levels were 0, 28, 86, 144, and 720 ppm. Homogeneity, stability, and dose verification were confirmed by analysis of diet. Observations of adult mortality and overt signs of toxicity were made daily. Adult food consumption was measured weekly and body weight was taken on Weeks 0, 2, 4, 6, 8, and at adult termination. Reproductive parameters were measured beginning at the onset of egg laying. Observations of chicks were made for 14 days following hatching.

II. RESULTS AND DISCUSSION

A. FINDINGS

There was one adult mortality in the control, one at 28.8 ppm, one at 144 ppm, and 6 at 720 ppm; each was considered incidental to treatment. There were no treatment-related mortalities, overt signs of toxicity, or treatment-related effects on body weight at any of the concentrations tested (Table 8). Feed consumption was reduced by 14% in the 720 ppm group relative to controls during the first 2 weeks of study (Dunnett's test, $p < 0.05$).

There were no apparent treatment-related effects on the reproductive parameters measured at 28, 86, 144, or 720 ppm DPX-MP062 (Dunnett's test, $p > 0.05$, Table 9 and Table 10).

Table 8
Summary of mean body weight (g) of adult bobwhite quail exposed to DPX-MP062 in the diet

Dose (ppm)	Sex	Week of the test						Total weight change (g)
		0	2	4	6	8	Adult termination	
Control	M	209	207	208	206	210	218	9
	F	208	206	208	205	207	230	22
28	M	213	209	212	209	213	217	5
	F	204	203	204	202	205	227	24
86	M	207	206	207	206	206	208	1
	F	205	203	204	203	206	224	18
144	M	208	206	207	205	208	213	4
	F	207	204	207	207	209	231	25
720	M	211	205	203	203	209	216	4
	F	205	201	201	200	203	227	20

Table 9
Summary of reproductive effects of DPX-MP062 on northern bobwhite quail

Reproductive parameter	Test group (dietary concentration in mg a.s./kg feed)				
	0	28	86	144	720
Number of replicates	15	15	16	15	10
Total eggs laid/group	631	656	769	659	475
Eggs cracked	21	8	13	31	11
Eggs set	534	576	676	555	410
Viable embryos	479	547	606	510	388
Live three-week embryos	474	546	604	507	387
Hatchlings	448	522	581	472	363
14-day-old survivors	426	477	528	432	334
Eggs laid/hen	42	44	48	44	48
Eggs laid/hen/day ^a	0.46	0.48	0.52	0.48	0.52
14-day-old survivors/hen	28	32	33	29	33

^a Based on 92 days of egg production

Table 10
Summary of reproductive effects, normalised as percentages, of DPX-MP062 on
northern bobwhite quail

Reproductive parameters	Test Group (dietary concentration in mg a.s./kg feed)				
	0	28	86	144	720
Number of replicates ^a	15	15	16	15	10
Total eggs laid ^a	631	656	769	659	475
Eggs laid/maximum laid	65	67	74	68	73
Eggs cracked/eggs laid	9	1	2	4	2
Viable embryos/eggs set	90	95	89	86	94
Live three-week embryos/viable embryos	99	100	100	99	100
Hatchlings/live 3-week embryos	94	95	95	92	93
14-day old survivors/hatchlings	94	91	91	91	91
Hatchlings/eggs set	84	90	85	79	80
14-day-old survivors/eggs set	79	83	77	72	79
Hatchlings/maximum set	50	58	61	53	60
14-day-old survivors/maximum set	47	53	55	48	56

^a Data are expressed as absolute numbers, not percentages.

III. CONCLUSIONS

There were no treatment-related effects on reproduction of northern bobwhite quail. The NOEC for bobwhite quail exposed to DPX-MP062 in the diet for 20 weeks (145 days) was 144 mg DPX-MP062/kg diet (ppm), based on reduced food intake at 720 ppm during the first 2 weeks of study.

([REDACTED] 1997b)

RMS comment

This study was assessed for previous Annex I inclusion and a NOEC of 720 mg DPX-MP062 (79:21)/kg feed equivalent to 75.7 mg DPX-MP062/kg bw/d was found. This study was conducted according to the current guideline OECD 206. Validity criteria were checked by RMS. The values reported by the applicant in the tables of the summary were different than those in the study report. The reason of that was not understood by RMS so they were corrected by RMS. Although there was an increase in the number of mortalities observed at the 720 ppm a.i. test concentration (6 birds died at 720 ppm), due to the nature of the lesions observed at necropsy, all mortalities were considered to be incidental. At the highest concentration tested (720 ppm), transient effects upon adult body weight loss and feed consumption during the first week of the study were observed. Both effects were most likely due to an initial aversion to diet containing the test substance. The effects found were supposed to be ecologically irrelevant (original DAR). The difference of bodyweight was not statistically significant (only food consumption was significantly different). RMS still considers the NOEC of 720 mg DPX-MP062 (79:21)/kg feed, equivalent to 75.7 mg DPX-MP062/kg bw/d, relevant and reliable. This is equivalent to 56.8 mg DPX-KN128/kg bw/d.

B.9.1.2. Effects on terrestrial vertebrates other than birds

B.9.1.2.1. Acute oral toxicity to mammals

Please refer to the mammalian toxicology section (B.6) for study summaries.

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 11. Studies were conducted with IN-JT333, as it is considered as a

toxicologically significant metabolite. Endpoints selected for use in the risk assessment are summarised in Table 11.

Table 11
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

^aSummarised in section Toxicology.

B.9.1.2.2. Long-term and reproduction toxicity to mammals

Please refer to the mammalian toxicology section (B.6) for study summaries.

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%). Details of mammalian studies are provided in the section Toxicology (see Table B.6.6-1).

Table 12
Mammalian toxicity endpoints of indoxacarb

Study	Test substance	Test species	Endpoints	Reference
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

^cProposed for ecotoxicological risk assessment.

B.9.1.3. Active substance bioconcentration in prey of birds and mammals

The octanol-water partition coefficient of indoxacarb (log P_{ow} >3) triggered a bioaccumulation study (AMR 3663-95) which has been previously submitted and is summarised in this document. The study resulted in a bioconcentration factor of 77.3 L/kg (whole fish) and a depuration constant of 0.0879, 0.103, 0.100, and 0.106 for the [indanone-1-¹⁴C] DPX-JW062 low dose, and [indanone-1-¹⁴C] DPX-JW062 high dose, [trifluoromethoxyphenyl-¹⁴C(U)] DPX-JW062 low dose, and [trifluoromethoxyphenyl-¹⁴C(U)] DPX-JW062 high dose treatment groups, respectively, per day.

B.9.1.4. Other data on effects on terrestrial vertebrate wildlife (birds, mammals, reptiles and amphibians)

According to the revised data requirements under regulation 1107/2009 (Commission Regulations (EU) 283/2013 and 284/2013 for the active ingredient and the plant protection products, respectively), the risk to amphibians and reptiles shall be addressed. Nevertheless, unlike birds and mammals, toxicity tests for amphibian and reptile species are not requested. In the EU there is no guidance or validated regulatory protocols yet available neither on the type of regulatory testing necessary nor how to conduct a risk assessment for amphibian and reptiles. According to the applicant, in the case of indoxacarb, there are no studies in the literature on the toxicity of this active ingredient on amphibians and reptiles.

In addition, the EFSA Guidance Document for aquatic organisms (EFSA, 2013) states that the rainbow trout is a good surrogate test species for predicting the acute toxicity for larval stages of amphibians living in the aquatic environment and that recent investigation indicate that this is also applicable for chronic toxicity.

In this condition data on other terrestrial vertebrates (reptile and amphibians) are not considered required.

B.9.1.5. Potential for endocrine disruption

No potential for endocrine disruption was indicated in the toxicology section. No effects on reproduction were observed in the multigeneration reproduction study conducted with DPX-JW062, in the developmental studies in rats with DPX-JW062, DPX-MP062, and DPX-KN128, or the developmental neurotoxicity study with DPX-KN128.

For birds there is no indication from the reproductive toxicity studies for an endocrine disrupting potential of indoxacarb. Indeed, no effects were observed in the reproductive toxicity studies up to the highest tested dose.

Therefore, indoxacarb is not considered to have an endocrine disrupting potential.

B.9.2. EFFECT ON AQUATIC ORGANISMS

B.9.2.1. Acute toxicity to fish

Report: [REDACTED] (1996); DPX-MP062 (approximately 75% DPX-KN128, 25% IN-KN127): Flow-through, acute, 96-hour LC₅₀ to rainbow trout, *Oncorhynchus mykiss*

DuPont Report No.: HLR 911-96

Guidelines: OECD 203, EEC 92/69 Annex V - Method C.1 (1992), USEPA 72-1 **Deviations:** None

Testing Facility: [REDACTED]

Testing Facility Report No.: HLR 911-96

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.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: DPX-MP062 technical
 Lot/Batch #: MP062-51A
 Purity: 94.5%
 Description: Off-white solid
 CAS#: None for DPX-MP062
 DPX-KN128: 173584-44-6
 Stability of test compound: Shown to be stable in the test system by analysis
2. Control: Dilution (laboratory well water) water
 Solvent control: Dimethylformamide (DMF)
 Test vehicle: Dilution (laboratory well water) water
 Toxic reference: None
3. Test organism: Rainbow trout
 Species: *Oncorhynchus mykiss*
 Age at dosing: Life stage: fingerling
 Weight: 0.597 to 2.747 g (weight at termination)
 Initial population: Five fish per test chamber
 Source: [REDACTED]
 Acclimation period: 128 days
 Diet: Pre-test (approx. 50 hr): unfed
 Test period: unfed
 Test chamber: Stainless steel aquaria (29.3 l × 29.3 w × 29.3 h cm) holding approximately 14 L of test solution (15 cm liquid depth)
 Test medium: [REDACTED], well water
4. Environmental conditions (in-life period)
 Temperature: 11.5 to 12.5°C (of test solution)
 Photoperiod: 16 hr light (approx. 398 to 409 lux) and 8 hr dark including 30 min transitional light (approx. 2 lux) preceding and following the 16-hr light interval

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
 26-September-1996 to 30-September-1996
2. Test system
 The acute toxicity of DPX-MP062 to fingerling rainbow trout, *Oncorhynchus mykiss*, was determined during a 96-hour exposure under flow-through, unaerated conditions in a laboratory study. Treatments consisted of dilution water and DMF (0.1 mL/L) controls, and 5 test concentrations (0.036, 0.073, 0.15, 0.29, and 0.65 mg mean measured DPX-MP062/L). There were 10 fish and two replicates (5 fish per replicate) per treatment concentration. The fish were acclimated for 128 days in continuously flowing dilution water at 13.1°C, and were not fed for 49 hours and 45 minutes prior to or during the test. Loading in the water control was 0.049 g/L at test conclusion. Observations of mortality and sublethal effects were made every 24 hours. Dissolved oxygen and pH were measured in both controls and all the test solutions at test start, before fish were added, and daily thereafter. Concentrations of the test substance in all test solutions, including the dilution water and DMF control solutions, were determined from samples taken at the beginning and end of the study. Results are expressed on the basis of the mean measured concentrations of DPX-MP062.

II. RESULTS AND DISCUSSION

A. FINDINGS

Summaries of cumulative mortality (Table 13) and sublethal effects (Table 14) are shown below. Sublethal effects were observed only in the 0.65 mg DPX-MP062/L test concentration. The LC₅₀ was determined graphically because of the nature of the mortality data.

Table 13
Observed mortality of rainbow trout, *Oncorhynchus mykiss*, exposed to DPX-MP062 for 96 hours in a flow-through, unaerated test

Mean measured concentration (mg DPX-MP062/L)	Cumulative mortality (%)			
	24 Hours ^a	48 Hours ^a	72 Hours ^a	96 Hours ^a
Water Control	0	0	0	10
DMF Control	0	0	0	0
0.036	0	0	0	0
0.073	0	0	0	0
0.15	0	0	0	0
0.29	0	0	0	0
0.65	0	20	30	50

^a Ten fish per test concentration at test start.

Table 14
Sublethal effects of DPX-MP062 on rainbow trout, *Oncorhynchus mykiss*, exposed for 96 hours in a flow-through, unaerated test

Mean measured concentration (mg DPX-MP062/L)	Number of fish with effect/total alive			
	24 Hours ^a	48 Hours ^a	72 Hours ^a	96 Hours ^a
Water Control	0/10	0/10	0/10	0/9
DMF Control	0/10	0/10	0/10	0/10
0.036	0/10	0/10	0/10	0/10
0.073	0/10	0/10	0/10	0/10
0.15	0/10	0/10	0/10	0/10
0.29	0/10	0/10	0/10	0/10
0.65	4 ^{b,c,d} /10	4 ^{b,c,d} /8	4 ^{b,c,d,e} /7	5 ^{b,c,d} /5

^a Ten fish per test concentration at test start

^b Gasping for air;

^c Lying on the bottom

^d Partial loss of equilibrium

^e Dark coloration

III. CONCLUSION

The 96-hour LC₅₀ of DPX-MP062 dissolved in 0.1 mL/L DMF in the rainbow trout was 0.65 mg DPX-MP062/L.

([REDACTED] 1996)

RMS comment

This study was assessed for previous Annex I inclusion and was conducted with the old material DPX-MP062. As new acute toxicity study is available on the same species (rainbow trout) for the new material DPX-KN128, this study is not considered essential. The validity was not checked.

Report: [REDACTED] (1997a); DPX-MP062 (approximately 75% DPX-KN128, 25% IN-KN127): Flow-through, acute, 96-hour LC₅₀ to bluegill sunfish, *Lepomis macrochirus*

DuPont Report No.: HLR 912-96, Revision No. 2

Guidelines: OECD 203, EEC 92/69 Annex V-Method C.1 (1992), U.S. EPA 72-1 **Deviations:** None

Testing Facility: [REDACTED]

Testing Facility Report No.: HLR 912-96, Revision No. 2

GLP: Yes

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

I. MATERIALS AND METHODS

A. MATERIALS

- | | |
|--|---|
| 1. Test material: | DPX-MP062 Technical |
| Lot/Batch #: | MP062-51A |
| Purity: | 94.5% |
| Description: | Off-white solid |
| CAS#: | None for DPX-MP062 |
| | DPX-KN128: 173584-44-6 |
| Stability of test compound: | Shown to be stable under conditions of test |
| 2. Control: | Dilution (laboratory well water) water |
| Solvent control: | N,N-dimethylformamide |
| Test vehicle: | Dilution (laboratory well water) water |
| Toxic reference: | None |
| 3. Test organism: | Bluegill sunfish |
| Species: | <i>Lepomis macrochirus</i> |
| Age at dosing: | Juveniles |
| Weight: | 0.156 to 0.392 g (weight at termination) |
| Initial population: | 5 fish per test chamber |
| Source: | [REDACTED], |
| Acclimation period: | 17 days |
| Diet: | Pre-test (approx. 53 hr): unfed |
| | Test period: unfed |
| Test chamber: | Stainless steel aquaria (29.5 l × 29.5 w × 29.5 h cm) holding approximately 14 L of test solution (17.5 cm liquid depth) |
| Test medium: | [REDACTED], well water |
| 4. Environmental conditions (in-life period) | |
| Temperature: | 19.8 to 20.2°C (of test solution) |
| Photoperiod: | 16 hr light (approximately 334 to 355 lux) and 8 hr dark including 35 min transitional light (approximately 2 lux) preceding and following the 16-hr light interval |

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed

15-November-1996 to 19-November-1996

2. Test system:

The acute toxicity of DPX MP062 to juvenile bluegill sunfish, *Lepomis macrochirus*, was determined during a 96-hour exposure under flow-through, unaerated conditions in a laboratory study. Treatments consisted of dilution water and dimethylformamide (0.1 mL/L) controls and test concentrations (0.10, 0.21, 0.41, 0.52, and 1.3 mg mean measured DPX MP062/L). There were 10 fish and 2 replicates (5 fish per replicate) per treatment concentration. The fish were acclimated for 17 days in continuously flowing dilution water at 19.7°C, and were not fed for approximately 53 hours prior to or during the test. Loading in the water control was 0.008 g/L at test conclusion. Observations of mortality and sublethal effects were made every 24 hours. Dissolved oxygen and pH were measured in both controls and all the test solutions at test start, before fish were added, and daily thereafter. Concentrations of the test substance in all test solutions, including the dilution water and dimethylformamide control solutions were determined from samples taken at the beginning and at the end of the study. Results are expressed on the basis of the mean measured concentrations of DPX-MP062.

II. RESULTS AND DISCUSSION

A. FINDINGS

Summaries of cumulative mortality (Table 15) and sublethal effects (Table 16) are shown below. Sublethal effects were observed in the 0.52 and 1.3 mg DPX-MP062/L concentrations. The LC₅₀ was determined graphically because of the nature of the mortality data.

Table 15
Observed mortality of bluegill sunfish, *Lepomis macrochirus*, exposed to DPX-MP062 for 96 hours in a flow-through, unaerated test

Mean measured concentration (mg DPX-MP062/L)	Cumulative mortality (%)			
	24 Hours ^a	48 Hours ^a	72 Hours ^a	96 Hours ^a
Water Control	0	0	0	0
DMF Control	0	0	0	0
0.10	0	0	0	0
0.21	0	0	0	0
0.41	0	0	0	0
0.52	0	0	0	10
1.3	0	10	30	60

^a Ten fish per test concentration at test start

Table 16
**Sublethal effects of DPX-MP062 on juvenile bluegill sunfish, *Lepomis macrochirus*,
exposed for 96 hours in a flow-through, unaerated test**

Mean measured concentration (mg DPX-MP062/L)	Number of fish with effect/total alive			
	24 Hours ^a	48 Hours ^a	72 Hours ^a	96 Hours ^a
Water Control	0/10	10/10	0/10	0/10
DMF Control	0/10	0/10	0/10	0/10
0.10	0/10	0/10	0/10	0/10
0.21	0/10	0/10	0/10	0/10
0.41	0/10	0/10	0/10	0/10
0.52	2 ^{b,c} /10	1 ^{b,c} /10	1 ^{b,c} /10	0/9
1.3	1 ^{b,c} /10	3 ^{b,c,1,d,e} /9	4 ^{b,c,1,f} /7	4 ^{b,c,f} /4

^a There were ten fish at test start for each concentration tested

^b Lying on the bottom

c Gasping for air

d Erratic swimming

e At the surface

f Lethargic.

III. CONCLUSION

The 96-hour LC₅₀ of DPX MP062 dissolved in 0.1 mL/L DMF in the bluegill sunfish was 0.90 mg DPX MP062/L.

(██████████ 1997a)

RMS comment

This study was assessed for previous Annex I inclusion and was conducted with the old material DPX-MP062 (79:21). The study was conducted according to the current guideline. The study is valid according to validity criteria. RMS noted that it was stated in the previous DAR that the highest concentrations used in this test were above the solubility of indoxacarb: 0.2 mg/L at 25°C (0.90 mg/L at 21°C according to the study report). However, as the endpoint of 0.90 mg DPX MP062/L is based on mean measured concentrations, it is considered acceptable.

Report: [REDACTED] (1997); Flow-through acute toxicity of DPX-MP062 to the sheepshead minnow, *Cyprinodon variegatus*

DuPont Report No.: HLO-1997-00090, Revision No. 1

Guidelines: U.S. EPA 72-3(a) **Deviations:** None

Testing Facility: _____

Testing Facility Report No.: 802-DU

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 DPX-MP062 to unfed juvenile sheepshead minnows, *Cyprinodon variegatus*, a marine/estuarine fish, was determined in an unaerated, flow-through, 96-hour dose response test. The test was conducted in accordance with U.S. EPA Pesticide Assessment Guideline, FIFRA 72-3(a).

Treatments consisted of a dilution water control, a solvent control (0.1 mL/L dimethylformamide), and five nominal concentrations of 0.058, 0.090, 0.14, 0.22, and 0.36 mg DPX-MP062/L. The corresponding mean measured concentrations of DPX-MP062 were 0.0532, 0.0910, 0.129, 0.188, and 0.374 mg/L.

The 96-hour LC₅₀ for *Cyprinodon variegatus* based on mortality and mean measured concentrations was >0.374 mg DPX-MP062/L, the highest tested concentration and approximately equal to the solubility limit of the test substance in seawater.

I. MATERIALS AND METHODS**A. MATERIALS**

- | | |
|--|--|
| 1. Test material: | DPX-MP062 technical |
| Lot/Batch #: | MP062-51A |
| Purity: | 94.54% |
| Description: | Off-white solid |
| CAS#: | 144171-61-9 |
| Stability of test compound: | Test substance appeared to be stable under the conditions of the study; no evidence of instability was observed. |
| 2. Control: | Dilution water (carbon filtered natural seawater) |
| Solvent control: | Dimethylformamide |
| Test vehicle: | Dilution water (carbon filtered natural seawater) |
| Toxic reference: | None |
| 3. Test organism: | Sheepshead minnow |
| Species: | <i>Cyprinodon variegatus</i> |
| Age at dosing: | Life stage: juvenile |
| Weight: | 0.33 g (wet weight of control organisms at termination) |
| Initial population: | 20 fish per treatment with 10 fish per replicate/test chamber |
| Source: | [REDACTED] |
| Acclimation period: | 14 days |
| Diet: | Pre-test (approx. 48 hr): unfed |
| | Test period: unfed |
| Test chamber: | 10 L glass aquaria containing 7 L of test solution (16 cm liquid depth) |
| Test medium: | ([REDACTED])
carbon filtered natural seawater |
| 4. Environmental conditions (in-life period) | |
| Temperature: | 21.2 to 22.5°C (of test solution) |
| Photoperiod: | 16 hr light (65 footcandles) and 8 hr dark including 15 minutes of transitional light preceding and following the 16-hr light interval |

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
27-November-1995 to 30-November-1996
2. Experimental treatments
The acute toxicity of DPX-MP062 to unfed juvenile sheepshead minnows, *Cyprinodon variegatus*, a marine/estuarine fish, was determined in an unaerated, flow-through, 96-hour dose-response test.

An initial range-finding test was conducted in which sheepshead minnows were exposed under static-renewal conditions to DPX-MP062 at nominal concentrations of 0 (control and solvent control), 0.010, 0.050, 0.10, 0.36, and 0.50 mg DPX-MP062/L. The media was renewed in all test vessels after 24, 48, and 72 hours. After 96 hours of exposure, survival was 90% at 0.010 mg/L, 80% at 0.050 and 0.10 mg/L, and 30% at 0.36 and 0.50 mg DPX-MP062/L. In a second range-finding test conducted under flow through conditions at 0, 0.058 and 0.36 mg DPX-MP062/L, survival was 100% at 0.058 mg/L and 0% at 0.36 mg/L DPX-MP062 after 96 hours of exposure.

In the definitive test, treatments consisted of a dilution water control, a solvent control (0.1 mL/L dimethylformamide), and five nominal concentrations of 0.058, 0.090, 0.14, 0.22, and 0.36 mg DPX-MP062/L. The highest tested nominal concentration was the solubility limit of the test substance in seawater. Two replicate control test chambers and two replicate test concentration chambers containing 10 fish each were exposed to each treatment concentration and control (total of 20 fish in the dilution water control and 20 fish in each test concentration).

3. Observations

Mortality and sublethal (behavioural) observations were made every 24 hours. Dead fish were removed from the test chambers when observed.

4. Statistics

The LC_{50} value was not statistically calculated as there was no adverse effect of 50% or greater on mortality, and therefore, the LC_{50} value was estimated to be greater than the highest concentration tested. The no observed effect concentration is the highest concentration of test substance that did not cause toxicant related mortalities or sublethal effects.

II. RESULTS AND DISCUSSION

A. FINDINGS

Mean measured concentrations of DPX-MP062 were 0.0532, 0.0910, 0.129, 0.188, and 0.374 mg DPX-MP062/L and ranged from 85 to 104% of nominal concentrations. The concentrations were stable throughout the test. All validation criteria were met for the study.

A summary of cumulative mortality and sublethal effects is presented in Table 17 and Table 18, respectively. There was 100% survival in the both controls with no sublethal effects noted. One sheepshead minnow exposed to 0.0532 mg/L DPX-MP062 exhibited lethargy after 48 hours of exposure, and one minnow exposed to 0.374 mg/L exhibited erratic swimming after 24 hours, and erratic swimming and loss of equilibrium after 96 hours of exposure. No other sublethal effects were observed during the definitive test.

Table 17
Mortality of sheepshead minnow, *Cyprinodon variegatus*, exposed to DPX-MP062 for 96 hours in an unaerated, flow-through, acute test

Mean, measured concentration of DPX-MP062 (mg/L)	Mortality (No. dead/No. at test start)							
	24 h		48 h		72 h		96 h	
	A ^a	B ^a	A	B	A	B	A	B
Water control	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
Solvent control	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
0.0532	0/10	1/10	0/10	1/10	0/10	1/10	0/10	1/10
0.0910	1/10	0/10	1/10	1/10	1/10	1/10	1/10	1/10
0.129	0/10	0/10	0/10	1/10	0/10	1/10	0/10	1/10
0.188	0/10	1/10	0/10	1/10	0/10	1/10	0/10	1/10
0.374	2/10	3/10	4/10	3/10	4/10	3/10	5/10	3/10

^a A and B represent replicates; each replicate contained 10 fish (total 20 fish per test concentration) at test start.

The report states that the LC₅₀ is expected to be only slightly above 0.374 mg/L, as 40% mortality occurred in the definitive study at this measured concentration and 70 and 100% mortality occurred at the 0.36 mg DPX-MP062/L nominal concentration in the initial and second range-finding studies, respectively. In addition, the report stated that the apparent variability of the results may be at least partially related to testing at the approximate solubility limit.

The study report included a solubility and stability report as an appendix. The highest tested concentration (0.36 mg DPX-MP062/L) was the reported solubility limit of the test substance in seawater.

Table 18
Observed sublethal effects of sheepshead minnow, *Cyprinodon variegatus*, exposed to DPX-MP062 for 96 hours in an unaerated, flow-through, acute test

Mean, measured concentration of DPX-MP062 (mg/L)	Sublethal effects (No. affected ^a /No. at test start)							
	24 h		48 h		72 h		96 h	
	A ^d	B ^d	A	B	A	B	A	B
Water control	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
Solvent control	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
0.0532	0/10	0/10	0/10	1 ^b /10	0/10	0/10	0/10	0/10
0.0910	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
0.129	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
0.188	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
0.374	1 ^a /10	0/10	0/10	0/10	0/10	0/10	1 ^c /10	0/10

^a Affected sheepshead minnow exhibited erratic swimming.

^b Affected sheepshead minnow exhibited lethargy.

^c Affected sheepshead minnow exhibited erratic swimming and loss of equilibrium.

^d A and B represent replicates; each replicate contained 10 fish (total 20 fish per test concentration) at test start.

III. CONCLUSION

The 96-hour LC₅₀ for the sheepshead minnow, *Cyprinodon variegatus*, based on mortality and mean measured concentrations was >0.374 mg DPX-MP062/L, the highest tested concentration and approximately equal to the solubility limit of the test substance in seawater.

The highest mean measured test concentration causing no observed effect was 0.188 mg DPX-MP062/L.

([REDACTED] 1997)

RMS comment

This study was conducted with the old material DPX-MP062. This study was not conducted according to the current guideline OECD 203. As a new acute toxicity study is available on rainbow trout for the new material DPX-KN128, this study is not considered essential. The validity was not checked.

Report: [REDACTED] (2010); Indoxacarb (DPX-KN128)
technical: A 96-hour flow-through acute toxicity test with the rainbow trout (*Oncorhynchus mykiss*)

DuPont Report No.: DuPont-29541

Guidelines: OECD 203 (1993), USEPA 850.1075 (1996) **Deviations:** None

Testing Facility: [REDACTED]

Testing Facility Report No.: 112A-312

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 (DPX-KN128) to unfed juvenile rainbow trout (*Oncorhynchus mykiss*), a cold freshwater fish, was determined in an unaerated, flow-through, 96-hour dose response test. The test was conducted based upon procedures outlined in the OECD Guideline for Testing of Chemicals, 203 (1993), *Fish, Acute Toxicity Test* and U.S. EPA OPPTS Guideline 850.1075, *Fish Acute Toxicity Test, Freshwater and Marine* (Draft, 1996). Treatments consisted of a dilution water control, a solvent control, and nominal indoxacarb concentrations of 0.013, 0.025, 0.050, 0.10, and 0.20 mg a.s./L. The corresponding mean measured concentrations of indoxacarb were 0.011, 0.022, 0.045, 0.090, and 0.17 mg a.s./L. The 96-hour LC₅₀ for *Oncorhynchus mykiss* based on mean, measured concentrations of indoxacarb active substance and mortality was greater than 0.17 mg a.s./L, the highest mean measured concentration tested and the apparent limit of solubility of indoxacarb.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Indoxacarb technical
 Lot/Batch #: KN128-215
 Purity: 98.4%
 Description: Solid
 CAS#: 173584-44-6
 Stability of test compound: The mean recovery of indoxacarb from freshwater stability samples was 101, 55, 37, and 53% of the 0-hour measured concentration after 3, 24, 48, and 96 hours, respectively.
2. Negative control: Dilution water (laboratory well water)
 Solvent control: HPLC grade dimethylformamide (0.1 mL/L)
 Test vehicle: 0.1 mL DMF/L in solvent control and treatment groups
 Toxic reference: Not applicable
3. Test organism: Rainbow Trout
 Species: *Oncorhynchus mykiss*
 Age at dosing: Juveniles
 Weight at dosing: Not specified
 Weight at termination: 0.56 to 0.81 g
 Source: [REDACTED]
 Acclimation period: At least 14 days
 Diet: Pre-test (approx. 48 hr): unfed
 Test period: unfed
 Test chamber: Stainless steel aquaria (25 L) holding 15 L of test solution (18 cm liquid depth)
 Water: [REDACTED], well water
4. Environmental conditions:
 Temperature: 12.1 to 12.2°C in test chambers; 11 to 12°C measured continuously in the negative control.
 Photoperiod: 16 hr light (162 lux at initiation) and 8 hr dark including a 30 min transitional period preceding and following the 16-hr light interval.

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
 19-April-2010 to 23-April-2010
2. Experimental treatments
 The acute toxicity of indoxacarb to unfed juvenile rainbow trout (*Oncorhynchus mykiss*), a cold freshwater fish, was determined in an unaerated, flow-through, 96-hour dose response test. Treatments consisted of a dilution water control, a solvent control (0.1 mL DMF/L) and five nominal concentrations of 0.013, 0.025, 0.050, 0.10, and 0.20 mg a.s./L. One control test chamber and a single test chamber per treatment level containing 7 fish each were utilised in the study.
3. Observations
 Mortality and behavioural observations were made at approximately 2.5 hours after initiation and then every 24 hours following initiation of exposure.
4. Statistics
 The absence of mortality in all of the treatment groups during the test, precluded the statistical calculation of LC₅₀ values at 24, 48, 72, and 96 hours. The highest mean, measured test concentration causing no mortality at test end and the lowest mean, measured test concentration

causing 100% mortality at test end were assessed by visual observation of the mortality and observation data.

II. RESULTS AND DISCUSSION

A. FINDINGS

Mean measured concentrations of indoxacarb during pretest diluter verification ranged from 85.9 to 95.6% of nominal concentrations. Mean measured concentrations of indoxacarb during the test were 0.011, 0.022, 0.045, 0.090, and 0.17 mg a.s./L and ranged from 85 to 90% of nominal concentrations. All validation criteria were met for the study. Summaries of the cumulative mortality and sublethal effects are presented in Table 19 and Table 20, respectively.

Table 19
Observed mortality of rainbow trout (*Oncorhynchus mykiss*) exposed to indoxacarb for 96 hours in an unaerated, flow-through, acute toxicity test

Mean, measured indoxacarb concentration (mg a.s./L)	Cumulative mortality (No. dead/No. at test start) ^{a,b}			
	24 hour	48 hour	72 hour	96 hour
Water Control (0.0)	0/7	0/7	0/7	0/7
Solvent Control (0.0)	0/7	0/7	0/7	0/7
0.011	0/7	0/7	0/7	0/7
0.022	0/7	0/7	0/7	0/7
0.045	0/7	0/7	0/7	0/7
0.090	0/7	0/7	0/7	0/7
0.17	0/7	0/7	0/7	0/7

^a One test chamber containing 7 fish at test start was tested at each concentration.

^b Note that at the 2.5-hour assessment period there were no mortalities or sublethal effects observed at any test concentration.

Table 20
Observed sublethal effects of rainbow trout (*Oncorhynchus mykiss*) exposed to indoxacarb for 96 hours in an unaerated, flow-through, acute toxicity test

Mean, measured indoxacarb concentration (mg a.s./L)	Sublethal effects (Number affected/Number alive) ^{a,b}			
	24 hour	48 hour	72 hour	96 hour
Water Control (0.0)	0/7	0/7	0/7	0/7
Solvent Control (0.0)	0/7	0/7	0/7	0/7
0.011	0/7	0/7	0/7	0/7
0.022	0/7	0/7	0/7	0/7
0.045	0/7	0/7	0/7	0/7
0.090	0/7	0/7	0/7	0/7
0.17	0/7	0/7	0/7	0/7

^a One test chamber containing 7 fish at test start was tested at each concentration.

^b Note that at the 2.5-hour assessment period there were no mortalities or sublethal effects observed at any test concentration.

III. CONCLUSION

The 96-hour LC₅₀ for *Oncorhynchus mykiss* based on mean measured concentrations of indoxacarb and mortality was greater than 0.17 mg a.s./L, the highest mean measured test concentration tested and the apparent limit of solubility of indoxacarb.

([REDACTED] 2010)

RMS comment

This study was conducted in compliance with the current guideline. The 96-hour LC₅₀ for *Oncorhynchus mykiss* based on mean measured concentrations of indoxacarb and mortality was greater than 0.17 mg a.s./L. This study is acceptable.

Report: [REDACTED] (1997b); IN-JT333-20: Flow-through, acute, 96-hour LC₅₀ to rainbow trout, *Oncorhynchus mykiss*

DuPont Report No.: HL-1997-00180

Guidelines: OECD 203, EEC 92/69 Annex V - Method C.1 (1992), USEPA 72-1 **Deviations:** None

Testing Facility: [REDACTED]

Testing Facility Report No.: HL-1997-00180

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.

I. MATERIALS AND METHODS

A. MATERIALS

- | | |
|-----------------------------|---|
| 1. Test material: | IN-JT333 technical metabolite |
| Lot/Batch #: | JT333-20 |
| Purity: | 98.7% by analysis |
| Description: | Beige solid |
| CAS#: | 144171-39-1 |
| Stability of test compound: | Shown to be stable in the test system by analysis |
| 2. Control: | Dilution (laboratory well water) water |
| Solvent control: | Dimethylformamide (DMF) |
| 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: | Water control: 0.33 to 0.92 g (weight at termination)
DMF control: 0.34 to 1.10 g (weight at termination) |
| | Initial population: | 5 fish per test chamber |
| | Source: | [REDACTED] |
| | Acclimation period: | 93 days |
| | Diet: | Pre-test (approx. 48 hr): unfed
Test period: unfed |
| | Test chamber: | Stainless steel aquaria (29.3 l × 29.3 w × 29.3 h cm) holding approximately 14 L of test solution (15 cm liquid depth) |
| | Test medium: | [REDACTED] well water |
| 4. | Environmental conditions (in-life period) | |
| | Temperature: | 11.2 to 12.3°C (of test solution) |
| | Photoperiod: | 16 hr light (approximately 322.8 lux) and 8 hr dark including 30 min transitional light (4.3 lux) preceding and following the 16-hr light interval |

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed

14-February-1997 to 18-February-1997

2. Experimental treatments

The acute toxicity of IN-JT333, a sediment metabolite of DPX-MP062, to fingerling rainbow trout, *Oncorhynchus mykiss*, was determined during a 96-hour exposure under flow-through, unaerated conditions in a laboratory study. Treatments consisted of dilution water and dimethylformamide (0.1 mL/L) controls, and 5 test concentrations (0.00049, 0.0012, 0.0052, 0.0090, and 0.029 mg mean measured IN-JT333/L). The solubility of IN-JT333 was 0.036 mg/L at 12°C. There were 10 fish and 2 replicates (5 fish per replicate) per treatment concentration. The fish were acclimated for 93 days in continuously flowing dilution water at 12.6°C, and were not fed for approximately 48 hours prior to or during the test. Loading in the water control was 0.017 g/L at test conclusion. Observations of mortality and sublethal effects were made every 24 hours. Dissolved oxygen and pH were measured in both controls and all the test solutions at test start, before fish were added, and daily thereafter. Concentrations of the test substance in all test solutions, including the dilution water and dimethylformamide control solutions, were determined from samples taken at the beginning and at the end of the study. Results are expressed on the basis of the mean measured concentrations of IN-JT333.

II. RESULTS AND DISCUSSION

A. FINDINGS

Summaries of cumulative mortality (Table 21) and sublethal effects (Table 22) are shown below. Sublethal effects were observed in only the 0.0052, 0.0090, and 0.029 mg IN-JT333/L concentrations. The LC₅₀ was determined graphically because of the nature of the mortality data.

Table 21
Observed mortality of rainbow trout, *Oncorhynchus mykiss*, exposed to IN-JT333 for 96 hours in a flow-through, unaerated test

Mean measured concentration (mg IN-JT333/L)	Cumulative mortality (%)			
	24 Hours ^a	48 Hours ^a	72 Hours ^a	96 Hours ^a
Water Control	0	0	0	10
DMF Control	0	0	0	0
0.00049	0	0	0	0
0.0012	0	0	0	0
0.0052	0	0	0	20
0.0090	0	0	20	30
0.029	10	40	50	50

^a Ten fish per test concentration at test start

Table 22
Sublethal effects of IN-JT333 on rainbow trout, *Oncorhynchus mykiss*, exposed for 96 hours in a flow-through, unaerated test

Mean measured concentration (mg IN-JT333/L)	Number of fish with effect/total alive			
	24 Hours ^a	48 Hours ^a	72 Hours ^a	96 Hours ^a
Water Control	0/10	0/10	0/10	0/9
DMF Control	0/10	0/10	0/10	0/10
0.00049	0/10	0/10	0/10	0/10
0.0012	0/10	0/10	0/10	0/10
0.0052	0/10	3 ^d /10	4 ^{b,d} /10	1 ^{b,c,d} , 1 ^{b,c} /8
0.0090	0/10	1 ^{b,c,d} /9	1 ^b /8	4 ^{b,d} , 1 ^{b,c,d} /7
0.029	5 ^{b,c,d} , 4 ^d /9	2 ^{b,d,e} , 4 ^{b,c,d} /6	5 ^{b,c,d} /5	5 ^{b,c,d,e} /5

^a Ten fish per test concentration at test start

^b Lying on the bottom

^c Lethargic

^d Partial loss of equilibrium

^e Gasping for air

III. CONCLUSION

The 96-hour LC₅₀ of IN-JT333 dissolved in 0.1 mL/L DMF in the rainbow trout was 0.029 mg/L, approximately equivalent to the limit of solubility of IN-JT333.

([REDACTED] 1997b)

RMS comment

This study was conducted in compliance with the current guideline. Because 50% mortality occurred at the highest mean measured test concentration of IN-JT333 (0.029 mg/L), it was not possible to statistically calculate a 96-hour LC₅₀ with 95% confidence limits. The 96-hour LC₅₀ for *Oncorhynchus mykiss* based on mean measured concentrations of IN-JT333 was 0.029 mg IN-JT333/L (determined graphically). This study is still considered acceptable.

Report: [REDACTED] (2014); IN-JU873: Acute toxicity to the rainbow trout, *Oncorhynchus mykiss*, determined under static-renewal test conditions

DuPont Report No.: DuPont-35826

Guidelines: OECD 203 (1992), OPPTS 850.1075 (1996) **Deviations:** None

Testing Facility: [REDACTED]

Testing Facility Report No.: 69137

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:

The acute toxicity of IN-JU873 to unfed *Oncorhynchus mykiss* was determined in a static-renewal, 96-hour test. The test was conducted in accordance with the Organization for Economic Cooperation and Development (OECD), July 17, 1992 OECD Guidelines for Testing of Chemicals, *Fish, Acute Toxicity Test*, OECD Guideline No. 203, 9 pp. and also the U.S. Environmental Protection Agency, Ecological Effects Test Guidelines, OPPTS 850.1075, *Fish Acute Toxicity Test, Freshwater and Marine*. Treatments consisted of a dilution water control, solvent control, and five nominal total formulation concentrations of 0.031, 0.063, 0.13, 0.25, and 0.50 mg IN-JU873/L. The 96-hour LC₅₀ value was >0.441 mg IN-JU873/L based on mean measured concentrations. No mean measured IN-JU873 concentration caused 100% mortality at test end. The highest mean measured IN-JU873 concentration causing 0% mortality at test end was 0.0184 mg/L.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: IN-JU873 technical metabolite
 Lot/Batch #: JU873-005
 Purity: 99.1%
 Description: Solid, crystalline
 CAS#: 144172-25-8
 Stability of test compound: Determined to not be stable in the test system
2. Control: Dilution (blended) water
 Solvent control: DMF
 Test vehicle: Dilution (blended) water
 Toxic reference: None
3. Test organism: Rainbow trout
 Species: *Oncorhynchus mykiss*
 Age at dosing: Juvenile
 Weight at dosing: 0.6049 to 0.9764 g
 Initial population: 7 fish per treatment level
 Source: [REDACTED]
 Acclimation period: >7 days
 Diet: Pre-test (approx. 48 hours): unfed
 Test period: unfed
 Test chamber: 21-L glass jar containing 18 L of test solution (32-cm test solution depth), covered with a glass lid.
 Test medium: [REDACTED] blended freshwater
4. Environmental conditions (in-life period)
 Temperature: 13.7 to 16.3°C (recirculating waterbath used to maintain test chamber temperature)
 Photoperiod: 16 hr photoperiod (892 lux) and 8 hr darkness which included 30 min transitional light preceding and following the 16-hr light interval
 pH: 8.0 to 8.6
 Dissolved oxygen: 6.6 to 10.4 mg O₂/L

B. STUDY DESIGN AND METHODS

1. In- life initiated/completed
 23-October-2013 to 27-October-2013
2. Experimental treatments
 The acute toxicity of IN-JU873 to unfed *Oncorhynchus mykiss* was determined in a static-renewal, 96-hour test. Treatments consisted of a dilution water control, solvent control, and five nominal concentrations of 0.031, 0.063, 0.13, 0.25, and 0.50 mg IN-JU873/L. Seven fish were used per test concentration and control. Treatments were not replicated.
3. Observations
 Mortality and behavioural observations were made at 6, 24, 48, 72, and 96 hours. Dead fish were removed from the test chambers when observed.
4. Statistics
 Estimates of EC₅₀ values and their 95% confidence limits were calculated using the Trimmed Spearman-Kärber method. The lowest concentration resulting in 100% immobility and highest concentration resulting in 0% immobility were assessed by visual observation.

II. RESULTS AND DISCUSSION

A. FINDINGS

The concentration of the IN-JU873 was measured in test solution samples collected at 0, 24, 72, and 96 hours of the definitive test. Freshly prepared solutions were samples for analysis at 0 and 72 hours. Spent solutions were samples for analysis at 24 and 96 hours. The measured concentrations of IN-JU873 in test substance treatment samples collected at initiation were 0.0280, 0.0590, 0.124, 0.252, and 0.490 mg/L or 90 to 101% of the nominal concentrations. The measured concentrations of IN-JU873 in test solutions at 24-hours were 0.00868, 0.0204, 0.0538, 0.135, and 0.373 mg/L or 28 to 75% of the nominal concentrations. The measured concentrations of IN-JU873 in test solutions at 72-hours were 0.0280, 0.0528, 0.121, 0.260, and 0.483 mg/L or 84 to 104% of the nominal concentrations. The measured concentrations of IN-JU873 in test solutions at 96-hours were 0.00886, 0.0153, 0.0590, 0.198, and 0.417 mg/L or 24 to 83% of the nominal concentrations. Mean measured concentrations of IN-JU873 during the exposure were 0.0184, 0.0369, 0.0895, 0.211, and 0.441 mg/L or 59 to 88% of the nominal concentrations. The dilution water control and solvent control solutions contained no detectable concentrations of IN-JU873. Recoveries from the QC fortifications ranged from 101 to 114% of the nominal concentrations.

All results from biological responses were based on mean measured concentrations of IN-JU873. Summaries of the cumulative mortality and sublethal effects are presented in Table 23 and Table 24, respectively.

Table 23
Observed mortality of rainbow trout, *Oncorhynchus mykiss*, exposed to IN-JU873 for 96 hours in a static acute test

Nominal IN-JU873 Concentration (mg/L)	Mean, Measured IN-JU873 Concentration (mg/L)	Cumulative Mortality/Number at Test Start ^a				
		6 Hours	24 Hours	48 Hours	72 Hours	96 Hours
0 (Control)	<LOD	0/7	0/7	0/7	0/7	0/7
0 (Solvent Control)	<LOD	0/7	0/7	0/7	0/7	0/7
0.031	0.0184	0/7	0/7	0/7	0/7	0/7
0.063	0.0369	0/7	0/7	0/7	2/7	2/7
0.13	0.0895	0/7	0/7	3/7	3/7	3/7
0.25	0.211	0/7	0/7	0/7	0/7	1/7
0.50	0.441	0/7	1/7	1/7	1/7	2/7

^a Test chambers contained seven rainbow trout each at test initiation.

Table 24
Observed sublethal effects of rainbow trout, *Oncorhynchus mykiss*, exposed to IN-JU873 for 96 hours in a static, acute test

Nominal IN-JU873 Concentration (mg/L)	Mean, Measured IN-JU873 Concentration (mg/L)	Number Affected/Number Alive ^a				
		6 Hours	24 Hours	48 Hours	72 Hours	96 Hours
0 (Control)	<LOD	0/7	0/7	0/7	0/7	0/7
0 (Solvent Control)	<LOD	0/7	0/7	0/7	0/7	0/7
0.031	0.0184	0/7	0/7	0/7	0/7	0/7
0.063	0.0369	0/7	0/7	2/7 ^f	5/5 ^j	4/5 ⁿ
0.13	0.0895	0/7	7/7 ^c	4/4 ^g	4/4 ^k	4/4 ^o
0.25	0.211	0/7	7/7 ^d	7/7 ^h	7/7 ^l	6/6 ^p
0.50	0.441	1/7 ^b	6/6 ^e	6/6 ⁱ	6/6 ^m	5/5 ^q

^a Test chambers contained seven rainbow trout each at test initiation.

^b One fish was discolored.

^c Three fish were discolored and exhibiting loss of equilibrium. Four fish were exhibiting loss of equilibrium.

^d Two fish were discolored and exhibiting loss of equilibrium. Two fish were exhibiting erratic swimming pattern and loss of equilibrium. Three fish were exhibiting loss of equilibrium.

^e One fish was discolored and exhibiting erratic swimming pattern and loss of equilibrium. Five fish were exhibiting loss of equilibrium.

^f Two fish were exhibiting loss of equilibrium.

^g Three fish were discolored and exhibiting loss of equilibrium. One fish was exhibiting loss of equilibrium.

^h Three fish were discolored and exhibiting loss of equilibrium. Three fish were exhibiting loss of equilibrium. One fish was discolored

ⁱ Two fish were discolored and exhibiting loss of equilibrium. Four fish were exhibiting loss of equilibrium.

^j Two fish were exhibiting erratic swimming pattern. Three fish were exhibiting loss of equilibrium.

^k Two fish were discolored and exhibiting loss of equilibrium. One fish was exhibiting loss of equilibrium. One fish was surfacing and exhibiting loss of equilibrium.

^l Three fish were discolored and laying on the bottom of the test chamber. Two fish were laying on the bottom of the test chamber. Two fish were exhibiting loss of equilibrium.

^m Two fish were discolored and exhibiting loss of equilibrium. Three fish were exhibiting loss of equilibrium. One fish was floating on the surface of the test solution.

ⁿ Two fish were discolored and exhibiting loss of equilibrium. Two fish were exhibiting loss of equilibrium.

^o Two fish were discolored and exhibiting loss of equilibrium. One fish was exhibiting loss of equilibrium. One fish was exhibiting erratic swimming pattern.

^p Three fish were discolored and laying on the bottom of the test chamber. One fish was floating on the surface of the test solution and exhibiting loss of equilibrium. Two fish were exhibiting loss of equilibrium.

^q Three fish were discolored and laying on the bottom of the test chamber. One fish was floating on the surface of the test solution and exhibiting loss of equilibrium. One fish was exhibiting loss of equilibrium.

III. CONCLUSION

IN-JU873 was assessed for acute toxicity to the rainbow trout, *Oncorhynchus mykiss*, in a 96-hour static-renewal test. The 24-, 48-, 72-, and 96-hour LC₅₀ value, based on mortality, was >0.441 mg IN-JU873/L, the highest concentration tested. No mean measured IN-JU873 concentration caused 100% mortality at test end. The highest mean measured IN-JU873 concentration causing 0% mortality at test end was 0.0184 mg/L.

[REDACTED] 2014)

RMS comment

This study was conducted in compliance with the current guideline. The 96-hour LC₅₀ for *Oncorhynchus mykiss* based on mean measured concentrations of IN-JU873 was greater than 0.441 mg/L. RMS notes

that severe sublethal effects were observed at tested concentrations above 0.0184 mg/L. This study is acceptable.

Report: [REDACTED] (2013); IN-KB687: Acute toxicity to the rainbow trout, *Oncorhynchus mykiss*, determined under static test conditions

DuPont Report No.: DuPont-35828

Guidelines: OECD 203 (1992), OPPTS 850.1075 (1996) **Deviations:** None

Testing Facility: [REDACTED]

Testing Facility Report No.: 69143

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 IN-KB687 to unfed *Oncorhynchus mykiss* was determined in a static, 96-hour test. The test was conducted in accordance with the Organization for Economic Cooperation and Development (OECD), 1992 OECD Guidelines for Testing of Chemicals, *Fish, Acute Toxicity Test*, OECD Guideline No. 203, and U.S. Environmental Protection Agency, Ecological Effects Test Guidelines, OPPTS 850.1075, *Fish Acute Toxicity Test, Freshwater and Marine*. Treatments consisted of a dilution water control and five nominal total formulation concentrations of 1.5, 3.0, 6.0, 12, and 24 mg IN-KB687/L. The 96-hour LC₅₀ value was 11.9 mg IN-KB687/L, based on mean measured concentrations. The lowest mean measured concentration causing 100% mortality at test end was 23.7 mg IN-KB687/L. The highest mean measured concentration causing 0% mortality at test end was 5.29 mg IN-KB687/L.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: IN-KB687 technical metabolite
 Lot/Batch #: KB687-002
 Purity: 99.8%
 Description: Solid powder
 CAS#: 177905-10-1
 Stability of test compound: Determined to be stable in the test system
2. Control: Dilution (laboratory well water) water
 Solvent control: None
 Test vehicle: Dilution (laboratory well water) water
 Toxic reference: None
3. Test organism: Rainbow trout
 Species: *Oncorhynchus mykiss*
 Age at dosing: Juvenile
 Weight at dosing: 0.7471 to 1.3231 g/fish
 Initial population: 7 fish per treatment level
 Source: [REDACTED]
 Acclimation period: >7 days
 Diet: Pre-test (approx. 24 hours): unfed
 Test period: unfed
 Test chamber: 21-L glass jar containing 18 L of test solution (32-cm test solution depth), covered with a glass lid.
 Test medium: [REDACTED] blended freshwater
4. Environmental conditions (in-life period)
 Temperature: 14.4 to 15.0°C (recirculating waterbath used to maintain test chamber temperature)
 Photoperiod: 16 hr photoperiod (1,034 lux) and 8 hr darkness which included 30 min transitional light preceding and following the 16-hr light interval
 pH: 7.9 to 8.4
 Dissolved oxygen: 6.9 to 10.3 mg O₂/L

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
 22-February-2013 to 26-February-2013
2. Experimental treatments
 The acute toxicity of IN-KB687 to unfed *Oncorhynchus mykiss* was determined in a static, 96-hour test. Treatments consisted of a dilution water control and five nominal concentrations of 1.5, 3.0, 6.0, 12, and 24 mg IN-KB687/L. Seven fish were used per test concentration and control. Treatments were not replicated.
3. Observations
 Mortality and behavioural observations were made at 6, 24, 48, 72, and 96 hours. Dead fish were removed from the test chambers when observed.
4. Statistics
 Estimates of LC₅₀ values and their 95% confidence limits were calculated using the probit or Spearman Karber method.

II. RESULTS AND DISCUSSION

A. FINDINGS

The concentration of IN-KB687 was measured in test solutions at 0 and 96 hours. Mean measured concentrations of IN-KB687 during the exposure were 1.37, 2.69, 5.29, 11.4, and 23.7 mg/L or 88 to 99% of the nominal concentrations. No residues of IN-KB687 were detected in the dilution water control solutions above the LOD of 0.00159 mg/L. Recoveries from the QC fortifications ranged from 103 to 106% of the nominal concentrations.

All results from biological responses were based on mean measured concentrations of IN-KB687. Summaries of cumulative mortality and sublethal effects are presented in Table 25 and Table 26, respectively.

Table 25
Observed mortality of rainbow trout, *Oncorhynchus mykiss*, exposed to IN-KB687 for 96 hours in a static acute test

Nominal IN-KB687 Concentration (mg/L)	Mean, Measured IN-KB687 Concentration (mg/L)	Cumulative Mortality/Number at Test Start ^a				
		6 Hours	24 Hours	48 Hours	72 Hours	96 Hours
0 (Control)	<LOD	0/7	0/7	0/7	0/7	0/7
1.5	1.37	0/7	0/7	0/7	0/7	0/7
3.0	2.69	0/7	0/7	0/7	0/7	0/7
6.0	5.29	0/7	0/7	0/7	0/7	0/7
12	11.4	3/7	3/7	3/7	3/7	3/7
24	23.7	7/7	7/7	7/7	7/7	7/7

^a Test chambers contained seven rainbow trout each at test initiation.

Table 26
Observed sublethal effects of rainbow trout, *Oncorhynchus mykiss*, exposed to IN-KB687 for 96 hours in a static, acute test

Nominal IN-KB687 Concentration (mg/L)	Mean, Measured IN-KB687 Concentration (mg/L)	Number Affected/Number Alive ^a				
		6 Hours	24 Hours	48 Hours	72 Hours	96 Hours
0 (Control)	<LOD	0/7	0/7	0/7	0/7	0/7
1.5	1.37	0/7	0/7	0/7	0/7	0/7
3.0	2.69	0/7	0/7	0/7	0/7	0/7
6.0	5.29	0/7	0/7	0/7	0/7	0/7
12	11.4	4/4 ^b	4/4 ^c	4/4 ^c	4/4 ^c	4/4 ^c
24	23.7	---	---	---	---	---

^a Test chambers contained seven rainbow trout each at test initiation.

^b Three fish were on the bottom of the test chamber exhibiting loss of equilibrium and irregular respiration. One fish was floating on the surface and exhibiting loss of equilibrium and irregular respiration.

^c Four fish were on the bottom of the test chamber, discolored, and exhibiting loss of equilibrium and irregular respiration.

Note: Dashes (---) denotes no sublethal effects due to total mortality.

III. CONCLUSION

IN-KB687 was assessed for acute toxicity to the rainbow trout, *Oncorhynchus mykiss*, in a 96-hour static test. The 24-, 48-, 72-, and 96-hour LC₅₀ value, based on mortality, was 11.9 mg IN-KB687/L with 95% confidence intervals of 9.00 and 15.8 mg IN-KB687/L. The lowest mean measured concentration causing 100% mortality at test end was 23.7 mg IN-KB687/L. The highest mean measured concentration causing 0% mortality at test end was 5.29 mg IN-KB687/L.

([REDACTED] 2013)

RMS comment

This study was conducted in compliance with the current guideline. The 96-hour LC₅₀ for *Oncorhynchus mykiss* based on mean measured concentrations of IN-KB687 was 11.9 mg/L. RMS notes that severe sublethal effects were observed at tested concentrations above 5.29 mg/L. This study is acceptable.

Report: [REDACTED] (1997); IN-KG433 technical: Flow-through, acute, 96-hour limit test to rainbow trout, *Oncorhynchus mykiss*

DuPont Report No.: HL-1997-00412

Guidelines: EEC Method C.1. (1992), OECD 203 (1992), USEPA 72-1 (1988) **Deviations:** None

Testing Facility: [REDACTED]

Testing Facility Report No.: HL-1997-00412

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 IN-KG433 technical metabolite, to unfed fingerling rainbow trout, *Oncorhynchus mykiss*, a coldwater fish, was determined in an unaerated, flow-through, 96-hour limit test. The test was conducted in accordance with (i) the Organisation for Economic Co-Operation and Development (OECD) Guideline for Testing Chemicals: 203; (ii) the European Economic Community 92/69 Annex V - Method C.1 (1992); and (iii) the United States Environmental Protection Agency, Pesticide Assessment Guidelines, Acute Toxicity Freshwater Fish, Subdivision E, 72-1.

Treatments consisted of a dilution water control, a solvent control (0.1 mL/L N,N-dimethylformamide), and one nominal concentration of 0.300 mg IN-KG433/L. The corresponding mean, measured concentration of IN-KG433 was 0.220 mg/L.

The 96-hour LC₅₀ for *Oncorhynchus mykiss*, based on mortality and mean measured concentrations, was >0.220 mg IN-KG433/L.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: IN-KG433 technical metabolite
 Lot/Batch #: IN-KG433-2 and IN-KG433-3^a
 Purity: 98.0% for both batches
 CAS#: 526224-31-7
 Description: Off-white solid
 Stability of test compound: In a separate study, IN-KG433 was shown to be stable at 0.2 mg/L in well water at 4°C for at least 72 hours. In this study, concentrations were maintained by flow-through.
 2. Control: Dilution (laboratory well water) water
 Solvent control: N,N-dimethylformamide ("DMF")
 Test vehicle: None
 Toxic reference: None
 3. Test organism: Rainbow trout
 Species: *Oncorhynchus mykiss*
 Age at dosing: Life stage: Fingerling
 Weight: Mean, 0.82 g (from pre-test loading weights)^b
 Initial population: Five fish per test chamber
 Source: [REDACTED]
 Acclimation period: The fish (purchased as eggs) were held in a holding tank at the laboratory for 135 days in continuously-flowing, approximately 12°C well water.
 Diet: Freshly-hatched brine shrimp and Purina Trout Chow
 Pre-test (approx. 72 hours): Unfed
 Test period: Unfed
 Test chamber: Stainless steel aquaria (29.3 × 29.3 × 29.3 cm) holding approx. 14 L of test solution (15 cm liquid depth)
 Test medium: [REDACTED] well water
 4. Environmental conditions (in-life period)
 Temperature: 11.8 to 12.3°C (of recirculating water-bath used to maintain test chamber temperature)
 Photoperiod: 16 hr photoperiod (442 to 484 lux) and 8 hr darkness
- ^a The Day -1 stock solutions were prepared with IN-KG433-3. Stock solutions used in the definitive study from Day 0 through Day 4 were prepared with IN-KG433-2. It was stated in the report that the use of two different batches of IN-KG433 did not affect the results or conclusions of the study.
- ^b Lengths and weights of surviving control fish were not measured at test end, which represented a protocol and test guideline deviation. The fish weights used to determine pre-test loading provide a representative mean weight (0.82 g) for individual fish used during the study. It was stated in the report that this deviation had no effect on the results or conclusions of the study.

B. STUDY DESIGN AND METHODS

1. Experimental start/completion
 28-March-1997 to 01-April-1997
2. Experimental treatments
 The acute toxicity of IN-KG433 to unfed fingerling rainbow trout, *Oncorhynchus mykiss*, a coldwater fish, was determined in an unaerated, flow-through, 96-hour limit test. Treatments consisted of a dilution water control, a solvent control (0.1 mL/L N,N-dimethylformamide), and one nominal concentration of 0.300 mg IN-KG433/L. The corresponding mean measured concentration of IN-KG433 was 0.220 mg/L. Two replicate test chambers with five fish in each (total of 10 fish) were used for the water and DMF controls. Three treatments (A-C) of the nominal 0.300 mg/L concentration of IN-KG433 (two replicates per treatment) were tested with five fish per replicate (total of 30 fish).

3. Observations

Mortality and sublethal (behavioural) observations were made at test start and every 24 hours thereafter.

4. Statistics

The LC₅₀ value was not statistically calculated as there was no adverse effect of 50% or greater on mortality. Therefore, the LC₅₀ value was estimated to be greater than the highest concentration tested.

II. RESULTS AND DISCUSSION

A. FINDINGS

Mean measured concentrations of IN-KG433 were 0.22, 0.25, and 0.19 mg/L (mean, 0.22 mg/L; recoveries ranged from 65 to 85% of the nominal concentration). All validation criteria were met for the study. Summaries of cumulative mortality and sublethal effects are presented in Table 27 and Table 28, respectively. The dilution water control group had 10% mortality. No sublethal effects were observed in the dilution water control fish. No mortality or sublethal effects were seen in the DMF solvent control fish.

Table 27
Observed mortality of rainbow trout, *Oncorhynchus mykiss*, exposed to IN-KG433 for 96 hours in an unaerated, flow-through, acute test

Mean, measured concentration of IN-KG433 (mg/L)	Cumulative mortality (No. dead/No. at test start)							
	24 h		48 h		72 h		96 h	
	A ^a	B ^a	A ^a	B ^a	A ^a	B ^a	A ^a	B ^a
Water Control	0/5	0/5	0/5	0/5	1/5	0/5	1/5	0/5
DMF Control	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
0.22 [A]	0/5	0/5	1/5	0/5	1/5	0/5	1/5	0/5
0.22 [B]	0/5	0/5	1/5	0/5	2/5	1/5	2/5	2/5
0.22 [C]	0/5	1 ^b /5	0/5	1 ^b /5	1/5	2/5	1/5	4 ^b /5

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

^b One fish was missing and presumed dead.

Table 28
Observed sublethal effects of rainbow trout, *Oncorhynchus mykiss*, exposed to IN-KG433 for 96 hours in an unaerated, flow-through, acute test

Mean measured concentration of IN-KG433 (mg/L)	Sublethal effects (No. affected/No. at test start)							
	24 h		48 h		72 h		96 h	
	A ^a	B ^a	A	B	A	B	A	B
Water Control	0/5	0/5	0/5	0/5	1 ^c /4	0/5	1 ^c /4	0/5
DMF Control	0/5	0/5	0/5	0/5	0/5	2/5	4 ^h /5	2 ^c /5
0.22	0/5	0/5	0/5	0/5	0/4	0/5	0/4	0/5
0.22	0/5	0/5	1 ^{c,d} /4	1 ^{c,d} /5	1 ^{c,d,f} /3	1 ^{d,f,g} /4	2 ^{f,g} /3	1 ^c /3
0.22	0/5	0/4 ^b	1 ^{c,d} /5	3 ^{e,f} /4 ^b	1 ^{d,f,g} /4	1 ^{d,f,g} 2 ^{d,e,f,g} /3 ^b	1 ^{c,f,g} /4	1 ^g /1 ^b

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

^b One fish was missing and presumed dead

^c At the surface

^d Partial loss of equilibrium

^e Lying on the bottom

^f Gasping for air

^g Erratic swimming

^h Lethargic

III. CONCLUSION

The 96-hour LC₅₀ for *Oncorhynchus mykiss*, based on mortality and mean measured concentrations, was >0.220 mg IN-KG433/L.

([REDACTED] 1997)

RMS comment

This study was conducted in compliance with the current guideline. The 96-hour LC₅₀ for *Oncorhynchus mykiss* based on mean measured concentrations of IN-KG433 was > 0.220 mg/L. This study is acceptable.

Report: [REDACTED] (2015a); IN-KN124: Acute toxicity to the rainbow trout, *Oncorhynchus mykiss*, determined under static-renewal test conditions

DuPont Report No.: DuPont-43113

Guidelines: OECD 203 (1992), OPPTS 850.1075 (1996) **Deviations:** None

Testing Facility: [REDACTED]

Testing Facility Report No.: 82059

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 IN-KN124 to unfed *Oncorhynchus mykiss* was determined in a static-renewal, 96-hour test. The test was conducted in accordance with the Organization for Economic Cooperation and Development (OECD), 1992 OECD Guidelines for Testing of Chemicals, Fish, Acute Toxicity Test, OECD Guideline No. 203 and the U.S. Environmental Protection Agency Ecological Effects Test Guidelines for fish acute toxicity testing, OPPTS 850.1075. Treatments consisted of a dilution water control, vehicle control, and nominal concentrations of 0.013, 0.025, 0.050, 0.10, and 0.20 mg IN-KN124/L. There was not a treatment causing 100% mortality at test end. The highest mean measured test concentration causing 0% mortality at test end was 0.0931 mg IN-KN124/L. The 96-hour LC₅₀ value was >0.0931 mg IN-KN124/L, the highest concentration tested.

I. MATERIALS AND METHODS**A. MATERIALS**

1. Test material:	IN-KN124 technical metabolite
Lot/Batch #:	KN124-001
Purity:	99.8%
Description:	solid
CAS#:	200568-73-6
Stability of test compound:	Determined to be unstable in the test system
2. Control:	Dilution water (freshwater)
Test vehicle:	Dimethylformamide (DMF)
Toxic reference:	None
3. Test organism:	Rainbow trout
Species:	<i>Oncorhynchus mykiss</i>
Age at dosing:	Juvenile
Weight at dosing:	0.7933 to 1.0066 g/fish
Initial population:	7 fish per treatment level
Source:	
Acclimation period:	>12 days
Diet:	Pre-test (approx. 48 hours): unfed
	Test period: unfed
Test chamber:	21-L glass jar containing 18 L of test solution (32-cm test solution depth), covered with a glass lid.
Test medium:	blended freshwater
4. Environmental conditions (in-life period)	
Temperature:	11.8 to 13.1°C (recirculating waterbath used to maintain test chamber temperature)
Photoperiod:	16 hr photoperiod (818 lux) and 8 hr darkness which included 30 min transitional light preceding and following the 16-hr light interval
pH:	6.4 to 7.1
Dissolved oxygen:	8.4 to 10.7 mg O ₂ /L (82 to 106% of saturation)

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
20-July-2015 to 24-July-2015
2. Experimental treatments

The acute toxicity of IN-KN124 to unfed *Oncorhynchus mykiss* was determined in a static-renewal, 96-hour test. Treatments consisted of a dilution water control, vehicle control, and nominal concentrations of 0.013, 0.025, 0.050, 0.10, and 0.20 mg IN-KN124/L. Seven fish were used per test concentration, vehicle control, and control. Treatments were not replicated.

3. Observations

Mortality and behavioral observations were made at 24, 48, 72, and 96 hours.

4. Statistics

Due to a lack of mortality in the blank control, vehicle control, and all test substance treatments, no statistical analyses were conducted.

II. RESULTS AND DISCUSSION

A. FINDINGS

The concentrations of IN-KN124 were measured in test solutions at 0, 24 (old) 72 (new), and 96 hours. Nominal concentrations were 0.013, 0.025, 0.050, 0.10, and 0.20 mg IN-KN124/L. The treatment mean measured concentrations of IN-KN124 during the 96-hour exposure were 0.00786, 0.0148, 0.0322, 0.0578, and 0.0931 mg IN-KN124/L, or 47 to 64% of the nominal concentrations. Recoveries from the IN-KN124 QC samples ranged from 92 to 103% of the nominal concentrations throughout the test.

All results from biological responses were based on mean measured IN-KN124 concentrations. Summaries of cumulative mortality and sublethal effects are presented in Table 29 and Table 30, respectively.

Table 29
Observed mortality of rainbow trout, *Oncorhynchus mykiss*, exposed to IN-KN124 for 96 hours in a static-renewal acute test

Mean Measured Concentration (mg IN-KN124/L)	Cumulative Mortality/Number at Test Start ^a			
	24 Hours	48 Hours	72 Hours	96 Hours
0 (Control)	0/7	0/7	0/7	0/7
0 (Vehicle Control)	0/7	0/7	0/7	0/7
0.00786	0/7	0/7	0/7	0/7
0.0148	0/7	0/7	0/7	0/7
0.0322	0/7	0/7	0/7	0/7
0.0578	0/7	0/7	0/7	0/7
0.0931	0/7	0/7	0/7	0/7

^a Test chambers contained seven rainbow trout each at test initiation.

Table 30
Observed sublethal effects of rainbow trout, *Oncorhynchus mykiss*, exposed to IN-KN124 for 96 hours in a static-renewal, acute test

Mean Measured Concentration (mg IN-KN124/L)	Number Affected/Number Alive ^a			
	24 Hours	48 Hours	72 Hours	96 Hours
0 (Control)	0/7	0/7	0/7	0/7
0 (Vehicle Control)	0/7	0/7	0/7	0/7
0.00786	0/7	0/7	0/7	0/7
0.0148	0/7	0/7	0/7	0/7
0.0322	0/7	0/7	0/7	0/7
0.0578	0/7	0/7	0/7	0/7
0.0931	0/7	0/7	0/7	0/7

^a Test chambers contained seven rainbow trout each at test initiation.

III. CONCLUSION

The 96-hour LC_{50} value was >0.0931 mg IN-KN124/L, the highest concentration tested. There was not a test treatment causing 100% mortality at test end. The highest mean measured test concentration causing 0% mortality at test end was 0.0931 mg IN-KN124/L.

([REDACTED] 2015a)

RMS comment

This study was conducted in compliance with the current guideline. The 96-hour LC_{50} value was >0.0931 mg IN-KN124/L based on mean measured concentrations. This study is acceptable.

Report: [REDACTED] (2015); IN-KN125: Acute toxicity to the rainbow trout, *Oncorhynchus mykiss*, determined under static-renewal test conditions

DuPont Report No.: DuPont-43104

Guidelines: OECD 203 (1992), OPPTS 850.1075 (1996) **Deviations:** None

Testing Facility: [REDACTED]

Testing Facility Report No.: 82062

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 IN-KN125 to unfed *Oncorhynchus mykiss* was determined in a static-renewal, 96-hour test. The test was conducted in accordance with the Organization for Economic Cooperation and Development (OECD), 1992 OECD Guidelines for Testing of Chemicals, Fish, Acute Toxicity Test, OECD Guideline No. 203 and the U.S. Environmental Protection Agency Ecological Effects Test Guidelines for fish acute toxicity testing, OPPTS 850.1075. Treatments consisted of a dilution water control, vehicle control, and nominal concentrations of 0.0013, 0.0025, 0.0050, 0.010, and 0.020 mg IN-KN125/L. There was not a treatment causing 100% mortality at test end. The highest mean measured test concentration causing 0% mortality at test end was 0.00587 mg IN-KN125/L. The 96-hour LC_{50} value was 0.0105 mg IN-KN125/L, with 95% confidence limits of 0.00746 and 0.0164 mg IN-KN125/L.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:	IN-KN125 technical metabolite
Lot/Batch #:	KN125-005
Purity:	99.4%
Description:	Solid
CAS#:	200568-74-7
Stability of test compound:	Determined to be unstable in the test system
2. Control:	Dilution water (pH-adjusted freshwater)
Test vehicle:	Dimethylformamide (DMF)
Toxic reference:	None
3. Test organism:	Rainbow trout
Species:	<i>Oncorhynchus mykiss</i>
Age at dosing:	Juvenile
Weight at dosing:	1.6173 to 2.0088 g/fish
Initial population:	7 fish per treatment level
Source:	
Acclimation period:	>12 days
Diet:	Pre-test (at least 48 hours): unfed Test period: unfed
Test chamber:	21-L glass jar containing 18 L of test solution (32-cm test solution depth), covered with a glass lid.
Test medium:	blended pH-adjusted freshwater
4. Environmental conditions (in-life period)	
Temperature:	11.7 to 12.5°C (recirculating waterbath used to maintain test chamber temperature)
Photoperiod:	16 hr photoperiod (1163 lux) and 8 hr darkness which included 30 min transitional light preceding and following the 16-hr light interval
pH:	6.4 to 7.0
Dissolved oxygen:	6.6 to 10.7 mg O ₂ /L (64 to 106% of saturation)

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
30-June-2015 to 04-July-2015
2. Experimental treatments
The acute toxicity of IN-KN125 to unfed *Oncorhynchus mykiss* was determined in a static-renewal, 96-hour test. Treatments consisted of a dilution water control, vehicle control, and nominal concentrations of 0.0013, 0.0025, 0.0050, 0.010, and 0.020 mg IN-KN125/L. Seven fish were used per test concentration, vehicle control, and control. Treatments were not replicated.
3. Observations
Mortality and behavioral observations were made at 24, 48, 72, and 96 hours.
4. Statistics
All statistical analyses were performed with SAS software, version 9.3.1 with Ecostats. Estimates of LC₅₀ values and their confidence limits were calculated using the probit method or the moving average angle estimate and 95% (Fieller) fiducial bounds.

II. RESULTS AND DISCUSSION

A. FINDINGS

The concentrations of IN-KN125 were measured in test solutions at 0, 24 (spent) 72 (new), and 96 hours. Nominal concentrations were 0.0013, 0.0025, 0.0050, 0.010, and 0.020 mg IN-KN125/L. The treatment mean measured concentrations of IN-KN125 during the 96-hour exposure were 0.000866, 0.00162, 0.00304, 0.00587, and 0.0122 mg IN-KN125/L, or 59 to 67% of the nominal concentrations. Recoveries from the IN-KN125 QC samples ranged from 85 to 104% of the nominal concentrations throughout the test.

All results from biological responses were based on mean measured IN-KN125 concentrations. Summaries of cumulative mortality and sublethal effects are presented in Table 31 and Table 32, respectively.

Table 31
Observed mortality of rainbow trout, *Oncorhynchus mykiss*, exposed to IN-KN125 for 96 hours in a static-renewal acute test

Mean Measured Concentration (mg IN-KN125/L)	Cumulative Mortality/Number at Test Start ^a			
	24 Hours	48 Hours	72 Hours	96 Hours
0 (Control)	0/7	0/7	0/7	0/7
0 (Vehicle Control)	0/7	0/7	0/7	0/7
0.000866	0/7	0/7	0/7	0/7
0.00162	0/7	0/7	0/7	0/7
0.00304	0/7	0/7	0/7	0/7
0.00587	0/7	0/7	0/7	0/7
0.0122	1/7	1/7	2/7	5/7

^a Test chambers contained seven rainbow trout each at test initiation.

Table 32
Observed sublethal effects of rainbow trout, *Oncorhynchus mykiss*, exposed to IN-KN125 for 96 hours in a static-renewal, acute test

Mean Measured Concentration (mg IN-KN125/L)	Number Affected/Number Alive ^a			
	24 Hours	48 Hours	72 Hours	96 Hours
0 (Control)	0/7	0/7	0/7	0/7
0 (Vehicle Control)	0/7	0/7	0/7	0/7
0.000866	0/7	0/7	0/7	0/7
0.00162	0/7	0/7	0/7	0/7
0.00304	0/7	1/7	1/7	1/7
0.00587	0/7	4/7	7/7	7/7
0.0122	6/6	6/6	5/5	2/2

^a Test chambers contained seven rainbow trout each at test initiation.

III. CONCLUSION

The 96-hour LC₅₀ value was 0.0105 mg IN-KN125/L, with 95% confidence limits of 0.00746 and 0.0164 mg IN-KN125/L. There was not a test treatment causing 100% mortality at test end. The highest mean measured test concentration causing 0% mortality at test end was 0.00587 mg IN-KN125/L.

([REDACTED] 2015)

RMS comment

This study was conducted in compliance with the current guideline. The 96-hour LC₅₀ value was 0.0105 mg IN-KN125/L based on mean measured concentrations. This study is acceptable.

Report: [REDACTED] (1999); IN-KT413, a metabolite of DPX-MP062: Acute, static, 96-hour toxicity test to rainbow trout, *Oncorhynchus mykiss*

DuPont Report No.: DuPont-1311

Guidelines: OECD 203, EEC 92/69 Annex V - Method C.1 (1992), USEPA 72-1 **Deviations:** None

Testing Facility: [REDACTED]

Testing Facility Report No.: 1662-DU

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.

I. MATERIALS AND METHODS

A. MATERIALS

- | | |
|--|--|
| 1. Test material: | IN-KT413 technical metabolite |
| Lot/Batch #: | KT413-3 |
| Purity: | 99.87% |
| Description: | Solid/ |
| CAS#: | Not available |
| 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: | 0.40 to 1.84 g (weight at termination) |
| Initial population: | 10 fish per test chamber |
| Source: | [REDACTED] |
| Acclimation period: | 34 days |
| Diet: | Pre-test (approx. 48 hr): unfed |
| | Test period: unfed |
| Test chamber: | Glass aquaria (40 l × 20 w × 25 h cm) holding approximately 15 L of test solution (20 cm liquid depth) |
| Test medium: | [REDACTED] deionised water |
| 4. Environmental conditions (in-life period) | |
| Temperature: | 11.3 to 12.6°C (of test solution) |
| Photoperiod: | 16 hr light (approximately 581 lux) and 8 hr dark including 15 min transitional light preceding and following the 16-hr light interval |

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed

04-November-1998 to 24-December-1998

2. Experimental treatments

The acute toxicity of IN-KT413, a water metabolite of DPX-MP062, to fingerling rainbow trout, *Oncorhynchus mykiss*, was determined in a 96-hour, static, unaerated limit test. Treatments consisted of a dilution water control and a mean measured concentration of 1.06 mg/L. The water concentration was selected to exceed 100 times the worst-case PEC_{sw}. Three replicates containing 10 fish were used in the treatment.

II. RESULTS AND DISCUSSION

A. FINDINGS

There were no mortality and no sublethal effects at the measured limit dose of 1.06 mg/L.

Table 33
Observed mortality and sublethal effects of rainbow trout, *Oncorhynchus mykiss*, exposed to IN-KT413 for 96 hours in a static, unaerated test

Mean measured concentration (mg IN-KT413/L)	Cumulative mortality (%)			Sublethal effects No. affected/No. alive		
	24 h	48 h	96 h	24 h	48 h	96 h
Water Control	0	0	0	0/10	0/10	0/10
1.06	0	0	0	0/10	0/10	0/10
	0	0	0	0/10	0/10	0/10
	0	0	0	0/10	0/10	0/10

III. CONCLUSION

The 96-hour LC₅₀ of IN-KT413 for the rainbow trout was >1.06 mg/L.

([REDACTED] 1999)

RMS comment

This study was assessed for previous Annex I inclusion and was conducted according to the current guideline. The study is valid according to validity criteria. It was stated in the addendum I that the actual concentrations were measured only at the beginning. RMS checked the study report and found that measures were also available at the end of the experiment showing that the concentration was maintained. The endpoint of >1.06 mg IN-KT413/L (based on mean measured concentrations) is acceptable.

Report: [REDACTED] (2013); IN-MK638: Acute toxicity to the rainbow trout, *Oncorhynchus mykiss*, determined under static test conditions

DuPont Report No.: DuPont-35827

Guidelines: OECD 203 (1992), OPPTS 850.1075 (1996) **Deviations:** None

Testing Facility: [REDACTED]

Testing Facility Report No.: 69140

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 IN-MK638 to unfed *Oncorhynchus mykiss* was determined in a static, 96-hour test. The test was conducted in accordance with the Organization for Economic Cooperation and Development (OECD), July 17, 1992 OECD Guidelines for Testing of Chemicals, Fish, Acute Toxicity Test, OECD Guideline No. 203, 9 pp. and U.S. Environmental Protection Agency, Ecological Effects Test Guidelines, OPPTS 850.1075, *Fish Acute Toxicity Test, Freshwater and Marine*. Treatments consisted of a dilution water control and six nominal concentrations of 1.9, 4.1, 9.1, 20, 45, and 100 mg IN-MK638/L. The corresponding mean, measured IN-MK638 concentrations were <LOD, 2.27, 4.15, 9.56, 20.8, 47.4, and 98.8 mg/L. The highest concentration causing no mortality was 9.56 mg IN-MK638/L. The lowest concentration causing 100% mortality at test end was 47.4 mg IN-MK638/L. The 96-hour LC₅₀ value was 28.0 mg/L based on mean measured IN-MK638 concentrations.

I. MATERIALS AND METHODS

A. MATERIALS

- | | |
|--|---|
| 1. Test material: | IN-MK638 technical metabolite |
| Lot/Batch #: | MK638-002 |
| Purity: | 99.9% |
| Description: | Solid, Crystalline |
| CAS#: | None |
| Stability of test compound: | Stable up to 96 Hours |
| 2. Control: | Dilution (laboratory well water) water |
| Solvent control: | None |
| Test vehicle: | Dilution (laboratory well water) water |
| Toxic reference: | None |
| 3. Test organism: | Rainbow trout |
| Species: | <i>Oncorhynchus mykiss</i> |
| Age at dosing: | Juvenile |
| Weight at dosing: | 0.6587 to 1.1611 g/fish |
| Initial population: | 7 fish per treatment level |
| Source: | |
| Acclimation period: | >12 days |
| Diet: | Pre-test (approx. 51 hr): unfed |
| | Test period: unfed |
| Test chamber: | 21-L glass jar containing 18 L of test solution (32-cm test solution depth), covered with a plastic Petri dish. |
| Test medium: | blended freshwater |
| 4. Environmental conditions (in-life period) | |
| Temperature: | 14.6 to 15.3°C (recirculating waterbath used to maintain test chamber temperature) |
| Photoperiod: | 16 hr photoperiod (989 lux) and 8 hr darkness which included 30 min transitional light preceding and following the 16-hr light interval |
| pH: | 7.76 to 8.54 |
| Dissolved oxygen: | 6.4 to 10.0 |

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed

18-February-2013 to 22-February-2013

2. Experimental treatments

The acute toxicity of IN-MK638 to unfed *Oncorhynchus mykiss* was determined in a static, 96-hour test. Treatments consisted of a dilution water control and six nominal concentrations of 1.9, 4.1, 9.1, 20, 45, and 100 mg IN-MK638/L. Seven fish were used per test concentration and control. Treatments were not replicated.

3. Observations

Mortality and behavioural observations were made at 6, 24, 48, 72, and 96 hours. Dead fish were removed from the test chambers when observed.

4. Statistics

Estimates of LC₅₀ values and their 95% confidence limits were calculated using the Untrimmed Spearman-Kärber method.

II. RESULTS AND DISCUSSION

A. FINDINGS

Total nominal concentrations were 1.9, 4.1, 9.1, 20, 45, and 100 mg IN-MK638/L. The corresponding mean measured concentrations of IN-MK638 were <LOD, 2.27, 4.15, 9.56, 20.8, 47.4, and 98.8 mg/L. No residues of IN-MK638 were detected in the controls above the LOD value of 0.00316 mg/L. Recoveries from the QC samples ranged from 103 to 112% of the nominal concentrations throughout the test.

The results demonstrate that IN-MK638 is stable in aquatic test media. All results from biological responses were based on mean measured concentrations of IN-MK638. Summaries of the cumulative mortality and sublethal effects are presented in Table 34 and Table 35, respectively.

Table 34
Observed mortality of rainbow trout, *Oncorhynchus mykiss*, exposed to IN-MK638 for 96 hours in a static, acute test

Nominal IN-MK638 Concentration (mg/L)	Mean, Measured IN-MK638 Concentration (mg/L)	Cumulative Mortality/Number at Test Start ^a				
		6 Hours	24 Hours	48 Hours	72 Hours	96 Hours
0 (Control)	<LOD, LOQ ^b	0/7	0/7	0/7	0/7	0/7
1.9	2.27	0/7	0/7	0/7	0/7	0/7
4.1	4.15	0/7	0/7	0/7	0/7	0/7
9.1	9.56	0/7	0/7	0/7	0/7	0/7
20	20.8	0/7	1/7	1/7	1/7	1/7
45	47.4	1/7	7/7	7/7	7/7	7/7
100	98.8	7/7	7/7	7/7	7/7	7/7

^a Test chambers contained seven rainbow trout each at test initiation.

^b The limit of detection (LOD) was 0.00316 mg/L. The limit of quantitation (LOQ) was 0.0105 mg/L.

Table 35
Observed sublethal effects of rainbow trout, *Oncorhynchus mykiss*, exposed to IN-MK638 for 96 hours in an aerated, static, acute test

Nominal IN-MK638 Concentration (mg/L)	Mean, Measured IN-MK638 Concentration (mg/L)	Number Affected/Number Alive a				
		6 Hours	24 Hours	48 Hours	72 Hours	96 Hours
0 (Control)	<LOD, LOQ ⁱ	0/7	0/7	0/7	0/7	0/7
1.9	2.27	0/7	0/7	0/7	0/7	0/7
4.1	4.15	0/7	0/7	0/7	0/7	0/7
9.1	9.56	1/7 ^b	6/7 ^c	1/7 ^b	1/7 ^b	1/7 ^b
20	20.8	4/7 ^d	6/6 ^e	6/6 ^f	6/6 ^g	6/6 ^g
45	47.4	6/6 ^h	-	-	-	-
100	98.8	-	-	-	-	-

^a Test chambers contained seven rainbow trout each at test initiation.

^b One fish was discolored.

^c Six fish were discolored.

^d Four fish were discolored.

^e One fish was on the bottom and discolored. Five fish were stunted, discolored, and lost equilibrium.

^f Two fish were on the bottom, discolored, and exhibited irregular respiration. Four fish were stunted, discolored, and exhibited irregular respiration.

^g Six fish were on the bottom, had lost equilibrium, were discolored, and exhibited irregular respiration.

^h Six fish were on the bottom, discolored, and lost equilibrium.

ⁱ The limit of detection (LOD) was 0.00316 mg/L. The limit of quantitation (LOQ) was 0.0105 mg/L.

Note: Dash (-) denotes no sublethal effects due to total mortality.

III. CONCLUSION

The highest concentration causing no mortality was 9.56 mg IN-MK638/L. The lowest concentration causing 100% mortality at test end was 47.4 mg IN-MK638/L. The 96-hour LC₅₀ value, based on mean measured IN-MK638 concentrations, was 28.0 mg/L.

[REDACTED] 2013)

RMS comment

This study was conducted in compliance with the current guideline. The 96-hour LC₅₀ for *Oncorhynchus mykiss* based on mean measured concentrations of IN-MK638 was 28.0 mg/L. RMS notes that one fish died at 20 mg/L and that all remaining fish showed strong effects (fish on the bottom, loss of equilibrium, discolored, irregular respiration). This study is acceptable.

Report: [REDACTED] (2013); IN-MK643: Acute toxicity to the rainbow trout, *Oncorhynchus mykiss*, determined under static test conditions

DuPont Report No.: DuPont-36162

Guidelines: OECD 203 (1992), OPPTS 850.1075 (1996) **Deviations:** None

Testing Facility: [REDACTED]

Test Facility Report No.: 69279

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 IN-MK643 to unfed *Oncorhynchus mykiss* was determined in a static, 96-hour test. The test was conducted in accordance with the Organization for Economic Cooperation and Development (OECD), July 17, 1992 OECD Guidelines for Testing of Chemicals, *Fish, Acute Toxicity Test*, OECD Guideline No. 203, 9 pp. and U.S. Environmental Protection Agency, Ecological Effects Test Guidelines, OPPTS 850.1075, *Fish Acute Toxicity Test, Freshwater and Marine*. Treatments consisted of a dilution water control and five nominal total formulation concentrations of 0.63, 1.3, 2.5, 5.0, and 10 mg IN-MK643/L. The 96-hour LC₅₀ value was 6.99 mg IN-MK643/L based on mean measured concentrations. The lowest mean measured concentration causing 100% mortality at test end was 10.7 mg IN-MK643/L. The highest mean measured concentration causing 0% mortality at test end was 4.57 mg IN-MK643/L.

I. MATERIALS AND METHODS

A. MATERIALS

- | | |
|--|---|
| 1. Test material: | IN-MK643 technical metabolite |
| Lot/Batch #: | MK643-002 |
| Purity: | 96.7% |
| Description: | Solid powder |
| CAS#: | 877681-12-4 |
| Stability of test compound: | Determined to be stable in the test system |
| 2. Control: | Dilution (laboratory well water) water |
| Solvent control: | None |
| Test vehicle: | Dilution (laboratory well water) water |
| Toxic reference: | None |
| 3. Test organism: | Rainbow trout |
| Species: | <i>Oncorhynchus mykiss</i> |
| Age at dosing: | Juvenile |
| Weight at dosing: | 0.5990 to 0.9315 g |
| Initial population: | 7 fish per treatment level |
| Source: | |
| Acclimation period: | >7 days |
| Diet: | Pre-test (approx. 48 hours): unfed |
| | Test period: unfed |
| Test chamber: | 21-L glass jar containing 18 L of test solution (32-cm test solution depth), covered with a glass lid. |
| Test medium: | blended freshwater |
| 4. Environmental conditions (in-life period) | |
| Temperature: | 14.0 to 15.1°C (recirculating waterbath used to maintain test chamber temperature) |
| Photoperiod: | 16 hr photoperiod (941 lux) and 8 hr darkness which included 30 min transitional light preceding and following the 16-hr light interval |
| pH: | 7.8 to 8.4 |
| Dissolved oxygen: | 6.6 to 10.6 mg O ₂ /L |

B. STUDY DESIGN AND METHODS

- In-life initiated/completed
30-January-2013 to 03-February-2013
- Experimental treatments
The acute toxicity of IN-MK643 to unfed *Oncorhynchus mykiss* was determined in a static, 96-hour test. Treatments consisted of a dilution water control and five nominal concentrations of 0.63, 1.3,

2.5, 5.0, and 10 mg IN-MK643/L. Seven fish were used per test concentration and control. Treatments were not replicated.

3. Observations

Mortality and behavioural observations were made at 6, 24, 48, 72, and 96 hours. Dead fish were removed from the test chambers when observed.

4. Statistics

Estimates of LC_{50} values and their 95% confidence limits were calculated using the probit or Trimmed Spearman-Kärber method.

II. RESULTS AND DISCUSSION

A. FINDINGS

The concentration of IN-MK643 was measured in test solutions at 0 and 96 hours. The measured concentrations of IN-MK643 in test substance treatment samples collected at initiation were 0.630, 1.29, 2.47, 4.89, and 10.5 mg/L or 98 to 105% of the nominal concentrations. The measured concentrations of IN-MK643 in test solutions at 96-hours were 0.630, 1.30, 2.58, 4.24, and 10.9 mg/L or 85 to 109% of the nominal concentrations. Mean measured concentrations of IN-MK643 during the exposure were 0.630, 1.30, 2.53, 4.57, and 10.7 mg/L or 91 to 107% of the nominal concentrations. No residues of IN-MK643 were detected in the dilution water control or solvent control solutions above the LOD of 0.00231 mg/L. Recoveries from the QC fortifications ranged from 104 to 116% of the nominal concentrations.

All results from biological responses were based on mean measured concentrations of IN-MK643. Summaries of cumulative mortality and sublethal effects are presented in Table 36 and Table 37, respectively.

Table 36
Observed mortality of rainbow trout, *Oncorhynchus mykiss*, exposed to IN-MK643 for 96 hours in a static acute test

Nominal IN-MK643 Concentration (mg/L)	Mean, Measured IN-MK643 Concentration (mg/L)	Cumulative mortality/Number at test start ^a				
		6 Hours	24 Hours	48 Hours	72 Hours	96 Hours
0 (Control)	<LOD	0/7	0/7	0/7	0/7	0/7
0.63	0.630	0/7	0/7	0/7	0/7	0/7
1.3	1.30	0/7	0/7	0/7	0/7	0/7
2.5	2.53	0/7	0/7	0/7	0/7	0/7
5.0	4.57	0/7	0/7	0/7	0/7	0/7
10	10.7	0/7	7/7	7/7	7/7	7/7

^a Test chambers contained seven rainbow trout each at test initiation.

Table 37
Observed sublethal effects of rainbow trout, *Oncorhynchus mykiss*, exposed to IN-MK643 for 96 hours in a static, acute test

Nominal IN-MK643 Concentration (mg/L)	Mean, Measured IN-MK643 Concentration (mg/L)	Number affected/Number alive ^a				
		6 Hours	24 Hours	48 Hours	72 Hours	96 Hours
0 (Control)	<LOD	0/7 ^e	0/7 ^e	0/7 ^e	0/7 ^e	0/7 ^e
0.63	0.630	0/7 ^e	0/7 ^e	0/7 ^e	0/7 ^e	0/7 ^e
1.3	1.30	0/7 ^e	0/7 ^e	0/7 ^e	0/7 ^e	0/7 ^e
2.5	2.53	0/7 ^e	3/7 ^c	0/7 ^e	0/7 ^e	4/7 ^f
5.0	4.57	0/7 ^e	7/7 ^d	7/7 ^d	7/7 ^e	7/7 ^g
10	10.7	7/7 ^b	--- ^e	--- ^e	--- ^e	--- ^e

^a Test chambers contained seven rainbow trout each at test initiation.

^b Seven fish were on the bottom, exhibiting loss of equilibrium and irregular respiration.

^c Three fish exhibited exophthalmia.

^d Four fish were on the bottom of test chamber exhibiting loss of equilibrium and exophthalmia. Two fish exhibited loss of equilibrium and exophthalmia, and one fish exhibited exophthalmia.

^e Six fish were on the bottom and exhibited loss of equilibrium and exophthalmia, and one fish exhibited exophthalmia.

^f Four fish exhibited exophthalmia.

^g Seven fish were on the bottom exhibiting loss of equilibrium and exophthalmia.

Note: Dashes (---) denotes no sublethal effects due to total mortality

III. CONCLUSION

IN-MK643 was assessed for acute toxicity to the rainbow trout, *Oncorhynchus mykiss*, in a 96-hour static test. The 24-, 48-, 72-, and 96-hour LC₅₀ value, based on mortality, were 6.99 mg IN-MK643/L with 95% confidence limits of 4.57 and 10.7 mg IN-MK643/L. The lowest mean measured concentration causing 100% mortality at test end was 10.7 mg IN-MK643/L. The highest mean measured concentration causing 0% mortality at test end was 4.57 mg IN-MK643/L.

([REDACTED] 2013)

RMS comment

This study was conducted in compliance with the current guideline. The study author calculated an LC₅₀ of 6.99 mg a.s./L by using the Untrimmed Spearman-Kärber method (because there was 0% mortality at 5 mg/L and 100% mortality at the next concentration 10 mg/L). According to OECD 203, the geometric mean of these two concentrations should be used. RMS calculated a geometric mean of 6.99 mg/L. The endpoint is therefore confirmed. RMS notes that no fish died at 5 mg/L however all fish showed strong effects (Seven fish were on the bottom exhibiting loss of equilibrium and exophthalmia). This study is acceptable.

Report: [REDACTED] (2003a); IN-MP819: Flow-through, acute, 96-hour LC₅₀ to rainbow trout, *Oncorhynchus mykiss*

DuPont Report No.: DuPont-11492

Guidelines: OECD 203, EEC 92/69 Annex V - Method C.1 (1992), U.S. EPA 72-1 **Deviations:** None

Testing Facility: [REDACTED]

Testing Facility Report No.: DuPont-11492

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.

I. MATERIALS AND METHODS

A. MATERIALS

- | | |
|--|--|
| 1. Test material: | IN-MP819 technical metabolite |
| Lot/Batch #: | MP819-002 |
| Purity: | 98.6% |
| Description: | Light yellow solid |
| CAS#: | Not available |
| Stability of test compound: | Shown to be stable under conditions of test |
| 2. Control: | Dilution (laboratory well water) water |
| Solvent control: | N,N-dimethylformamide |
| 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: | Water control: 0.38 to 0.72 g (weight at termination) |
| | DMF control: 0.35 to 0.69 g (weight at termination) |
| Initial population: | 5 fish per test chamber |
| Source: | |
| Acclimation period: | 37-41 days |
| Diet: | Pre-test (approx. 26 hr): unfed |
| | Test period: unfed |
| Test chamber: | Stainless steel aquaria (30 l × 14.5 w × 30 h cm) holding approximately 7.5 L of test solution (16.5 cm liquid depth) |
| Test medium: | well water |
| 4. Environmental conditions (in-life period) | |
| Temperature: | 11.3 to 11.5°C (of test solution) |
| Photoperiod: | 16 hr light (approximately 368 to 471 lux) and 8 hr dark including 30 min transitional light (104 to 148 lux) preceding and following the 16-hr light interval |

B. STUDY DESIGN AND METHODS

1. Study initiated/completed
14-March-2003 to 06-June-2003
2. Experimental treatments
The acute toxicity of IN-MP819 to unfed fingerling rainbow trout, *Oncorhynchus mykiss*, was determined in an unaerated, flow-through, 96-hour test. Treatments consisted of a dilution water control, a 0.1 mL/L solvent control (N,N-dimethylformamide), and nominal concentrations of 0.022, 0.044, 0.088, 0.175, and 0.350 mg IN-MP819/L. Two replicate(s) containing 10 fish were exposed to each treatment concentration and control. Test solutions were maintained between 11.3 and 11.5°C.

II. RESULTS AND DISCUSSION

A. FINDINGS

Summaries of cumulative mortality and sublethal effects are presented in Table 38 and Table 39, respectively. Mean measured concentrations of IN-MP819 were 0.024, 0.044, 0.086, 0.193, and 0.368 mg/L and ranged from 99 to 112% of nominal concentrations. The dilution water control group had 10% mortality. No sublethal effects were observed in the dilution water control fish. No mortality or sublethal effects were seen in the DMF solvent control fish. The highest mean measured concentration causing no mortality at test end was 0.086 mg/L. The lowest mean measured concentration causing 100% mortality at test end was greater than 0.368 mg/L, the highest concentration tested. Mean measured concentrations of IN-MP819 were used for calculation of LC_{50} values. The 96-hour LC_{50} , based on the mean measured concentrations of IN-MP819 and mortality, was greater than 0.368 mg/L.

Table 38
Observed mortality of rainbow trout, *Oncorhynchus mykiss*, exposed to IN-MP819 for 96 hours in an unaerated, flow-through, acute test

Mean measured concentration of IN-MP819 (mg/L)	Cumulative mortality (No. dead/No. at test start)							
	24 h		48 h		72 h		96 h	
	A ^a	B ^a	A ^a	B ^a	A ^a	B ^a	A ^a	B ^a
Water Control	0/5	0/5	0/5	0/5	0/5	0/5	0/5	1/5
DMF Control	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
0.024	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
0.044	0/5	0/5	0/5	0/5	0/5	0/5	0/5	1/5
0.086	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
0.193	0/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5
0.368	0/5	0/5	0/5	0/5	0/5	1/5	1/5	3/5

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

Table 39
Observed sublethal effects of rainbow trout, *Oncorhynchus mykiss*, exposed to IN-MP819 for 96 hours in an unaerated, flow-through, acute test

Mean measured concentration of IN-MP819 (mg/L)	Sublethal effects (No. affected/No. at test start)							
	24 h		48 h		72 h		96 h	
	A ^g	B ^g	A	B	A	B	A	B
Water Control	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/4
DMF Control	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
0.024	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
0.044	0/5	0/5	0/5	0/5	0/5	0/4	1 ^a /5	2 ^a /4
0.086	0/5	0/5	0/5	0/5	1 ^e /5	2 ^e /5	1 ^e /5	2 ^e /5
0.193	0/5	0/5	0/5	0/5	1 ^{de} ,4 ^d /5	2 ^{de} ,3 ^d /5	4 ^{be} /4	5 ^{be} /5
0.368	0/5	0/5	2 ^{ef} /5	3 ^{ef} /5	1 ^{bc} ,4 ^{def} /5	3 ^{bc} ,1 ^{bde} /4	4 ^{cb} /4	2 ^{cb} /2

^a Erratic swimming;

^b Laboured respiration

^c Lying on the bottom;

^d Lethargic;

^e Partial loss of equilibrium

^f Rapid respiration

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

III. CONCLUSION

The 96-hour LC₅₀, based on mean measured concentrations of IN-MP819 dissolved in 0.1mL/L DMF and mortality, was greater than 0.368 mg/L.

() 2003a)

RMS comment

This study was assessed for previous Annex I inclusion and was conducted according to the current guideline. The study is valid according to validity criteria. The LC50 of >0.368 mg IN-MP819/L (based on mean measured concentrations) is acceptable. RMS notes all remaining fish showed strong effects at concentrations of 0.193 mg /L and above (laboured respiration, partial loss of equilibrium, fish lying on the bottom).

Report: () (2003b); IN-MS775: Static, acute, 96-hour limit test to rainbow trout, *Oncorhynchus mykiss*

DuPont Report No.: DuPont-12091

Guidelines: OECD 203, EEC 92/69 Annex V - Method C.1 (1992), USEPA 72-1 **Deviations:** None

Testing Facility: ()

Testing Facility Report No.: DuPont-12091

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.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: IN-MS775 technical metabolite
 Lot/Batch #: MS775-002
 Purity: 99.7%
 Description: Light yellow solid
 CAS#: Not available
 Stability of test compound: Shown to be stable in the test system by analysis
2. Control: Dilution (laboratory well water) water
 Solvent control: N,N-dimethylformamide
 Test vehicle: Dilution (laboratory well water) water
 Toxic reference: None
3. Test organism: Rainbow trout
 Species: *Oncorhynchus mykiss*
 Age at dosing: Life stage: fingerling
 Weight: Water control: 0.12 to 0.19 g (weight at termination)
 DMF control: 0.10 to 0.18 g (weight at termination)
 Initial population: 10 fish per test chamber
 Source: [REDACTED]
 Acclimation period: 22-26 days
 Diet: Pre-test (approx. 27 hr): unfed
 Test period: unfed
 Test chamber: Stainless steel aquaria (30 l × 30 w × 30 h cm) holding approximately 15 L of test solution (17.5 cm liquid depth)
 Test medium: [REDACTED] well water
4. Environmental conditions (in-life period)
 Temperature: 11.0 to 11.2°C (of test solution)
 Photoperiod: 16 hr light (approximately 221 to 549 lux) and 8 hr dark including 30 min transitional light (35 to 227 lux) preceding and following the 16-hr light interval

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
 06-October-2003 to 10-October-2003
2. Experimental treatments
 The acute toxicity of IN-MS775 to unfed fingerling rainbow trout, *Oncorhynchus mykiss*, was determined in an unaerated, static, 96-hour limit test. Treatments consisted of a dilution water control, a 0.1 mL/L solvent control (N,N-dimethylformamide), and a nominal concentration of 0.0065 mg IN-MS775/L, the water solubility limit of IN-MS775 in DMF solvent. Three replicate(s) containing 10 fish in each chamber (total of 10 fish in each control and 30 fish in the limit test concentration) were exposed to each treatment concentration and control. Test solutions were maintained between 11.0 and 11.2°C

II. RESULTS AND DISCUSSION

A. FINDINGS

Summaries of cumulative mortality and sublethal effects are presented in Table 40 and Table 41, respectively. Mean measured concentration of IN-MS775 was 0.00396 mg/L. No mortality or sublethal effects were seen at the 0.00396 µg/L mean measured concentration of IN-MS775 or in the dilution control at the end of the 96-hour limit test. Mortality in the DMF control was 10% (one out of ten) at the end of 96 hours. No sublethal effects were observed in surviving fish in the DMF control at the end of 96 hours.

The 96-hour LC₅₀, based on the mean measured concentration of IN-MS775 and mortality, was greater than 0.00396 mg/L.

Table 40
Observed mortality of rainbow trout, *Oncorhynchus mykiss*, exposed to IN-MS775 for 96 hours in an unaerated, static, acute test

Mean measured concentration of IN-MS775 (mg/L)	Cumulative mortality (No. dead/No. at test start)			
	24 h	48 h	72 h	96 h
Water Control	0/10	0/10	0/10	0/10
DMF Control	0/10	1/10	1/10	1/10
0.00396 ^a	0/10	0/10	0/10	0/10
0.00396 ^a	0/10	0/10	0/10	0/10
0.00396 ^a	0/10	0/10	0/10	0/10

^a The 3 replicate test chambers contained 10 fish each at test start.

Table 41
Observed sublethal effects of rainbow trout, *Oncorhynchus mykiss*, exposed to IN-MS775 for 96 hours in an unaerated, static, acute test

Mean, measured concentration of IN-MS775 (mg a.s./L)	Sublethal effects (No. affected/No. at test start)			
	24 h	48 h	72 h	96 h
Water Control	0/10	0/10	0/10	0/10
DMF Control	0/10	0/9	0/9	0/9
0.00396 ^a	0/10	0/10	0/10	0/10
0.00396 ^a	0/10	0/10	0/10	0/10
0.00396 ^a	0/10	0/10	0/10	0/10

^a The 3 replicate test chambers contained 10 fish each at test start.

III. CONCLUSION

The 96-hour LC₅₀, based on mean, measured concentrations of IN-MS775 and mortality, was greater than 0.00396 mg/L.

(b) (2003b)

RMS comment

This study was assessed for previous Annex I inclusion and was conducted according to the current guideline. The study is valid according to validity criteria. The LC₅₀ of >0.00396 mg IN-MS775/L (based on mean measured concentrations) is acceptable.

Report: (b) (2015); IN-U8E24: Acute toxicity to the rainbow trout, *Oncorhynchus mykiss*, determined under static test conditions

DuPont Report No.: DuPont-43485

Guidelines: OECD 203 (1992), OPPTS 850.1075 (1996), EPA 712-C-96-118 (1996) **Deviations:** None

Testing Facility: [REDACTED]

Test Facility Report No.: 82065

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 IN-U8E24 to unfed *Oncorhynchus mykiss* was determined in a static, 96-hour test. The test was conducted in accordance with the Organization for Economic Cooperation and Development (OECD), July 17, 1992 OECD Guidelines for Testing of Chemicals, Fish, Acute Toxicity Test, OECD Guideline No. 203, 9 pp. and U.S. Environmental Protection Agency, Ecological Effects Test Guidelines, OPPTS 850.1075, Fish Acute Toxicity Test, Freshwater and Marine. Treatments consisted of a dilution water (blank) control and five nominal concentrations of 3.8, 7.5, 15, 30, and 60 mg IN-U8E24/L. The 96-hour LC₅₀ value, based on mortality and mean measured test concentrations, was 46.5 mg IN-U8E24/L, with best estimates of 95% confidence limits of 32.7 and 66.1 mg IN-U8E24/L. The lowest mean measured concentration causing 100% mortality at test end was 66.1 mg IN-U8E24/L. The highest mean measured concentration causing 0% mortality at test end was 32.7 mg IN-U8E24/L.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: IN-U8E24 technical metabolite
 Lot/Batch #: U8E24-000
 Purity: 90.7%
 Description: Solid
 CAS#: Not Provided
 Stability of test compound: Stable
2. Control: Dilution (blended) water
 Test vehicle: None
 Toxic reference: None
3. Test organism: Rainbow trout
 Species: *Oncorhynchus mykiss*
 Age at dosing: Juvenile
 Weight at dosing: 0.6475 to 1.1659 g
 Initial population: 7 fish per treatment level
 Source: [REDACTED]
 Acclimation period: >7 days
 Diet: Pre-test (approx. 48 hours): unfed
 Test period: unfed
 Test chamber: 21-L glass jar containing 18 L of test solution (32-cm test solution depth), covered with a clear glass lid.
 Test medium: [REDACTED] blended freshwater
4. Environmental conditions (in-life period)
 Temperature: 12.0 to 12.8°C (recirculating waterbath used to maintain test chamber temperature)
 Photoperiod: 16 hr photoperiod (561 lux) and 8 hr darkness which included 30 min transitional light preceding and following the 16-hr light interval
 pH: 7.7 to 8.4
 Dissolved oxygen: 6.7 to 10.7 mg O₂/L

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed

20 July 2015 to 24 July 2014

2. Experimental treatments

The acute toxicity of IN-U8E24 to unfed *Oncorhynchus mykiss* was determined in a static, 96-hour test. Treatments consisted of a dilution water (blank) control and five nominal concentrations of 3.8, 7.5, 15, 30, and 60 mg IN-U8E24/L. Seven fish were used per test concentration and blank control. Treatments were not replicated.

3. Observations

Mortality and behavioural observations were made at 24, 48, 72, and 96 hours. Dead fish were removed from the test chambers when observed.

4. Statistics

All statistical analyses were performed with SAS software version 9.3. Estimates of EC₅₀ values and their 95% confidence limits were calculated using the probit method or untrimmed Spearman-Kärber method. When there was no evidence of questionable convergence, the probit method was selected for reporting. When this criterion was not achieved, untrimmed Spearman-Kärber method was selected for reporting.

II. RESULTS AND DISCUSSION

A. FINDINGS

The concentration of the IN-U8E24 was measured in fresh test solution samples collected at 0 hour (initiation) and in test solution samples at 48 and 96 hours (termination) of the definitive test. The measured concentrations of IN-U8E24 in test substance treatment samples collected at initiation were 4.04, 7.68, 16.0, 32.7, and 65.2 mg IN-U8E24/L or 102 to 109% of the nominal concentrations. The measured concentrations of IN-U8E24 in test solutions at 48-hours were 3.99, 7.73, 15.7, 32.4, and 65.3 mg IN-U8E24/L or 103 to 109% of the nominal concentrations. The measured concentrations of IN-U8E24 in test solutions at 96-hours were 4.10, 7.87, 16.2, 33.0, and 67.8 mg IN-U8E24/L or 105 to 113% of the nominal concentrations. Mean measured concentrations of IN-U8E24 during the 96-hour exposure were 4.04, 7.76, 16.0, 32.7, and 66.1 mg IN-U8E24/L or 103 to 110% of the nominal concentrations.

All results from biological responses were based on mean measured concentrations of IN-U8E24. Summaries of cumulative mortality and sublethal effects are presented in Table 42 and Table 43, respectively.

Table 42
Observed mortality of rainbow trout, *Oncorhynchus mykiss*, exposed to IN-U8E24 for 96 hours in a static acute test

Nominal IN-U8E24 Concentration (mg IN-U8E24/L)	Mean Measured IN-U8E24 Concentration (mg IN-U8E24/L)	Cumulative Mortality/Number at Test Start ^a			
		24 Hours	48 Hours	72 Hours	96 Hours
0 (Control)	<LOD	0/7	0/7	0/7	0/7
3.8	4.04	0/7	0/7	0/7	0/7
7.5	7.76	0/7	0/7	0/7	0/7
15	16.0	0/7	0/7	0/7	0/7
30	32.7	0/7	0/7	0/7	0/7
60	66.1	0/7	0/7	4/7	7/7

^a Test chambers contained seven rainbow trout each at test initiation.

Table 43
Observed sublethal effects of rainbow trout, *Oncorhynchus mykiss*, exposed to IN-U8E24 for 96 hours in a static, acute test

Nominal IN-U8E24 Concentration (mg IN-U8E24/L)	Mean Measured IN-U8E24 Concentration (mg IN-U8E24/L)	Number Affected/Number Alive ^a			
		24 Hours	48 Hours	72 Hours	96 Hours
0 (Control)	<LOD	7 N/7	7 N/7	7 N/7	7 N/7
3.8	4.04	7 N/7	7 N/7	7 N/7	7 N/7
7.5	7.76	7 N/7	7 N/7	7 N/7	7 N/7
15	16.0	7 N/7	7 N/7	7 N/7	7 N/7
30	32.7	7 N/7	7 N/7	7 N/7	1 B, DC, LE; 6 N/7
60	66.1	2 B, LE; 5N/7	2 B, DC, LE; 5N/7	2 B, DC, LE, I; 1 DC, LE/3	0/0*

^a Test chambers contained seven rainbow trout each at test initiation.

* Statistical significance as compared to the control (Fisher's One-tailed Exact Test)

Key: N = Normal, B = On Bottom, DC = Discoloration, I = Irregular Respiration, LE = Loss of Equilibrium

III. CONCLUSION

IN-U8E24 was assessed for acute toxicity to the rainbow trout, *Oncorhynchus mykiss*, in a 96-hour static test. The 24- and 48-hour LC₅₀ value, based on mortality and mean measured test concentrations, was >66.1 mg IN-U8E24/L, the highest test substance treatment. The 72-hour LC₅₀ value, based on mortality and mean measured test concentrations, was 60.5 mg IN-U8E24/L, with best estimates of 95% confidence limits of 40.4 and 90.6 mg IN-U8E24/L. The 96-hour LC₅₀ value, based on mortality and mean measured test concentrations, was 46.5 mg IN-U8E24/L, with best estimates of 95% confidence limits of 32.7 and 66.1 mg IN-U8E24/L. The lowest mean measured concentration causing 100% mortality at test end was 66.1 mg IN-U8E24/L. The highest mean measured concentration causing 0% mortality at test end was 32.7 mg IN-U8E24/L.

([REDACTED] 2015)

RMS comment

This study was conducted in compliance with the current guideline. The 96-hour LC₅₀ value was 46.5 mg IN-U8E24/L based on mean measured concentrations. This study is acceptable.

Report: [REDACTED] (2015b); IN-UYG24: Acute toxicity to the rainbow trout, *Oncorhynchus mykiss*, determined under static test conditions

DuPont Report No.: DuPont- 43422

Guidelines: OECD 203 (1992), OPPTS 850.1075 (1996) **Deviations:** None

Testing Facility: _____

Test Facility Report No.: 82068

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 IN-UYG24 to unfed *Oncorhynchus mykiss* was determined in a static, 96-hour test. The test was conducted in accordance with the Organization for Economic Cooperation and Development (OECD), July 17, 1992 OECD Guidelines for Testing of Chemicals, Fish, Acute Toxicity Test, OECD Guideline No. 203 and U.S. Environmental Protection Agency, Ecological Effects Test Guidelines, OPPTS 850.1075, Fish Acute Toxicity Test, Freshwater and Marine. Treatments consisted of a dilution water (blank) control and five nominal concentrations of 7.5, 15, 30, 60, and 120 mg IN-UYG24/L. The 96-hour LC₅₀ value was >115 mg IN-UYG24/L based on mean measured concentrations. No mean measured IN-UYG24 concentration caused 100% mortality at test end. The highest mean measured IN-UYG24 concentration causing 0% mortality at test end was 115 mg IN-UYG24/L.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:	IN-UYG24 technical metabolite
Lot/Batch #:	UYG24-001
Purity:	96.1%
Description:	Solid
CAS#:	Not Provided
Stability of test compound:	Stable
2. Control:	Dilution (blended) water
Vehicle control:	None
Test vehicle:	Dilution (blended) water
Toxic reference:	None
3. Test organism:	Rainbow trout
Species:	<i>Oncorhynchus mykiss</i>
Age at dosing:	Juvenile
Weight at dosing:	0.6475 to 1.1659 g
Initial population:	7 fish per treatment level
Source:	
Acclimation period:	>12 days
Diet:	Pre-test (approx. 48 hours): unfed
	Test period: unfed
Test chamber:	21-L glass jar containing 18 L of test solution (32-cm test solution depth), covered with a clear glass lid.
Test medium:	blended freshwater
4. Environmental conditions (in-life period)	
Temperature:	11.9 to 12.6°C (recirculating waterbath used to maintain test chamber temperature)
Photoperiod:	16 hr photoperiod (533 lux) and 8 hr darkness which included 30 min transitional light preceding and following the 16-hr light interval
pH:	7.6 to 8.5
Dissolved oxygen:	6.4 to 10.2 mg O ₂ /L (62 to 101% saturation)

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
20 July 2015 to 24 July 2015
2. Experimental treatments
The acute toxicity of IN-UYG24 to unfed *Oncorhynchus mykiss* was determined in a static, 96-hour test. Treatments consisted of a dilution water (blank) control and five nominal concentrations of 7.5,

15, 30, 60, and 120 mg IN-UYG24/L. Seven fish were used per test concentration and blank control. Treatments were not replicated.

3. Observations

Mortality and behavioral observations were made at 24, 48, 72, and 96 hours.

4. Statistics

Due to a lack of mortality in the blank control and all test substance treatments, no statistical analyses were conducted.

II. RESULTS AND DISCUSSION

A. FINDINGS

The concentration of the IN-UYG24 was measured in fresh test solution samples collected at 0 hour (initiation), 48 hours, and 96 hours (termination) of the definitive test. The measured concentrations of IN-UYG24 in test substance treatment samples collected at initiation were 7.10, 14.1, 29.1, 57.2, and 114 mg IN-UYG24/L or 94 to 97% of the nominal concentrations. The measured concentrations of IN-UYG24 in test substance treatment samples collected at 48 hours were 7.43, 14.3, 29.7, 59.3, and 117 mg IN-UYG24/L or 95 to 99% of the nominal concentrations. The measured concentrations of IN-UYG24 in test solutions at 96 hours were 7.18, 14.3, 29.2, 58.8, and 115 mg IN-UYG24/L or 95 to 98% of the nominal concentrations. Mean measured concentrations of IN-UYG24 during the exposure were 7.24, 14.2, 29.3, 58.4, and 115 mg IN-UYG24/L or 95 to 98% of the nominal concentrations. The dilution water (blank) control solution contained no detectable concentrations of IN-UYG24. Recoveries of IN-UYG24 from the QC samples during the definitive test ranged from 96 to 108% of the nominal concentrations.

All results from biological responses were based on mean measured concentrations of IN-UYG24. Summaries of cumulative mortality and sublethal effects are presented in Table 44 and Table 45, respectively

Table 44
Observed mortality of rainbow trout, *Oncorhynchus mykiss*, exposed to IN-UYG24 for 96 hours in a static acute test

Nominal IN-UYG24 Concentration (mg IN-UYG24/L)	Mean Measured IN-UYG24 Concentration (mg IN-UYG24/L)	Cumulative Mortality/Number at Test Start ^a			
		24 Hours	48 Hours	72 Hours	96 Hours
0 (Control)	<LOD	0/7	0/7	0/7	0/7
7.5	7.24	0/7	0/7	0/7	0/7
15	14.2	0/7	0/7	0/7	0/7
30	29.3	0/7	0/7	0/7	0/7
60	58.4	0/7	0/7	0/7	0/7
120	115	0/7	0/7	0/7	0/7

^a Test chambers contained seven rainbow trout each at test initiation.

Table 45
Observed sublethal effects of rainbow trout, *Oncorhynchus mykiss*, exposed to IN-UYG24 for 96 hours in a static, acute test

Nominal IN-UYG24 Concentration (mg IN-UYG24/L)	Mean Measured IN-UYG24 Concentration (mg IN-UYG24/L)	Number Affected/Number Alive ^a			
		24 Hours	48 Hours	72 Hours	96 Hours
0 (Control)	<LOD	0/7	0/7	0/7	0/7
7.5	7.24	0/7	0/7	0/7	0/7
15	14.2	0/7	0/7	0/7	0/7
30	29.3	0/7	0/7	0/7	0/7
60	58.4	0/7	0/7	0/7	0/7
120	115	0/7	0/7	0/7	0/7

^a Test chambers contained seven rainbow trout each at test initiation.

III. CONCLUSION

IN-UYG24 was assessed for acute toxicity to the rainbow trout, *Oncorhynchus mykiss*, in a 96-hour static test. The 24-, 48-, 72-, and 96-hour LC₅₀ values, based on mortality and mean measured test concentrations, were >115 mg IN-UYG24/L, the highest concentration tested. No mean measured IN-UYG24 concentration caused 100% mortality at test end. The highest mean measured IN-UYG24 concentration causing 0% mortality at test end was 115 mg IN-UYG24/L.

([REDACTED] 2015b)

RMS comment

This study was conducted in compliance with the current guideline. The 96-hour LC₅₀ value was >115 mg IN-UYG24/L based on mean measured concentrations. This study is acceptable.

B.9.2.2. Long-term and chronic toxicity to fish

Report: [REDACTED] (1997); DPX-MP062 (approximately 75% DPX-KN128, 25% IN-KN127): Early life-stage toxicity to rainbow trout, *Oncorhynchus mykiss*

DuPont Report No.: HLR 598-96, Revision No. 1

Guidelines: OECD 210, USEPA 72-4 **Deviations:** None

Testing Facility: [REDACTED]

Testing Facility Report No.: HLR 598-96 Revision No. 1

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.

I. MATERIALS AND METHODS

A. MATERIALS

- | | |
|-----------------------------|---|
| 1. Test material: | DPX-MP062 technical |
| Lot/Batch #: | MP062-51A |
| Purity: | 94.5% |
| Description: | Off-white solid |
| CAS#: | None for DPX-MP062. |
| | 173584-44-6 for DPX-KN128, insecticidally active isomer. |
| Stability of test compound: | Shown to be stable under the conditions of the test |
| 2. Control: | Dilution (laboratory well water) water |
| Solvent control: | N,N-dimethylformamide |
| 3. Test animals: | Rainbow trout |
| Species: | <i>Oncorhynchus mykiss</i> |
| Age at dosing: | Less than 24 h post fertilization. |
| Source: | Unfertilised eggs and sperm (CRARO) via [REDACTED] |
| | [REDACTED] |
| Acclimation period: | Approximately 18 hours |
| Diet: | Brine shrimp and Purina® Trout Chow® |
| Test chamber: | Glass aquaria (40 l × 20 w × 25 h cm) holding approximately 7 L of test solution (18 cm liquid depth), fitted with a screen mesh-covered overflow pipe. Before thinning, embryos were held in aquaria in glass embryo cups (212 mL, 5.5 cm diameter) with screen mesh bottoms attached with silicone adhesive |
| Water: | Tap water |
| 4. Environmental conditions | |
| Dissolved Oxygen | 6.9 - 11.6°mg/L |
| pH | 7.1 to 7.6 |
| Temperature: | 8 - 12°C |
| Photoperiod: | 16 hour photoperiod (approximately 140 lux) and 8 hour darkness which included 30 minutes of transitional light (approximately 2 lux) |

B. STUDY DESIGN AND METHODS

- In-life initiated/completed
22-August-1996 to 20-November-1996

2. Experimental treatments

The effects of DPX-MP062 on the early life stages of rainbow trout (*Oncorhynchus mykiss*) were determined under continuous-flow conditions for 90 days. A total of 80 fertilised embryos per treatment (40/replicate; 2 replicates/treatment) were exposed to dilution water and dimethylformamide (0.1 mL/L) controls or 0.014, 0.025, 0.047, 0.071, 0.15, and 0.25 mg mean measured DPX-MP062/L. Unaerated test solutions were delivered intermittently (approximately 144 1-litre volume additions every 24 hours) using a modified Mount and Brungs proportional diluter. Embryos, larvae, and juvenile fish were maintained in 20 litre glass vessels containing approximately 7 L of test solution. On Day 46, larvae were thinned to 15 larvae/replicate and 2 replicates/treatment and fed newly hatched brine shrimp. On Day 62 through and including Day 89, the fish were fed Purina® Trout Chow® once daily. Temperature was maintained at a mean of 10.5°C. Observations of mortality and sublethal effects were made daily throughout the study duration of 90 days and length and weight were taken at the end of the study. Results are presented based on mean measured concentrations of test substance.

II. RESULTS AND DISCUSSION

A. FINDINGS

Hatching, survival, abnormalities, and growth data are summarised in Table 46 and Table 47.

Table 46
Summary of hatching, survival, and sublethal effects (hatch to thinning) of DPX-MP062
in an early life stage test with rainbow trout

Mean measured concentrations (mg DPX-MP062/L)	Mean hatching day		Hatch (%) ^a	Survival ^b		Abnormalities ^b	
	(Start) ^a	(End) ^a		no. alive/total	(%)	no. affected/no. alive	(%)
Water Control	30	32	90	72/72	100	1/72	1.4
DMF Control	30	32	96	77/77	100	2/77	2.6
0.014	30	32	89	71/71	100	1/71	1.4
0.025	30	32	99	77/79	97	2/77	2.6
0.047	30	32	89	71/71	100	1/71	1.4
0.071	29	31	94	74/75	99	1/74	1.4
0.15	30	32	89	71/71	100	0/71	0
0.25	30	32	94	75/75	100	1/75	1.3

^a Based on Day 40 data

^b Based on Day 46 data.

Table 47
Summary of fingerling mortality, growth, and sublethal effects (thinning to test end) of DPX-MP062
in an early life stage test with rainbow trout

Mean measured concentrations (mg DPX-MP062/L)	Mortality ^a		Abnormalities ^a		Mean Length (cm)	Mean Wet Weight (g)
	No. dead/total	%	No. affected/no. alive	%		
Water Control	0/30	0	2/30	7	4.5	1.2231
DMF Control	1/30	3	1/29	3	4.5	1.2822
0.014	0/30	0	1/30	3	4.4	1.1739
0.025	0/30	0	0/30	0	4.4	1.1999
0.047	0/30	0	0/30	0	4.4	1.1953
0.071	0/30	0	0/30	0	4.5	1.3067
0.15	0/30	0	0/30	0	4.4	1.1755
0.25	8/30	27*	22/22	100*	4.0	0.9590

^a Based on 90-day data

* Significantly different from controls (p <0.05, Dunnett's ANOVA)

III. CONCLUSION

The 90-day NOEC of DPX-MP062 in a trout early life stage test was 0.15 mg DPX-MP062/L, based on statistically significant differences in total surviving fingerlings, total observed abnormalities to fingerlings after 90 days of exposure, and fingerling standard length and weight at 90 days.

() 1997)

RMS comment

This study was submitted in the original DAR. This study was conducted in compliance with the current guideline. The 90-day NOEC of DPX-MP062 (79:21) in a trout early life stage test was 0.15 mg DPX-MP062/L based on mean measured concentrations. This study is still considered acceptable.

Report: () (2014); Indoxacarb (DPX-KN128): Early life-stage toxicity test with the fathead minnow, *Pimephales promelas*, under flow-through conditions

DuPont Report No.: DuPont-41426

Guidelines: OECD 210 (2013), U.S. EPA 850.1400 (1996) **Deviations:** None

Testing Facility: ()

Testing Facility Report No.: 81344

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 early life-stage toxicity of fathead minnow (*Pimephales promelas*) exposed to indoxacarb (DPX-KN128) was determined in a 28-day post-hatch flow-through test. The test was conducted in accordance with the U.S. EPA, Office of Chemical Safety and Pollution Prevention (OCSPP), Ecological Effects Test Guideline

850.1400 and the Organization for Economic Cooperation and Development (OECD), Guideline 210. Treatments consisted of a dilution water control, a vehicle control (100 µL DMF/L), and five nominal concentrations of 0.010, 0.020, 0.040, 0.080, and 0.16 mg a.s./L. Based on mean measured concentrations of indoxacarb, the NOEC value for egg hatchability was 0.129 mg a.s./L (the highest concentration tested), and for post-hatch survival, standard length, and blotted wet weight, the NOEC was 0.0675 mg a.s./L.

I. MATERIALS AND METHODS

A. MATERIALS

- | | |
|--|--|
| 1. Test material: | Indoxacarb technical |
| Lot/Batch #: | KN128-374 |
| Purity: | 98.8% |
| Description: | White solid |
| CAS#: | 173584-44-6 |
| Stability of test compound: | Stable at normal temperatures and storage conditions |
| 2. Control: | Dilution water (laboratory freshwater) |
| Solvent control: | Dimethylformamide (DMF) |
| Test vehicle: | Dilution water (laboratory freshwater) |
| Toxic reference: | None |
| 3. Test organism: | Fathead minnow |
| Species: | <i>Pimephales promelas</i> |
| Age at dosing: | <24 hours |
| Initial population: | 20 embryos per test chamber, with the exception of one control chamber that contained 21 |
| Source: | In-house culture |
| Diet: | Brine shrimp nauplii and/or salmon starter at least twice daily except 24 hours prior to termination |
| Test chamber: | Glass aquaria measuring approximately 14 cm wide by 23.5 cm long by 16.5 cm high with a test solution depth of 12 cm |
| 4. Environmental conditions (in-life period) | |
| Temperature: | 24.6 to 25.4°C for fry |
| Photoperiod: | 16 hr photoperiod (422 to 594 lux) and 8 hr darkness which included 30 min transitional light preceding and following the 16-hr light interval |

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
19-September-2014 to 22-October-2014
2. Experimental treatments
The early life-stage toxicity of indoxacarb on the fathead minnow, *Pimephales promelas* was determined in an unaerated, flow-through, 28-day post-hatch test. Treatments consisted of a dilution water control, vehicle control (100 µL DMF/L), and five nominal test substance concentrations of 0.010, 0.020, 0.040, 0.080, and 0.16 mg a.s./L. Twenty embryos, with the exception of one control replicate, were used per replicate with four replicates per test concentration and control group. The one exception contained 21 embryos.
3. Observations
On a daily basis during incubation, the embryos were counted and dead embryos were removed and discarded. Survival of hatched fry was monitored daily by visually inspecting each test chamber and any behavioural or physical changes, including abnormalities, were recorded. At the end of the 28-day post-hatch exposure, all surviving fry were measured for standard length (*i.e.*, tip of the snout to the caudal peduncle) using a millimeter scale and blotted wet weight using an electronic balance.

Temperature, pH, and dissolved oxygen concentration were measured in all replicates of the test substance treatments and control groups at test initiation, weekly throughout the test, and at termination of the definitive test. The concentration of indoxacarb was measured in test solution samples collected from the control and each treatment prior to the definitive test initiation (Day -1), and on days 0, 7, 14, 21, 28, and 33 of the definitive test. The analysis of the samples for indoxacarb during the test was based on an analytical method provided by the Sponsor and validated at [REDACTED] prior to the definitive test initiation.

4. Statistics

Experimental units, on which observations or measurements were made, were the replicated test chambers. All statistical analyses were performed using SAS software (version 9.3 for Windows) and Ecostats. Prior to comparisons of the treatment groups to the control group, the control and vehicle control were compared to determine if differences between control groups were statistically significant. Since there was no statistical difference between the control and vehicle control, the control and vehicle control groups were pooled. All statistical comparisons were made to this pooled control group. Inferences of statistical significance were based upon a $p = 0.05$ unless otherwise noted.

A one-way analysis of variance (ANOVA) was performed to identify LOEC and NOEC values for all endpoints. If the data were consistent with a monotone concentration-response, then the LOEC and NOEC values were determined by the step-down Jonckheere Terptra test ($p = 0.05$) with the alternate hypothesis being that the mean for the measured endpoint parameter was reduced in comparison to the control mean. If the data for a given endpoint parameter were not consistent with a monotone concentration-response, a Shapiro-Wilk test and Levene test were conducted to test for normality and homogeneity of variance, respectively, over treatments at each time point. If the results from the Shapiro-Wilk's and Levene's tests indicated normality and homogeneity (*i.e.*, $p > 0.01$), Dunnett's test was performed on the non-transformed raw data. In instances of non-normality or heterogeneity (*i.e.*, $p < 0.01$), a Dunn's test was performed. The maximum acceptable toxicant concentration (MATC) was determined, when possible, by calculating the geometric mean of the NOEC and the LOEC values for the biological parameters.

II. RESULTS AND DISCUSSION

A. FINDINGS

The mean measured concentrations of indoxacarb in the control, vehicle control, and test substance treatments during the study were <LOD (control), <LOD (vehicle control), 0.00808, 0.0158, 0.0324, 0.0675, and 0.129 mg a.s./L, which represented 79 to 84% of the nominal concentrations. No residues of indoxacarb were detected in the control or vehicle control above the LOD of 0.00291 mg a.s./L. All test acceptability criteria were met.

Egg hatch began in the control and 0.0324 mg a.s./L treatment on study Day 3. Hatch in the vehicle control, 0.00808, 0.0158, 0.0675, and 0.129 mg a.s./L treatments began on study Day 4. Day 0 post-hatch (*i.e.*, $\geq 95\%$ hatch) in the control treatment was determined to be Day 5. Hatch was completed in all treatment replicates between study days 4 and 5. Overall hatching success in the control treatment was 99% which met the acceptability criterion for this endpoint. Hatching success in the test substance treatments ranged from 99% in the vehicle control, and 0.0324, 0.0675, and 0.129 mg a.s./L treatments to 100% in the 0.00808 and 0.0158 mg a.s./L treatment. There was no statistically significant hatch reduction in success, or delays in time to start or completion of hatch observed in the test substance treatments as compared to the pooled control.

Post-hatch survival in the control treatment was 93% which met the acceptability criterion for this endpoint. Post-hatch survival in the vehicle control treatment was 99%. Post-hatch survival in the test substance treatments were 95, 95, 96, 96, and 0% in the 0.00808, 0.0158, 0.0324, 0.0675, and 0.129 mg a.s./L treatments, respectively. There was a statistically significant reduction in post-hatch survival in the 0.129 mg a.s./L test substance treatment as compared to the pooled control.

Mean standard length was 19.4, 19.3, 19.6, 19.5, 20.0, 19.9 mm in the control, vehicle control, 0.00808, 0.0158, 0.0324, and 0.0675 mg a.s./L treatments, respectively. There was a statistically significant increase in mean standard length in the 0.0324 and 0.0675 mg a.s./L test substance treatments as compared to the pooled control. However, the increased standard length at these treatments was not considered to be biologically significant. There was no statistically significant reduction in mean standard length in any of the test substance treatments as compared to the pooled control. Mean blotted wet weight was 0.123, 0.124, 0.129, 0.124, 0.128, and 0.129 g in the control, vehicle control, 0.00808, 0.0158, 0.0324, and 0.0675 mg a.s./L treatments, respectively. There were no statistically significant reduction of mean standard length and mean blotted wet weight as compared to the control. There were no fry surviving the exposure to 0.129 mg a.s./L. Therefore, no fish from this treatment were available for length or weight measurements at test termination.

A summary of hatching and survival is presented Table 48.

Table 48
Summary of observed mortality of *Pimephales promelas* exposed to indoxacarb in a flow-through test

Mean measured indoxacarb concentration (mg a.s./L)	Hatch (No. of hatched fry/initial No. of embryos)				Survival (No. of surviving fry/total no. of hatched fry)			
	A	B	C	D	A	B	C	D
Control	20/20	20/20	20/21	20/20	19/20	19/20	17/20	19/20
Vehicle Control	20/20	20/20	19/20	20/20	20/20	19/20	19/19	20/20
0.00808	20/20	20/20	20/20	20/20	19/20	20/20	19/20	18/20
0.0158	20/20	20/20	20/20	20/20	20/20	18/20	18/20	20/20
0.0324	19/20	20/20	20/20	20/20	18/19	19/20	20/20	19/20
0.0675	20/20	20/20	19/20	20/20	19/20	20/20	19/19	18/20
0.129	20/20	19/20	20/20	20/20	0/20	0/19	0/20	0/20

III. CONCLUSION

Based on mean measured concentrations of indoxacarb, the NOEC value for egg hatchability was 0.129 mg a.s./L, and for post-hatch survival, standard length, and blotted wet weight, the NOEC was 0.0675 mg a.s./L.

(██████████ 2014)

RMS comment

This study was conducted according to the current guideline. The study is valid according to validity criteria. The NOEC of 0.0675 mg DPX-KN128/L (based on mean measured concentrations) is acceptable.

Report: ██████████ (1997); Early life stage toxicity of DPX-MP062 to the sheepshead minnow, *Cyprinodon variegatus*

DuPont Report No.: HLO-1997-00091, Revision No. 1

Guidelines: U.S. EPA 72-4 **Deviations:** None

Testing Facility: ██████████

Testing Facility Report No.: 806-DU

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 effects of DPX-MP062 on the early life stages of sheepshead minnow (*Cyprinodon variegatus*), embryos, larvae, and juveniles, were assessed in a flow-through, 35-day early life stage toxicity test in accordance with the appropriate Good Laboratory Practice standards and test guidelines, U.S. EPA Pesticide Assessment Guideline, Subdivision E, 72-4.

Dilution water control, solvent control (0.1 mL/L dimethylformamide) and nominal test item concentrations of 0.022, 0.047, 0.090, 0.18, and 0.36 mg DPX-MP062/L were used during the study. The corresponding mean, measured concentrations were 0.0169, 0.0417, 0.0750, 0.157, and 0.316 mg DPX-MP062/L.

The NOEC for sheepshead minnows exposed to DPX-MP062 was 0.0169 mg/L, based on mean measured concentration and mortality (survival of fish post hatch). The LOEC (lowest observed effect concentration) and MATC (maximum acceptable toxicant concentration), were 0.0417 and 0.0265 mg/L, respectively, based on mean measured concentration and mortality.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: DPX-MP062 technical
 Lot/Batch #: MP062-51A
 Purity: 94.54%
 Description: Off-white solid
 CAS#: 144171-61-9
 Stability of test compound: Test substance appeared to be stable under the conditions of the study; no evidence of instability was observed.
2. Control: Dilution water (carbon filtered natural seawater)
 Solvent control: Dimethylformamide
3. Test animals: Sheepshead minnow
 Species: *Cyprinodon variegatus*
 Age at dosing: Less than 24 h post fertilization.
 Source: Eggs, [REDACTED]
 Acclimation period: Not applicable. The embryos were used for the definitive test the day of arrival at the laboratory.
 Diet: Newly hatched brine shrimp, *Artemia salina nauplii*, were fed 2-3 times per day, beginning on Day 3, except during the final 24 hours of the test.
 Test chamber: Embryos were exposed in cages that consisted of glass cylinders (8 cm high x 8 cm diameter) that were closed at one end with Nitex® screen. After thinning the fish were kept in 20 L glass aquaria (approximately 20 cm in width, 30 cm in length, and 26 cm in height) that contained approximately 15 L of test solution (water depth was ~ 18 cm)
 Water: Carbon filtered natural seawater
4. Environmental conditions
 Dissolved Oxygen 4.4 to 7.7 mg/L
 pH 7.6 to 8.2
 Temperature: 29.0 to 30.9°C
 Photoperiod: 16 Hour light (40 foot-candles) and 8 hour night with 15 minute transition period.

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed

22-October-1996 to 16-December-1996

2. Experimental treatments

The effects of DPX-MP062 on the early life stages of sheepshead minnows (*Cyprinodon variegatus*) were determined under unaerated, flow-through conditions for 35 days (32 days post hatch). A dilution water control, solvent control (0.1 mL/L N,N-dimethylformamide), and five nominal test item concentrations of 0.022, 0.047, 0.090, 0.18, and 0.36 mg DPX-MP062/L were used during the study. A total of 80 eggs per treatment were exposed per concentration. After hatching (Day 3 of exposure) fish were thinned to a total of 30 fish per test item concentration (15 fish per replicate, two replicates per concentration) at test start. Test solutions were maintained between 29.0 and 30.9 °C. Analytical verification of DPX-MP062 concentrations were made on test solutions sampled on Days 0, 7, 14, 21, 28, and 35.

3. Observations

The number of surviving organisms and the occurrence of sublethal effects (loss of equilibrium, erratic swimming, loss of reflex, excitability, discoloration, or change in appearance or behaviour) were determined visually and recorded initially and at 24 hour intervals. Dead test organisms were removed when first observed. At the conclusion of the test, surviving fish were quickly sacrificed, blotted on paper towels, and immediately weighed and measured.

4. Statistics

Data that were statistically analysed included: 1) percent of healthy and unhealthy larvae and juveniles 7, 14, 21, 28, and 32 days after hatching, 2) the mean total length of surviving fish at the end of the test, and 3) the mean wet weight of surviving fish at the end of the test. The mortality of the test organisms after 48 hours of exposure and the percent of healthy and unhealthy embryos hatched, were not statistically analysed due to 100% survival and hatch in all replicates at all tested concentrations. The time to first feeding was not statistically analysed because all fish fed when first presented with food. Time to hatch, start and end, was the same for each treatment and the control, so statistical analyses were not warranted.

Control and solvent control data were compared with a parametric "t" test. In those cases where no significant differences were observed at the 95% confidence level (Day 28 and 32 post hatch survival and sublethal effects, mean wet weight, and mean total lengths), control and solvent control data were pooled prior to subsequent statistical analyses. In those cases where the "t" test could not be performed due to a lack of variance (Day 7, 14, and 21 post hatch survival and sublethal effects) treatment data were compared to the control data. The Shapiro-Wilk's test was used to determine if data were normally distributed, and Bartlett's test was used to determine if variances were homogeneous. A one-way analysis of variance (ANOVA) and Dunnett's or Bonferroni's test was then used to compare treatment and control means. All calculations were performed using mean measured concentrations of the active ingredient. Survival and sublethal effects data were arc sine [square root(Y)] transformed prior to statistical analysis. Because no survival occurred at the two highest tested concentrations from Day 7 through the test termination, data from these treatments were assumed to be different from the control and not included in the statistical analyses of the survival, sublethal effect, length, and weight data. Length and weight data from test vessels with a mean measured concentration of 0.0750 mg/L were assumed to be different from the control and not included in the statistical analyses due to complete mortality in the replicate 1 test vessel.

The no observed effect level (NOEL) is the highest tested concentration at which a measured biological parameter is not statistically different (at the 95% confidence level) than the control. The lowest observed effect level (LOEL) is the lowest tested concentration at which any measured biological parameter is statistically different from the control and above which all concentrations are significantly different. The maximum acceptable toxicant concentration (MATC) is calculated as the geometric mean of the NOEL and the LOEL.

II. RESULTS AND DISCUSSION

A. FINDINGS

Mean measured concentrations of DPX-MP062 were ND (not detected at or above the limit of quantitation of 0.0100 mg/L; water control and solvent control), 0.0169, 0.0417, 0.0750, 0.157, and 0.316 mg/L, and ranged from 77 to 89% of nominal concentrations. DPX-MP062 was stable throughout the test. All chemical and physical parameters for the 35-day study were within acceptable ranges.

A summary of survival and sublethal effects of DPX-MP062 in an early life stage test with sheepshead minnows, *Cyprinodon variegatus*, at 48 hours, at hatch (Day 3), and at 7, 14, 21, 28, and 32 days post-hatch is presented in Table 49. A summary of total lengths and wet weights are presented in Table 50.

There was 100% hatching in the water and solvent control test vessels. The number of live, normal control fish at 32 days post-hatch was at least 93% in each water and solvent control chamber. At the end of the test, water and solvent control fish had an average wet weight (blotted) of 272 and 286 mg, respectively, and an average total length of 24 and 24 mm, respectively. The relative standard deviation of the weights of surviving fish in the control test chambers was less than 40%. Maximum loading rate during the toxicity test was approximately 0.29 g/L at any time and 0.026 g/L/24 hours. Sublethal effects, including loss of equilibrium and erratic swimming, were noted at 0.0417 mg/L on Days 18, 19, 20, 21, 27, 29, and 30 of the definitive test. These effects were not observed at any other time during the test. No sublethal effects other than the relative size of test organisms as visually observed were noted at any other time or concentration.

The most sensitive biological endpoint was survival of fish at 14, 21, 28, and 32 days post-hatch. The NOEC for fish exposed for 35 days to DPX-MP062 was 0.0169 mg/L (Dunnett's ANOVA, $p < 0.05$). The LOEL was 0.0417 mg/L DPX-MP062. Survival and sublethal effects at 48 hours and at hatch, time to first feeding, and the time to hatch were not significantly different from the controls at any tested concentration. Survival and sublethal effects 7 days post hatch, total length of surviving fish, and wet weight of surviving fish were significantly different than the controls at 0.0750, 0.157, and 0.316 mg/L DPX-MP062.

Table 49
Summary of mortality and sublethal effects of DPX-MP062 in an early life stage test with sheepshead minnows, *Cyprinodon variegatus*, at 48 hours, at hatch (Day 3), and at 7, 14, 21, 28, and 32 days post-hatch

Mean measured concentration of DPX-MP062 (mg/L)	Replicates	Percent mortality at 48 hours	Percent mortality at hatch Day 3	Percent mortality (days post hatch)				
				7	14	21	28	32
ND ^a (water control)	1	0	0	7	7	7	7	7
	2	0	0	7	7	7	7	7
ND (solvent control)	1	0	0	0	0	0	0	0
	2	0	0	0	0	0	7	7
0.0169	1	0	0	7	7	7	7	13
	2	0	0	13	13	13	13	13
0.0417	1	0	0	40	60	73	87	87
	2	0	0	20	60	60	80	80
0.0750	1	0	0	87	100	100	100	100
	2	0	0	60	73	73	73	73
0.157	1	0	0	100	100	100	100	100
	2	0	0	100	100	100	100	100
0.316	1	0	0	100	100	100	100	100
	2	0	0	100	100	100	100	100

^a ND = not detected at or above the limit of quantitation of 0.0100 mg/L

Table 50
Total lengths and wet weights of sheepshead minnows, *Cyprinodon variegatus*, at the end of the toxicity test with DPX-MP062

Mean, measured concentrations of DPX-MP062 (mg/L)	Replicates	Total length (mm) Mean ± Std. Dev.	Wet weight (mg) Mean ± Std. Dev.
ND ^a (water control)	1	23.6 ± 1.75	273 ± 52.9
	2	23.5 ± 1.66	272 ± 56.5
ND (solvent control)	1	23.3 ± 1.68	281 ± 59.2
	2	23.7 ± 1.68	292 ± 59.2
0.0169	1	24.6 ± 2.04	326 ± 114
	2	24.5 ± 1.88	307 ± 70.8
0.0417	1	27.2 ± 4.53	561 ± 84.1
	2	25.7 ± 1.53	495 ± 185
0.0750 ^b	1	--- ^c	---
	2	26.1 ± 1.65	444 ± 97.1

^a ND = not detected at or above the limit of quantitation of 0.0100 mg/L.

^b No fish survived at concentrations above 0.0750 mg/L

^c A dash indicates that no fish survived in this replicate

III. CONCLUSION

Exposure of embryonic, larval, and juvenile sheepshead minnows, *Cyprinodon variegatus*, to DPX-MP062 dissolved in 0.1 mL/L DMF, resulted in a 35-day NOEC of 0.0169 mg DPX-MP062/L based on mean, measured concentrations and mortality.

The most sensitive measured biological endpoints were the survival of fish at 14, 21, 28, and 32 days post hatch.

([REDACTED] 1997)

RMS comment

This study was not conducted in accordance with the current guideline and was conducted with the old material DPX-MP062. RMS notes that the temperature (30.0 ± 0.403°C) seems rather high even for this species. RMS considers that it is not known if the effects at 0.0169 mg/L are significant or not because no statistical analysis was provided. As new toxicity study is available for the new material DPX-KN128, this study is not considered essential.

Report: [REDACTED] (2014); IN-JT333: Early life-stage toxicity test with the fathead minnow, *Pimephales promelas*, under flow-through conditions

DuPont Report No.: DuPont-41669

Guidelines: OECD 210 (1992), U.S. EPA 850.1400 (1996) **Deviations:** None

Testing Facility: [REDACTED]

Testing Facility Report No.: 81345

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 early life-stage toxicity of fathead minnow (*Pimephales promelas*) exposed to IN-JT333 was determined in a 28-day post-hatch flow-through test. The test was conducted in accordance with the U.S. EPA, Office of Chemical Safety and Pollution Prevention (OCSPP), Ecological Effects Test Guideline 850.1400 and the Organization for Economic Cooperation and Development (OECD), Guideline 210. Treatments consisted of a dilution water control, a vehicle control, and five nominal concentrations of 1.6, 3.1, 6.3, 13, and 25 µg IN-JT333/L. Based on mean measured concentrations of IN-JT333, the NOEC value for egg hatchability was 17.0 µg IN-JT333/L. Based on mean measured concentrations of IN-JT333, the NOEC value for post-hatch survival, standard length, and blotted wet weight was 2.42 µg IN-JT333/L.

I. MATERIALS AND METHODS**A. MATERIALS**

- | | |
|--|---|
| 1. Test material: | IN-JT333 technical metabolite |
| Lot/Batch #: | JT333-023 |
| Purity: | 99.6% |
| Description: | Solid |
| CAS#: | 144171-39-1 |
| Stability of test compound: | Stable at ambient temperatures |
| 2. Control: | Dilution water (laboratory freshwater) |
| Solvent control: | Dimethylformamide (DMF) |
| Test vehicle: | Dilution water (laboratory freshwater) |
| Toxic reference: | None |
| 3. Test organism: | Fathead minnow |
| Species: | <i>Pimephales promelas</i> |
| Age at dosing: | <24 hours |
| Initial population: | 25 embryos per test chamber |
| Source: | In-house culture |
| Diet: | Brine shrimp nauplii and/or salmon starter at least twice daily except 24 hours prior to termination |
| Test chamber: | Glass aquaria measuring approximately 14 cm wide by 23 cm long by 16.5 cm high with a test solution depth of 12.5 cm |
| 4. Environmental conditions (in-life period) | |
| Temperature: | 24.3 to 25.9°C for fry |
| Photoperiod: | 16 hr photoperiod (579 lux) and 8 hr darkness which included 30 min transitional light preceding and following the 16-hr light interval |

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
12-November-2014 to 15-December-2014
2. Experimental treatments
The early life-stage toxicity of IN-JT333 on the fathead minnow, *Pimephales promelas*, was determined in an unaerated, flow-through, 28-day post-hatch test. Treatments consisted of a dilution water control, vehicle control, and five nominal concentrations of 0 (control), 0 (vehicle control, 50 µL DMF/L), 1.6, 3.1, 6.3, 13, and 25 µg IN-JT333/L. Twenty-five embryos were used per replicate with four replicates per test concentration, vehicle control, and control.
3. Observations
On a daily basis during incubation, the embryos were counted and dead embryos were removed and discarded. Survival of hatched fry was monitored daily by visually inspecting each test chamber and any behavioural or physical changes, including abnormalities, were recorded. At the end of the

28-day post-hatch exposure, all surviving fry were measured for standard length (*i.e.*, tip of the snout to the caudal peduncle) using a millimeter scale and blotted wet weight using an electronic balance.

Temperature, pH, and dissolved oxygen concentration were measured in all replicates of the test substance treatments and control groups at test initiation, weekly throughout the test, and at termination of the definitive test. Additional water quality measurements were collected on Day 9 when 100% mortality was observed in the 25 µg a.s./L nominal treatment and on Day 29 to monitor dissolved oxygen concentration. The concentration of IN-JT333 was measured in test solution samples collected from the control, vehicle control, and each treatment prior to the definitive test initiation (Day -4), and on days 0, 7, 9, 12, 21, 28, and 33 of the definitive test. Samples were collected from all four replicates of the vehicle control on Day 9, and from all four replicates of the control and vehicle control on days 12, 21, 28 and 33. The analysis of the samples for IN-JT333 during the test was based on an analytical method provided by the Sponsor and validated at [REDACTED] prior to the definitive test initiation.

4. Statistics

Experimental units, on which observations or measurements were made, were the replicated test chambers. All statistical analyses were performed using SAS software (version 9.3 for Windows) and Ecostats. Prior to comparisons of the treatment groups to the control group, the control and vehicle control were compared to determine if differences between control groups were statistically significant. Since there were no statistical differences between the control and vehicle control treatment for any endpoints, the control and vehicle control groups were pooled. All statistical comparisons were made against the pooled control group. Inferences of statistical significance were based upon a $p = 0.05$ unless otherwise noted.

A one-way analysis of variance (ANOVA) was performed to identify LOEC and NOEC values for all endpoints. If the data were consistent with a monotone concentration-response, then the LOEC and NOEC values were determined by the step-down Jonckheere Terpstra test ($p \leq 0.05$) with the alternate hypothesis that the mean for the measured endpoint parameter was reduced in comparison to the control mean. If the data for a given endpoint parameter were not consistent with a monotone concentration-response, a Shapiro-Wilk test and Levene test were conducted to test for normality and homogeneity of variance, respectively, over treatments at each time point. If the results from the Shapiro-Wilk's and Levene's tests indicated normality and homogeneity (*i.e.*, $p > 0.01$), Dunnett's test was performed on the non-transformed raw data. In instances of non-normality or heterogeneity (*i.e.*, $p < 0.01$), a Dunn's test was performed.

Because data for proportion surviving and blotted wet weight were consistent with a monotone concentration-response, the NOEC and LOEC values for this parameter were determined by using a Jonckheere-Terpstra test. Data for standard length was not consistent with a monotone dose-response, but the data satisfied the assumptions of normality and homogeneity of variance. Therefore, the NOEC and LOEC value for this parameter was determined using a Dunnett's test. Data for proportion hatching, and hatch completion were not consistent with a monotone dose-response, and the data did not satisfy the assumption of normality. Therefore, the NOEC and LOEC value for these parameters was determined using a Dunn's test. The maximum acceptable toxicant concentration (MATC) was determined, when possible, by calculating the geometric mean of the NOEC and the LOEC values for the biological parameters.

II. RESULTS AND DISCUSSION

A. FINDINGS

The mean measured concentrations of IN-JT333 in the control, vehicle control, and test substance treatments during the study were <LOD (control), <LOD (vehicle control), 1.26, 2.42, 4.79, 9.05, and 17.0 µg IN-JT333/L, which represented 68 to 79% of the nominal concentrations. With the single exception noted below, no residues of IN-JT333 were detected in the control or vehicle control above the LOD of 0.00563 µg/L. The weight of evidence from the vehicle control treatment over the course of the study indicates that the measured value at test termination (study day 33; 0.0523 µg IN-JT333/L) from the

vehicle control replicate B sample was not representative of test substance contamination in the test chamber. All test acceptability criteria were met.

Egg hatch began in the control, vehicle control, and all test treatments on study Day 4. Day 0 post-hatch (*i.e.*, $\geq 95\%$ hatch) in the control treatment was determined to be Day 5. Hatch was completed in all treatment replicates between study days 4 and 5. Overall hatching success in the control treatment was 100% which met the acceptability criterion for this endpoint. Hatching success in the vehicle control was 99%. Hatching success in the test substance treatments ranged from 98% 17.0 μg IN-JT333/L treatment to 99% in the 1.26, 2.42, 4.79, and 9.05 $\mu\text{g}/\text{L}$ treatments. There was no statistically significant hatch success or time to completion of hatch observed in the test substance treatments as compared to the pooled control.

Post-hatch survival in the control treatment was 93% which met the acceptability criterion for this endpoint. Post-hatch survival for the vehicle control was 95%. Post-hatch survival in the test substance treatments were 97, 86, 29, 0, and 0% in the 1.26, 2.42, 4.79, 9.05, and 17.0 μg IN-JT333/L treatments, respectively. There was a statistically significant reduction in post-hatch survival in the 4.79, 9.05, and 17.0 $\mu\text{g}/\text{L}$ test substance treatments as compared to the pooled control.

Mean standard length was 22.9, 22.7, 22.7, 22.5, and 25.9 mm in the control, vehicle control, 1.26, 2.42, and 4.79 μg IN-JT333/L treatments, respectively. Mean blotted wet weight was 0.1028, 0.1116, 0.1145, 0.1198, and 0.1896 g in the control, vehicle control, 1.26, 2.42, and 4.79 $\mu\text{g}/\text{L}$ treatments, respectively. Due to 100% mortality, no fish were available for standard length measurements in the 9.05 and 17.0 $\mu\text{g}/\text{L}$ test substance treatments at test termination. The 4.79 $\mu\text{g}/\text{L}$ treatment was excluded from analysis for standard length and blotted wet weight because of a statistically significant reduction in post-hatch fry survival in this treatment. There was no statistically significant reduction of mean standard length and mean blotted wet weight in the 1.26 and 2.42 μg IN-JT333/L test substance treatments as compared to the pooled control.

A summary of hatching and survival is presented in Table 51.

Table 51
Summary of observed mortality of *Pimephales promelas* exposed to IN-JT333 in a flow-through test

Mean Measured IN-JT333 Concentration ($\mu\text{g a.s.}/\text{L}$)	Hatch (No. of hatched fry/initial no. of embryos)				Survival (No. of surviving fry/total no. of hatched fry)			
	A	B	C	D	A	B	C	D
Control	25/25	25/25	25/25	25/25	22/25	24/25	23/25	24/25
Vehicle Control	25/25	24/25	25/25	25/25	24/25	21/24	24/25	25/25
1.26	25/25	24/25	25/25	25/25	24/25	23/24	25/25	24/25
2.42	24/25	25/25	25/25	25/25	20/24	19/25	24/25	22/25
4.79	25/26	25/25	25/25	25/25	9/25	6/25	7/25	7/25
9.05	24/25	25/25	25/25	25/25	0/24	0/25	0/25	0/25
17.0	24/25	25/25	25/25	24/25	0/24	0/25	0/25	0/24

III. CONCLUSION

Based on mean measured concentrations of IN-JT333, the NOEC value for egg hatchability was 17.0 $\mu\text{g}/\text{L}$. Based on mean measured concentrations of IN-JT333, the NOEC value for post-hatch survival, standard length, and blotted wet weight was 2.42 $\mu\text{g}/\text{L}$.

([REDACTED] 2014)

RMS comment

This study was conducted according to the current guideline. The study is valid according to validity criteria. The NOEC of 2.42 µg IN-JT333/L (based on mean measured concentrations) is acceptable. An EC10 value of 2.49 µg IN-JT333/L was calculated by RMS (95% confidence intervals: 2.22-2.99). This EC10 value is reliable.

Report: [REDACTED] (2002); Bioconcentration and metabolism of [indanone-1-¹⁴C]DPX-JW062 and [trifluoromethoxyphenyl](U)-¹⁴C]DPX-JW062 in fish (a racemic mixture of DPX-KN128 and IN-KN127)

DuPont Report No.: AMR 3663-95, Revision No. 1

Guidelines: USEPA 165-4 (1982), OECD 305 (Draft) (1992) **Deviations:** None

Testing Facility: [REDACTED]

Testing Facility Report No.: 42910

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.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: DPX-JW062 technical
 Lot/Batch #: JW062-119
 Purity: 99.6%, by analysis
 Description: White powder
 CAS#: 144171-61-9
 Stability of test compound: Shown to be stable under the conditions of the test
2. Radiolabel test material: ^{14}C DPX-JW062
 Lot/Batch #: [Indanone-1- ^{14}C]DPX-JW062: 451
 [Trifluoromethoxyphenyl(U)- ^{14}C]: DPX-JW062456
 Radiochemical purity: [Indanone-1- ^{14}C]DPX-JW062: >95%
 [Trifluoromethoxyphenyl(U)- ^{14}C]DPX-JW062: >95%
 Specific activity: [Indanone-1- ^{14}C]DPX-JW062: 52.0 $\mu\text{Ci}/\text{mg}$
 [Trifluoromethoxyphenyl(U)- ^{14}C]DPX-JW062: 51.7 $\mu\text{Ci}/\text{mg}$
 Stability of test compound: The test material was stable for at least 28 days at room temperature.
3. Control: Dilution (laboratory well water) water
 Solvent control: N,N-dimethylformamide
4. Test animals: Bluegill sunfish
 Species: *Lepomis macrochirus*
 Age/Life stage at dosing: Approximately 1 year old
 Weight at termination of exposure: $6.87 \pm 1.5\text{g}$
 Source: [REDACTED]
 Acclimation period: 5 days
 Diet: Not given
 Test medium: Tap water, *ad libitum*
 Test chamber: 100-L aquaria
4. Environmental conditions
 Temperature: $22 \pm 1^\circ\text{C}$
 Photoperiod: 16 hour light:8 hour dark, 30 minute transition

B. STUDY DESIGN AND METHODS

1. In life initiate/completed
 25-November-1995 to 20-August-2002

2. Experimental treatments

The bioconcentration potential of [indanone-1- ^{14}C]DPX-JW062 (IND) and [trifluoromethoxyphenyl(U)- ^{14}C]DPX-JW062 (TMP) was determined in bluegill sunfish (*Lepomis macrochirus*). DPX-JW062 is a racemic (1:1) mixture of the insecticidally active S-enantiomer DPX-KN128 and the insecticidally inactive R-enantiomer IN-KN127. Fish were exposed to mean measured concentrations of 0.010 and 0.100 mg/L (10 and 100 $\mu\text{g}/\text{L}$) of [indanone-1- ^{14}C]DPX-JW062 or [trifluoromethoxyphenyl(U)- ^{14}C]DPX-JW062 in a flow-through system. Treated fish were exposed for 28 days followed by a 21-day depuration period. Water and fillet samples were analysed for total radioactive residues and for concentrations of DPX-JW062, DPX-KN128, IN-KN127, and the metabolite IN-JT333 and its enantiomers IN-KN124 (insecticidally inactive) and IN-KN125 (insecticidally active). From these data, rates of bioconcentration and depuration of total residues in whole fish were calculated and steady state bioconcentration factors for DPX-JW062, DPX-KN128, and IN-KN127 were determined.

II. RESULTS AND DISCUSSION

A. FINDINGS

Test organism observations: No adverse effects were observed in treated fish at either dose of [^{14}C]DPX-JW062 during the course of this study. One fish in the low dose IND- treatment group was removed due to an observed eye injury which was not considered dose-related.

Dose administration: Radioassay and chromatographic analyses confirmed the stability of [^{14}C]DPX-JW062 during the 28-day exposure phase of the study. Dose solutions were analysed on Days 2 and 28 of exposure. The radiochemical purity of the low dose solutions was 98.7–99.9%, while the percent radiochemical purity for the high dose solutions was 99.4–100%.

Bioconcentration phase: The concentrations of total radioactive residues (TRR) in fish tissue are shown in Table 52 through Table 55 for the low and high dose treatment groups for the IND-labeled and TMP-labeled DPX-JW062 treated fish. The TRRs found in water samples are shown in Table 56. A steady-state plateau for whole fish was achieved on approximately Day 21 of exposure. The uptake rate constants were 141, 120, 137, and 120 for the IND low dose, IND high dose, TMP low dose, and TMP high dose treatment groups, respectively. These rate constants were determined by the BIOFAC[®] computer program.

Depuration phase: After 21 days of depuration, approximately 66% of the TRR in fillets from the low dose (0.01 mg/L) IND-treatment group had depurated while about 69% of the TRR in viscera depurated. In whole fish, these data resulted in 68.8% depuration 21 days after exposure was terminated. For the high dose IND-dosed group, the percent depuration for fillet, viscera, and whole fish were 70.2, 71.7, and 71.4%, respectively. The time to 95% depuration was calculated to be 34.1 and 29.0 days, respectively. For the low dose TMP-treated group, 67.5% of the TRR in fillet depurated at 21 days after exposure was stopped and 73.4% of the TRR in viscera had depurated within that same period. For whole fish, calculations indicated 72.6% of the dose depurated during the 21-day depuration phase. For the high dose (0.100 mg/L) TMP-treated group, the percent depuration for fillet, viscera, and whole fish were 76.8, 80.2, and 79.7%, respectively. The time to 95% depuration was calculated to be 29.9 and 28.3 days, respectively. The average times to 50 and 90% depuration were 7 and 21.8 days, respectively, as calculated by BIOFAC[®]. The depuration rate constant (day^{-1}) was 0.0879, 0.103, 0.100, and 0.106 for the IND low dose, IND high dose, TMP low dose, and TMP high dose treatment groups, respectively. The TRR data are shown in Table 57 through Table 60.

Characterisation of radiolabeled residues in fish tissues: Identification of parent and metabolites was confirmed by HPLC retention time matching with reference standards using reverse phase HPLC methods, HPLC retention time matching using chiral chromatography of isolated residues, and/or by LC/MS/MS.

Percent TRR and TRR extracted, in $\mu\text{g/kg}$, for fillet samples from low dose groups are shown in Table 61. At 21 days of exposure, 98.4% of the TRR (3806 $\mu\text{g/kg}$) was extracted from the IND-label while 92.6% (3197 $\mu\text{g/kg}$) was extracted from fillets of fish exposed to the TMP-label. After 28 days of exposure, the percent TRRs were 86.3% (3822 $\mu\text{g/kg}$) and 99.2% (3684 $\mu\text{g/kg}$), respectively. At Day 21 of the depuration phase, 78.1% of the TRR (1172 $\mu\text{g/kg}$) was extracted for the IND-label and 81.5% of TRR (985 $\mu\text{g/kg}$) was extracted for the TMP-label.

The concentrations of DPX-JW062 and its metabolites in fillet extracts are shown in Table 62 and Table 63 from fish exposed to the low dose of IND- and TMP-treated groups, respectively. During the exposure phase, parent is the largest ^{14}C -residue, representing 62.7% (2425 $\mu\text{g/kg}$) and 58.5% (2019 $\mu\text{g/kg}$) of the TRR for the IND- and TMP-extracts on Day 21 of exposure, respectively. On Day 28 of the exposure phase, parent represented 53.6 and 61.6% (2374 and 2288 $\mu\text{g/kg}$), respectively. IN-JT333 was the only other significant ($>10\%$ of TRR) residue. It represented 23.7 and 24.4% (917 and 842 $\mu\text{g/kg}$) of the TRR on Day 21 of the exposure phase, respectively, and 22.2 and 27.8% (983 and 1032 $\mu\text{g/kg}$) on Day 28. A number of other metabolites were identified: IN-ML811, IN-KG433, IN-KT319, IN-JU873, and three unknowns. Each of their concentrations was low ($<4.5\%$ of TRR, $<155 \mu\text{g/kg}$) and not all metabolites were present in both extracts. Even though these metabolites contained both ring systems that were radiolabeled, fish-to-fish variation and low concentrations explain why some of the metabolites were not

present in both extracts. Furthermore, these metabolites had no insecticidal activity or had low ($<0.001 \mu\text{g/kgDPX-JW062}$) insecticidal activity. After 21 days of depuration, parent comprised 15.8% of the TRR ($237 \mu\text{g/kg}$) in the IND-labeled fillet extract and 9.4% ($113 \mu\text{g/kg}$) of the TRR for the TMP-labeled extract. The concentration of IN-JT333 increased to 61.8% ($927 \mu\text{g/kg}$) and 70.2% ($848 \mu\text{g/kg}$) of the TRR for IND- and TMP-extracts, respectively. No other metabolites were detected in the extracts of fillets after the 21-day depuration period.

Percent TRR and TRR extracted in $\mu\text{g/kg}$ for viscera samples from low dose groups are shown in Table 64. In general, TRR values in viscera were higher than in fillet. This trend was expected considering the higher fat content of viscera (versus fillet) and the high K_{ow} value of DPX-MP062, about 45000. At 21 days of exposure, 95.1% of the TRR ($17874 \mu\text{g/kg}$) was extracted from the IND-label, while 102.1% ($20342 \mu\text{g/kg}$) was extracted from fillets of fish treated exposed to the TMP-label. After 28 days of exposure, the percent TRRs were 96.5 and 90.7% (17367 and $16506 \mu\text{g/kg}$), respectively. At Day 21 of the depuration phase, 93.8% of the TRR ($5170 \mu\text{g/kg}$) was extracted for the IND-label and 95.9% ($4650 \mu\text{g/kg}$) was extracted for the TMP-label.

The trends noted above regarding concentrations of parent and IN-JT333 in fillet extracts were similar to those concentrations, in terms of percent TRR, found in viscera extracts. The concentrations in $\mu\text{g/kg}$ were higher as expected due to higher TRR values. The data are shown in Table 65 and Table 66. Parent was the major ^{14}C -residue found in viscera during the exposure phase, accounting for 56.1 to 65.4% (10210 to $13030 \mu\text{g/kg}$) of the TRR. IN-JT333 was the only significant metabolite (in terms of percent TRR) found in the viscera, representing 23.6 to 30.2% of the TRR (4436 to $5818 \mu\text{g/kg}$). The same metabolites found in fillet were also observed in viscera; no additional metabolites were detected. For the IND-label, their concentration ranged from 0.3% of TRR (IN-KG433, $56 \mu\text{g/kg}$) to 4.8% of TRR (IN-ML811, $902 \mu\text{g/kg}$). For the TMP-label, IN-ML811, IN-KT319, IN-JU873, and one unknown were detected. IN-KG433 was detected in the 28-day sample only. The concentrations of these were between 0.4 and 2.4% of TRR (73 and $478 \mu\text{g/kg}$). At Day 21 of the depuration phase, DPX-JW062 represented 18.5 and 9.9% of the TRR (1019 and $479 \mu\text{g/kg}$) for the IND- and TMP-extracts, respectively. IN-JT333 represented 72.5 and 85.5% (3997 and $4148 \mu\text{g/kg}$) of the TRR, respectively.

Since DPX-JW062 and IN-JT333, the insecticidally active metabolite, were each composed of one insecticidally active enantiomer (DPX-KN128 and IN-KN125, respectively) and one insecticidally inactive isomer (IN-KN127 and IN-KN124, respectively), it was of interest to determine whether there was any preferential distribution, metabolism, and/or excretion of enantiomers.

Dose solutions and isolated fractions of DPX-JW062 and IN-JT333 were analysed by chiral HPLC chromatography to determine the ratio of each residue's enantiomers. Day 28 water samples and isolated fractions of parent and IN-JT333 from Days 21 and 28 (exposure phase) and Day 21 (depuration phase) were all analysed using chiral HPLC. Results from analysis of the water samples indicated that the enantiomeric ratio of DPX-KN128 to IN-KN127 did not change throughout the exposure phase. It still was a racemic or 1:1 mixture. Chiral analysis of parent isolated from fillet and viscera extracts of Day 21 exposure phase samples indicated that the ratio of DPX-KN128 to IN-KN127 was about 5:95. This ratio was approximately the same for IN-KN125 and IN-KN124, the enantiomers of IN-JT333. These ratios for parent and IN-JT333 were similar for Day 28 exposure phase and Day 21 depuration phase samples, indicating that there was no preferential uptake or depuration of enantiomers. The data indicated there was no accumulation of the insecticidally active enantiomers of parent (DPX-KN128) and metabolite IN-JT333 (IN-KN125), though there was accumulation of the inactive enantiomers of both of these (IN-KN127 and IN-KN124, respectively).

DPX-JW062 underwent N-decarboxylation to form IN-JT333 as the major metabolite in tissues. It represented about 22–28% of the TRR in fillet and approximately 23–30% of TRR in viscera during the exposure phase. IN-JT333 had been identified in soil, livestock, and the rat. Other metabolites that were identified were IN-JU873, IN-KG433, IN-KT319, and IN-ML811. All of these metabolites had been previously identified in soil, livestock, and/or the rat. Each of these represented less than 10% of TRR and usually $<3\%$ (usually $<0.05 \text{ ppm}$).

Steady-State Bioconcentration Factors (BCFs): The worst-case steady-state bioconcentration factors are shown in Table 67. The average steady-state BCF in whole fish for DPX-JW062 was 950.3 based on extraction TRR data from Day 21 and 28 samples. The average steady-state BCF (from the same samples) for DPX-KN128, the insecticidally active enantiomer of parent, was 77.3; the BCF for IN-KN127 (insecticidally inactive) was 1848.

Table 52
Total radioactive residue (TRR) calculated as [indanone-1-¹⁴C]DPX-JW062 equivalents in the low-dose treatment group (Group I, 10 µg/L) during the exposure phase

Study day (exposure phase)	Total Radioactive Residue (µg/kg) in:		
	Fillets ^a	Viscera	Whole fish ^b
0	NA	NA	NA
0.17	322	942	670
1	890	3717	2479
3	1237	5768	3783
7	2822	12060	8014
14	4439	18047	12087
21	3868	18795	12257
28	4429	17997	12054

^a TRR values from combustion data

^b Whole Fish TRR calculated as (µg/kg in fillet × 0.438) + (µg/kg in viscera × 0.562), where 0.438 and 0.563 equal the weight percent/100 that fillet and viscera, respectively, represent of the whole fish

Table 53
Total radioactive residue (TRR) calculated as [indanone-1-¹⁴C]DPX-JW062 equivalents in the high-dose treatment group (Group I, 100 µg/L) during the exposure phase

Study day (exposure phase)	Total Radioactive Residue (µg/kg) in:		
	Fillet ^a	Viscera	Whole fish ^b
0	NA	NA	NA
0.17	3048	8373	6067
1	8056	29406	20161
3	11458	52357	34648
7	27364	97274	67003
14	31873	113890	78377
21	31242	133350	89137
28	33287	128010	86995

^a TRR values from combustion data

^b Whole Fish TRR calculated as (µg/kg in fillet × 0.433) + (µg/kg in viscera × 0.567), where 0.433 and 0.567 equal the weight percent/100 that fillet and viscera, respectively, represent of the whole fish

Table 54
Total radioactive residue (TRR) calculated as [trifluoromethoxyphenyl(U)-¹⁴C]DPX-JW062 equivalents in the low-dose treatment group (Group II, 10 µg/L) during the exposure phase

Study day (exposure phase)	Total Radioactive Residue (µg/kg) in:		
	Fillet	Viscera	Whole fish ^a
0	NA	NA	NA
0.17	316	852	620
1	903	3620	2446
3	1343	7217	4679
7	3348	13750	9256
14	4747	17257	11853
21	3452	19924	12808
28	3714	18199	11941

^a Whole Fish TRR calculated as (µg/kg in fillet × 0.432) + (µg/kg in viscera × 0.568), where 0.432 and 0.568 equal the weight percent/100 that fillet and viscera, respectively, represent of the whole fish

Table 55
Total radioactive residue (TRR) calculated as [trifluoromethoxyphenyl(U)-¹⁴C]DPX-JW062 equivalents in the high-dose treatment group (Group II, 100 µg/L) during the exposure phase

Study day (exposure phase)	Total Radioactive Residue (µg/kg) in:		
	Fillet	Viscera	Whole fish ^a
0	NA	NA	NA
0.17	2595	7196	5222
1	6023	23195	15828
3	11659	44365	30334
7	17368	84325	55600
14	42280	114390	83455
21	29938	135460	90191
28	30524	125690	84864

^a Whole Fish TRR calculated as (µg/kg in fillet × 0.429) + (µg/kg in viscera × 0.571), where 0.429 and 0.571 equal the weight percent/100 that fillet and viscera, respectively, represent of the whole fish

Table 56
Total radioactive residue calculated as DPX-JW062 equivalents in test water during exposure phase

Study day (exposure phase)	Total Radioactive Residue (TRR) in µg/L, DPX-JW062 equivalents			
	[Indanone-1- ¹⁴ C]DPX-JW062 treated aquaria		[Trifluoromethoxyphenyl(U)- ¹⁴ C] DPX-JW062 treated aquaria	
	10 µg/L Dose group (low dose)	100 µg/L Dose group (high dose)	10 µg/L Dose group	100 µg/L Dose group
0	10.8	101	12.2	93.3
0.17	6.95	73.8	9.26	70.6
1	7.14	72.4	9.47	73.2
3	9.23	87.3	10.6	96.9
7	10.2	88.6	11.4	88.5
14	8.51	83.2	11.6	92.2
21	10.4	83.4	11.0	90.4
28	9.48	83.5	9.26	86.0
Mean of all samples	9.08	84.2	10.6	86.4
Mean of samples through 14 days	8.81	84.4	10.8	85.8
Mean of samples through 21 days	9.03	84.2	10.8	86.4

Table 57
**Total radioactive residue in fish tissues during the 21-day depuration period calculated as
¹⁴C-DPX-JW062 equivalents from [indanone-1-¹⁴C]DPX-JW062 low dose (10 µg/L) treatment group**

Study day	[Indanone-1- ¹⁴ C]DPX-JW062 low dose					
	Fillet		Viscera		Whole fish	
	Concentration in µg/kg	Percent depuration ^a	Concentration in µg/kg	Percent depuration	Concentration in µg/kg ^b	Percent depuration ^b
28 ^c	4429	NA	17997	NA	12054	NA
1	3080	30.5	16758	6.9	10767	10.7
3	2398	45.9	14196	15.3	9028	25.1
7	1777	59.9	9918	30.1	6353	47.3
10	1817	59.0	8863	10.6	5777	52.1
14	1389	68.6	7428	58.7	4783	60.3
21	1501	66.1	5512	69.4	3755	68.8

^a % Depuration = $\frac{[(\text{concentration on Day 28 of exposure}) - (\text{concentration on depuration Day X})] \times 100\%}{(\text{concentration on Day 28 of exposure})}$

^b Whole fish concentration = $[\text{concentration in fillet} \times 0.438] + [\text{concentration in viscera} \times 0.562]$

^c Last day of exposure to treated water

Table 58
Total radioactive residue in fish tissues during the 21-day depuration period calculated as
¹⁴C-DPX-JW062 equivalents from [indanone-1-¹⁴C]DPX-JW062 high dose (100 µg/L) treatment group

Study day	[Indanone-1- ¹⁴ C]DPX-JW062 high dose					
	Fillet		Viscera		Whole fish ^b	
	Concentration in µg/kg	Percent depuration ^a	Concentration in µg/kg	Percent depuration	Concentration in µg/kg	Percent depuration
28 ^c	33287	NA	128010	NA	86995	NA
1	24223	27.2	93577	26.9	63547	27.0
3	20346	38.9	93253	27.2	61684	29.1
7	14111	57.6	63291	50.6	41996	51.7
10	12299	63.1	57724	54.9	38055	56.3
14	9021	72.9	45386	64.5	29640	65.9
21	9904	70.2	36280	71.7	24859	71.4

^a % Depuration = $\frac{[(\text{concentration on Day 28 of exposure}) - (\text{concentration on depuration Day X})]}{(\text{concentration on Day 28 of exposure})} \times 100\%$

^b Whole fish concentration = $[\text{concentration in fillet} \times 0.433] + [\text{concentration in viscera} \times 0.567]$

^c Last day of exposure to treated water

Table 59
Total radioactive residue in fish tissues during the 21-day depuration period calculated as
¹⁴C-DPX-JW062 equivalents from [trifluoromethoxyphenyl(U)-¹⁴C]DPX-JW062
low dose (10 µg/L) treatment group

Study day	[Trifluoromethoxyphenyl(U)- ¹⁴ C]DPX-JW062 low dose					
	Fillet		Viscera		Whole fish ^a	
	Concentration in µg/kg	Percent depuration ^b	Concentration in µg/kg	Percent depuration	Concentration in µg/kg	Percent depuration
28 ^c	3714	NA	18199	NA	11937	NA
1	2696	27.4	18093	0.6	11441	4.2
3	2516	32.3	11279	38.0	7493	37.2
7	1706	54.1	9868	45.8	6342	46.9
10	1272	65.8	8261	54.6	5242	56.1
14	1300	65.0	6448	64.6	4224	64.6
21	1208	67.5	4849	73.4	3276	72.6

^a Whole fish concentration = $[\text{concentration in fillet} \times 0.432] + [\text{concentration in viscera} \times 0.568]$

^b % Depuration = $\frac{[(\text{concentration on Day 28 of exposure}) - (\text{concentration on depuration Day X})]}{(\text{concentration on Day 28 of exposure})} \times 100\%$

^c Last day of exposure to treated water

Table 60
Total radioactive residue in fish tissues during the 21-day depuration period calculated as
¹⁴C-DPX-JW062 equivalents from [trifluoromethoxyphenyl(U)-¹⁴C]DPX-JW062
 high dose (100 µg/L) treatment group

Study day	[Trifluoromethoxyphenyl(U)- ¹⁴ C]DPX-JW062 high dose					
	Fillet		Viscera		Whole fish ^a	
	Concentration in µg/kg	Percent depuration ^b	Concentration in µg/kg	Percent depuration	Concentration in µg/kg	Percent depuration
28 ^c	30524	NA	125690	NA	84908	NA
1	23931	21.6	125310	0.3	81818	3.6
3	18057	40.8	94017	25.2	61430	27.7
7	10357	66.1	63497	49.5	40700	52.1
10	7084	76.8	40706	67.6	26282	69.0
14	9741	68.1	57832	54.0	37201	56.2
21	7088	76.8	24857	80.2	17234	79.7

^a Whole fish concentration = [concentration in fillet × 0.429] + [concentration in viscera × 0.571]

^b % Depuration = $\frac{[(\text{concentration on Day 28 of exposure}) - (\text{concentration on depuration Day X})]}{(\text{concentration on Day 28 of exposure})} \times 100\%$

^c Last day of exposure to treated water

Table 61
Percent and µg/kg extracted from fillet samples from fish exposed to a low dose (10 µg/L) of
¹⁴C-DPX-JW062

[Indanone-1- ¹⁴ C]DPX-JW062							
		Exposure phase				Depuration phase	
		Day 21 TRR=3868 µg/kg ^a		Day 28 TRR=4429 µg/kg		Day 21 TRR=1501 µg/kg	
		% TRR ext.	µg/kg ext. ^b	% TRR ext.	µg/kg ext.	% TRR ext.	µg/kg ext.
ACN	Replicate 1	95.1	3678	81.3	3601	76.1	1142
	Replicate 2	94.9	3671	85.0	3765	—	
	Average	95.0	3675	83.2	3685	—	
ACN/Water	Replicate 1	3.6	139	3.2	142	2.0	30
	Replicate 2	3.2	124	3.0	133	—	
	Average	3.4	132	3.1	137	—	
	Avg. total extracted	98.4	3806	86.3	3822	78.1	1172
Unextracted	Replicate 1	8.9	344	8.2	363	11.2	168
	Replicate 2	7.9	306	7.8	345	—	
	Average	8.4	325	8.0	354	—	
Total recovery		106.8	4131	94.3	4177	89.3	1340
[Trifluoromethoxyphenyl(U)- ¹⁴ C]DPX-JW062							
		TRR=3452 µg/kg		TRR=3714 µg/kg		TRR=1208 µg/kg	
ACN	Replicate 1	91.6	3162	97.1	3606	80.7	975
	Replicate 2	90.1	3110	97.7	3629	—	
	Average	90.9	3138	97.4	3617	—	
ACN/Water	Replicate 1	1.8	62	1.8	67	0.8	9.7
	Replicate 2	1.6	55	1.8	67	—	
	Average	1.7	59	1.8	67	—	
	Avg. Total Extracted	92.6	3197	99.2	3684	81.5	985
Unextracted	Replicate 1	0.4	14	0.3	11	0.7	8.5
	Replicate 2	0.4	14	0.4	15	—	
	Average	0.4	14	0.4	15	—	
Total recovery		93.0	3210	99.6	3699	82.2	993

^a TRR values from combustion data.

^b µg/kg Extracted = %TRR Extracted/100 × TRR in µg/kg.

Table 62
Concentration of ^{14}C -DPX-JW062 and metabolites in extracts of fillet samples from fish exposed to [indanone-1- ^{14}C]DPX-JW062 at 10 $\mu\text{g/L}$ (low dose)

Peak ^a	Residue	Concentration in %TRR and $\mu\text{g/kg}$, ppb (DPX-JW062 equivalents)					
		Exposure phase				Depuration phase	
		Day 21 TRR=3868 $\mu\text{g/kg}$		Day 28 TRR=4429 $\mu\text{g/kg}$		Day 21 TRR=1501 $\mu\text{g/kg}$	
		% TRR ^b	$\mu\text{g/kg}$ ^c	% TRR	$\mu\text{g/kg}$	% TRR	$\mu\text{g/kg}$
A1	IN-ML811	1.8	70	1.6	71	ND ^d	—
A2	Unknown	2.4	93	1.0	44	ND	—
A3	Unknown	0.6	23	0.7	31	ND	—
B	Unknown	0.9	35	0.5	22	ND	—
C	IN-KG433	0.8	31	0.6	27	ND	—
D	IN-KT319	4.0	155	4.0	177	ND	—
E	IN-JU873	1.8	70	2.0	89	ND	—
F	DPX-JW062	62.7	2425	53.6	2374	15.8	237
G	IN-JT333	23.7	917	22.2	983	61.8	927
Total identified		98.7	3818	86.2	3818	77.6	1164
Total extracted		98.4	3806	86.3	3822	78.1	1172

^a Peak numbers from HPLC radiochromatograms

^b %TRR = average %TRR extracted (Table 61)/100 \times % of injected ^{14}C each peak represents

^c $\mu\text{g/kg}$ = %TRR/100 \times TRR, $\mu\text{g/kg}$

^d ND = not detected

Table 63
Concentration of ^{14}C -DPX-JW062 and metabolites in extracts of fillet samples from fish exposed to [trifluoromethoxy]phenyl(U)- ^{14}C]DPX-JW062 at 10 $\mu\text{g/L}$ (low dose)

Peak ^a	Residue	Concentration in %TRR and $\mu\text{g/kg}$, ppb (DPX-JW062 equivalents)					
		Exposure phase				Depuration phase	
		Day 21 TRR=3452 $\mu\text{g/kg}$		Day 28 TRR=3714 $\mu\text{g/kg}$		Day 21 TRR=1208 $\mu\text{g/kg}$	
		% TRR ^b	$\mu\text{g/kg}$ ^c	% TRR	$\mu\text{g/kg}$	% TRR	$\mu\text{g/kg}$
A1	IN-ML811	ND ^d		0.3	11	—	—
A2	Unknown	ND		ND		—	—
A3	Unknown	ND		0.3	11	—	—
B	Unknown	2.5	86	1.9	71	—	—
C	IN-KG433	0.7	24	0.7	26	—	—
D	IN-KT319	4.5	155	4.5	167	—	—
E	IN-JU873	1.9	66	2.3	85	—	—
F	DPX-JW062	58.5	2019	61.6	2288	9.4	113
G	IN-JT333	24.4	842	27.8	1032	70.2	848
Total identified		92.5	3193	99.4	3692	79.6	962
Total extracted		92.6	3197	99.2	3684	81.5	985

^a Peak numbers from HPLC radiochromatograms

^b %TRR = average %TRR extracted (Table 61)/100 x % of injected ^{14}C each peak represents

^c $\mu\text{g/kg}$ = %TRR/100 x TRR in $\mu\text{g/kg}$

^d ND = not detected

Table 64
Percent and µg/kg extracted from viscera samples from fish exposed to a low dose (10 µg/L) of
¹⁴C-DPX-JW062

[Indanone-1- ¹⁴ C]DPX-JW062							
		Exposure phase				Depuration phase	
		Day 21 TRR=18795 µg/kg		Day 28 TRR=17997 µg/kg		Day 21 TRR=5512 µg/kg	
		% TRR ext.	µg/kg ext. ^a	% TRR ext.	µg/kg ext.	% TRR ext.	µg/kg ext.
ACN	Replicate 1	94.5	17761	92.5	16647	92.4	5093
	Replicate 2	91.5	17197	95.4	17169	—	
	Average	93.0	17479	94.0	16917	—	
ACN/Water	Replicate 1	2.1	395	2.4	432	1.4	77
	Replicate 2	2.1	395	2.5	450	—	
	Average	2.1	395	2.5	450	—	
	Avg. total extracted	95.1	17874	96.5	17367	93.8	5170
Unextracted	Replicate 1	3.9	733	4.8	864	6.1	336
	Replicate 2	3.9	733	4.5	810	—	
	Average	3.9	733	4.7	846	—	
Total recovery		99.0	18607	101.2	18213	99.9	5506
[Trifluoromethoxyphenyl(U)- ¹⁴ C]DPX-JW062							
		TRR=19924 µg/kg		TRR=18199 µg/kg		TRR=4849 µg/kg	
ACN	Replicate 1	103.0	20522	90.2	16743	95.1	4611
	Replicate 2	98.9	19705	89.5	16288	—	
	Average	101.0	20123	89.9	16352	—	
ACN/Water	Replicate 1	1.1	219	0.8	146	0.8	39
	Replicate 2	1.0	199	0.8	146	—	
	Average	1.1	219	0.8	146	—	
	Avg. total extracted	102.1	20342	90.7	16506	95.9	4650
Unextracted	Replicate 1	0.4	80	0.3	55	0.5	24
	Replicate 2	0.4	80	0.3	55	—	
	Average	0.4	80	0.3	55	—	
Total recovery		102.5	20422	91.0	16561	96.4	4674

^a µg/kg Extracted = %TRR extracted/100 × TRR in µg/kg.

Table 65
Concentration of ^{14}C -DPX-JW062 and metabolites in extracts of viscera samples from fish exposed to [indanone-1- ^{14}C]DPX-JW062 at 10 $\mu\text{g/L}$ (low dose)

Peak ^a	Residue	Concentration in %TRR and $\mu\text{g/kg}$, ppb (DPX-JW062 equivalents)					
		Exposure phase				Depuration phase	
		Day 21 TRR=18795 $\mu\text{g/kg}$		Day 28 TRR=17997 $\mu\text{g/kg}$		Day 21 TRR=5512 $\mu\text{g/kg}$	
		% TRR ^b	$\mu\text{g/kg}$ ^c	% TRR	$\mu\text{g/kg}$	% TRR	$\mu\text{g/kg}$
A1	IN-ML811	4.8	902	4.8	864	—	—
A2	Unknown	0.5	94	ND ^d		—	—
A3	Unknown	0.4	75	ND		—	—
B	Unknown	0.4	75	ND		—	—
C	IN-KG433	0.3	56	0.3	54	—	—
D	IN-KT319	2.1	395	1.9	342	—	—
E	IN-JU873	1.0	188	1.4	252	—	—
F	DPX-JW062	62.2	11690	58.0	10438	18.5	1019
G	IN-JT333	23.6	4436	30.2	5435	72.5	3997
Total identified		95.3	17912	96.5	17367	91.0	5016
Total extracted		95.1	17874	96.5	17367	93.8	5171

^a Peak numbers from HPLC Radiochromatograms

^b %TRR = average %TRR extracted (Table 64)/100 \times % of injected ^{14}C each peak represents

^c $\mu\text{g/kg}$ = %TRR/100 \times TRR in $\mu\text{g/kg}$

^d ND = not detected

Table 66
Concentration of ^{14}C -DPX-JW062 and metabolites in extracts of viscera samples from fish exposed to [trifluoromethoxyphenyl(U)- ^{14}C]DPX-JW062 at 10 $\mu\text{g/L}$ (low dose)

Peak ^a	Residue	Concentration in %TRR and $\mu\text{g/kg}$, ppb (DPX-JW062 equivalents)					
		Exposure phase				Depuration phase	
		Day 21 TRR=19924 $\mu\text{g/kg}$		Day 28 TRR=18199 $\mu\text{g/kg}$		Day 21 TRR=4849 $\mu\text{g/kg}$	
		% TRR ^b	$\mu\text{g/kg}$ ^c	% TRR	$\mu\text{g/kg}$	% TRR	$\mu\text{g/kg}$
A1	IN-ML811	1.5	299	1.0	182	—	—
A2	Unknown	ND ^d		ND		—	—
A3	Unknown	ND		ND		—	—
B	Unknown	2.2	438	1.2	218	—	—
C	IN-KG433	ND		0.4	73	—	—
D	IN-KT319	2.4	478	2.0	364	—	—
E	IN-JU873	1.2	239	1.2	218	—	—
F	DPX-JW062	65.4	13030	56.1	10210	9.9	479
G	IN-JT333	29.2	5818	29.0	5278	85.5	4148
Total identified		101.9	19924	90.9	16543	95.4	4627
Total extracted		102.1	20342	90.7	16506	95.9	4650

^a Peak numbers from HPLC radiochromatograms

^b %TRR = average %TRR extracted (Table 64)/100 \times % of injected ^{14}C each peak represents

^c $\mu\text{g/kg}$ = %TRR/100 \times TRR in $\mu\text{g/kg}$

^d ND = not detected

Table 67
Bioconcentration factors for DPX-JW062, DPX-KN128, and IN-KN127

Measured parameters	Average value	Indanone-1- ^{14}C treatment group		Trifluoromethoxyphenyl (U)- ^{14}C treatment group	
Dose ($\mu\text{g/L}$)		10	100	10	100
BIOFAC [®] BCF (k_1/k_2) ^a	1316	1603	1162	1361	1136
Steady-state BCF for DPX-JW062 from extraction TRR of Day 21 and 28 samples in whole fish	950.3	981.5	NA ^b	919.0	NA ^b
Steady-state BCF for DPX-KN128 from extraction TRR of Day 21 and 28 samples in whole fish	77.3	76.0	NA ^b	78.5	NA ^b
Steady-state BCF for IN-KN127 from extraction TRR of Day 21 and 28 samples in whole fish	1848	1897	NA ^b	1798	NA ^b

^a Where k_1 is the uptake rate [$(\mu\text{g/kg in fish})/(\mu\text{g/L in water})$] = ~141, 120, 137, and 120 and k_2 is the depuration rate (day⁻¹) = ~0.0879, 0.103, 0.100 and 0.106 for the IND low dose, IND high dose, TMP low dose, and TMP high dose, respectively.

^b NA means not applicable

III. CONCLUSION

The average steady-state BCF in whole fish for DPX-JW062 was 950.3 L/kg based on extraction TRR data from Day 21 and 28 samples. The average steady-state BCF (from the same samples) for DPX-KN128, the insecticidally active enantiomer of parent, was 77.3 L/kg; the BCF for IN-KN127 (insecticidally inactive) was 1848 L/kg.

A steady-state plateau for whole fish was achieved on approximately Day 21 of exposure. The uptake rate constants were 141, 120, 137, and 120 for the IND low dose, IND high dose, TMP low dose, and TMP high dose treatment groups, respectively. The depuration rate constant (day^{-1}) was 0.0879, 0.103, 0.100, and 0.106 for the IND low dose, IND high dose, TMP low dose, and TMP high dose treatment groups, respectively. The average times to 50 and 90% depuration were 7 and 21.8 days, respectively.

These results indicated that there was no bioaccumulation of the insecticidally active enantiomer DPX-KN128 during the exposure phase while there was some bioaccumulation of the insecticidally inactive enantiomer of DPX-JW062, IN-KN127. However, DPX-JW062 residues did depurate upon cessation of dosing, with 95% of the residues gone within 30 days.

([REDACTED] 2002)

RMS comment

The bioconcentration in fish study AMR 3663-95, Revision No. 1 was conducted under guidelines U.S. EPA 165-4 (1982), and OECD 305 (Draft) (1992).

This study was assessed for previous Annex I inclusion and was conducted with the old material DPX-JW062 (racemic (1:1) mixture of the S-enantiomer DPX-KN128 and the R-enantiomer IN-KN127). RMS notes that the study summary presented above was initially not the one of the study report but a revision of this latter by the applicant. The values reported by the applicant in the tables of the summary were different than those in the study report. The reason of that was not understood by RMS so they were corrected by RMS. Rates of bioconcentration and depuration of total residues in whole fish were calculated and steady state bioconcentration factors for DPX-JW062, DPX-KN128, and IN-KN127 were determined. The average steady-state BCF in whole fish for DPX-JW062 was 950.3 L/kg (measured value). The average steady-state BCF for DPX-KN128 was 77.3 L/kg (measured value); the BCF for IN-KN127 was 1848 L/kg.

B.9.2.3. Potential for endocrine disruption

Effects of DPX-KN128 were studied in an early life-stage test (ELS) with the fathead minnow. No effects on egg hatchability or on post-hatch survival, standard length, and blotted wet weight at test termination were seen at the concentration of 0.0675 mg a.s./L. There were no indications for adverse endocrine activity observed in fish.

B.9.2.4. Acute toxicity to aquatic invertebrates

Report: Hoke, R.A. (1996); DPX-MP062 (approximately 75% DPX-KN128, 25% IN-KN127): Static acute, 48-hour EC_{50} to *Daphnia magna*

DuPont Report No.: HLR 603-96

Guidelines: OECD 202, EEC 92/69 Annex V - Method C.2 (1992), U.S. EPA 72-2 **Deviations:** None

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

Testing Facility Report No.: HLR 603-96

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.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: DPX-MP062 technical
 Lot/Batch #: MP062-51A
 Purity: 94.5%
 Description: Off-white solid
 CAS#: None for DPX-MP062
 DPX-KN128: 173584-44-6 for, insecticidally active isomer
 Stability of test compound: Shown to be stable in the test system by analysis
2. Control: Daphnid dilution water (DaDW), which consisted of filtered and aerated laboratory well water that had been conditioned by flowing through a tank containing adult fathead minnows.
 Solvent control: N,N-dimethylformamide
 Test vehicle: DaDW
 Toxic reference: None
3. Test organism: *Daphnia magna*
 Species: *Daphnia magna*
 Age at dosing: <24 hours
 Initial population: 5 daphnids per test chamber
 Source: Laboratory, in-house culture (Laboratory: Haskell Laboratory, Newark, Delaware)
 Diet: Unfed during test
 Test chamber: 250-mL Pyrex beaker containing 200 mL of test solution (6.4-cm test solution depth), covered with a plexiglass plate
4. Environmental conditions (in-life period)
 Dissolved oxygen: 8.3 to 9.1 mg/L
 pH: 7.5 to 8.5
 Temperature: 19.4 to 21.7°C
 Photoperiod: 16 hr photoperiod (355 to 422 lux) and 8 hr darkness which included 30 min transitional light (0.11 to 0.22 lux) preceding and following the 16-hr light interval

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
 03-July-1996 to 05-July-1996

2. Experimental treatments

The acute toxicity of DPX-MP062 to *Daphnia magna* was tested in a laboratory study. *Daphnia magna* neonates (less than 24 hours old) were randomly distributed into each of 8 test concentration groups, a dilution water control group, and a 0.1 mL/L dimethylformamide control group (5 daphnids/replicate, 4 replicates/test concentration and controls). *Daphnia magna* were exposed for 48 hours under static, unaerated conditions (mean temperature 20.6°C; 16 hour light/8 hour dark). Mean measured concentrations of DPX-MP062 tested were 0.0094, 0.022, 0.043, 0.084, 0.19, 0.35, 0.53, and 0.67 mg/L. The daphnids used in this test were collected from 23-day-old parent daphnids and were not fed during the test. The loading rate was 40 mL test solution per daphnid. Dissolved oxygen and pH were measured in both controls and in all test solutions at the beginning of the test, before the daphnids were added, and at the end of the test. Concentration of the test substance in the test solutions, including the water and dimethylformamide controls, was determined from samples taken at the beginning and at the end of the study. Observations of immobility were made every 24 hours.

II. RESULTS AND DISCUSSION

A. FINDINGS

A summary of the findings is presented in Table 68. The LC₅₀ and 95% confidence intervals were calculated by the moving-angle method.

Table 68
Summary of percentage observed immobility of *Daphnia magna* exposed to DPX-MP062 in a static, unaerated test

Mean measured concentrations (mg DPX-MP062/L)	24 Hours				48 Hours			
	1 ^a	2	3	4	1	2	3	4
Dilution Water Control	20	0	0	0	20	0	0	0
DMF Control ^b	0	0	0	0	0	0	0	0
0.0094	0	0	0	0	0	0	0	0
0.022	0	0	0	0	0	0	0	0
0.043	0	0	0	0	0	0	0	0
0.084	0	0	0	0	0	0	0	0
0.19	0	0	0	0	0	0	0	0
0.35	20	0	0	0	20	0	0	0
0.53	0	0	0	0	0	0	0	0
0.67	0	0	0	0	80	80	80	60

^a 1, 2, 3, and 4 represent replicate test vessels containing 5 daphnids each at test start

^b DMF = dimethylformamide

III. CONCLUSION

The 48-hour EC₅₀ of DPX-MP062 with 0.1 mL/L DMF solvent to *Daphnia magna* was 0.60 mg DPX-MP062/L. The highest concentration with no immobility was 0.19 mg DPX-MP062/L, based on immobility.

(Hoke, R. A., 1997)

RMS comment

This study was assessed for previous Annex I inclusion and was conducted with the old material DPX-MP062. As new acute toxicity study is available on the same species (*Daphnia magna*) for the new material DPX-KN128, this study is not considered essential.

Report: Gallagher, S.P., Kendall, T.Z., Krueger, H.O. (2010); Indoxacarb (DPX-KN128) technical: A 48-hour flow-through acute toxicity test with the cladoceran (*Daphnia magna*)

DuPont Report No.: DuPont-29542

Guidelines: OECD 202 (2004), OPPTS 850.1010 (1996) **Deviations:** None

Testing Facility: Wildlife International, Ltd., Easton, Maryland, USA

Testing Facility Report No.: 112A-311A

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 (DPX-KN128) to unfed *Daphnia magna* was determined in an unaerated, flow-through, 48-hour test. The test was conducted in accordance with the OECD Guideline for Testing of Chemicals, 202, *Daphnia sp. Acute Immobilization Test* and U.S. Environmental Protection Agency Series 850 - Ecological Effects Test Guidelines, and OPPTS Number 850.1010, *Aquatic Invertebrate Acute Toxicity Test, Freshwater Daphnids*. Treatments consisted of a dilution water control, a solvent control and nominal indoxacarb concentrations of 0.013, 0.025, 0.050, 0.10, and 0.20 mg a.s./L. The corresponding mean, measured concentrations of indoxacarb were 0.011, 0.021, 0.044, 0.077, and 0.17 mg a.s./L. The 48-hour EC₅₀ in *Daphnia magna* based on mean measured concentrations of indoxacarb active substance and immobility was greater than 0.17 mg a.s./L, the highest concentration tested and the apparent limit of solubility of indoxacarb.

I. MATERIALS AND METHODS

A. MATERIALS

- | | |
|------------------------------|---|
| 1. Test material: | Indoxacarb technical |
| Lot/Batch #: | KN128-215 |
| Purity: | 98.4% |
| Description: | Solid |
| CAS#: | 173584-44-6 |
| Stability of test compound: | The mean recovery of DPX-KN128 from freshwater stability samples was 101, 55, 37, and 53% of the 0-hour measured concentration after 3, 24, 48, and 96 hours, respectively. |
| 2. Negative control: | Dilution water (UV sterilised laboratory well water) control |
| Solvent control: | HPLC grade dimethylformamide (0.1 mL/L) |
| Test vehicle: | 0.1 mL DMF/L in solvent control and treatment groups |
| Toxic reference: | Not applicable |
| 3. Test organism: | Cladoceran |
| Species: | <i>Daphnia magna</i> |
| Age/life stage at dosing: | <24 hours |
| Initial population: | 10 daphnids per test chamber; 2 replicate test chambers |
| Source: | Wildlife International, Ltd. in-house culture |
| Diet: | Unfed during test |
| Test chamber: | 300-mL glass beaker (7.8-cm test solution depth) |
| 4. Environmental conditions: | Dissolved oxygen: ≥6.5 mg/L (≥72% of saturation) |
| | pH: 8.1 to 8.2 |
| Temperature: | 19.9 to 20.0°C in test chambers; approx. 20°C measured continuously in an adjacent container of water. |
| Photoperiod: | 16 hr light (311 lux at initiation) and 8 hr dark including 30 min transitional period preceding and following the 16-hr light interval. |

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
26-April-2010 to 28-April-2010

2. Experimental treatments

The acute toxicity of indoxacarb to unfed *Daphnia magna* (<24-hour old) was determined in an unaerated, flow-through, 48-hour test. Treatments consisted of a dilution water control, a solvent control (0.1 mL DMF/L), and mean measured test concentrations of 0.011, 0.021, 0.044, 0.07, and 0.17 mg a.s./L. Ten daphnids were used per replicate with two replicates per test concentration and control.

3. Observations

Immobility and behavioural observations were made at approximately 2.5 hours after initial exposure and then every ~24 hours following initiation of exposure.

4. Statistics

There was less than 50% immobility in any of the treatment groups at the 24- and 48-hour exposure, which precluded the statistical calculation of an EC₅₀ values at 24 and 48 hours. The highest mean measured test concentration causing no immobility at test end and the lowest mean measured test concentration causing 100% immobility at test end were assessed by visual observation of the immobility and observation data.

II. RESULTS AND DISCUSSION

A. FINDINGS

Mean measured concentrations of indoxacarb during pretest diluter verification ranged from 75.2 to 87.3% of nominal concentrations. Mean measured concentrations indoxacarb during the test were 0.011, 0.021, 0.044, 0.077, and 0.17 mg a.s./L and ranged from 77 to 87% of nominal concentrations. All validation criteria were met for the study. Summaries of the cumulative immobility and sublethal effects are presented in Table 69 and Table 70, respectively.

Table 69
Summary of observed immobility of unfed *Daphnia magna* exposed to indoxacarb for 48 hours in an unaerated, flow-through, acute test

Mean Measured Test Concentration (mg a.s./L)	Immobility (No. immobile/No. at test start) ^a			
	24 Hours ^b		48 Hours	
	A	B	A	B
Dilution water control (0.0)	0/10	0/10	0/10	0/10
Solvent control (0.0)	0/10	0/10	0/10	0/10
0.011	0/10	0/10	1/10	0/10
0.021	0/10	0/10	0/10	0/10
0.044	0/10	0/10	0/10	0/10
0.077	0/10	0/10	1/10	0/10
0.17	0/10	0/10	3/10	5/10

^a A–B represent replicate test chambers containing 10 daphnids each at test start

^b There were no immobile daphnids noted at the 2.5-hour observation interval.

Table 70
Summary of sublethal effects of unfed *Daphnia magna* exposed to indoxacarb for 48 hours
in an unaerated, flow-through, acute test

Mean Measured Test Concentration (mg a.s./L)	Number affected/Number alive ^a			
	24 Hours ^b		48 Hours	
	A	B	A	B
Dilution Water Control (0.0)	0/10	0/10	0/10	0/10
Solvent Control (0.0)	0/10	0/10	0/10	0/10
0.011	4 ^c /10	3 ^c /10	3 ^c /9	0/10
0.021	2 ^e /10	0/10	0/10	0/10
0.044	2 ^e /10	2 ^e /10	1 ^e /10	1 ^e /10
0.077	1 ^e /10	2 ^e /10	0/9	3 ^e /10
0.17	10 ^e /10	10 ^e /10	2 ^d ;5 ^e /7	3 ^d ;2 ^e /5

^a A–B represent replicate test chambers containing 10 daphnids each at test start

^b There were no treatment-related sublethal effects noted at the 2.5 hour observation interval.

^c Daphnid trapped at water surface but appears normal after submersion below water surface.

^d Daphnid trapped at water surface and appears lethargic after submersion below water surface.

^e Lethargic.

III. CONCLUSION

The 48-hour EC₅₀ in *Daphnia magna* based on mean, measured concentrations of indoxacarb and immobility was greater than 0.17 mg a.s./L, the highest concentration tested and the apparent limit of solubility of indoxacarb.

(Gallagher, S.P., Kendall, T.K., Krueger, H.O., 2010)

RMS comment

This study was conducted in compliance with the current guideline. The 48-hour EC₅₀ for *Daphnia magna* based on mean measured concentrations of indoxacarb was > 0.17 mg a.s./L. This study is acceptable.

Report: Rebstock, M. (2014); IN-JU873: 48-Hour static-renewal, acute toxicity test with the Cladoceran, *Daphnia magna*

DuPont Report No.: DuPont-35829

Guidelines: OECD 202 (2004), OPPTS 850.1010 (1996) **Deviations:** None

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

Testing Facility Report No.: 69136

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 IN-JU873 to unfed *Daphnia magna* neonates, less than 24 hours old at test start, was determined in an unaerated, 48-hour, static-renewal test. Daphnids were from at least the 3rd brood of at least 12 day-old parents. The test was conducted in accordance with the OECD Guideline for the Testing of Chemicals No. 202 and U.S. EPA Ecological Effects Test Guidelines OPPTS 850.1010. Treatments consisted of a dilution water control, solvent control and five nominal concentrations of 0.031, 0.063, 0.13, 0.25, and 0.50 mg IN-JU873/L. Mean measured concentrations were <LOD (control), <LOD (solvent control), 0.0180, 0.0494, 0.108, 0.214, and 0.433 mg IN-JU873/L, and ranged from 58 to 87% of the nominal concentrations of IN-JU873. No sublethal effects were observed in any treatment in the study. No IN-JU873 treatment concentrations caused 100% immobility at test end. No IN-JU873 treatment concentration caused 0% immobility at test end.

I. MATERIALS AND METHODS**A. MATERIALS**

1. Test material:	IN-JU873 technical metabolite
Lot/Batch #:	JU873-005
Purity:	99.1%
Description:	Solid, crystalline
CAS#:	144172-25-8
Stability of test compound:	Determined to not be stable in the test system
2. Control:	Dilution (blended) water
Test solvent:	DMF
Toxic reference:	None
3. Test organism:	Cladoceran
Species:	<i>Daphnia magna</i>
Age at dosing:	Neonates (<24 hours old)
Weight at dosing:	NA
Initial population:	5 daphnids per test chamber/20 daphnids per treatment
Source:	In house culture
Acclimation period:	Continuous culture
Diet:	Test period: unfed
Test chamber:	250-mL glass container containing ~200 mL of test solution (6.5-cm test solution depth), covered with a plastic Petri dish
4. Test medium:	ABC blended freshwater
Environmental conditions (in-life period)	
Temperature:	19.6 to 20.0°C
Photoperiod:	16 hr photoperiod (720 lux) and 8 hr darkness which included 30 min transitional light preceding and following the 16-hr light interval
Dissolved oxygen:	8.2 to 9.0 mg/L (94 to 103% saturation)
pH:	8.4 to 8.5

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
29-October-2013 to 31-October-2013

2. Experimental treatments

The acute toxicity of IN-JU873 to unfed *Daphnia magna* (<24-hour old) was determined in an unaerated, static-renewal, 48-hour test. Daphnids were from at least the 3rd brood of at least 12 day-old parents. Treatments consisted of a dilution water control, solvent control, and five nominal concentrations of 0.031, 0.063, 0.13, 0.25, and 0.50 mg IN-JU873/L. Five daphnids were used per replicate with four replicates per test concentration and control.

3. Observations

Immobility and sublethal (behavioural) observations were made every 24 hours.

4. Statistics

Estimates of EC₅₀ values and their 95% confidence limits were calculated using the Trimmed Spearman-Kärber method. The lowest concentration resulting in 100% immobility and highest concentration resulting in 0% immobility were assessed by visual observation.

II. RESULTS AND DISCUSSION

A. FINDINGS

Mean measured concentrations were <LOD (control), <LOD (solvent control), 0.0180, 0.0494, 0.108, 0.214, and 0.433 mg IN-JU873/L, and ranged from 58 to 87% of the nominal concentrations of IN-JU873. No sublethal effects were observed in any treatment in the study. Summaries of observed immobility and sublethal effects are presented in Table 71 and Table 72, respectively.

Table 71
Observed immobility of the Cladoceran, *Daphnia magna*, exposed to IN-JU873 for 48 hours in an unaerated, static-renewal, acute test

Mean, Measured IN-JU873 Concentration (mg/L)	Cumulative immobility (Number immobile/Number at test start) ^{a,b}								Mean % Immobile (after 48-hours)
	24 Hours				48 Hours				
	A	B	C	D	A	B	C	D	
0 (Control)	0/5	0/5	0/5	1/5	0/5	0/5	0/5	1/5	5
0 (Solvent Control)	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0
0.0180	0/5	0/5	1/5	0/5	0/5	0/5	1/5	2/5	15
0.0494	0/5	1/5	0/5	0/5	1/5	2/5	1/5	1/5	25
0.108	1/5	1/5	1/5	0/5	1/5	2/5	3/5	0/5	30
0.214	1/5	1/5	1/5	1/5	2/5	2/5	1/5	2/5	35
0.433	3/5	0/5	1/5	2/5	3/5	2/5	3/5	3/5	55

^a Immobile. No observed movement of appendages or postabdomen within 15 seconds after gentle agitation of the test chamber or gentle disturbance of the daphnid itself. Affected numbers are cumulative.

^b Replicate test chambers contained 5 daphnids each for a total of 20 per concentration at test initiation

Table 72
Observed sublethal effects of the Cladoceran, *Daphnia magna*, exposed to IN-JU873 for 48 hours in an unaerated, static-renewal, acute test

Mean Measured IN-JU873 Concentration (mg/L)	Sublethal effects/Number alive ^a							
	24 Hours				48 Hours			
	A	B	C	D	A	B	C	D
0 (Control)	0/5	0/5	0/5	0/4	0/5	0/5	0/5	0/4
0 (Solvent Control)	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
0.0180	0/5	0/5	0/4	0/5	0/5	0/5	0/4	0/3
0.0494	0/5	0/4	0/5	0/5	0/4	0/3	0/4	0/4
0.108	0/4	0/4	0/4	0/5	0/4	0/3	0/2	0/5
0.214	0/4	0/4	0/4	0/4	0/3	0/3	0/4	0/3
0.433	0/2	0/5	0/4	0/3	0/2	0/3	0/2	0/2

^a Replicate test chambers contained 5 daphnids each for a total of 20 per concentration at test initiation.

III. CONCLUSIONS

The 48-hour EC₅₀ value based on mean measured concentrations of IN-JU873 and immobility was 0.379 mg/L, with lower and upper 95% confidence limits of 0.201 and 0.717 mg/L, respectively. No IN-JU873 treatment concentrations caused 100% immobility at test end. No IN-JU873 treatment concentration caused 0% immobility at test end.

(Rebstock, M., 2014)

RMS comment

This study was conducted in compliance with the current guideline. The 48-hour EC₅₀ value based on mean measured concentrations of IN-JU873 was 0.379 mg/L. This study is acceptable.

Report: Hoke, R.A. (1997); IN-JT333-20: Static-renewal, acute, 48-hour EC₅₀ to *Daphnia magna*

DuPont Report No.: HL-1997-00006

Guidelines: OECD 202, EEC 92/69 Annex V - Method C.2 (1992), U.S. EPA 72-2 **Deviations:** None

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

Testing Facility Report No.: HL-1997-00006

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.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: IN-JT333 technical metabolite
 Lot/Batch #: JT333-20
 Purity: 98.7%
 Description: Beige solid
 CAS#: 144171-39-1
 Stability of test compound: Shown to be stable in the test system by analysis.
2. Control: Laboratory well water that had been filtered, aerated, and
 Solvent control: conditioned by flowing through a tank containing adult
 fathead minnows (DaDW).
 N,N-dimethylformamide (DMF)
 Test vehicle: DaDW
 Toxic reference: None
3. Test organism:
 Species: *Daphnia magna*
 Age at dosing: <24 hours
 Initial population: 5 daphnids per test chamber
 Source: Laboratory, in-house culture (Laboratory: Haskell
 Laboratory, Newark, Delaware)
 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)
 Dissolved oxygen 8.2 to 8.9 mg/L
 pH 7.0 to 8.7
 Temperature: 19.7 to 21.2°C
 Photoperiod: 16 hr photoperiod (369 to 421 lux) and 8 hr darkness which
 included 30 min transitional light (6 to 8 lux) preceding and
 following the 16-hr light interval

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
 03-December-1996 to 05-December-1996

2. Test system

The acute toxicity of IN-JT333, a sediment metabolite of DPX-MP062, to *Daphnia magna* was tested in a laboratory study. *Daphnia magna* neonates (less than 24 hours old) were randomly distributed into each of seven test concentration groups, a dilution water control group, and a 0.1 mL/L dimethylformamide control group (five daphnids/replicate, four replicates/test concentration and controls). *Daphnia magna* were exposed for 48 hours under static, unaerated conditions (mean temperature 20.2°C; 16 hour light/8 hour dark). Mean measured concentrations of IN-JT333 tested were 0.00012, 0.00032, 0.00055, 0.0012, 0.0024, 0.012, and 0.029 mg/L. The solubility of IN-JT333 in water is 0.036 mg/L (with 0.1 mL/L dimethylformamide [DMF]). The daphnids used in this test were collected from 12-day-old parent daphnids and were not fed during the test. The loading rate was 40 mL test solution per daphnid. Dissolved oxygen and pH were measured in both controls and in all test solutions at the beginning of the test, before the daphnids were added, and at the end of the test. Concentration of the test substance in the test solutions, including the water and DMF controls, was determined from samples taken at the beginning and at the end of the study. Observations of immobility were made every 24 hours.

II. RESULTS AND DISCUSSION

A. FINDINGS

There was no immobility observed in the controls and only 2/10 at the highest concentration of IN-JT333. Thus an LC₅₀ could not be calculated. The highest concentration was approximately the limit of solubility for IN-JT333 in the test medium. A summary of the findings is presented in Table 73.

Table 73
Summary of percentage observed immobility of *Daphnia magna* exposed to IN-JT333 in a static, unaerated test

Mean measured concentrations (mg IN-JT333/L)	24 Hours				48 Hours			
	1 ^a	2	3	4	1	2	3	4
Dilution water control	0	0	0	0	0	0	0	0
DMF control ^b	0	0	0	0	0	0	0	0
0.00012	0	0	0	0	0	0	0	0
0.00032	0	0	0	0	0	0	0	0
0.00055	0	0	0	0	0	0	0	0
0.0012	0	0	0	0	0	0	0	0
0.0024	0	0	0	0	0	0	0	0
0.012	0	0	0	0	0	0	0	0
0.029	0	0	0	0	0	0	0	20

^a 1, 2, 3, and 4 represent replicate test vessels containing 5 daphnids each at test start

^b Dimethylformamide

III. CONCLUSION

The 48-hour EC₅₀ of IN-JT333 with DMF solvent to *Daphnia magna* was much greater than 0.029 mg IN-JT333/L, the solubility limit with DMF. The highest concentration with no immobility was 0.012 mg IN-JT333/L.

(Hoke, R.A., 1997)

RMS comment

This study was submitted in the original DAR. This study was conducted in compliance with the current guideline. The 48-hour EC₅₀ of IN-JT333 (with DMF solvent) to *Daphnia magna* was > 0.029 mg IN-JT333/L based on mean measured concentrations. This study is still considered acceptable.

Report: Gaertner, K. (2013); IN-KB687: 48-Hour static, acute toxicity test with the Cladoceran, *Daphnia magna*

DuPont Report No.: DuPont-35831

Guidelines: OECD 202 (2004), OPPTS 850.1010 (1996) **Deviations:** None

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

Testing Facility Report No.: 69142

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 IN-KB687 to unfed *Daphnia magna* neonates, less than 24 hours old at test start, was determined in an unaerated, 48-hour, static test. The test was conducted in accordance with the OECD Guideline for the Testing of Chemicals No. 202, and U.S. EPA Ecological Effects Test Guidelines OPPTS 850.1010. Treatments consisted of a dilution water control and five nominal concentrations of 1.5, 3.0, 6.0, 12, and 24 mg IN-KB687/L. Mean measured concentrations (0 to 48 h) were <LOD, 1.66, 2.82, 5.79, 11.3, and 24.0 mg/L, respectively, and recovery ranged from 94 to 111% of nominal concentrations of IN-KB687. No sublethal effects were observed in any treatment in the study. The 48-hour EC₅₀ value was 7.83 mg IN-KB687/L (95% confidence intervals of 6.57 and 9.32 mg/L) based on mean measured concentrations and immobility. The lowest test treatment concentration causing 100% immobility at test end was 24.0 mg IN-KB687/L. The highest concentration causing 0% immobility at test end was 2.82 mg IN-KB687/L.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:	IN-KB687 technical metabolite
Lot/Batch #:	KB687-002
Purity:	99.8%
Description:	Solid, powder
CAS#:	177905-10-1
Stability of test compound:	Stable in the test system
2. Control:	Dilution (blended) water
Test solvent:	None
Toxic reference:	None
3. Test organism:	Cladoceran
Species:	<i>Daphnia magna</i>
Age at dosing:	Neonates (<24 hours old)
Weight at dosing:	NA
Initial population:	5 daphnids per test chamber/20 daphnids per treatment
Source:	In house culture
Acclimation period:	Continuous culture
Diet:	Test period: unfed
Test chamber:	250-mL glass container containing 200 mL of test solution (6.5-cm test solution depth), covered with a plastic lid
4. Test medium:	ABC blended freshwater
Environmental conditions (in-life period)	
Temperature:	19.1 to 20.1°C
Photoperiod:	16-h photoperiod (618 lux) and 8-h darkness which included 30 min transitional light preceding and following the 16-h light interval
Dissolved oxygen:	8.3 to 9.3 mg/L
pH:	8.3 to 8.6

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
26-February-2013 to 28-February-2013
2. Experimental treatments
The acute toxicity of IN-KB687 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 total

formulation concentrations of 1.5, 3.0, 6.0, 12, and 24 mg IN-KB687/L. Five daphnids were used per replicate with four replicates per test concentration and control.

3. Observations

Immobility and sublethal (behavioural) observations were made every 24 hours.

4. Statistics

Estimates of EC₅₀ values and their 95% confidence limits were calculated using the probit method or Untrimmed Spearman-Kärber method. The lowest concentration resulting in 100% immobility and highest concentration resulting in 0% immobility were assessed by visual observation.

II. RESULTS AND DISCUSSION

A. FINDINGS

Mean measured concentrations (0 to 48 h) were <LOD, 1.66, 2.82, 5.79, 11.3 and 24.0 mg/L respectively, and recovery ranged from 94 to 111% of nominal concentrations of IN-KB687. All validation criteria were met for the study. Summaries of observed immobility and sublethal effects are presented in Table 74 and Table 75, respectively.

Table 74
Observed immobility of the Cladoceran, *Daphnia magna*, exposed to IN-KB687 for 48 hours
in an unaerated, static, acute test

Nominal Concentration (mg/L)	Mean, measured IN-KB687 concentration (mg/L)	Cumulative immobility (Number immobile/Number at test start) ^a							
		24 Hours				48 Hours			
		A	B	C	D	A	B	C	D
Dilution water control	Dilution water control (ND) ^b	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
1.5	1.66	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
3.0	2.82	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
6.0	5.79	0/5	0/5	0/5	0/5	0/5	2/5	2/5	0/5
12	11.3	3/5	4/5	2/5	4/5	3/5	4/5	5/5	5/5
24	24.0	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5

^a A, B, C, and D represent replicates; each replicate contained 5 daphnids (total 20 daphnids per test concentration) at test start.

^b ND denotes not detected. At 0 hour, the limit of detection (LOD) for IN-KB687 was 0.00159 mg/L. The limit of quantitation (LOQ) was 0.00530 mg/L.

Table 75
Observed sublethal effects of the Cladoceran, *Daphnia magna*, exposed to IN-KB687 for 48 hours
in an unaerated, static, acute test

Nominal Concentration (mg/L)	Mean, measured IN-KB687 concentration (mg/L)	Sublethal Effects/Number Alive ^a							
		24 Hours				48 Hours			
		A	B	C	D	A	B	C	D
Dilution water control	Dilution water control (ND) ^b	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
1.5	1.66	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
3.0	2.82	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
6.0	5.79	0/5	0/5	0/5	0/5	0/5	0/3	0/3	0/5
12	11.3	0/2	0/1	0/3	0/1	0/2	0/1	0/0	0/0
24	24.0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0

^a A, B, C, and D represent replicates; each replicate contained 5 daphnids (total 20 daphnids per test concentration) at test start.

^b ND denotes not detected. At 0 hour, the limit of detection (LOD) for IN-KB687 was 0.00159 mg/L. The limit of quantitation (LOQ) was 0.00530 mg/L.

III. CONCLUSIONS

The 48-hour EC₅₀ value was 7.83 mg IN-KB687/L (95% confidence intervals of 9.32 and 6.57 mg/L) based on mean measured concentrations and immobility. The lowest test treatment concentration causing 100% immobility at test end was 24.0 mg IN-KB687/L. The highest concentration causing 0% immobility at test end was 2.82 mg IN-KB687/L.

(Gaertner, K., 2013)

RMS comment

This study was conducted in compliance with the current guideline. The 48-hour EC₅₀ value was 7.83 mg IN-KB687/L based on mean measured concentrations. This study is acceptable.

Report: Hoke, R.A. (1997); IN-KG433 technical: Static-renewal, acute, 48-hour limit test to *Daphnia magna*

DuPont Report No.: HLO-1997-00363

Guidelines: EEC Method C.2. (1992), OECD 202 (1984), USEPA 72-2 (1988) **Deviations:** None

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

Testing Facility Report No.: HLO-1997-00363

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 IN-KG433 technical to unfed *Daphnia magna* was determined in an unaerated, static-renewal, 48-hour limit test. The test was conducted in accordance with (i) the Organisation for Economic

Co-Operation and Development (OECD) Guideline for Testing Chemicals: 202; (ii) the European Economic Community 92/69 Annex V - Method C.2 (1992); and (iii) the United States Environmental Protection Agency Pesticide Assessment Guidelines Subdivision E, 72-2.

Treatments consisted of a dilution water control, a solvent control (0.1 mL/L N,N-dimethylformamide), and a nominal concentration of 0.300 mg IN-KG433/L. The corresponding mean measured concentration was 0.23 mg IN-KG433/L, essentially the limit of solubility for IN-KG433 in the test medium.

The test chambers were renewed with freshly prepared solutions at 24 hours. No immobility or sublethal effects were observed at the nominal 300 mg IN-KG433/L, or in the control groups, during the 48-hour limit test.

The 48-hour EC₅₀ for *Daphnia magna*, based on immobility and mean measured concentrations was >0.23 mg IN-KG433/L.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: IN-KG433 technical metabolite
 Lot/Batch #: KG433-3
 Purity: 98.0%
 CAS#: 526224-31-7
 Description: Off-white solid
 Stability of test compound: In a separate study, the stability of IN-KG433 was demonstrated in HEPES-buffered daphnid dilution water with recoveries >80% of nominal (0.2 mg/L) after 48 hours at 22°C and after 72 hours at 4°C. Analytical recovery of IN-KG433 in unbuffered Haskell well water was <70% of nominal (0.2 mg/L) after 24 hours at 22°C.
2. Control: Dilution (laboratory well water) water
 Solvent control: N,N-dimethylformamide (DMF, 0.1 mL/L in water buffered with HEPES)
 Test vehicle: Dilution (laboratory well water) water
 Toxic reference: None
3. Test organism: *Daphnia magna*
 Species: *Daphnia magna*
 Age at dosing: <24 hours
 Initial population: Five daphnids per test chamber
 Source: Laboratory, in-house culture (Haskell Laboratory, Newark, Delaware)
 Diet: Green algal species, *S. capricornutum* and *A. falcatus*
 Test period: Unfed
 Test chamber: 250-mL Pyrex beaker containing 200 mL of test solution (6.8-cm test solution depth), covered with a plexiglass plate
4. Environmental conditions (in-life period)
 Temperature: 20.1 to 20.8°C (of recirculating water-bath used to maintain test chamber temperature)
 Photoperiod: 16 hours light (1645- 1780 Lux) and 8 hours darkness, including 25 minutes of transitional light (~2 Lux) between the light and dark intervals.

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
 26-March-1997 to 28-March-1997

2. Experimental treatments

The acute toxicity of IN-KG433 to unfed *Daphnia magna* (<24 hours old) was determined in an unaerated, static-renewal, 48-hour limit test. Treatments consisted of a dilution water control, a 2.6 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES)-buffered solvent control (0.1 mL/L N,N-dimethylformamide, "DMF"), and a nominal concentration of 0.300 mg IN-KG433/L. The corresponding mean measured concentration was 0.23 mg IN-KG433/L, essentially the limit of solubility for IN-KG433 in the test medium. Each replicate (six per test substance concentration) contained five daphnids, for a total of 30 daphnids per test concentration. For the two controls, each replicate (four per control) contained five daphnids, for a total of 20 daphnids per control. The control and test solutions were renewed at 24 hours.

3. Observations

Immobility and sublethal (behavioural) observations were made every 24 hours.

4. Statistics

The EC₅₀ value was not statistically calculated as there was no adverse effect of 50% or greater on immobility. Therefore, the EC₅₀ value was estimated to be greater than the highest concentration tested.

II. RESULTS AND DISCUSSION

A. FINDINGS

The mean measured value of IN-KG433 was 0.23 mg/L, 77% of the targeted nominal concentration. The data indicated lower amounts of IN-KG433 after 24 hours. The mean measured value for the "old" test solutions from Day 1 and 2 were 67 and 73%, respectively. All measured values of IN-KG433 were within 1.5 times of the lowest value for all replicates within a concentration. These data indicate that IN-KG433 concentrations were maintained at acceptable levels throughout the definitive test. Control solutions showed no detectable levels of IN-KG433. All validation criteria were met for the study.

A summary of observed immobility is presented in the table shown below. No immobility or sublethal effects were observed at the nominal IN-KG433 concentration of 0.300 mg/L during the 48-hour limit test.

Table 76
Observed immobility of unfed *Daphnia magna* exposed to IN-KG433 for 48 hours in an unaerated, static-renewal, acute test

Mean, measured IN-KG433 concentration (mg/L)	Immobility (No. immobile/No. at test start) ^a											
	24 hours						48 hours					
	A	B	C	D	E	F	A	B	C	D	E	F
Dilution water control (0.0)	0/5	0/5	0/5	0/5	--- ^b	---	0/5	0/5	0/5	0/5	---	---
Solvent Control (0.0)	0/5	0/5	0/5	0/5	---	---	0/5	0/5	0/5	0/5	---	---
0.23	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5

^a A–F represent replicate test chambers containing five daphnids each at test start.

^b "---" = not applicable (the two controls had four replicates).

III. CONCLUSION

The 48-hour EC₅₀ based on immobility and mean measured concentrations was >0.23 mg IN-KG433/L, the highest concentration tested and the solubility limit in water. The highest concentration with no immobility was 0.23 mg IN-KG433/L.

(Hoke, R.A., 1997)

RMS comment

This study was conducted in compliance with the current guideline. The 48-hour EC₅₀ based on mean measured concentrations was >0.23 mg IN-KG433/L. This study is acceptable.

Report: Goudie, O.J. (2015); IN-KN124: 48-hour static-renewal, acute toxicity test with the cladoceran, *Daphnia magna*

DuPont Report No.: DuPont-43106

Guidelines: USEPA 850.1010 (1996), OECD 202 (2004) **Deviations:** None

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

Testing Facility Report No.: 82058

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 IN-KN124 to the cladoceran, *Daphnia magna*, was determined in a 48-hour static-renewal test. The test was conducted in accordance with U.S. EPA (OPPTS 850.1010) Guideline and the OECD Guidelines for the Testing of Chemicals: Guideline No. 202. The study was conducted with nominal concentrations of 0.013, 0.025, 0.050, 0.10, and 0.20 mg IN-KN124/L, a vehicle control, and a dilution water control at a temperature range of 20.0 to 20.7°C. Four replicates with five daphnids per replicate were used for each of the test substance concentrations and controls. Exposure of *Daphnia magna* to the dilution water control, vehicle control, and nominal concentrations of 0.013, 0.025, 0.050, 0.10, and 0.20 mg IN-KN124/L resulted in 0% immobility at the end of 48 hours. Mean measured concentrations (0 to 48 hours) of IN-KN124 were <LOQ (control), <LOQ (vehicle control), 0.00923, 0.0169, 0.0304, 0.0551, and 0.106 mg IN-KN124/L, and ranged from 53 to 71% of the nominal concentrations of IN-KN124. No sublethal effects were observed in any treatment in the study. The 48-hour EC₅₀ value, determined from immobility data, was >0.106 mg IN-KN124/L with no calculable estimates of 95% confidence limits, based on mean measured concentrations. The lowest mean measured concentration causing 100% immobility at test end was >0.106 mg IN-KN124/L. The highest mean measured concentration causing 0% immobility at test end was 0.106 mg IN-KN124/L.

I. MATERIALS AND METHODS**A. MATERIALS**

- | | |
|-----------------------------|---|
| 1. Test material: | IN-KN124 technical metabolite |
| Lot/Batch #: | KN124-001 |
| Purity: | 99.8% |
| Description: | Solid |
| CAS#: | 200568-73-6 |
| Stability of test compound: | Determined to be unstable in the test system |
| 2. Control: | Dilution (blended) water |
| Test solvent: | Dimethylformamide (DMF) |
| Toxic reference: | None |
| 3. Test organism: | Cladoceran |
| Species: | <i>Daphnia magna</i> |
| Age at dosing: | Neonates (<24 hours old) |
| Weight at dosing: | NA |
| Initial population: | 5 daphnids per test chamber/20 daphnids per treatment |

Source:	In house culture
Acclimation period:	Continuous culture
Diet:	Test period: unfed
Test chamber:	250-mL glass container containing ~200 mL of test solution (6.5-cm test solution depth), covered with a plastic Petri dish
Test medium:	ABC blended freshwater
4. Environmental conditions (in-life period)	
Temperature:	20.0 to 20.7°C
Photoperiod:	16 hr photoperiod (643 lux) and 8 hr darkness which included 30 min transitional light preceding and following the 16-hr light interval
Dissolved oxygen:	8.3 to 8.8 mg/L (95 to 101% saturation)
pH:	6.8 to 7.4

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed

08-July-2015 to 10-July-2015

2. Experimental treatments

The acute toxicity of IN-KN124 to unfed *Daphnia magna* (<24-hour old) was determined in an unaerated, static-renewal, 48-hour test. Daphnids were from the 19th brood of at least 25 day-old parents. Treatments consisted of a dilution water control, vehicle control, and five nominal concentrations of 0.013, 0.025, 0.050, 0.10, and 0.20 mg IN-KN124/L. Five daphnids were used per replicate with four replicates per test concentration, vehicle control, and control.

3. Observations

Immobility and sublethal (behavioral) observations were made every 24 hours.

4. Statistics

No immobility or other adverse effects were observed in any of the test substance treatments and controls. Therefore, statistical analyses were not performed. The EC₅₀ value was reported as greater than the highest mean measured treatment concentration.

II. RESULTS AND DISCUSSION

A. FINDINGS

Mean measured concentrations (0 to 48 hours) were <LOD (control), <LOD (vehicle control), 0.00923, 0.0169, 0.0304, 0.0551, and 0.106 mg IN-KN124/L, and ranged from 53 to 71% of the nominal concentrations of IN-KN124. No sublethal effects were observed in any treatment in the study. Summaries of observed immobility and sublethal effects are presented in Table 77 and Table 78, respectively.

Table 77
Observed immobility of the Cladoceran, *Daphnia magna*, exposed to IN-KN124 for 48 hours in an unaerated, static-renewal, acute test

Mean Measured IN-KN124 Concentration (mg IN-KN124/L)	Cumulative immobility ^a (Number immobile/Number at test start ^b)								Mean % Immobile (after 48-hours)
	24 Hours				48 Hours				
	A	B	C	D	A	B	C	D	
0 (Control)	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0
0 (Vehicle Control)	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0
0.00923	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0
0.0169	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0
0.0304	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0
0.0551	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0
0.106	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0

^a Immobile. No observed movement of appendages or postabdomen within 15 seconds after gentle agitation of the test chamber or gentle disturbance of the daphnid itself. Affected numbers are cumulative.

^b Replicate test chambers contained 5 daphnids each for a total of 20 per concentration at test initiation.

Table 78
Observed sublethal effects of the Cladoceran, *Daphnia magna*, exposed to IN-KN124 for 48 hours in an unaerated, static-renewal, acute test

Mean Measured IN-KN124 Concentration (mg IN-KN124/L)	Sublethal Effects/Number Alive ^a							
	24 Hours				48 Hours			
	A	B	C	D	A	B	C	D
0 (Control)	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
0 (Vehicle Control)	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
0.00923	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
0.0169	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
0.0304	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
0.0551	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
0.106	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5

^a Replicate test chambers contained 5 daphnids each for a total of 20 per concentration at test initiation.

III. CONCLUSION

The 48-hour EC₅₀ value, based on immobility, was >0.106 mg IN-KN124/L with no calculable estimates of 95% confidence limits. The lowest mean measured concentration causing 100% immobility at test end was >0.106 mg IN-KN124/L. The highest mean measured concentration causing 0% immobility at test end was 0.106 mg IN-KN124/L.

(Goudie, O.J., 2015)

RMS comment

This study was conducted in compliance with the current guideline. The 48-hour EC₅₀ value was >0.106 mg IN-KN124/L based on mean measured concentrations. This study is acceptable.

Report: Mays, C. (2013); IN-KN125: 48-hour static-renewal, acute toxicity test with the cladoceran, *Daphnia magna*

DuPont Report No.: DuPont-43105

Guidelines: USEPA 850.1010 (1996), OECD 202 (2004) **Deviations:** None

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

Testing Facility Report No.: 82061

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 IN-KN125 to the cladoceran, *Daphnia magna*, was determined in a 48-hour static-renewal test. The test was conducted in accordance with U.S. EPA (OPPTS 850.1010) Guideline and the OECD Guidelines for the Testing of Chemicals: Guideline No. 202. The study was conducted with nominal concentrations of 0.013, 0.025, 0.050, 0.10, and 0.20 mg IN-KN125/L, a vehicle control, and a dilution water control at a temperature range of 19.2 to 20.5°C. Four replicates with five daphnids per replicate were used for each of the test substance concentrations and controls. Exposure of *Daphnia magna* to the dilution water control, vehicle control, and nominal concentrations of 0.013, 0.025, 0.050, 0.10, and 0.20 mg IN-KN125/L resulted in 0, 0, 0, 35, 20, and 40% immobility at the end of 48 hours. Arithmetic mean measured concentrations (0 to 48 hours) of IN-KN125 were <LOQ (control), <LOQ (control), 0.00961, 0.0190, 0.0329, 0.0526, and 0.121 mg IN-KN125/L, and ranged from 53 to 76% of the nominal concentrations of IN-KN125. No sublethal effects were observed in any treatment in the study. The 48-hour EC₅₀ value, determined from immobility data, was >0.121 mg IN-KN125/L, the highest concentration tested, with 95% confidence limits of 0.0826 and 0.611 mg IN-KN125/L, based on arithmetic mean measured concentrations. There was no arithmetic mean measured concentration causing 100% immobility at test end. The highest arithmetic mean measured concentration causing 0% immobility at test end was 0.0190 mg IN-KN125/L.

I. MATERIALS AND METHODS

A. MATERIALS

- | | |
|-----------------------------|---|
| 1. Test material: | IN-KN125 technical metabolite |
| Lot/Batch #: | KN125-005 |
| Purity: | 99.4% |
| Description: | Solid |
| CAS#: | 200568-74-7 |
| Stability of test compound: | Determined to be unstable in the test system |
| 2. Control: | Dilution (blended) water |
| Test solvent: | Dimethylformamide (DMF) |
| Toxic reference: | None |
| 3. Test organism: | Cladoceran |
| Species: | <i>Daphnia magna</i> |
| Age at dosing: | Neonates (<24 hours old) |
| Weight at dosing: | NA |
| Initial population: | 5 daphnids per test chamber/20 daphnids per treatment |
| Source: | In house culture |
| Acclimation period: | Continuous culture |
| Diet: | Test period: unfed |

Test chamber:	250-mL glass container containing ~200 mL of test solution (6.5-cm test solution depth), covered with a plastic Petri dish
4. Test medium:	ABC blended freshwater
Environmental conditions (in-life period)	
Temperature:	19.2 to 20.5°C
Photoperiod:	16 hr photoperiod (684 lux) and 8 hr darkness which included 30 min transitional light preceding and following the 16-hr light interval
Dissolved oxygen:	8.1 to 9.9 mg/L (95 to 111% saturation)
pH:	6.5 to 7.3

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
02-July-2015 to 04-July-2015
2. Experimental treatments
The acute toxicity of IN-KN125 to unfed *Daphnia magna* (<24-hour old) was determined in an unaerated, static-renewal, 48-hour test. Daphnids were from at least the 7th brood of at least 18 day-old parents. Treatments consisted of a dilution water control, vehicle control, and five nominal concentrations of 0.013, 0.025, 0.050, 0.10, and 0.20 mg IN-KN125/L. Five daphnids were used per replicate with four replicates per test concentration and control.
3. Observations
Immobility and sublethal (behavioural) observations were made every 24 hours.
4. Statistics
All statistical analyses were performed with SAS software and ECOSTATS (DuPont Ver V9X64 2013.01.01). Estimates of EC₅₀ values and their 95% confidence limits were calculated using the probit method.

II. RESULTS AND DISCUSSION

A. FINDINGS¹⁵

Arithmetic mean measured concentrations (0 to 48 hours) were <LOD (control), <LOD (control), 0.00961, 0.0190, 0.0329, 0.0526, and 0.121 mg IN-KN125/L, and ranged from 53 to 76% of the nominal concentrations of IN-KN125. No sublethal effects were observed in any treatment in the study. Summaries of observed immobility and sublethal effects are presented in Table 79 and Table 80, respectively.

Table 79
Observed immobility of the Cladoceran, *Daphnia magna*, exposed to IN-KN125 for 48 hours in an unaerated, static-renewal, acute test

Arithmetic mean Measured IN-KN125 Concentration (mg IN-KN125/L)	Cumulative immobility ^a (Number immobile/Number at test start ^b)								Mean % Immobile (after 48-hours)
	24 Hours				48 Hours				
	A	B	C	D	A	B	C	D	
0 (Control)	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0
0 (Vehicle Control)	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0
0.00961	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0
0.0190	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0
0.0329	1/5	2/5	2/5	0/5	2/5	3/5	2/5	0/5	35
0.0526	0/5	0/5	0/5	0/5	1/5	2/5	0/5	1/5	20
0.121	0/5	0/5	0/5	0/5	2/5	3/5	2/5	1/5	40

^a Immobile. No observed movement of appendages or postabdomen within 15 seconds after gentle agitation of the test chamber or gentle disturbance of the daphnid itself. Affected numbers are cumulative.

^b Replicate test chambers contained 5 daphnids each for a total of 20 per concentration at test initiation.

Table 80
Observed sublethal effects of the Cladoceran, *Daphnia magna*, exposed to IN-KN125 for 48 hours in an unaerated, static-renewal, acute test

Mean Measured IN-KN125 Concentration (mg IN-KN125/L)	Sublethal Effects /Number Alive ^a							
	24 Hours				48 Hours			
	A	B	C	D	A	B	C	D
0 (Control)	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
0 (Vehicle Control)	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
0.00961	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
0.0190	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
0.0329	0/4	0/3	0/3	0/5	0/3	0/2	0/3	0/5
0.0526	0/5	0/5	0/5	0/5	0/4	0/3	0/5	0/4
0.121	0/5	0/5	0/5	0/5	0/3	0/2	0/3	0/4

^a Replicate test chambers contained 5 daphnids each for a total of 20 per concentration at test initiation.

III. CONCLUSION

The 48-hour EC₅₀ value, determined from immobility data, was >0.121 mg IN-KN125/L, the highest concentration tested, with 95% confidence limits of 0.0826 and 0.0611 mg IN-KN125/L. There was no arithmetic mean measured concentration causing 100% immobility at test end. The highest arithmetic mean measured concentration causing 0% immobility at test end was 0.0190 mg IN-KN125/L.

(Mays, C., 2015)

RMS comment

This study was conducted in compliance with the current guideline. The 48-hour EC₅₀ value was >0.121 mg IN-KN125/L based on mean measured concentrations. This study is acceptable.

Report: Boeri, R.L., Magazu, J.P., Ward, T.J. (1999); IN-KT413, a metabolite of DPX-MP062: Acute, static, 48-hour toxicity (EC₅₀) test to *Daphnia magna*

DuPont Report No.: DuPont-1309

Guidelines: OECD 202, EEC 92/69 Annex V - Method C.2 (1992), USEPA 72-2 **Deviations:** None

Testing Facility: T.R. Wilbury Laboratories, Inc., Marblehead, Massachusetts, USA

Testing Facility Report No.: 1663-DU

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.

I. MATERIALS AND METHODS

A. MATERIALS

- | | |
|--|---|
| 1. Test material: | IN-KT413 technical metabolite |
| Lot/Batch #: | KT413-3 |
| Purity: | 99.87% |
| Description: | Solid |
| CAS#: | Not available |
| 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: | 10 daphnids per test chamber |
| Source: | Laboratory, in-house culture (Laboratory: T.R. Wilbury Laboratories, Marblehead, Massachusetts) |
| Diet: | Unfed during test |
| Test chamber: | 300-mL Pyrex beaker containing 250 mL of test solution (9-cm test solution depth), covered with a glass plate or plastic sheet |
| 4. Environmental conditions (in-life period) | |
| Dissolved oxygen | 8.7 to 9.1 mg/L |
| pH | 7.5 to 8.0 |
| Temperature: | 19.2 to 20.7°C (of recirculating waterbath used to maintain test chamber temperature) |
| Photoperiod: | 16 hr photoperiod (approximately 398 lux) and 8 hr darkness which included 15 min transitional light preceding and following the 16-hr light interval |

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
04-November-1998 to 24-December-1998

2. Experimental treatments

The acute toxicity of IN-KT413, a water metabolite of DPX-MP062, to less than 24-hour old *Daphnia magna* was determined in an unaerated, 48-hour static test. Treatments consisted of a dilution water control and mean measured concentrations of 0.13, 0.22, 0.36, 0.59, and 0.967 mg IN-KT413/L. The highest dose level was selected to exceed 100 times the predicted environmental concentrations in surface water. Ten daphnids were used per replicate with two replicates per test concentration and control. Observations of immobility were made every 24 hours. Water solubility of IN-KT413 is approximately 50 to 100 g/L.

II. RESULTS AND DISCUSSION

A. FINDINGS

A summary of the findings is presented in Table 81. There were no dosage-related immobility or sublethal effects at doses below 0.967 mg IN-KT413/L. The mean observed immobility was 15% at 0.967 mg IN-KT413/L. The highest dosage causing no immobility was 0.59 mg IN-KT413/L. Dosages used in this study did not elicit 100% immobility.

Table 81
Summary of percentage observed immobility and sublethal effects of *Daphnia magna* exposed to IN-KT413 in an unaerated 48-hour flow-through test

Mean measured concentrations (mg IN-KT413/L)	Cumulative immobility and sublethal effect at 24 hours (%)		Cumulative immobility and sublethal effects at 48 hours (%)	
	Replicate Number		Replicate Number	
	1 ^a	2 ^a	1 ^a	2 ^a
Dilution Water Control	0	0	0	0
0.13	0	0	0	0
0.22	0	0	0	0
0.36	0	0	0	0
0.59	0	0	0	0
0.967	0	0	30	0

^a 5 *Daphnia* per replicate with 10 *Daphnia* per concentration

III. CONCLUSION

The 48-hour EC₅₀ of IN-KT413 to *Daphnia magna* was >0.967 mg IN-KT413/L. The highest concentration with no immobility was 0.967 mg IN-KT413/L.

(Boeri, R. L., Magazu, J. P., Ward, T. J., 1999)

RMS comment

This study was submitted in the DAR (AD1 2001). This study was conducted in compliance with the current guideline. It was stated in the addendum I that the actual concentrations were measured only at the beginning. RMS checked the study report and found that measures were also available at the end of the experiment showing that the concentration was maintained. The 48-hour EC₅₀ of IN-KT413 to *Daphnia magna* was >0.967 mg IN-KT413/L, based on mean measured concentrations. This study is still considered acceptable.

Report: Holou, M. (2013); IN-MK638: 48-Hour static, acute toxicity test with the Cladoceran, *Daphnia magna*

DuPont Report No.: DuPont-35830

Guidelines: OECD 202 (2004), OPPTS 850.1010 (1996) **Deviations:** None

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

Testing Facility Report No.: 69139

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 IN-MK638 to unfed *Daphnia magna* neonates, less than 24 hours old at test start, was determined in an unaerated, 48-hour, static test. The test was conducted in accordance with the OECD Guideline for the Testing of Chemicals No. 202, and U.S. EPA Ecological Effects Test Guidelines OPPTS 850.1010. Treatments consisted of a dilution water control and five nominal concentrations of 6.3, 13, 25, 50, and 100 mg IN-MK638/L. Mean measured concentrations of IN-MK638 were 6.17, 13.3, 25.5, 51.9, and 105 mg/L and ranged from 98 to 105% of nominal active substance concentrations. The 48-hour EC₅₀ value was 80.0 mg IN-MK638/L (95% confidence intervals of 69.6 and 92.2 mg/L) based on mean measured concentrations and immobility. None of the test treatment concentrations caused 100% immobility at test end. The highest concentration causing 0% immobility at test end was 6.17 mg IN-MK638/L.

I. MATERIALS AND METHODS

A. MATERIALS

- | | |
|---|---|
| 1. Test material: | IN-MK638 technical metabolite |
| Lot/Batch #: | MK638-002 |
| Purity: | 99.9% |
| Description: | Solid, Crystalline |
| CAS#: | 82971-90-2 |
| Stability of test compound: | Stable up to 96 Hours |
| 2. Control: | Dilution (blended) water |
| Test solvent: | None |
| Toxic reference: | None |
| 3. Test organism: | Cladoceran |
| Species: | <i>Daphnia magna</i> |
| Age at dosing: | Neonates (<24 hours old) |
| Weight at dosing: | NA |
| Initial population: | 5 daphnids per test chamber/20 daphnids per treatment |
| Source: | In house culture |
| Acclimation period: | Continuous culture |
| Diet: | Test period: unfed |
| Test chamber: | 250-mL glass container containing 200 mL of test solution (6.5-cm test solution depth), covered with a plastic lid |
| 4. Test medium: | ABC blended freshwater |
| Environmental conditions (in-life period) | |
| Temperature: | 19.4 to 19.9°C |
| Photoperiod: | 16 hr photoperiod (667 lux) and 8 hr darkness which included 30 min transitional light preceding and following the 16-hr light interval |
| Dissolved oxygen: | 8.2 to 9.1 mg/L |
| pH: | 8.1 to 8.6 |

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed

26-February-2013 to 28-February-2013

2. Experimental treatments

The acute toxicity of IN-MK638 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 6.3, 13, 25, 50, and 100 mg IN-MK638/L. Five daphnids were used per replicate with four replicates per test concentration and control.

3. Observations

Immobility and sublethal (behavioural) observations were made every 24 hours.

4. Statistics

Estimates of EC₅₀ values and their 95% confidence limits were calculated using the Trimmed Spearman-Kärber method. The lowest concentration resulting in 100% immobility and highest concentration resulting in 0% immobility were assessed by visual observation.

II. RESULTS AND DISCUSSION

A. FINDINGS

Mean, measured concentrations of IN-MK638 were 6.17, 13.3, 25.5, 51.9, and 105 mg/L and ranged from 98 to 105% of nominal active substance concentrations. All validation criteria were met for the study. Summaries of observed immobility and sublethal effects are presented in Table 82 and Table 83, respectively.

Table 82
Observed immobility of the Cladoceran, *Daphnia magna*, exposed to IN-MK638 for 48 hours in an unaerated, static, acute test

Nominal Concentration (mg/L)	Mean, measured IN-MK638 concentration (mg/L)	Cumulative immobility (Number immobile/Number at test start) ^a							
		24 Hours				48 Hours			
		A	B	C	D	A	B	C	D
Dilution water control	<LOD, LOQ ^b	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
6.3	6.17	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
13	13.3	0/5	0/5	0/5	1/5	0/5	0/5	0/5	1/5
25	25.5	0/5	0/5	0/5	1/5	0/5	0/5	0/5	1/5
50	51.9	0/5	0/5	0/5	0/5	1/5	1/5	0/5	0/5
100	105	0/5	0/5	3/5	2/5	3/5	3/5	4/5	5/5

^a A, B, C, and D represent replicates; each replicate contained 5 daphnids (total 20 daphnids per test concentration) at test start.

^b The limit of detection (LOD) for IN-MK638 was 0.00316 mg/L throughout the course of the study. The limit of quantitation (LOQ) was 0.0105 mg/L.

Table 83
Observed sublethal effects of the Cladoceran, *Daphnia magna*, exposed to IN-MK638 for 48 hours in an unaerated, static, acute test

Nominal Concentration (mg/L)	Mean, measured IN-MK638 concentration (mg/L)	Sublethal Effects/Number Alive ^a							
		24 Hours				48 Hours			
		A	B	C	D	A	B	C	D
Dilution water control	<LOD, LOQ ^b	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
6.3	6.17	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
13	13.3	0/5	0/5	0/5	0/4	0/5	0/5	0/5	0/4
25	25.5	0/5	0/5	0/5	0/4	0/5	0/5	0/5	0/4
50	51.9	0/5	0/5	0/5	0/5	0/4	0/4	0/5	0/5
100	105	0/5	0/5	0/2	0/3	0/2	0/2	0/1	0/0

^a Replicate test chambers contained 5 daphnids each for a total of 20 per concentration at test initiation.

^b The limit of detection (LOD) for IN-MK638 was 0.00316 mg/L throughout the course of the study. The limit of quantitation (LOQ) was 0.0105 mg/L.

III. CONCLUSIONS

The 48-hour EC₅₀ value was 80.0 mg IN-MK638/L (95% confidence intervals of 69.6 and 92.2 mg/L) based on mean measured concentrations and immobility. None of the test treatment concentrations caused 100% immobility at test end. The highest concentration causing 0% immobility at test end was 6.17 mg IN-MK638/L.

(Holou, M., 2013)

RMS comment

This study was conducted in compliance with the current guideline. The 48-hour EC₅₀ value was 80.0 mg IN-MK638/L based on mean measured concentrations. This study is acceptable.

Report: Bergfield, A. (2013); IN-MK643: 48-Hour static, acute toxicity test with the Cladoceran, *Daphnia magna*

DuPont Report No.: DuPont-36163

Guidelines: OECD 202 (2004), OPPTS 850.1010 (1996) **Deviations:** None

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

Testing Facility Report No.: 69278

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:

The acute toxicity of IN-MK643 to unfed *Daphnia magna* neonates, less than 24 hours old at test start, was determined in an unaerated, 48-hour, static test. Daphnids were from at least the 11th brood of at least 21 day-old parents. The test was conducted in accordance with the OECD Guideline for the Testing of Chemicals No. 202,

and U.S. EPA Ecological Effects Test Guidelines OPPTS 850.1010. Treatments consisted of a dilution water control and five nominal concentrations of 6.3, 13, 25, 50, and 100 mg IN-MK643/L. Mean measured concentrations (0 to 48 hours) were <LOD (control), 6.16, 12.7, 24.8, 49.1, and 96.5 mg/L, and ranged from 97 to 99% of the nominal concentrations of IN-MK643. No sublethal effects were observed in any treatment in the study. The 48-hour EC₅₀ value based on mean measured concentrations of IN-MK643 and immobility was 34.1 mg/L, with lower and upper 95% confidence limits of 32.3 and 36.0 mg/L, respectively. The lowest concentration to cause 100% immobility at test end was 49.1 mg IN-MK643/L. The highest concentration causing 0% immobility at test end was 12.7 mg IN-MK643/L.

I. MATERIALS AND METHODS

A. MATERIALS

- | | |
|---|---|
| 1. Test material: | IN-MK643 technical metabolite |
| Lot/Batch #: | MK643-002 |
| Purity: | 96.7% |
| Description: | Solid powder |
| CAS#: | 877681-12-4 |
| Stability of test compound: | Determined to be stable in the test system |
| 2. Control: | Dilution (blended) water |
| Test solvent: | None |
| Toxic reference: | None |
| 3. Test organism: | Cladoceran |
| Species: | <i>Daphnia magna</i> |
| Age at dosing: | Neonates (<24 hours old) |
| Weight at dosing: | NA |
| Initial population: | 5 daphnids per test chamber/20 daphnids per treatment |
| Source: | In house culture |
| Acclimation period: | Continuous culture |
| Diet: | Test period: unfed |
| Test chamber: | 125-mL glass container containing ~80 mL of test solution (21-cm test solution depth), covered with a plastic Petri dish |
| 4. Test medium: | ABC blended freshwater |
| Environmental conditions (in-life period) | |
| Temperature: | 19.1 to 19.6°C |
| Photoperiod: | 16 hr photoperiod (652 lux) and 8 hr darkness which included 30 min transitional light preceding and following the 16-hr light interval |
| Dissolved oxygen: | 8.1 to 8.8 mg/L |
| pH: | 8.3 to 8.7 |

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
20-February-2013 to 22-February-2013
2. Experimental treatments
The acute toxicity of IN-MK643 to unfed *Daphnia magna* (<24-hour old) was determined in an unaerated, static, 48-hour test. Daphnids were from at least the 11th brood of at least 21 day-old parents. Treatments consisted of a dilution water control and five nominal concentrations of 6.3, 13, 25, 50, and 100 mg IN-MK643/L. Five daphnids were used per replicate with four replicates per test concentration and control.
3. Observations
Immobility and sublethal (behavioural) observations were made every 24 hours.

4. Statistics

Estimates of EC₅₀ values and their 95% confidence limits were calculated using the Trimmed Spearman-Kärber method. The lowest concentration resulting in 100% immobility and highest concentration resulting in 0% immobility were assessed by visual observation.

II. RESULTS AND DISCUSSION

A. FINDINGS

Mean measured concentrations (0 to 48 hours) were <LOD (control), 6.16, 12.7, 24.8, 49.1, and 96.5 mg/L, and ranged from 97 to 99% of the nominal concentrations of IN-MK643. No sublethal effects were observed in any treatment in the study. Summaries of observed immobility and sublethal effects are presented in Table 84 and Table 85, respectively.

Table 84
Observed immobility of the Cladoceran, *Daphnia magna*, exposed to IN-MK643 for 48 hours in an unaerated, static, acute test

Mean Measured IN-MK643 Concentration (mg/L)	Cumulative immobility (Number immobile/Number at test start) ^{a,b}								Mean % Immobile (after 48-hours)
	24 Hours				48 Hours				
	24 Hours	48 Hours	24 Hours	48 Hours	24 Hours	48 Hours	24 Hours	48 Hours	
0 (Control)	0/5	0/5	0/5	0/5	1/5	0/5	0/5	0/5	5
6.16	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0
12.7	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0
24.8	0/5	0/5	0/5	1/5	0/5	0/5	0/5	1/5	5
49.1	4/5	4/5	4/5	3/5	5/5	5/5	5/5	5/5	100
96.5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	100

^a Immobile. No observed movement of appendages or postabdomen within 15 seconds after gentle agitation of the test chamber or gentle disturbance of the daphnid itself. Affected numbers are cumulative.

^b Replicate test chambers contained 5 daphnids each for a total of 20 per concentration at test initiation.

Table 85
Observed sublethal effects of the Cladoceran, *Daphnia magna*, exposed to IN-MK643 for 48 hours in an unaerated, static, acute test

Mean Measured IN-MK643 Concentration (mg/L)	Sublethal Effects/Number Alive ^a							
	24 Hours				48 Hours			
	A	B	C	D	A	B	C	D
Dilution water control	0/5	0/5	0/5	0/5	0/4	0/5	0/5	0/5
6.16	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
12.7	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
24.8	0/5	0/5	0/5	0/4	0/5	0/5	0/5	0/4
49.1	0/1	0/1	0/1	0/2	0/0	0/0	0/0	0/0
96.5	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0

^a Replicate test chambers contained 5 daphnids each for a total of 20 per concentration at test initiation.

III. CONCLUSIONS

The 48-hour EC₅₀ value based on mean measured concentrations of IN-MK643 and immobility was 34.1 mg/L, with lower and upper 95% confidence limits of 32.3 and 36.0 mg/L, respectively. The lowest concentration to cause 100% immobility at test end was 49.1 mg IN-MK643/L. The highest concentration causing 0% immobility at test end was 12.7 mg IN-MK643/L.

(Bergfield, A., 2013)

RMS comment

This study was conducted in compliance with the current guideline. The 48-hour EC₅₀ value based on mean measured concentrations of IN-MK643 was 34.1 mg/L. This study is acceptable.

Report: Samel, A. (2003a); IN-MP819: Flow-through, acute, 48-hour EC₅₀ to *Daphnia magna*

DuPont Report No.: DuPont-11491

Guidelines: OECD 202, EEC 92/69 Annex V - Method C.2 (1992), USEPA 72-2 **Deviations:** None

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

Testing Facility Report No.: DuPont-11491

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.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: IN-MP819 technical metabolite
 Lot/Batch #: MP819-002
 Purity: 98.6%
 Description: Light yellow solid
 CAS#: Not available
 Stability of test compound: Shown to be stable in the test system by analysis.
2. Control: Aerated, filtered well water with hardness adjusted with CaCl_2 .
 Solvent control: N,N-dimethylformamide (DMF)
 Test vehicle: Aerated, filtered well water with hardness adjusted with CaCl_2 .
 Toxic reference: None
3. Test organism: *Daphnia magna*
 Species: *Daphnia magna*
 Age at dosing: <24 hours
 Initial population: 10 daphnids per test chamber
 Source: Laboratory, in-house culture (Haskell Laboratory, Newark, Delaware)
 Diet: Unfed during test
 Test chamber: 1.5-L Pyrex[®] beaker containing 1000 mL of test solution (13.5- to 14-cm test solution depth) with a side port covered with Nitex[®] netting.
4. Environmental conditions (in-life period)
 Dissolved oxygen: 8.7 to 8.9 mg/L
 pH: 7.7 to 7.9
 Temperature: 19.8 to 20.0°C (of recirculating waterbath used to maintain test chamber temperature)
 Photoperiod: 16 hr photoperiod (350 to 523 lux) and 8 hr darkness which included 30 min transitional light (100 to 155 lux) preceding and following the 16-hr light interval

B. STUDY DESIGN AND METHODS

1. Study initiated/completed
 28-February-2003 to 22-May-2003
2. Experimental treatments
 The acute toxicity of IN-MP819 to unfed *Daphnia magna* (<24-hour old) was determined in an unaerated, flow-through, 48-hour test. Treatments consisted of a dilution water control, dimethylformamide (DMF) control, and nominal concentrations of 0.022, 0.044, 0.088, 0.175, and 0.350 mg IN-MP819/L. Ten daphnids were used per replicate with two replicates per test concentration and control. Water solubility of IN-MP819 is 0.303 to 0.514 mg/L in 0.1 mL/L DMF.

II. RESULTS AND DISCUSSION

A. FINDINGS

A summary of the findings is presented in Table 86. Mean measured concentrations of IN-MP819 in 0.1 mL/L DMF solvent were 0.021, 0.039, 0.071, 0.138, and 0.311 mg IN-MP819/L and ranged from 80 to 95% of nominal concentrations. No immobility was observed in the dilution water and DMF control daphnids. The highest mean measured concentration causing no immobility at test end was less than 0.021 mg IN-MP819/L. The lowest mean measured concentration causing 100% immobility at test end was 0.311 mg IN-MP819/L. Mean measured IN-MP819 concentrations were used for calculation of EC_{50} .

values by moving average angle method (24-hr EC₅₀) or probit analysis (48-hr EC₅₀). The 48-hour EC₅₀, based on mean measured concentrations of IN-MP819 and immobility, was 0.06 mg/L.

Table 86
Summary of observed immobility and sublethal effects of unfed *Daphnia magna* exposed to IN-MP819 for 48 hours in an unaerated, flow-through, acute test

Mean measured concentrations of IN-MP819 (mg/L)	Immobility (no. immobile/no. at test start)			
	24 Hours		48 Hours	
	A ^c	B ^c	A	B
Dilution water control	0/10	0 ^{3a} /10	0/10	0/10
DMF control	0 ^{5a} /10	0 ^{3a} /10	0/10	0 ^{1a} /10
0.021	4/10	0/10	4/10	0/10
0.039	1/10	0/10	2 ^{2b} /10	0 ^{1a} /10
0.071	3 ^{2b} /10	1 ^{3b} /10	6 ^{4b} /10	9 ^{1b} /10
0.138	3 ^{3b} /10	1 ^{6b} /10	6 ^{4b} /10	9 ^{1b} /10
0.311	5 ^{5b} /10	7 ^{3b} /10	10/10	10/10

^a Daphnids floating at surface, superscript numbers indicate the number of daphnids with this sublethal effect.

^b Daphnids lethargic, superscript numbers indicate the number of daphnids with this sublethal effect.

^c A and B represent replicate test vessels containing 10 daphnids.

III. CONCLUSION

The 48-hour EC₅₀ of IN-MP819 with 0.1 mL/LDMF solvent to *Daphnia magna* was 0.06 mg IN-MP819/L based on mean measured test concentration and immobility. The highest concentration with no mortalities was <0.021 mg IN-MP819/L, based on immobility.

(Samel, A., 2003a)

RMS comment

This study was submitted in the original DAR. This study was conducted in compliance with the current guideline. The 48-hour EC₅₀ of IN-MP819 (with 0.1 mL/LDMF solvent) to *Daphnia magna* was 0.06 mg IN-MP819/L based on mean measured test concentration. This study is still considered acceptable.

Report: Samel, A. (2003b); IN-MS775: Static, acute, 48-hour EC₅₀ to *Daphnia magna*

DuPont Report No.: DuPont-12090

Guidelines: OECD 202, EEC 92/69 Annex V - Method C.2 (1992), USEPA 72-2 **Deviations:** None

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

Testing Facility Report No.: DuPont-12090

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.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: IN-MS775 technical metabolite
 Lot/Batch #: MS775-002
 Purity: 99.7%
 Description: Light yellow solid
 CAS#: Not available
 Stability of test compound: Shown to be stable in the test system by analysis
2. Control: Dilution (laboratory well water that had been filtered, aerated,
 and hardness adjusted with CaCl₂) water
 Solvent control: N,N-dimethylformamide
 Test vehicle: Dilution (laboratory well water that had been filtered, aerated,
 and hardness adjusted with CaCl₂) water
 Toxic reference: None
3. Test organism: *Daphnia magna*
 Species: <24 hours
 Age at dosing: 5 daphnids per test chamber
 Initial population: Laboratory, in-house culture (Laboratory: Haskell
 Source: Laboratory, Newark, Delaware)
 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)
 Dissolved oxygen 8.1 to 9.1 mg/L
 pH 7.8 to 8.0
 Temperature: 20.3 to 20.9°C (of recirculating waterbath used to maintain
 test chamber temperature)
 Photoperiod: 16 hr photoperiod (473 to 883 lux) and 8 hr darkness which
 included 30 min transitional light (17 to 76 lux) preceding and
 following the 16-hr light interval

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
 07-October-2003 to 09-October-2003
2. Experimental treatments
 The acute toxicity of IN-MS775 to unfed *Daphnia magna* (<24-hour old) was determined in an unaerated, static, 48-hour test. Treatments consisted of a dilution water control, a 0.1 mL/L solvent control (N,N-dimethylformamide) and nominal concentrations of 0.0222, 0.00229, 0.00338, 0.005, and 0.0065 mg IN-MS775/L. Five daphnids were used per replicate with four replicates per test concentration and control. Water solubility of IN-MS775 is 0.0064 mg/L in 0.1 mL/L DMF.

II. RESULTS AND DISCUSSION

A. FINDINGS

A summary of the findings is presented in Table 87. Mean measured concentrations of IN-MS775 were 0.0022, 0.00273, 0.00347, 0.00467, and 0.00567 mg/L and ranged from 87 to 94% of nominal concentrations. The 24-hour EC₅₀, based on mean measured concentrations of IN-MS775, was greater than 0.00567 mg/L. The 48-hour EC₅₀, based on mean measured concentration of IN-MS775 and immobility, was greater than 0.00567 mg/L. The highest mean measured concentration causing no immobility at test end was 0.00567 mg/L.

Table 87
Summary of observed immobility and sublethal effects of unfed *Daphnia magna* exposed to IN-MS775 for 48 hours in an unaerated, static, acute test

Mean measured concentrations of IN-MS775 (mg/L)	Immobility (No. immobile/No. at test start)							
	24 Hours				48 Hours			
	A ^c	B ^c	C ^c	D ^c	A ^c	B ^c	C ^c	D ^c
Dilution Water Control	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
DMF Control	0 ^{1b} /5	0/5	0/5	0/5	0 ^{1b} /5	0/5	0/5	0/5
0.00202	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
0.00273	0/5	0/5	0/5	0/5	0 ^{1a} /5	0/5	0 ^{1a} /5	0 ^{1a} /5
0.00347	0 ^{1b} /5	0/5	0/5	0/5	0/5	0 ^{1a} /5	0 ^{1a} /5	0 ^{1a} /5
0.00467	0/5	0 ^{1b} /5	0/5	0/5	0 ^{1a} /5	0 ^{1a} /5	0 ^{2a} /5	0 ^{2a} /5
0.00567	0/5	0/5	0/5	0/5	0 ^{2a} /5	0 ^{2a} /5	0 ^{2a} /5	0 ^{4a} /5

^a Daphnids lethargic, superscript numbers indicate the number of daphnids with this sublethal effect.

^b Daphnid floating at surface, superscript numbers indicate the number of daphnids with this sublethal effect.

^c Replicate test chambers contained 5 daphnids each (total 20 daphnids per concentration) at test start.

III. CONCLUSION

The 48-hour EC₅₀ of IN-MS775 with 0.1 mL/L DMF solvent to *Daphnia magna* was >0.00567 mg/L, the highest concentration tested based on mean measured concentrations and immobility. The highest concentration with no immobility was 0.00567 mg IN-MS775/L.

(Samel, A., 2003b)

RMS comment

This study was submitted in the original DAR (AD3). The 48-hour EC₅₀ of IN-MS775 (with 0.1 mL/L DMF solvent) to *Daphnia magna* was >0.00567 mg/L based on mean measured concentrations. This study is still considered acceptable.

Report: Bradbury, N. (2015a); IN-U8E24: 48-Hour static, acute toxicity test with the Cladoceran, *Daphnia magna*

DuPont Report No.: DuPont-43486

Guidelines: OECD 202 (2004), USEPA 850.1010 (1996) **Deviations:** None

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

Testing Facility Report No.: 82064

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:

The acute toxicity of IN-U8E24 to unfed *Daphnia magna* neonates, less than 24 hours old at test start, was determined in an unaerated, 48-hour, static test. Daphnids were from the 15th brood of at least 21 day-old parents. The test was conducted in accordance with the OECD Guideline for the Testing of Chemicals No. 202, and U.S. EPA Ecological Effects Test Guidelines OPPTS 850.1010. Treatments consisted of a dilution water control and five nominal concentrations of 0.80, 1.5, 3.0, 6.0, and 12 mg IN-U8E24/L. Measured concentrations (0 hour) of IN-U8E24 were <LOQ (control), 0.924, 1.68, 3.36, 6.74, and 13.6 mg IN-U8E24/L, and ranged from 112 to 116% of the nominal concentrations. Mean measured concentrations (48 hours) of IN-U8E24 were <LOQ (control), 0.892, 1.65, 3.30, 6.54, and 13.4 mg IN-U8E24/L, and ranged from 109 to 112% of the nominal concentrations. Overall mean measured concentrations IN-U8E24 were <LOQ (control), 0.908, 1.67, 3.33, 6.64, and 13.5 mg IN-U8E24/L, and ranged from 111 to 114% of the nominal concentrations. No residues of IN-U8E24 were detected in the dilution water control solution above the LOQ of 0.0421 mg IN-U8E24/L. Recoveries for IN-U8E24 from the QC fortifications ranged from 97-112% of the nominal concentrations. No sublethal effects were observed in any treatment in the study. The 24- and 48-hour EC₅₀ value, based on immobility, was >12 mg IN-U8E24/L, the highest concentration tested. There was no concentration that caused 100% immobility at test end. The highest nominal concentration causing 0% immobility at test end was 12 mg IN-U8E24/L.

I. MATERIALS AND METHODS**A. MATERIALS**

- | | |
|--|---|
| 1. Test material: | IN-U8E24 technical metabolite |
| Lot/Batch #: | U8E24-000 |
| Purity: | 90.7% |
| Description: | Solid |
| CAS#: | None |
| Stability of test compound: | Determined to be stable in the test system |
| 2. Control: | Dilution (blended) water |
| Toxic reference: | None |
| 3. Test organism: | Cladoceran |
| Species: | <i>Daphnia magna</i> |
| Age at dosing: | Neonates (<24 hours old) |
| Weight at dosing: | NA |
| Initial population: | 5 daphnids per test chamber/20 daphnids per treatment |
| Source: | In house culture |
| Acclimation period: | Continuous culture |
| Diet: | Test period: unfed |
| Test chamber: | 250-mL glass container containing ~200 mL of test solution (6.5-cm test solution depth), covered with a plastic Petri dish |
| Test medium: | ABC blended freshwater |
| 4. Environmental conditions (in-life period) | |
| Temperature: | 20.2 to 20.3°C |
| Photoperiod: | 16 hr photoperiod (536 lux) and 8 hr darkness which included 30 min transitional light preceding and following the 16-hr light interval |
| Dissolved oxygen: | 8.3 to 8.7 mg/L (95 to 100% saturation) |
| pH: | 8.4 to 8.5 |

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
12 June 2015 to 14 June 2015
2. Experimental treatments
The acute toxicity of IN-U8E24 to unfed *Daphnia magna* (<24-hour old) was determined in an unaerated, static, 48-hour test. Daphnids were from the 15th brood of at least 21 day-old parents.

Treatments consisted of a dilution water control and five nominal concentrations of 0.80, 1.5, 3.0, 6.0, and 12 mg IN-U8E24/L. Five daphnids were used per replicate with four replicates per test concentration and control.

3. Observations

Immobility and sublethal (behavioral) observations were made every 24 hours.

4. Statistics

Due to a lack of immobility in the control and all test substance treatments, no statistical analyses were necessary.

II. RESULTS AND DISCUSSION

A. FINDINGS

The concentration of IN-U8E24 was measured in test solutions at 0 and 48 hours. Measured concentrations (0 hour) of IN-U8E24 were <LOQ (control), 0.924, 1.68, 3.36, 6.74, and 13.6 mg IN-U8E24/L, and ranged 112 to 116% of the nominal concentrations. Mean measured concentrations (48 hours) of IN-U8E24 were <LOQ (control), 0.892, 1.65, 3.30, 6.54, and 13.4 mg IN-U8E24/L, and ranged from 109 to 112% of the nominal concentrations. Overall mean measured concentrations IN-U8E24 were <LOQ (control) 0.908, 1.67, 3.33, 6.64, and 13.5 mg IN-U8E24/L, and ranged from 111 to 114% of the nominal concentrations. No residues of IN-U8E24 were detected in the dilution water control solution above the LOQ of 0.0421 mg IN-U8E24/L. Recoveries for IN-U8E24 from the QC fortifications ranged from 84 to 112% of the nominal concentrations. No sublethal effects were observed in any treatment in the study. Summaries of observed immobility and sublethal effects are presented in Table 88 and Table 89, respectively.

Table 88
Observed immobility of the Cladoceran, *Daphnia magna*, exposed to IN-U8E24 for 48 hours in an unaerated, static, acute test

IN-U8E24 Nominal Concentration (mg IN-U8E24/L)	Cumulative immobility (Number immobile/Number at test start) ^{a,b}								Mean % Immobile (after 48-hours)
	24 Hours				48 Hours				
	A	B	C	D	A	B	C	D	
0 (Control)	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0
0.80	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0
1.50	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0
3.0	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0
6.0	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0
12	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0

^a Immobile. No observed movement of appendages or postabdomen within 15 seconds after gentle agitation of the test chamber or gentle disturbance of the daphnid itself. Affected numbers are cumulative.

^b Replicate test chambers contained 5 daphnids each for a total of 20 per concentration at test initiation.

Table 89
Observed sublethal effects of the Cladoceran, *Daphnia magna*, exposed to IN-U8E24 for 48 hours in an unaerated, static, acute test

IN-U8E24 Nominal Concentration (mg IN-U8E24/L)	Sublethal Effects/Number Alive ^a							
	24 Hours				48 Hours			
	A	B	C	D	A	B	C	D
0 (Control)	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
0.80	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
1.50	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
3.0	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
6.0	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
12	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5

^a Replicate test chambers contained 5 daphnids each for a total of 20 per concentration at test initiation.

III. CONCLUSION

The 48-hour EC₅₀ value, based on immobility, was >12 mg IN-U8E24/L, the highest concentration tested. There was no concentration causing 100% immobility at test end. The highest nominal concentration causing 0% immobility at test end was 12 mg IN-U8E24/L.

(Bradbury, N., 2015a)

RMS comment

This study was conducted in compliance with the current guideline. The 48-hour EC₅₀ value was >12 mg IN-U8E24/L based on nominal concentrations. This study is acceptable.

Report: Bradbury, N. (2015b); IN-UYG24: 48-Hour static, acute toxicity test with the Cladoceran, *Daphnia magna*

DuPont Report No.: DuPont-43423

Guidelines: OECD 202 (2004), USEPA 850.1010 (1996) **Deviations:** None

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

Testing Facility Report No.: 82067

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:

The acute toxicity of IN-UYG24 to unfed *Daphnia magna* neonates, less than 24 hours old at test start, was determined in an unaerated, 48-hour, static test. Daphnids were from at least the 14th brood of 22 day-old parents. The test was conducted in accordance with the OECD Guideline for the Testing of Chemicals No. 202, and U.S. EPA Ecological Effects Test Guidelines OPPTS 850.1010. Treatments consisted of a dilution water control and five nominal concentrations of 7.5, 15, 30, 60, and 120 mg IN-UYG24/L. Measured concentrations (0 hour) of IN-UYG24 were <LOD (control), 7.16, 14.8, 29.4, 59.0, and 115 mg IN-UYG24/L, and ranged from

95 to 99% of the nominal concentrations. Mean measured concentrations (48 hours) of IN-UYG24 were <LOD (control), 6.99, 14.1, 28.8, 59.3, and 111 mg IN-UYG24/L, and ranged from 93 to 99% of the nominal concentrations. Overall mean measured concentrations IN-UYG24 were <LOD (control), 7.08, 14.5, 29.1, 59.2, and 113 mg IN-UYG24/L, and ranged from 94 to 99% of the nominal concentrations. No residues of IN-UYG24 were detected in the dilution water control solution above the LOD of 0.0147 mg IN-UYG24/L. Recoveries for IN-UYG24 from the QC fortifications ranged from 73 to 92% of the nominal concentrations. No sublethal effects were observed in any treatment in the study. The 24- and 48-hour EC₅₀ value, based on immobility, was >120 mg IN-UYG24/L, the highest concentration tested. There was no concentration that caused 100% immobility at test end. The highest nominal concentration causing 0% immobility at test end was 120 mg IN-UYG24/L.

I. MATERIALS AND METHODS

A. MATERIALS

- | | |
|--|---|
| 1. Test material: | IN-UYG24 technical metabolite |
| Lot/Batch #: | UYG24-001 |
| Purity: | 96.1% |
| Description: | Solid |
| CAS#: | Not assigned |
| Stability of test compound: | Determined to be stable in the test system |
| 2. Control: | Dilution (blended) water |
| Toxic reference: | None |
| 3. Test organism: | Cladoceran |
| Species: | <i>Daphnia magna</i> |
| Age at dosing: | Neonates (<24 hours old) |
| Weight at dosing: | NA |
| Initial population: | 5 daphnids per test chamber/20 daphnids per treatment |
| Source: | In house culture |
| Acclimation period: | Continuous culture |
| Diet: | Test period: unfed |
| Test chamber: | 250-mL glass container containing ~200 mL of test solution (6.5-cm test solution depth), covered with a plastic Petri dish |
| Test medium: | ABC blended freshwater |
| 4. Environmental conditions (in-life period) | |
| Temperature: | 20.4 to 20.8°C |
| Photoperiod: | 16 hr photoperiod (373 lux) and 8 hr darkness which included 30 min transitional light preceding and following the 16-hr light interval |
| Dissolved oxygen: | 8.2 to 8.5 mg/L (95 to 100% saturation) |
| pH: | 8.4 to 8.5 |

B. STUDY DESIGN AND METHODS

- In-life initiated/completed
10 June 2015 to 12 June 2015
- Experimental treatments
The acute toxicity of IN-UYG24 to unfed *Daphnia magna* (<24-hour old) was determined in an unaerated, static, 48-hour test. Daphnids were from at least the 14th brood of 22 day-old parents. Treatments consisted of a dilution water control and five nominal concentrations of 7.5, 15, 30, 60, and 120 mg IN-UYG24/L. Five daphnids were used per replicate with four replicates per test concentration and control.
- Observations
Immobility and sublethal (behavioral) observations were made every 24 hours.

4. Statistics

Due to a lack of immobility in the control and all test substance treatments, no statistical analyses were necessary.

II. RESULTS AND DISCUSSION

A. FINDINGS

The concentration of IN-UYG24 was measured in test solutions at 0 and 48 hours. Measured concentrations (0 hour) of IN-UYG24 were <LOD (control), 07.16, 14.8, 29.4, 59.0, and 115 mg IN-UYG24/L, and ranged from 95 to 99% of the nominal concentrations. Mean measured concentrations (48 hours) of IN-UYG24 were <LOD (control), 6.99, 14.1, 28.8, 59.3, and 111 mg IN-UYG24/L, and ranged from 93 to 99% of the nominal concentrations. Overall mean measured concentrations IN-UYG24 were <LOD (control), 7.08, 14.5, 29.1, 59.2, and 113 mg IN-UYG24/L, and ranged from 94 to 99% of the nominal concentrations. No residues of IN-UYG24 were detected in the dilution water control solution above the LOD of 0.0147 mg IN-UYG24/L. Recoveries for IN-UYG24 from the QC fortifications ranged from 73 to 92% of the nominal concentrations. No sublethal effects were observed in any treatment in the study. Summaries of observed immobility and sublethal effects are presented in Table 90 and Table 91, respectively.

Table 90
Observed immobility of the Cladoceran, *Daphnia magna*, exposed to IN-UYG24 for 48 hours in an unaerated, static, acute test

Nominal IN-UYG24 Concentration (mg IN-UYG24/L)	Cumulative immobility (Number immobile/Number at test start) ^{a,b}								Mean % Immobile (after 48-hours)
	24 Hours				48 Hours				
	A	B	C	D	A	B	C	D	
0 (Control)	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0
7.5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0
15	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0
30	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0
60	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0
120	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0

^a Immobile. No observed movement of appendages or postabdomen within 15 seconds after gentle agitation of the test chamber or gentle disturbance of the daphnid itself. Affected numbers are cumulative.

^b Replicate test chambers contained 5 daphnids each for a total of 20 per concentration at test initiation.

Table 91
Observed sublethal effects of the Cladoceran, *Daphnia magna*, exposed to IN-UYG24 for 48 hours in an unaerated, static, acute test

Nominal IN-UYG24 Concentration (mg IN-UYG24/L)	Sublethal Effects/Number Alive ^a							
	24 Hours				48 Hours			
	A	B	C	D	A	B	C	D
0 (Control)	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
7.5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
15	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
30	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
60	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
120	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5

^a Replicate test chambers contained 5 daphnids each for a total of 20 per concentration at test initiation.

III. CONCLUSION

The 48-hour EC₅₀ value, based on immobility, was >120 mg IN-UYG24/L, the highest concentration tested. There was no concentration causing 100% immobility at test end. The highest nominal concentration causing 0% immobility at test end was 120 mg IN-UYG24/L.

(Bradbury, N., 2015b)

RMS comment

This study was conducted in compliance with the current guideline. The 48-hour EC₅₀ value was >120 mg IN-UYG24/L based on nominal concentrations. This study is acceptable.

Report: Ward, T.J., Magazu, J.P., Boeri, R.L. (1997); Flow-through acute toxicity of DPX-MP062 to the mysid, *Mysidopsis bahia*

DuPont Report No.: HLO-1997-00205, Revision No. 1

Guidelines: USEPA 72-3(b) **Deviations:** None

Testing Facility: T.R. Wilbury Laboratories, Inc., Marblehead, Massachusetts, USA

Testing Facility Report No.: 803-DU

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:

The acute toxicity of DPX-MP062 to the mysid shrimp, *Mysidopsis bahia*, was determined in an unaerated, flow-through, 96-hour test. The test was conducted in accordance with U.S. EPA Pesticide Assessment Guideline FIFRA 72-3(b).

Treatments consisted of a dilution water control, a solvent control (0.1 mL/L dimethylformamide), and five nominal concentrations of 0.027, 0.045, 0.072, 0.11, and 0.18 mg DPX-MP062/L. The corresponding mean measured concentrations of DPX-MP062 were 0.0234, 0.0373, 0.0610, 0.0910, and 0.158 mg DPX-MP062/L.

The median 96-hour LC₅₀ for *Mysidopsis bahia* based on mortality and mean measured concentrations was 0.0542 mg DPX-MP062/L with a 95% confidence interval of 0.0443 to 0.0657 mg DPX-MP062/L (calculated by probit analysis).

The 96-hour no observed effect concentration (NOEC) was <0.0234 mg DPX-MP062/L.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: DPX-MP062 technical
 Lot/Batch #: MP062-51A
 Purity: 94.54%
 Description: Off-white solid
 CAS#: 144171-61-9
 Stability of test compound: Test substance appeared to be stable under the conditions of the study; no evidence of instability was observed.
2. Control: Dilution water (carbon filtered natural seawater)
 Solvent control: Dimethylformamide
 Test vehicle: Dilution water (carbon filtered natural seawater)
 Toxic reference: None
3. Test organism: *Mysidopsis bahia*
 Species: *Mysidopsis bahia*
 Age at dosing: <24 hours
 Initial population: 10 mysids per test chamber and two replicates of each treatment (n=20 for each treatment)
 Source: Laboratory, in-house culture (original brood-stock obtained from Aquatic BioSystems, Fort Collins, Colorado, USA)
 Diet: (prior to and during test) Brine shrimp, *Artemia salina nauplii*
 Test chamber: 9.5 liter glass aquaria that contained 7.0 L of test solution (water depth was approximately 17 cm)
4. Environmental conditions (in-life period)
 Dissolved oxygen: 5.5 to 7.9 mg/L
 pH: 7.6 to 8.1
 Temperature: 21.4 to 22.5°C (mean = 21.9°C)
 Photoperiod: 16 hr photoperiod (65 foot-candles) and 8 hr darkness which included 15 minutes of transitional light preceding and following the 16-hr light interval

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
 12-August-1996 to 18-December-1996
2. Experimental treatments
 The acute toxicity of DPX-MP062 to *Mysidopsis bahia* (<24-hours old) was determined in an unaerated, flow-through, 96-hour test, after a range-finding experiment. The range-finding test was conducted under static conditions at nominal concentrations of 0, 0.010, 0.050, 0.10, 0.36, and 0.50 mg DPX-MP062/L. After 96 hours of exposure, survival was 100% in the water control and at 0.10 mg/L, 90% in the solvent control and at 0.010 and 0.050 mg DPX-MP062/L, and 0% at 0.36 and 0.50 mg/L DPX-MP062. Insoluble material was not observed in any test vessel during the test.

In the definitive test, treatments consisted of a dilution water control, a solvent control (0.1 mL/L dimethylformamide), and five nominal concentrations of 0.027, 0.045, 0.072, 0.11, and 0.18 mg DPX-MP062/L. The corresponding mean measured concentrations of DPX-MP062 were 0.0234, 0.0373, 0.0610, 0.0910, and 0.158 mg DPX-MP062/L. Ten mysids were used per replicate with two replicates per test concentration and control.

3. Observations

The number of surviving organisms and the occurrence of sublethal effects (loss of equilibrium, erratic swimming, and lethargy) were determined visually and recorded initially and after 24, 48, 72, and 96 hours. Dissolved oxygen, pH, salinity, and temperature were measured and recorded daily in each test chamber. The temperature in a control test vessel was recorded continuously during the test.

4. Statistics

The probit method was used to calculate the LC_{50} values at 48, 72, and 96 hours and the slope of the 96 hour concentration-response curve. The 24 hour LC_{50} could not be calculated because greater than 50% survival occurred at each tested concentration of DPX-MP062. The no observed effect concentration is the highest concentration of test substance that did not cause toxicant related mortalities or sublethal effects.

II. RESULTS AND DISCUSSION

A. FINDINGS

Mean measured concentrations of DPX-MP062 were 0.0234, 0.0373, 0.0610, 0.0910, and 0.158 mg DPX-MP062/L and ranged from 83 to 88% of nominal concentrations. All validation criteria were met for the study. A summary of observed immobility and sublethal effects is presented in Table 92 below.

There was 100% survival in the controls. Several mysids exposed to 0.0610 and 0.158 mg DPX-MP062/L exhibited lethargy and a loss of equilibrium at 48 hours, lethargy and erratic swimming at 72 hours, and lethargy at 96 hours. Several mysids exposed to 0.0910 mg DPX-MP062/L exhibited lethargy and erratic swimming at 72 hours and lethargy at 96 hours. No other sublethal effects were observed during the definitive test.

Table 92
Summary of mortality and sublethal effects of *Mysidopsis bahia* exposed to DPX-MP062 for 96 hours in an unaerated, flow-through, acute test

Mean, measured concentrations of DPX-MP062 (mg/L)	Mortality																			
	Number dead										Number with sublethal effects ^b									
	0 h		24 h		48 h		72 h		96 h		0 h		24 h		48 h		72 h		96 h	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Water control ^a	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Solvent control ^a	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.0234	0	0	1	0	1	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0
0.0373	0	0	1	1	1	1	1	1	2	2	0	0	0	0	0	0	0	0	0	0
0.0610	0	0	0	1	0	1	1	2	8	6	0	0	0	0	4	0	4	3	2	4
0.0910	0	0	0	1	1	3	4	4	9	6	0	0	0	0	0	0	6	6	1	4
0.158	0	0	2	3	4	8	5	9	10	9	0	0	0	0	6	2	5	0	-- ^c	1

Note: A and B represent replicate test vessels containing 10 mysids.

^a ND = none detected at or above the limit of quantitation of 0.0100 mg/L.

^b Affected mysids exhibited lethargy and a loss of equilibrium at 48 hours, lethargy and erratic swimming at 72 hours, and lethargy at 96 hours.

^c All animals dead, thus no data on sublethal effects in this replicate.

III. CONCLUSION

The median 96-hour LC₅₀ for *Mysidopsis bahia* based on mortality and mean measured concentrations was 0.0542 mg DPX-MP062/L (calculated by probit analysis):

Time	LC ₅₀ (mg/L)	95% Confidence Intervals (mg/L)	
		Lower	Upper
24 hours	>0.158	---	---
48 hours	0.160	0.114	0.354
72 hours	0.112	0.0876	0.169
96 hours	0.0542	0.0443	0.0657

The 96-hour no observed effect concentration (NOEC) was <0.0234 mg DPX-MP062/L.

(Ward, T.J., Magazu, J.P., Boeri, R.L., 1997)

RMS comment

This study was not conducted in accordance with the current guideline and was conducted with the old material DPX-MP062. As new toxicity study on the same species is available for the new material DPX-KN128, this study is not considered essential.

Report: Dinehart, S. (2014a); Indoxacarb (DPX-KN128): Acute toxicity with the mysid shrimp, *Americamysis bahia*, determined under flow-through test conditions

DuPont Report No.: DuPont-38440

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

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

Testing Facility Report No.: 80402

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:

The acute toxicity of indoxacarb (DPX-KN128) with the mysid shrimp, *Americamysis bahia*, was determined in a 96-hour flow-through test. The test was conducted in accordance with the U.S. EPA Ecological Effects Test guidelines, OPPTS 850.1035.

The test substance, indoxacarb, contains 98.8% DPX-KN128 by analysis. The study was conducted with five target nominal concentrations of indoxacarb (0.013, 0.025, 0.050, 0.10, and 0.20 mg a.s./L), a vehicle control, and a dilution water control at a temperature range of 24.1 to 25.0°C. Ten mysids were used per test substance concentration, vehicle control, 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 <LOD, 0.0165, 0.0320, 0.0545, and 0.126 mg a.s./L, or from <LOD to 66% of the target nominal concentrations. After 96 hours of exposure, mortality was 0, 0, 5, 10, 0, 10, and 10% in the 0 (control), 0 (vehicle control), <LOD, 0.0165, 0.0320, 0.0545, and 0.126 mg a.s./L treatments, respectively. No sub-lethal effects were observed at any concentration. The highest mean measured concentration for which mortality did not differ to a biologically

meaningful extent from the control (*i.e.*, $\leq 10\%$) was 0.126 mg a.s./L. The 96-hour LC_{50} was estimated to be >0.126 mg a.s./L (the highest level tested). The 95% confidence intervals could not be determined.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Indoxacarb technical
 Lot/Batch #: KN128-374
 Purity: 98.8%
 Description: Solid powder
 CAS#: 173584-44-6
 Stability of test compound: Not stable in the test system
2. Control: Dilution (laboratory saltwater) water
 Vehicle control: dimethylformamide (DMF)
 Test vehicle: Dilution (laboratory saltwater) water
 Toxic reference: None
3. Test organism: Mysid Shrimp
 Species: *Americamysis bahia*
 Age at dosing: <24 hours
 Initial population: 5 mysids per retention basket, 2 retention baskets per replicate, 2 replicates per treatment for a total of 20 mysids per treatment
 Source: ABC Laboratories, in-house culture
 Diet: Fed *ad libitum* during test
 Test chambers: Glass aquaria with ~4-L solution volume
4. Environmental conditions (in-life period)
 Temperature: 24.1 to 25.0°C (of test chambers)
 Photoperiod: 14 hr photoperiod (426 to 490 lux) and 10 hr darkness which included two-30 min transitional light periods.

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
 27-December-2013 to 31-December-2013
2. Experimental treatments
 The acute toxicity of indoxacarb to *Americamysis bahia* was determined in a flow-through, 96-hour test. Treatments consisted of a dilution water control, a vehicle control, and five target nominal concentrations of 0.013, 0.025, 0.050, 0.10, and 0.20 mg a.s./L. Ten 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 LC_{50} values and their 95% confidence limits were calculated using the probit method and Trimmed 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

Target nominal concentrations were 0.013, 0.025, 0.050, 0.10, and 0.20 mg indoxacarb/L. The treatment mean measured concentrations of indoxacarb during the 96 hour exposure were <LOD, 0.0165, 0.0320, 0.0545, and 0.126 mg a.s./L, or from <LOD to 66% of the target nominal concentrations. Recoveries from the indoxacarb QC samples ranged from 94 to 103% of the target nominal concentrations throughout the test.

All results from biological responses were based on mean measured concentrations of indoxacarb. A summary of cumulative mortality is presented in Table 93.

Table 93
Observed mortality of mysid shrimp, *Americamysis bahia*, exposed to indoxacarb for 96 hours in a flow-through acute test

Mean Measured indoxacarb Concentration (mg a.s./L)	Cumulative Mortality/Number at Test Start																Mean % Mortality
	24 Hours				48 Hours				72 Hours				96 Hours				
	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2	
0 (Control)	0/5	0/5	0/5	0/5	0/5	0/5	0/5 ^a	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0
0 (V. Control)	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	0/5	0/5	0
<LOD	0/5	0/5	0/5	0/5 ^a	0/5	0/5	0/5	0/5 ^a	0/5	0/5	0/5	0/5 ^a	0/5	0/5	0/5	1/5	5
0.0165	0/5	0/5	0/5	0/5	1/5	0/5	0/5	0/5	1/5	0/5	0/5	0/5	2/5	0/5	0/5	0/5	10
0.0320	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	0/5	0/5	0
0.0545	0/5	0/5	0/5	0/5	0/5	0/5	2/5	0/5	0/5	0/5	2/5	0/5	0/5	0/5	2/5	0/5	10
0.126	0/5	0/5	0/5 ^a	0/5 ^a	0/5	0/5	0/5 ^a	0/5 ^a	0/5	0/5	0/5 ^a	0/5 ^a	0/5	0/5	1/5	1/5	10

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

Notes: Five mysids were added to each retention basket at test initiation, two retention baskets were placed in each test chamber.

LOD=0.00408 µg/mL

III. CONCLUSION

Indoxacarb was assessed for acute toxicity with the mysid shrimp, *Americamysis bahia*, in a 96-hour flow-through test. After 96 hours of exposure, mortality was 0, 0, 5, 10, 0, 10, and 10% in the 0 (control), 0 (vehicle control), <LOD, 0.0165, 0.0320, 0.0545, and 0.126 mg a.s./L treatments, respectively. No sub-lethal effects were observed at any concentration. The highest mean measured concentration for which mortality did not differ to a biologically meaningful extent from the control (*i.e.*, $\leq 10\%$) was 0.126 mg a.s./L. The 96-hour LC₅₀ was estimated to be >0.126 mg a.s./L (the highest concentration tested) based on mean measured indoxacarb concentrations, and appropriate 95% confidence intervals could not be estimated.

(Dinehart, S., 2014a)

RMS comment

This study was conducted in compliance with the current guideline. RMS noted that no data on sublethal effects were provided in the study report and the applicant was asked to provide information on sublethal effects, if any. The applicant answered that no effects were observed at any concentration. The 96-hour LC₅₀ was estimated to be >0.126 mg a.s./L based on mean measured indoxacarb concentrations. This study is acceptable.

Report: Dinehart, S. (2013d); IN-JT333: Acute toxicity with the mysid shrimp, *Americamysis bahia*, determined under static-renewal conditions

DuPont Report No.: DuPont-36489

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

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

Testing Facility Report No.: 69577

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 IN-JT333 with the mysid shrimp, *Americamysis bahia*, was determined in a 96-hour static-renewal test. The test was conducted in accordance with the U.S. EPA Ecological Effects Test guidelines, OPPTS 850.1035.

The test substance, IN-JT333, contains 98.8% IN-JT333 by analysis. The study was conducted with five nominal concentrations of IN-JT333 (0.013, 0.025, 0.050, 0.10, and 0.20 mg/L), a vehicle control, and a dilution water control at a temperature range of 24.4 to 25.1°C. Five mysids were used per test substance concentration, vehicle control, and dilution water control replicate, for a total of 20 mysids per treatment. The treatment mean measured concentrations of IN-JT333 during the 96 hour exposure were 0.00724, 0.0180, 0.0314, 0.0629, and 0.121 mg/L, or 56 to 72% of the nominal concentrations. After 96 hours of exposure, mortality was 5, 0, 0, 0, 10, 25, and 100% in the 0 (control), 0 (vehicle), 0.00724, 0.0180, 0.0314, 0.0629, and 0.121 mg IN-JT333/L treatments, respectively. The highest mean measured concentration causing no mortality at test end was 0.0180 mg IN-JT333/L. The lowest mean measured concentration causing 100% mortality at test end was 0.121 mg IN-JT333/L. The 96-hour LC₅₀ was estimated to be 0.070 mg IN-JT333, with 95% confidence intervals of 0.060 and 0.082 mg IN-JT333/L.

I. MATERIALS AND METHODS

A. MATERIALS

- | | |
|--|--|
| 1. Test material: | IN-JT333 technical metabolite |
| Lot/Batch #: | JT333-017 |
| Purity: | 98.8% |
| Description: | Solid powder |
| CAS#: | 144171-39-1 |
| Stability of test compound: | Not stable in the test system |
| 2. Control: | Dilution (laboratory saltwater) water |
| Vehicle control: | dimethylformamide (DMF) |
| 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: | 24.4 to 25.1°C (of test chambers) |
| Photoperiod: | 14 hr photoperiod (509 lux) and 10 hr darkness which included two-30 min transitional light periods. |

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
27-June-2013 to 01-July-2013
2. Experimental treatments
The acute toxicity of IN-JT333 to *Americamysis bahia* was determined in a static-renewal, 96-hour test. Treatments consisted of a dilution water control, a vehicle control, and five nominal concentrations of 0.013, 0.025, 0.050, 0.10, and 0.20 mg IN-JT333/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 0.013, 0.025, 0.050, 0.10, and 0.20 mg IN-JT333/L. The treatment mean measured concentrations of IN-JT333 during the 96 hour exposure were 0.00724, 0.0180, 0.0314, 0.0629, and 0.121 mg IN-JT333/L, or 56 to 72% of the nominal concentrations. Recoveries from the IN-JT333 QC samples ranged from 73 to 90% of the nominal concentrations throughout the test.

All results from the biological responses were based on mean measured concentrations of IN-JT333. A summary of the cumulative mortality and sublethal effects are presented in Table 94.

Table 94
Observed mortality of mysid shrimp, *Americamysis bahia*, exposed to IN-JT333 for 96 hours in a static-renewal acute test

Mean measured IN-JT333 concentration (mg/L)	Cumulative mortality/Number at test start																Mean % mortality
	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)	0/5	0/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5	0/5	0/5	1/5	0/5	0/5	0/5	1/5	5
0 (vehicle control)	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	0/5	0/5	0
0.00724	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	0/5	0/5	0
0.0180	0/5	0/5	0/5	0/5	0/5	0/5	0/5 ^a	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0
0.0314	0/5	0/5	0/5	0/5	0/5 ^b	0/5 ^c	1/5 ^c	0/5 ^b	0/5 ^b	0/5 ^b	1/5 ^c	0/5 ^b	0/5 ^d	0/5 ^e	2/5 ^a	0/5	10
0.0629	0/5 ^c	1/5 ^d	0/5 ^a	0/5 ^e	1/5 ^c	2/5 ^d	1/5 ^c	0/5 ^b	1/5 ^c	2/5 ^d	1/5 ^c	0/5 ^b	1/5 ^c	2/5 ^d	1/5 ^c	1/5 ^c	25
0.121	1/5 ^c	2/5 ^d	2/5 ^d	0/5 ^b	5/5	5/5	4/5 ^a	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	100

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

^a One mysid was lethargic.

^b Five mysids were lethargic.

^c Four mysids were lethargic.

^d Three mysids were lethargic.

^e Two mysids were lethargic.

III. CONCLUSION

IN-JT333 was assessed for acute toxicity with the mysid shrimp, *Americamysis bahia*, in a 96-hour static-renewal test. After 96 hours of exposure, mortality was 5, 0, 0, 0, 10, 25, and 100% in the 0 (control), 0 (vehicle), 0.00724, 0.0180, 0.0314, 0.0629, and 0.121 mg IN-JT333/L treatments, respectively. The highest mean measured concentration causing no mortality at test end was 0.0180 mg IN-JT333/L. The lowest mean measured concentration causing 100% mortality at test end was 0.121 mg IN-JT333/L. The 96-hour LC₅₀ was estimated to be 0.070 mg IN-JT333/L, with 95% confidence intervals of 0.060 and 0.082 mg IN-JT333/L.

(Dinehart, S., 2013d)

RMS comment

This study was conducted in compliance with the current guideline. The 96-hour LC₅₀ was estimated to be 0.070 mg IN-JT333/L based on mean measured concentrations. This study is acceptable.

Report: Dinehart, S. (2013e); IN-JU873: Acute toxicity with the mysid shrimp, *Americamysis bahia*, determined under static-renewal conditions

DuPont Report No.: DuPont-36474

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

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

Testing Facility Report No.: 69578

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 IN-JU873 with the mysid shrimp, *Americamysis bahia*, was determined in a 96-hour static-renewal test. The test was conducted in accordance with the U.S. EPA Ecological Effects Test guidelines, OPPTS 850.1035.

The test substance, IN-JU873, contains 99.1% IN-JU873 by analysis. The study was conducted with six nominal concentrations of IN-JU873 (0.065, 0.13, 0.25, 0.50, 1.0 and 2.0 mg/L), a vehicle control, and a dilution water control at a temperature range of 24.5 to 26.0°C. Five mysids were used per test substance concentration, vehicle control, and dilution water control replicate, for a total of 20 mysids per treatment. The treatment mean measured concentrations of IN-JU873 during the 96 hour exposure were 0.0350, 0.0948, 0.186, 0.411, 0.809, and 1.47 mg/L, or 54 to 82% of the nominal concentrations. After 96 hours of exposure, mortality was 0, 0, 0, 0, 5, 0, and 20% in the 0 (control), 0 (vehicle), 0.0350, 0.0948, 0.186, 0.411, 0.809, and 1.47 mg IN-JU873/L treatments, respectively. The highest mean measured concentration causing no mortality at test end was 0.411 mg IN-JU873/L. The 96-hour LC₅₀ was estimated to be >1.47 mg IN-JU873/L (the highest level tested). The 95% confidence intervals could not be determined.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:	IN-JU873 technical metabolite
Lot/Batch #:	JU873-005
Purity:	99.1%
Description:	Solid crystalline
CAS#:	144172-25-8
Stability of test compound:	Not stable in the test system
2. Control:	Dilution (laboratory saltwater) water
Vehicle control:	dimethylformamide (DMF)
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:	24.6 to 26.0°C (of test chambers)
Photoperiod:	14 hr photoperiod (417 lux) and 10 hr darkness which included two-30 min transitional light periods.

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
22-August-2013 to 26-August-2013
2. Experimental treatments
The acute toxicity of IN-JU873 to *Americamysis bahia* was determined in a static-renewal, 96-hour test. Treatments consisted of a dilution water control, a vehicle control, and six nominal

concentrations of 0.065, 0.13, 0.25, 0.50, 1.0, and 2.0 mg IN-JU873/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 LC_{50} 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 0.065, 0.13, 0.25, 0.50, 1.0, and 2.0 mg IN-JU873/L. The treatment mean measured concentrations of IN-JU873 during the 96 hour exposure were 0.0350, 0.0948, 0.186, 0.411, 0.809, and 1.47 mg IN-JU873/L, or 54 to 82% of the nominal concentrations. Recoveries from the IN-JU873 QC samples ranged from 86 to 109% of the nominal concentrations throughout the test.

All results from biological responses were based on mean measured concentrations of IN-JU873. A summary of cumulative mortality and sublethal effects are presented in Table 95.

Table 95
Observed mortality of mysid shrimp, *Americamysis bahia*, exposed to IN-JU873 for 96 hours
in a static-renewal acute test

Mean measured IN-JU873 concentration (mg/L)	Cumulative mortality/Number at test start																Mean % mortality
	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)	0/5	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	0/5	0/5	0
0 (V. Control)	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	0/5	0/5	0
0.0350	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	0/5	0/5	0
0.0948	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	0/5	0/5	0
0.186	0/5	0/5	0/5 ^a	0/5 ^a	0/5	0/5	1/5	0/5 ^a	0/5 ^a	0/5 ^d	1/5 ^d	0/5 ^a	0/5 ^c	0/5 ^c	1/5 ^b	0/5 ^c	5
0.411	0/5	0/5 ^a	0/5	0/5	0/5	0/5 ^a	0/5 ^a	0/5 ^d	0/5 ^e	0/5 ^d	0/5 ^e	0/5 ^e	0/5 ^c	0/5 ^c	0/5 ^c	0/5 ^c	0
0.809	0/5 ^b	0/5	0/5 ^b	0/5	0/5 ^d	0/5 ^a	0/5 ^e	1/5 ^d	0/5 ^d	0/5 ^a	0/5 ^e	1/5 ^d	0/5 ^c	0/5 ^c	0/5 ^c	1/5 ^b	5
1.47	1/5 ^b	0/5 ^c	1/5 ^b	0/5 ^c	1/5 ^b	1/5 ^b	1/5 ^b	0/5 ^c	1/5 ^e	1/5 ^b	1/5 ^b	0/5 ^c	1/5 ^b	1/5 ^b	2/5 ^e	0/5 ^c	20

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

- ^a One mysid was lethargic.
- ^b Four mysids were lethargic.
- ^c Five mysids were lethargic.
- ^d Two mysids were lethargic.
- ^e Three mysids were lethargic.

III. CONCLUSION

IN-JU873 was assessed for acute toxicity with the mysid shrimp, *Americamysis bahia*, in a 96-hour static-renewal test. After 96 hours of exposure, mortality was 0, 0, 0, 0, 5, 0, 5, and 20% in the 0 (control), 0 (vehicle), 0.0350, 0.0948, 0.186, 0.411, 0.809, and 1.47 mg IN-JU873/L treatments, respectively. The highest mean measured concentration causing no mortality at test end was 0.411 mg IN-JU873/L. The 96-hour LC_{50} was estimated to be >1.47 mg IN-JU873/L, and appropriate 95% confidence intervals could not be estimated.

(Dinehart, S., 2013e)

RMS comment

This study was conducted in compliance with the current guideline. The 96-hour LC_{50} was estimated to be >1.47 mg IN-JU873/L based on mean measured concentrations. This study is acceptable.

Report: Dinehart, S. (2013c); IN-KB687: Acute toxicity with the mysid shrimp, *Americamysis bahia*, determined under static-renewal test conditions

DuPont Report No.: DuPont-36477

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

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

Testing Facility Report No.: 69579

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 IN-KB687 with the mysid shrimp, *Americamysis bahia*, was determined in a 96-hour static-renewal test. The test was conducted in accordance with the U.S. EPA Ecological Effects Test guidelines, OPPTS 850.1035.

The test substance, IN-KB687, contains 99.8% IN-KB687 by analysis. The study was conducted with five concentrations of IN-KB687 (1.5, 3.0, 6.0, 12, and 24 mg/L) and a dilution water control at a temperature range of 24.1 to 25.4°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 IN-KB687 during the 96 hour exposure were 1.26, 2.55, 5.45, 10.0, and 23.6 mg/L, or 83 to 98% of the nominal concentrations. After 96 hours of exposure, mortality was 0, 5, 0, 5, 100, and 100% in the 0 (control), 1.26, 2.55, 5.45, 10.0, and 23.6 mg IN-KB687/L treatments, respectively. The highest mean measured concentration causing no mortality at test end was 2.55 mg IN-KB687/L. The lowest mean measured concentration causing 100% mortality at test end was 10.0 mg IN-KB687/L. The 96 hour LC_{50} was estimated to be 7.20 mg IN-KB687/L, with 95% confidence intervals of 6.67 and 7.76 mg IN-KB687/L.

I. MATERIALS AND METHODS

A. MATERIALS

- | | |
|--|--|
| 1. Test material: | IN-KB687 technical metabolite |
| Lot/Batch #: | KB687-002 |
| Purity: | 99.8% |
| Description: | Solid powder |
| CAS#: | 177905-10-1 |
| Stability of test compound: | Not stable in the test system |
| 2. Control: | Dilution water (laboratory saltwater) |
| Solvent control: | None |
| Test vehicle: | Dilution water (laboratory saltwater) |
| 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 ad libitum during test |
| Test chambers: | 500-mL glass jars with 250-mL solution volume |
| 4. Environmental conditions (in-life period) | |
| Temperature: | 24.1 to 25.4°C (of test chambers) |
| Photoperiod: | 14 hr photoperiod (492 lux) and 10 hr darkness which included two-30 min transitional light periods. |

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
15-March-2013 to 19-March-2013
2. Experimental treatments
The acute toxicity of IN-KB687 to *Americamysis bahia* was determined in a static, 96-hour test. Treatments consisted of a dilution water control and five nominal concentrations of 1.5, 3.0, 6.0, 12, and 24 mg/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 9.1). Estimates of LC₅₀ values and their 95% confidence limits were calculated using the probit method and Trimmed 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 Spearman-Kärber method was selected for reporting.

II. RESULTS AND DISCUSSION

A. FINDINGS

Nominal concentrations were 1.5, 3.0, 6.0, 12, and 24 mg IN-KB687/L. The treatment mean measured concentrations of IN-KB687 during the 96 hour exposure were 1.26, 2.55, 5.45, 10.0, and 23.6 mg/L, or 83 to 98% of the nominal concentrations. Recoveries from the IN-KB687 QC samples ranged from 83 to 99% of the nominal concentrations throughout the test.

All results from biological responses were based on mean measured concentrations of IN-KB687. A summary of the cumulative mortality and sublethal effects are presented in Table 96.

Table 96
Observed mortality and sublethal effects of mysid shrimp, *Americamysis bahia*, exposed to IN-KB687 for 96 hours in a static-renewal acute test

Mean, measured IN-KB687 Concentration (mg/L)	Cumulative mortality/Number at test start																Mean % Mortality
	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)	0/5	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	0/5	0/5	0
1.26	0/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5	0/5	0/5	1/5	0/5	0/5	0/5	1/5	0/5	5
2.55	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	0/5	0/5	0
5.45	1/5	0/5	0/5	0/5	1/5	0/5 ^a	0/5 ^a	0/5	1/5	0/5	0/5	0/5	1/5	0/5	0/5	0/5	5
10.0	5/5	4/5 ^a	4/5 ^a	4/5 ^a	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
23.6	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	5/5	5/5	100

^a Sublethal observation: One mysid shrimp lethargic

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

III. CONCLUSION

IN-KB687 was assessed for acute toxicity with the mysid shrimp, *Americamysis bahia*, in a 96-hour static-renewal test. After 96 hours of exposure, mortality was 0, 5, 0, 5, 100, and 100% in the 0 (control), 1.26, 2.55, 5.45, 10.0, and 23.6 mg IN-KB687/L treatments, respectively. The highest mean measured concentration causing no mortality at test end was 2.55 mg IN-KB687/L. The lowest mean measured concentration causing 100% mortality at test end was 10.0 mg IN-KB687/L. The 96 hour LC₅₀ was estimated to be 7.20 mg IN-KB687/L with 95% confidence intervals of 6.67 and 7.76 mg IN-KB687/L.

(Dinehart, S., 2013c)

RMS comment

This study was conducted in compliance with the current guideline. The 96 hour LC₅₀ was estimated to be 7.20 mg IN-KB687/L based on mean measured concentrations. This study is acceptable.

Report: Dinehart, S. (2014b); IN-KG433: Acute toxicity with the mysid shrimp, *Americamysis bahia*, determined under static-renewal test conditions

DuPont Report No.: DuPont-36478

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

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

Testing Facility Report No.: 69580

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 IN-KG433 with the mysid shrimp, *Americamysis bahia*, was determined in a 96-hour static-renewal test. The test was conducted in accordance with the U.S. EPA Ecological Effects Test guidelines, OPPTS 850.1035.

The test substance, IN-KG433, contains 99.9% active substance by analysis. The study was conducted with a single concentration of IN-KG433 (0.20 mg/L) and a dilution water control at a temperature range of 25.3 to 26.9°C. Ten mysids were used per test substance concentration and dilution water control replicate, for a total of 30 mysids per treatment. The treatment nominal concentration of IN-KG433 during the 96 hour exposure was 0.20 mg a.s./L. After 96 hours of exposure, mortality was 0% in both the 0 (control) and 0.20 mg IN-KG433/L treatment. The highest nominal concentration causing no mortality at test end was 0.20 mg IN-KG433/L. The 96 hour LC₅₀ was estimated to be >0.20 mg IN-KG433/L.

I. MATERIALS AND METHODS**A. MATERIALS**

- | | |
|--|---|
| 1. Test material: | IN-KG433 technical metabolite |
| Lot/Batch #: | KG433-004 |
| Purity: | 99.9% |
| Description: | Solid powder |
| CAS#: | Not available |
| Stability of test compound: | Not stable in the test system |
| 2. Control: | Dilution water (laboratory saltwater) |
| Solvent control: | None |
| Test vehicle: | Dilution water (laboratory saltwater) |
| Toxic reference: | None |
| 3. Test organism: | Mysid Shrimp |
| Species: | <i>Americamysis bahia</i> |
| Age at dosing: | <24 hours |
| Initial population: | 10 mysids per test chamber, 3 replicates per treatment for a total of 30 mysids per treatment |
| Source: | ABC Laboratories, in-house culture |
| Diet: | Fed ad libitum during test |
| Test chambers: | 1-L glass jars with 500-mL solution volume |
| 4. Environmental conditions (in-life period) | |
| Temperature: | 25.3 to 26.9°C (of test chambers) |
| Photoperiod: | 14 hr photoperiod (438 lux) and 10 hr darkness |

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
28-February-2014 to 04-March-2014
2. Experimental treatments
The acute toxicity of IN-KG433 to *Americamysis bahia* was determined in a static-renewal, 96-hour test. Treatments consisted of a dilution water control and a nominal concentration of 0.20 mg IN-KG433/L. Ten mysids were used per test concentration and control replicate for a total of 30 mysids per treatment.
3. Observations
Mortality and behavioural observations were made at 24, 48, 72, and 96 hours. No mortalities or sublethal effects were observed in any treatment.

4. Statistics

Due a lack of mortality or sublethal effects in the control and test substance treatment, no statistical analyses were necessary.

II. RESULTS AND DISCUSSION**A. FINDINGS**

The definitive test was completed as a limit test with a nominal test substance treatment concentration of 0.20 mg IN-KG433/L. The treatment measured concentrations of IN-KG433 during the 96 hour exposure were <LOD, LOQ. Recoveries from the IN-KG433 QC samples ranged from 111 to 153% of the nominal concentrations throughout the test.

All results from biological responses were based on nominal concentrations of IN-KG433. A summary of the cumulative mortality and sublethal effects are presented in Table 97.

Table 97
Observed mortality and sublethal effects of mysid shrimp, *Americamysis bahia*, exposed to IN-KG433 for 96 hours in a static-renewal acute test

Nominal IN-KG433 Concentration (mg/L)	Cumulative Mortality/Number at Test Start												Mean % Mortality
	24 Hours			48 Hours			72 Hours			96 Hours			
	A	B	C	A	B	C	A	B	C	A	B	C	
0 (Control)	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0
0.20	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0

Notes: Test chambers contained ten mysid shrimp each at test initiation. No sublethal effects were observed.

III. CONCLUSION

IN-KG433 was assessed for acute toxicity with the mysid shrimp, *Americamysis bahia*, in a 96-hour static-renewal test. After 96 hours of exposure, mortality was 0% in both the 0 (control) and 0.20 mg IN-KG433/L treatment. The highest nominal concentration causing no mortality at test end was 0.20 mg IN-KG433/L. The 96 hour LC₅₀ was estimated to be >0.20 mg IN-KG433/L.

(Dinehart, S., 2014b)

RMS comment

This study was conducted in compliance with the current guideline. However the test item IN-KG433 was not found (<LOD) at 0 and 48 hours and nominal concentrations were used. This study cannot be considered reliable and a new study is required. A new study has been scheduled by the notifier.

Report: Brougher, D.S., Martin, K.H., Gallagher, S.P., Bodle, E.S. (2014); IN-KT413: A 96-hour static acute toxicity test with the saltwater mysid (*Americamysis bahia*)

DuPont Report No.: DuPont-38347

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

Testing Facility: Wildlife International Ltd. (USA), Easton, Maryland, USA

Testing Facility Report No.: 112A-482

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 IN-KT413 to <24 hour old neonate saltwater mysid, *Americamysis bahia*, was determined in an unaerated, static, 96-hour dose response test. The test was conducted in accordance with the U.S. EPA Series 850 – Ecological Effects Test Guidelines, OPPTS Number 850.1035: *Mysid Acute Toxicity Test*. Treatments consisted of a dilution water control and five nominal IN-KT413 concentrations of 0.63, 1.3, 2.5, 5.0 and 10 mg IN-KT413/L. The mean measured concentrations were 0.49, 1.1, 2.0, 3.3, and 6.2 mg IN-KT413/L, representing 78, 85, 80, 66 and 62% of nominal IN-KT413 concentrations, respectively. The 96-hour LC₅₀ was 2.8 mg IN-KT413/L, based on mean measured concentrations of IN-KT413 and mortality. The highest mean measured test concentration causing no mortality was 2.0 mg IN-KT413/L. The lowest mean measured test concentration causing 100% mortality was 6.2 mg IN-KT413/L.

I. MATERIALS AND METHODS

A. MATERIALS

- | | | |
|----|-----------------------------|--|
| 1. | Test material: | IN-KT413 technical metabolite |
| | Lot #/Batch #: | KT413-003 |
| | Purity: | 99.7% |
| | Description: | Solid, Powder |
| | CAS # | Not applicable |
| | Stability of test compound: | Shown to be stable under the conditions of the test. |
| 2. | Controls: | Dilution water (filtered saltwater) |
| | Test vehicle: | Not applicable |
| | Toxic reference: | Not applicable |
| 3. | Test organism: | Mysid |
| | Species: | <i>Americamysis bahia</i> |
| | Age/life stage at dosing: | <24 hour neonates |
| | Source: | Wildlife International cultures |
| | Acclimation period: | At least 14 days |
| | Diet: | Culture: At least 2x daily |
| | | Test period: 2x daily |
| | Test chamber: | 2 L glass beaker holding 1 L of test solution (8.0 cm liquid depth) |
| | Water: | 20 ppt filtered saltwater |
| 4. | Environmental conditions: | Dissolved oxygen: ≥ 6.9 mg/L ($\geq 94\%$ of saturation); |
| | | pH: 7.8–8.1 |
| | Temperature: | 23.4–25.2°C in test chambers; 25.27–26.89°C measured continuously in a container of water adjacent to the test chambers. |
| | Photoperiod: | 16 hr light (308 lux at initiation) and 8 hr dark including 30 min transitional period preceding and following the 16-hr light interval. |

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
26-September-2013 to 30-September-2013

2. Experimental treatments

The acute toxicity of IN-KT413 to <24 hour old neonate mysid, *Americamysis bahia*, was determined in an unaerated, static, 96-hour dose response test. Treatments consisted of a dilution water control and five nominal IN-KT413 concentrations of 0.63, 1.3, 2.5, 5.0, and 10 mg IN-KT413/L. The test concentrations had mean measured IN-KT413 concentrations of 0.49, 1.1, 2.0, 3.3, and 6.2 mg IN-KT413/L, respectively. Duplicate test chambers were maintained in each treatment and control group, containing ten mysids each (total of twenty mysids in the negative control and in each test concentration).

3. Observations

Mortality and behavioural observations were made at approximately 4 hours and every 24 hours following initiation of exposure (± 1 hour).

4. Statistics

The mortality data were analysed using the computer program of C. E. Stephan. The program was designed to calculate the LC_{50} value and the 95% confidence interval by probit analysis, the moving average method, and binomial probability with nonlinear interpolation. In this study, nonlinear interpolation was used to calculate the 48 and 96-hour LC_{50} values and binomial probability was used to calculate the 95% confidence intervals. Binomial probability was used to calculate the 72 hour LC_{50} value and binomial probability was used to calculate the 95% confidence intervals. The absence of mortality in any of the IN-KT413 treatment groups at the 24 hour observation interval precluded the statistical calculation of LC_{50} values at 24 hours. The no-mortality concentration, NOEC, the highest mean measured test concentration causing no mortality at test end and the lowest mean measured test concentration causing 100% mortality at test end were determined by visual interpretation of the mortality and observation data.

II. RESULTS AND DISCUSSION

A. FINDINGS

Nominal concentrations of IN-KT413 of 0.63, 1.3, 2.5, 5.0 and 10 mg IN-KT413/L had equivalent mean measured IN-KT413 concentrations of 0.49, 1.1, 2.0, 3.3 and 6.2 mg IN-KT413/L, respectively. Mean, measured concentrations of IN-KT413 in the test solutions ranged from 62 to 85% of nominal concentrations during the test. Summaries of cumulative mortality and sublethal effects are presented in Table 98 and Table 99 respectively.

Table 98
Observed mortality of saltwater mysid (*Americamysis bahia*) exposed to
IN-KT413 for 96 hours in an unaerated, static, acute test

Mean, measured IN-KT413 Concentration (mg/L)	Cumulative mortality (No. dead/No. at test start) ^a									
	~4 Hours		24 Hours		48 Hours		72 Hours		96 Hours	
	A	B	A	B	A	B	A	B	A	B
Negative control (0.0)	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
0.49	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
1.1	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
2.0	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
3.3	0/10	0/10	0/10	0/10	0/10	0/10	6/10	4/10	9/10	8/10
6.2	0/10	0/10	0/10	0/10	9/10	9/10	10/10	10/10	10/10	10/10

^a Two replicate test chambers containing 10 mysids at test start were tested at each concentration.

Table 99
Observed sublethal effects of saltwater mysid (*Americamysis bahia*) exposed to
IN-KT413 for 96 hours in an unaerated, static, acute test

Mean, measured IN-KT413 Concentration (mg/L)	Sublethal effects (Number affected/Number alive) ^a									
	~4 Hours		24 Hours		48 Hours		72 Hours		96 Hours	
	A	B	A	B	A	B	A	B	A	B
Negative control (0.0)	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
0.49	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
1.1	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
2.0	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	1 ^d /10	3 ^d /10
3.3	0/10	0/10	0/10	0/10	1 ^d /10	2 ^d /10	4 ^d /4	3 ^c 3 ^d /6	1 ^c /1	2 ^c /2
6.2	0/10	0/10	1 ^c 4 ^d /10	1 ^b 3 ^d /10	1 ^c /1	1 ^c /1	--	--	--	--

^a Two replicate test chambers containing 10 mysids at test start were tested at each concentration.

^b Surfacing.

^c Lethargic.

^d Erratically swimming.

III. CONCLUSION

The 96-hour LC₅₀ for saltwater mysids was 2.8 mg IN-KT413/L, based on mean measured concentrations of IN-KT413 in the treatment solutions and mortality. The highest mean measured test concentration causing no mortality was 2.0 mg IN-KT413/L. The lowest mean measured test concentration causing 100% mortality was 6.2 mg IN-KT413/L.

(Brougher, D.S., Martin, K.H., Gallagher, S.P., Bodle, E.S., 2014)

RMS comment

This study was conducted in compliance with the current guideline. The 96-hour LC₅₀ for saltwater mysids was 2.8 mg IN-KT413/L, based on mean measured concentrations of IN-KT413. This study is acceptable.

Report: Dinehart, S. (2013b); IN-MK638: Acute toxicity with the mysid shrimp, *Americamysis bahia*, determined under static test conditions

DuPont Report No.: DuPont-36475

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

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

Testing Facility Report No.: 69581

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 IN-MK638 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, IN-MK638, contains 99.9% IN-MK638 by analysis. The study was conducted with six nominal concentrations of IN-MK638 (3.3, 6.5, 13, 25, 50, and 100 mg IN-MK638/L) and a dilution water control at a temperature range of 24.0 to 25.2°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 IN-MK638 during the 96-hour exposure were 3.20, 6.43, 13.2, 24.3, 49.9, and 104 mg IN-MK638/L, or 97 to 104% of the nominal concentrations. After 96 hours of exposure, mortality was 0, 0, 0, 5, 15, 55, and 100% in the 0 (control), 3.20, 6.43, 13.2, 24.3, 49.9, and 104 mg IN-MK638/L treatments, respectively. The highest mean measured concentration causing no mortality at test end was 6.43 mg IN-MK638/L. The lowest mean measured concentration causing 100% mortality at test end was 104 mg IN-MK638/L. The 96-hour LC₅₀ was estimated to be 41.1 mg IN-MK638/L, with 95% confidence intervals of 33.4 and 51.8 mg IN-MK638/L.

I. MATERIALS AND METHODS**A. MATERIALS**

- | | |
|--|--|
| 1. Test material: | IN-MK638 technical metabolite |
| Lot/Batch #: | MK638-002 |
| Purity: | 99.9% |
| Description: | Crystalline solid |
| CAS#: | Not available |
| Stability of test compound: | 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 ad libitum during test |
| Test chambers: | 500-mL glass jars with 250-mL solution volume |
| 4. Environmental conditions (in-life period) | |
| Temperature: | 24.0 to 25.2°C (of test chambers) |
| Photoperiod: | 14 hr photoperiod (441 lux) and 10 hr darkness which included two-30 min transitional light periods. |

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
11-March-2013 to 15-March-2013
2. Experimental treatments
The acute toxicity of IN-MK638 to *Americamysis bahia* was determined in a static, 96-hour test. Treatments consisted of a dilution water control and five nominal concentrations of 3.3, 6.5, 13, 25, 50, and 100 mg/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 LC_{50} 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 3.3, 6.5, 13, 25, 50, and 100 mg IN-MK638/L. The treatment mean measured concentrations of IN-MK638 during the 96 hour exposure were 3.20, 6.43, 13.2, 24.3, 49.9, and 104 mg IN-MK638/L, or 97 to 104% of the nominal concentrations. Recoveries from the IN-MK638 QC samples ranged from 104 to 111% of the nominal concentrations throughout the test.

All results from biological responses were based on mean measured concentrations of IN-MK638. A summary of the cumulative mortality and sublethal effects are presented in Table 100.

Table 100
Observed mortality of mysid shrimp, *Americamysis bahia*, exposed to IN-MK638 for 96 hours in a static acute test

Mean Measured IN-MK638 Concentration (mg/L)	Cumulative Mortality/Number at Test Start																Mean % Mortality
	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)	0/5	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	0/5	0/5	0
3.20	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	0/5	0/5	0
6.43	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	0/5	0/5	0
13.2	0/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5	0/5	0/5	1/5	0/5	0/5	0/5	1/5	0/5	5
24.3	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5	0/5	1/5	1/5	1/5	0/5	15
49.9	0/5	2/5	1/5	2/5	1/5	3/5	2/5	3/5	1/5	3/5	3/5	3/5	1/5	3/5	4/5	3/5	55
104	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	5/5	5/5	100

Notes: Test chambers contained five mysid shrimp each at test initiation. No sublethal effects were observed.

III. CONCLUSION

IN-MK638 was assessed for acute toxicity with the mysid shrimp, *Americamysis bahia*, in a 96-hour static test. After 96 hours of exposure, mortality was 0, 0, 0, 5, 15, 55, and 100% in the 0 (control), 3.20, 6.43, 13.2, 24.3, 49.9, and 104 mg IN-MK638/L treatments, respectively. The highest mean measured concentration causing no mortality at test end was 6.43 mg IN-MK638/L. The lowest mean measured concentration causing 100% mortality at test end was 104 mg IN-MK638/L. The 96-hour LC_{50} was estimated to be 41.1 mg IN-MK638/L, with 95% confidence intervals of 33.4 and 51.8 mg IN-MK638/L.

(Dinehart, S., 2013b)

RMS comment

This study was conducted in compliance with the current guideline. The 96-hour LC₅₀ was estimated to be 41.1 mg IN-MK638/L based on mean measured concentrations. This study is acceptable.

Report: Dinehart, S. (2013a); IN-MK643: Acute toxicity with the mysid shrimp, *Americamysis bahia*, determined under static test conditions

DuPont Report No.: DuPont-36476

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

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

Testing Facility Report No.: 69582

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 IN-MK643 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, IN-MK643, contains 96.7% IN-MK643 by analysis. The study was conducted with five concentrations of IN-MK643 (6.5, 13, 25, 50, and 100 mg/L) and a dilution water control at a temperature range of 24.2 to 25.1°C. Five mysids were used per test substance concentration and dilution water control replicate, for a total of 20 mysids per treatment. The mean measured concentrations of IN-MK643 during the 96 hour exposure were 6.19, 12.3, 25.0, 47.7, and 95.1 mg/L, or 95 to 100% of the nominal concentrations. After 96 hours of exposure, mortality was 0, 0, 10, 100, 100, and 100% in the 0 (control), 6.19, 12.3, 25.0, 47.7, and 95.1 mg IN-MK643/L treatments, respectively. Survival of the control organisms (100%) therefore satisfied the acceptability criteria stated in the protocol and the OPPTS 850.1035 guideline. The highest mean measured concentration causing no mortality at test end was 6.19 mg IN-MK643/L. The lowest mean measured concentration causing 100% mortality at test end was 25.0 mg IN-MK643/L. The 96-hour LC₅₀ was estimated to be 16.4 mg/L, with 95% confidence intervals of 14.9 and 18.0mg/L.

I. MATERIALS AND METHODS

A. MATERIALS

- | | |
|--|--|
| 1. Test material: | IN-MK643 technical metabolite |
| Lot/Batch #: | MK643-002 |
| Purity: | 96.7% |
| Description: | Powdered solid |
| CAS#: | 877681-12-4 |
| Stability of test compound: | 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 ad libitum during test |
| Test chambers: | 500-mL glass jars with 200-mL solution volume |
| 4. Environmental conditions (in-life period) | |
| Temperature: | 24.2 to 25.1°C (of test chambers) |
| Photoperiod: | 14 hr photoperiod (486 lux) and 10 hr darkness which included two-30 min transitional light periods. |

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
18-March-2013 to 22-March-2013
2. Experimental treatments
The acute toxicity of IN-MK643 to *Americamysis bahia* was determined in a static, 96-hour test. Treatments consisted of a dilution water control and five nominal concentrations of 6.5, 13, 25, 50, and 100 mg/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 6.5, 13, 25, 50, and 100 mg IN-MK643/L. The treatment mean measured concentrations of IN-MK643 during the 96 hour exposure were 6.19, 12.3, 25.0, 47.7, and 95.1 mg

IN-MK643/L, or 95 to 100% of the nominal concentrations. Recoveries from the IN-MK643 QC samples ranged from 102 to 105% of the nominal concentrations throughout the test.

All results from biological responses were based on mean measured concentrations of IN-MK643. A summary of the cumulative mortality and sublethal effects are presented in Table 101.

Table 101
Observed mortality and sublethal effects of mysid shrimp, *Americamysis bahia*, exposed to IN-MK643 for 96 hours in a static acute test

Mean measured IN-MK643 concentration (mg/L)	Cumulative mortality/Number at test start																Mean % mortality
	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)	0/5	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	0/5	0/5	0
6.19	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	0/5	0/5	0
12.3	0/5	0/5	0/5	0/5	1/5	0/5	0/5	0/5	2/5	0/5	0/5	0/5	2/5	0/5	0/5	0/5	10
25.0	5/5	5/5	5/5	4/5 ^a	5/5	5/5	5/5	4/5 ^a	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	100
47.7	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	5/5	5/5	100
95.1	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	5/5	5/5	100

^a One mysid shrimp lethargic

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

III. CONCLUSION

IN-MK643 was assessed for acute toxicity with the mysid shrimp, *Americamysis bahia*, in a 96-hour static test. After 96 hours of exposure, mortality was 0, 0, 10, 100, 100, and 100% in the 0 (control), 6.19, 12.3, 25.0, 47.7, and 95.1 mg IN-MK643/L treatments, respectively. The highest mean measured concentration causing no mortality at test end was 6.19 mg IN-MK643/L. The lowest mean measured concentration causing 100% mortality at test end was 25.0 mg IN-MK643/L. The 96-hour LC₅₀ was estimated to be 16.4 mg IN-MK643/L, with 95% confidence intervals of 14.9 and 18.0mg IN-MK643/L.

(Dinehart, S., 2013a)

RMS comment

This study was conducted in compliance with the current guideline. The 96-hour LC₅₀ was estimated to be 16.4 mg IN-MK643/L based on mean measured concentrations. This study is acceptable.

B.9.2.5. Long-term and chronic toxicity to aquatic invertebrates

Report: Hoke, R.A. (1997a); DPX-MP062 (approximately 75% DPX-KN128, 25% IN-KN127): Chronic toxicity to *Daphnia magna*

DuPont Report No.: HLR 597-96, Revision No. 1

Guidelines: USEPA 72-4 (1988), OECD 202 (1984) **Deviations:** None

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

Testing Facility Report No.: HLR 597-96

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.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: DPX-MP062 technical
 Lot/Batch #: MP062-51A
 Purity: 94.5%
 Description: Off-white solid
 CAS#: None for DPX-MP062
 DPX-KN128: 173584-44-6
 Stability of test compound: Shown to be stable in the test system by analysis
2. Untreated control: Dilution (laboratory well water that was aerated, filtered, and allowed to flow through a tank containing adult fathead minnows) water
 Solvent (positive) control: N,N-dimethylformamide
 Test vehicle: Dilution (laboratory well water that was aerated, filtered, and allowed to flow through a tank containing adult fathead minnows) water
 Toxic reference: None
3. Test organism
 Species: *Daphnia magna*
 Age at dosing: <24 hours old
 Initial population: 1 or 5 daphnids per test chamber
 Source: Haskell Laboratory, in-house culture
 Acclimation period: Less than 24 hours
 Diet: *Selenastrum capricornutum* and *Ankistrodesmus falcatus* every 48 hours
 Test chamber: 250-mL Pyrex beaker containing 200 mL of test solution (approximately 6.5-cm test solution depth), covered with Plexiglas
4. Environmental conditions (in-life period)
 Dissolved oxygen: 6.2 to 10.3 mg/L
 pH: 7.0 to 8.6
 Temperature: 20.1 to 21.1°C (of recirculating waterbath used to maintain test chamber temperature)
 Photoperiod: 16 hr photoperiod (316 to 426 lux) and 8 hr darkness which included 30 min transitional light (6.5 to 8.6 lux) preceding and following the 16-hr light interval

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
 06-September-1996 to 27-September-1996
2. Experimental treatments
 The effects of DPX-MP062 on the growth and reproduction of *Daphnia magna* were assessed in an unaerated, 21-day static-renewal test. Ten replicates were used per treatment. Seven replicates contained 1 daphnid each and 3 replicates contained 5 daphnids each. Treatments consisted of a dilution water control, a HEPES-buffered dilution water control, a solvent [0.1 mL/L dimethylformamide, DMF] control, and mean measured concentrations of 0.0026, 0.0055, 0.012, 0.023, 0.042, and 0.090 mg DPX-MP062/L. Immobilisation, sublethal effects, and production of

young were observed daily. Results are expressed on the basis of mean measured DPX-MP062 concentrations.

II. RESULTS AND DISCUSSION

A. FINDINGS

A summary of immobility and reproductive parameters is shown in Table 102. The 21-day EC₅₀, based on adult immobility and/or death, was greater than 0.090 mg/L DPX-MP062. The NOEC based on the first day of reproduction and total number of live young was greater than 0.090 mg/L. The NOEC based on total number of immobile young at 21 days was 0.042 mg/L DPX-MP062. The 21-day NOEC for length of surviving adults was greater than 0.090 mg/L DPX-MP062.

Table 102
Summary of test endpoints following exposure of *Daphnia magna* to DPX-MP062 for 21 days

Mean measured concentrations (mg DPX-MP062/L)	Replicates	Mean % adult survival ^a	Mean 1 st day of reproduction ^b	Mean total live young per female ^c	Mean total immobile young ^d	Mean adult length (mm) ^e
Dilution Water Control	1-7	100	7	215	0	4.71
	8-10	100	NA ^f	NA	NA	4.61
HEPES-Water Control	1-7	100	7.1	210	0.1	4.81
	8-10	100	NA	NA	NA	4.62
DMF Control	1-7	100	7.9	211	0	4.76
	8-10	93	NA	NA	NA	4.66
0.0026	1-7	100	7.3	237	0	4.87
	8-10	100	NA	NA	NA	4.65
0.0055	1-7	86	7.4	235	0	4.73
	8-10	93	NA	NA	NA	4.60
0.012	1-7	100	7.1	209	0	4.86
	8-10	100	NA	NA	NA	4.66
0.023	1-7	100	7.1	227	0	5.04
	8-10	100	NA	NA	NA	4.56
0.042	1-7	100	7.3	226	0	4.81
	8-10	87	NA	NA	NA	4.49
0.090	1-7	100	7.6	187	0.7 ^g	4.94
	8-10	100	NA	NA	NA	4.49

^a Percent of adult daphnids alive in all replicates at the end of the test (immobility was synonymous with death)

^b First day that reproduction was observed in replicates 1-7

^c Mean of live young produced per surviving female over 21 days

^d Sum of immobile young produced per surviving female over 21 days

^e Mean length of surviving adults

^f NA indicates that data were not available (data for survival only)

^g Statistically significant, $p \leq 0.05$

III. CONCLUSION

The 21-day NOEC (no observable effect concentration) and MATC (maximum acceptable toxicant concentration) for DPX-MP062, based on mean measured concentrations and all endpoints evaluated was greater than 0.090 mg/L for *Daphnia magna* neonates exposed for 21 days under static renewal conditions.

(Hoke, R. A., 1997a)

RMS comment

The reproductive and development toxicity to *Daphnia magna* study HL-597-96, Revision No.1 was conducted under guidelines OECD 202, and U.S. EPA 72-4. The applicant indicates that it meets the current guideline (OECD 211).

This study was assessed for previous Annex I inclusion and was conducted with the old material DPX-MP062. As new chronic toxicity study is available on the same species (*Daphnia magna*) for the new material DPX-KN128, this study is not considered essential.

Report: Hoke, R.A. (1997b); DPX-MP062 (approximately 75% DPX-KN128, 25% IN-KN127): Chronic toxicity to *Daphnia magna*

DuPont Report No.: HL-1997-00912

Guidelines: OECD 202, USEPA 72-4 **Deviations:** None

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

Testing Facility Report No.: HL-1997-00912

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.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: DPX-MP062 technical
 Lot/Batch #: MP062-51A
 Purity: 94.5%
 Description: Off-white solid
 CAS#: None for DPX-MP062
 DPX-KN128: 173584-44-6
 Stability of test compound: Shown to be stable under the conditions of the test
2. Untreated control: Dilution (laboratory well water) water
 Solvent (positive) control: N,N-dimethylformamide (DMF)
 Test vehicle: Dilution (laboratory well water) water
 Toxic reference: None
3. Test organism
 Species: *Daphnia magna*
 Age at dosing: <24 hours old
 Initial population: 10 daphnids per test chamber
 Source: Laboratory, in-house culture: Haskell Laboratory, Newark, Delaware
 Diet: Two green algal species, *Ankistrodesmus falcatus* and *Selenastrum capricornutum*, each Monday, Wednesday, and Friday at a rate of 125000 cells/mL of each species (total 250000 cells/mL).
 Test chamber: 250-mL Pyrex[®] beaker containing 200 mL of test solution (approximately 6.4-cm test solution depth), covered with a glass plate
 Environmental conditions (in-life period)
4. Dissolved oxygen 2.9 to 13.7 mg/L
 pH 6.9 to 8.7
 Temperature: 19.6 to 21.3°C (of recirculating waterbath used to maintain test chamber temperature) in replicates A–G
 19.6 to 21.4°C (of recirculating waterbath used to maintain test chamber temperature) in replicates H–J
 Photoperiod: 16 hr photoperiod (827 to 927 lux) and 8 hr darkness which included 30 min transitional light (6 to 9 lux) preceding and following the 16-hr light interval

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
 18-September-1997 to 09-October-1997
2. Test system
 The effects of DPX-MP062 on the growth and reproduction of *Daphnia magna* were assessed in an unaerated, 21-day static-renewal test. Ten replicates were used per treatment. Seven replicates contained one daphnid each and three replicates contained five daphnids each. Treatments consisted of a dilution water control, a HEPES-buffered solvent (0.1 mL/L dimethylformamide, [DMF]) control, and mean measured concentrations of 0.0024, 0.0050, 0.011, 0.019, 0.036, 0.075, and 0.19 mg DPX-MP062/L. Immobilisation, sublethal effects, and production of young were observed daily. Results are expressed on the basis of mean, measured DPX-MP062 concentrations.

II. RESULTS AND DISCUSSION

A. FINDINGS

A summary of immobility and reproductive parameters is shown in Table 103. The 21-day NOEC of DPX-MP062 in *Daphnia magna* was 0.075 mg/L, based on immobile neonates by an exact Jonkheere's test ($p < 0.05$).

Table 103
Summary of test endpoints following exposure of *Daphnia magna* to DPX-MP062 for 21 days

Mean measured concentrations (mg DPX-MP062/L)	Mean % adult survival ^a	Mean 1 st day of reproduction ^b	Mean total live young per female ^c	Mean total immobile young ^d	Mean adult length (mm) ^e
Dilution water control	91	8	133	0	4.4
HEPES-DMF control	100	8	159	0	4.7
0.0024	100	8	122	0	4.4
0.0050	100	8	140	0	4.6
0.011	100	8	166	0	4.7
0.019	100	8	155	0	4.7
0.036	100	8	153	0	4.7
0.075	100	8	158	0.4 ^f	4.7
0.19	95	8	116	10	4.5

^a Percent of adult daphnids alive in all replicates at the end of the test (immobility was synonymous with death)

^b First day that reproduction was observed in replicates A–G

^c Mean of live young produced per surviving female over 21 days

^d Sum of immobile young produced per surviving female over 21 days

^e Mean length of surviving adults in replicates A–G

^f $p > 0.05$

III. CONCLUSION

The 21-day NOEC and LOEC of DPX-MP062 in *Daphnia magna* were 0.075 and 0.19 mg DPX-MP062/L, respectively.

(Hoke, R.A., 1997b)

RMS comment

The reproductive and development toxicity to *Daphnia magna* study HL-1997-00912 was conducted under guidelines OECD 202, and U.S. EPA 72-4. The applicant indicates that it meets the current guideline (OECD 211).

This study was assessed for previous Annex I inclusion and was conducted with the old material DPX-MP062. As new chronic toxicity study is available on the same species (*Daphnia magna*) for the new material DPX-KN128, this study is not considered essential.

Report: Lamichhane, K. (2014); Indoxacarb (DPX-KN128): Static renewal, chronic toxicity test with the cladoceran, *Daphnia magna*

DuPont Report No.: DuPont-41661

Guidelines: OECD 211 (2012), OPPTS 850.1300 (1996) **Deviations:** None

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

Testing Facility Report No.: 81343

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 chronic toxicity of indoxacarb (DPX-KN128) to the cladoceran, *Daphnia magna*, was determined in a 21-day static renewal test. The test was conducted in accordance with OECD Guideline No. 211 and U.S. EPA OPPTS 850.1300 test guidelines.

The study was conducted with seven nominal concentrations of indoxacarb (0.00065, 0.0013, 0.0025, 0.0050, 0.010, 0.020, and 0.040 mg a.s./L), a vehicle control, a buffered control, and a dilution water control, at a temperature range of 19.8 to 20.6°C. One daphnid was used per replicate with ten replicates per treatment, for a total of 10 organisms per treatment.

The concentration of indoxacarb was measured at initiation, in spent test solutions on days 2, 8, 16, and 21, and in fresh test solutions on days 6, 14, and 20 of the definitive test. The mean measured concentrations of indoxacarb during the 21-day exposure were 0.000514, 0.000984, 0.00200, 0.00411, 0.00858, 0.0170, and 0.0351 mg a.s./L or 76 to 88% of nominal. No residues of indoxacarb were detected in the dilution water control, buffered control, or vehicle control solutions above the LOD of 0.0000198 mg a.s./L. Recoveries from the QC fortifications ranged from 85 to 112% of the nominal concentrations during the exposure. The biological response results are based on the mean measured indoxacarb concentrations.

Based on survival, the total number of live young per live adult, and adult length, the 21-day NOEC and LOEC based on mean measured concentrations were 0.0351 and >0.0351 mg a.s./L, respectively, the highest concentration tested. A statistically significant reduction in adult length was observed, with a NOEC and LOEC of 0.00858 and 0.0170 mg a.s./L, respectively. However, this decrease was determined to not be biologically significant. Therefore, the NOEC and LOEC based on adult length and mean measured concentrations is 0.0351 and >0.0351 mg a.s./L, respectively.

21 day EC₅₀ values for all parameters measured was determined to be >0.0351 mg a.s./L.

The MATC based on the survival, reproduction, length, and dry weight endpoints could not be calculated. The MATC based on the body length endpoint was calculated to be 0.0121 mg a.s./L. However, this is not considered relevant since it is the geometric mean of a NOEC and LOEC that are not considered biologically significant.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Indoxacarb technical
 Lot/Batch #: KN128-374
 Purity: 98.8%
 Description: White powder
 Stability of test compound: Stable in the test system based on indoxacarb recoveries from test solutions from the range-finding test
2. Control: Dilution water (laboratory blended fresh water) and Buffered dilution water (~7.0 pH)
 Solvent control: Dimethylformamide (DMF)
 Test vehicle: Dilution water (laboratory blended fresh water)
 Toxic reference: None
3. Test organism: Cladoceran
 Species: *Daphnia magna*
 Age at dosing: Neonates (<24-hrs old)
 Initial population: 1 daphnid per test chamber/10 daphnids per treatment
 Source: In-house culture
 Diet: Test period: algal suspension
 (*Pseudokirchneriella subcapitata*) plus a prepared invertebrate food solution consisting of wheat grass, salmon starter, and yeast suspension
 Test chamber: 125-mL glass jar containing 80 mL of test solution (3.5-cm test solution depth), covered with plastic Petri dish
4. Environmental conditions (in-life period)
 Temperature: 19.8 to 20.6°C (of replicate test chambers)
 Photoperiod: 16 hr photoperiod (481 lux) and 8 hr darkness which included 30 min transitional light preceding and following the 16-hr light interval
 Dissolved oxygen: 7.7 to 9.0 mg/L (89 to 103% saturation) in fresh solution
 4.4 to 8.1 mg/L (52 to 93% saturation) in old solution
 pH: 7.1 to 8.5

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
 01-October-2014 to 22-October-2014
2. Experimental treatments
 The chronic toxicity of indoxacarb to *Daphnia magna* was determined in a static renewal, 21-day test. Treatments consisted of a dilution water control, buffered control, vehicle control, and seven nominal concentrations of 0 (control), 0 (buffered control), 0 (vehicle control), 0.00065, 0.0013, 0.0025, 0.0050, 0.010, 0.020, and 0.040 mg a.s./L. One daphnid was used per replicate with ten replicates per test concentration and control.
3. Observations
 Observations were made daily on the number of surviving adult daphnids, occurrence of abnormalities, and production of neonates.
4. Statistics
 All statistical analyses were performed using SAS software version 9.3 and Ecostats. All statistical evaluations for the test parameters were performed against the pooled (buffered and vehicle) control treatment. Inferences of statistical significance were based upon a $p = 0.05$ unless otherwise noted.

A one-way analysis of variance (ANOVA) was performed to identify LOEC and NOEC values, based on reproduction, body length, and dry weight data. If the data were consistent with a monotone concentration-response, then the LOEC and NOEC values were determined by the step-down Jonckheere Terpstra test ($p = 0.05$) where the alternate hypothesis was that the mean for the measured endpoint parameter (*i.e.*, reproduction, body length, and body weight) was reduced in comparison to the control. Where the data were not consistent with a monotone concentration-response, a Shapiro-Wilk test and Levene test were conducted to test for normality and homogeneity of variance, respectively, over treatments at each time point. If the results from the Shapiro-Wilk's and Levene's tests indicated normality and homogeneity (*i.e.*, $p > 0.01$), Dunnett's test was performed on the non-transformed raw data. In instances of non-normality or heterogeneity (*i.e.*, $p < 0.01$), a Dunn's test was performed.

Statistical analysis of the mean measured concentrations versus the data on measured endpoint parameters (*i.e.*, reproduction, body length, and dry weight) was performed to estimate the EC_x statistics along with the 95% confidence limits. For the EC_x statistics, acceptable criteria for estimation were not met due to the following: (i) the 95% confidence interval reported for EC_x did not contain zero and was not overly wide, (ii) the 95% confidence interval for the predicted mean at EC_x did not contain the control mean, and (iii) there was no significant lack-of-fit of regression model to the data. Therefore, EC_{50} estimates and 95% confidence intervals were not statistically calculable. Where possible, the point estimates of the maximum acceptable toxicant concentration (MATC) were calculated as the geometric mean of the NOEC and LOEC values of the most sensitive endpoints.

II. RESULTS AND DISCUSSION

A. FINDINGS

The concentration of indoxacarb was measured at initiation, in spent test solutions on Days 2, 8, 16, and 21, and in fresh test solutions on Days 6, 14, and 20 of the definitive test. The mean measured concentrations of indoxacarb during the 21-day exposure were 0.000514, 0.000984, 0.00200, 0.00411, 0.00858, 0.0170, and 0.0351 mg a.s./L or 76 to 88% of nominal. No residues of indoxacarb were detected in the dilution water control, buffered control, or vehicle control solutions above the LOD of 0.0000198 mg a.s./L. Recoveries from the QC fortifications ranged from 85 to 112% of the nominal concentrations during the exposure. The biological response results are based on the mean measured indoxacarb concentrations.

Based on survival and mean measured concentrations, the 21-day NOEC and LOEC were 0.0351 and >0.0351 mg a.s./L, respectively, the highest concentration tested. The NOEC and LOEC, based on number of total young or total number of live young per live adult, and mean measured concentrations, were 0.0351 and >0.0351 mg a.s./L, respectively. Based on dry weight and mean measured concentrations, the 21 day NOEC and LOEC were 0.0351 and >0.0351 mg a.s./L, respectively. A statistically significant reduction in adult length was observed, with a NOEC and LOEC of 0.00858 and 0.0170 mg a.s./L, respectively. However, this decrease was determined to not be biologically significant. Therefore, the NOEC and LOEC based on adult length and mean measured concentrations is 0.0351 and >0.0351 mg a.s./L, respectively.

Table 104
Summary of survival, reproduction, and growth of *Daphnia magna* exposed to indoxacarb during a 21-day static renewal test

Mean measured concentration (mg a.s./L)	21-day survival (%)	Mean day of first brood	Mean live young per live adult	Day 21 mean length (mm)	Day 21 mean weight (mg)
Control	100	8	178.5	4.1	0.825
Buffered control	100	8	179.3	4.1	0.809
Vehicle control	80	8	196.3	4.1	0.747
0.000514	80	8	181.9	4.0	0.848
0.000984	100	8	192.4	4.1	0.819
0.00200	90	8	179.6	4.0	0.840
0.00411	90	8	196.9	4.0	0.772
0.00858	100	8	185.4	4.0	0.773
0.0170	100	9	180.3	4.0 *	0.776
0.0351	100	8	182.5	4.0 *	0.757

* Statistically significant reduction as compared to the control (Jonckheere-Terpstra test; $p \leq 0.05$); however, this reduction is not considered biologically significant

III. CONCLUSION

The relevant test acceptability criteria were met for this study. The water-quality characteristics in the fresh solution remained within the tolerance limits set forth in the protocol. Survival of the dilution water control daphnids was 100%. The average young per control parent present at test termination was 179 and no ephippia were produced by control animals.

Based on survival and mean measured concentrations, the 21-day NOEC and LOEC were 0.0351 and >0.0351 mg a.s./L, respectively.

The NOEC and LOEC, based on number of total young or total number of live young per live adult, and mean measured concentrations, were 0.0351 and >0.0351 mg a.s./L, respectively.

A statistically significant reduction in adult length was observed, with a NOEC and LOEC of 0.00858 and 0.0170 mg a.s./L, respectively. However, this decrease was determined to not be biologically significant. Therefore, the NOEC and LOEC based on adult length and mean measured concentrations is 0.0351 and >0.0351 mg a.s./L, respectively.

Based on dry weight and mean measured concentrations, the 21-day NOEC and LOEC were 0.0351 and >0.0351 mg a.s./L, respectively.

The MATC based on the survival, reproduction, and dry weight endpoints could not be calculated. The MATC based on the body length endpoint was calculated to be 0.0121 mg a.s./L; however, this is not considered relevant since it is the geometric mean of a NOEC and LOEC that are not considered biologically significant.

(Lamichhane, K., 2014)

RMS comment

This study was conducted in compliance with the current guideline. The 21-day NOEC was 0.0351 mg a.s./L based on mean measured concentrations. This study is acceptable.

Report: Boeri, R.L., Wyskiel, D.C., Ward, T.J. (2003); IN-KT413: Chronic, static-renewal toxicity to the daphnid, *Daphnia magna*

DuPont Report No.: DuPont-12041

Guidelines: OECD 211 (1998), USEPA 850.1300 (1996) **Deviations:** None

Testing Facility: T.R. Wilbury Laboratories, Inc., Marblehead, Massachusetts, USA

Testing Facility Report No.: 2547-DU

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 effects of IN-KT413 technical metabolite on the growth and reproduction of *Daphnia magna* (<24-hour old) were assessed in an unaerated, static-renewal, 21-day test. The test was conducted according to (i) the Organisation for Economic Co-Operation and Development (OECD) Guideline for Testing Chemicals: 211 (1998), and (ii) U.S. OPPTS 850.1300 – Public Draft (1996).

Treatments consisted of a dilution water control and five nominal concentrations of 0.50, 1.0, 2.0, 4.0, and 8.0 mg IN-KT413/L. The corresponding mean measured concentrations of IN-KT413 were 0.49, 1.0, 1.9, 3.9, and 7.6 mg IN-KT413/L.

The 21-day NOEC (no observed effect concentration), lowest observed effect concentration (LOEC), and MATC (maximum acceptable toxicant concentration), for IN-KT413 based on mean measured concentrations and adult immobilization, the number of live, mobile neonates per surviving adult, the day of first brood, the average length of surviving adult daphnids, and the average dry weight of surviving adult daphnids, were 3.9, 7.6, and 5.4 mg/L, respectively, for *Daphnia magna* exposed for 21 days under static-renewal conditions.

The 21 day EC₅₀ based on adult daphnid immobility was 5.8 mg/L IN-KT413, with a 95% confidence interval of 4.5 to 7.8 mg/L.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: IN-KT413 technical metabolite
Lot/Batch #: KT413-3
Purity: 97%
Description: Solid powder
CAS#: Not available
Stability of test compound: Shown to be stable in the test system under the conditions and duration of the test by analysis.
2. Untreated control: Dilution water (deionised and hardness adjusted)
Test vehicle: Dilution water (deionised and hardness adjusted)
Toxic reference: None
3. Test organism
Species: *Daphnia magna*
Age at dosing: <24 hours old
Initial population: 1 Daphnids per test chamber
Source: Laboratory, in-house culture (T.R. Wilbury Laboratories)
original culture was obtained on November 6, 2001 from
Aquatic BioSystems, Inc., Fort Collins, Colorado, USA

Acclimation period: None
Diet: Mixture of yeast, alfalfa, trout chow (~5 mg/L dry weight) and
S. capricornutum at a final concentration of ~10⁸ cells/L
(carbon content of feeding ration, ~0.2 mg/daphnid/day).

Test chamber: 250-mL Pyrex beaker containing 200 mL of test solution
(7-cm test solution depth), covered with clear plastic
4. Environmental conditions (in-life period)
Temperature: 18.4 to 20.1°C (mean 19.3°C)
Photoperiod: 16 hours light (~350 lux) and 8 hours darkness including
15 minutes of transitional light (~10 lux) between the dark and
light intervals

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
20-January-2003 to 13-March-2003
2. Experimental treatments

An 11-day range-finding test was conducted during January 20 to 31, 2003, using a dilution water control and nominal IN-KT413 concentrations of 0.1, 1.0, 10, and 120 mg/L, with five daphnids in each of two replicates per test group. After 11 days of exposure, the respective survival rates were 100, 100, 90, 0, and 0%. After 11 days of exposure, the respective live young production totalled 201, 210, 259, 0, and 0. No immobile young were observed at any concentration. No insoluble material was observed. Concentrations for the 21-day life cycle study were based on the results of the 11-day, static-renewal, range-finding study

In the definitive test, the effects of IN-KT413 on the growth and reproduction of *Daphnia magna* (<24-hour old) were assessed in an unaerated, static-renewal, 21-day test. Treatments consisted of a dilution water control and nominal concentrations of 0.50, 1.0, 2.0, 4.0, and 8.0 mg IN-KT413/L. Ten replicates were used per treatment, with one daphnid per replicate. Test concentrations were renewed every Monday, Wednesday, and Friday.
3. Observations

Observations were made daily of the number of surviving adult daphnids, occurrence of abnormalities, and production of live or immobile young. Length and dry weight of surviving adult daphnids were determined at test end (21 days).

4. Statistics

Immobilization of first generation daphnids, the average number of mobile young per surviving daphnid, day of first brood per surviving daphnid, length, and dry weight were statistically analysed. Abnormal effects of surviving first generation daphnids (other than length and weight) were not observed at the end of the test, so these data did not warrant statistical analysis. Immobilization of first generation daphnids data were arc sine square root transformed prior to statistical analysis.

A Chi-squared test was used to determine that data were normally distributed for mobile young per daphnid, average length, and average dry weight and non-normal for immobilization and day of first brood. Bartlett's test was used to determine that variances were homogeneous for mobile young per daphnid, average length, and average dry weight, and heteroscedastic for day of first brood, and first generation immobilization data. A parametric ANOVA and Bonferroni's test were used to compare treatments to the control for mobile young per daphnid, length and dry weight data. A nonparametric Williams test was used to compare treatments to the control mean for day of first brood, and first generation immobilization data. All calculations were performed using the mean measured concentrations of test substance.

The NOEC is the highest tested concentration at which no measured biological parameter was significantly different from the control. The lowest observed effect concentration (LOEC) is the lowest tested concentration at which any measured biological parameter was significantly different than the control. The MATC was calculated as the geometric mean of the NOEC and the LOEC. The 21 day EC₅₀ was calculated using the probit method with mean measured concentrations of test substance and the number of live, mobile adult daphnids.

II. RESULTS AND DISCUSSION

A. FINDINGS

Analytical verification of IN-KT413 concentrations was made on test solutions sampled on Day 0 and at regular intervals during the study. Mean measured concentrations of IN-KT413 were 0.49, 1.0, 1.9, 3.9, and 7.6 mg/L and ranged from 95 to 100% of nominal concentrations. All chemical and physical parameters for the 21-day study were within acceptable ranges.

A summary of percent adult survival, mean first day of reproduction, total live young produced per surviving female, total immobile young produced per surviving female, length and dry weight of surviving adults is shown in Table 105.

Table 105
Summary of test endpoints following exposure of *Daphnia magna* to IN-KT413 for 21 days

Mean, measured concentrations of IN-KT413 (mg/L)	Mean % adult survival ^a	Mean first day of reproduction ^b	Mean total live young ^c	Mean total immobile young ^d	Mean adult length (mm)	Mean adult dry weight (mg)
Water Control	100	10	160	0	4.4	0.95
0.49	100	10	166	0	4.4	0.91
1.0	100	10	175	0	4.4	0.80
1.9	100	10	165	0	4.4	0.75
3.9 ^e	90	10	144	0	4.2	0.89
7.6 ^f	20	14	78	0	3.5	0.31

^a Percent of adult daphnids alive at the end of the test (immobility was synonymous with death)

^b First day that reproduction was observed in the replicates

^c Mean of live young produced per surviving female

^d Mean of immobile young produced per surviving female

^e NOEC based on immobilisation of first generation daphnids, Williams test, p<0.05

^f LOEC based on immobilisation of first generation daphnids, Williams test, p<0.05

III. CONCLUSION

The 21-day NOEC (no observed effect concentration), lowest observed effect concentration (LOEC), and MATC (maximum acceptable toxicant concentration), for IN-KT413 based on mean measured concentrations and adult immobilization, the number of live, mobile neonates per surviving adult, the day of first brood, the average length of surviving adult daphnids, and the average dry weight of surviving adult daphnids, were 3.9, 7.6, and 5.4 mg/L, respectively, for *Daphnia magna* exposed for 21 days under static-renewal conditions.

(Boeri, R.L., Wyskiel, D.C., Ward, T.J., 2003)

RMS comment

This study was conducted in compliance with the current guideline. The 21-day NOEC was 3.9 mg IN-KT413/L based on mean measured concentrations, at which 10% effect was observed. This study is acceptable.

Report: Boeri, R.L., Magazu, J.P., Ward, T.J. (1997); Chronic toxicity of DPX-MP062 to the mysid, *Mysidopsis bahia*

DuPont Report No.: HLO-1997-00206, Revision No. 1

Guidelines: U.S. EPA 72-4 **Deviations:** None

Testing facility: T.R. Wilbury Laboratories, Inc., Marblehead, Massachusetts 01945, USA

Testing Facility Report No.: 805-DU

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 effects of DPX-MP062 technical on the growth and reproduction of the mysid shrimp, *Mysidopsis bahia* (<24-hours old), were assessed in an unaerated, flow-through, 28-day test. The test was conducted in accordance with the appropriate Good Laboratory Practice standards and U.S. EPA Pesticide Assessment Guideline Subdivision E, 72-4 (c). Treatments consisted of a dilution water control, a solvent control (0.1 mL/L dimethylformamide; DMF), and five nominal concentrations of 0.023, 0.047, 0.090, 0.18, and 0.36 mg DPX-MP062/L. The corresponding mean, measured concentrations of DPX-MP062 were 0.0184, 0.0407, 0.0847, 0.183, and 0.349 mg DPX-MP062/L.

The 28-day NOEC for *Mysidopsis bahia* based on mean, measured concentrations and survival of first generation mysids after 28-days exposure was 0.0184 mg DPX-MP062/L.

The LOEC (lowest observed effect concentration) and MATC (maximum acceptable toxicant concentration), were 0.047 and 0.0274 mg DPX-MP062/L, respectively, based on mean measured concentration and survival of first generation mysids after 28-days exposure.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: DPX-MP062 technical
 Lot/Batch #: MP062-51
 Purity: 94.54%
 Description: Off-white solid
 CAS#: 144171-61-9
 Stability of test compound: Test substance appeared to be stable under the conditions of the study; no evidence of instability was observed.
2. Untreated control: Dilution water (carbon filtered natural seawater)
 Solvent (positive) control: Dimethylformamide
 Test vehicle: Dilution water (carbon filtered natural seawater)
 Toxic reference: None
3. Test organism
 Species: *Mysidopsis bahia*
 Age at dosing: <24 hours old
 Initial population: 15 mysids per retention chamber (30 mysids per replicate)
 Source: Laboratory, in-house culture (originally obtained from Aquatic BioSystems, Inc., Fort Collins, Colorado, USA)
 Acclimation period: 14 days
 Diet: Newly hatched brine shrimp, *Artemia salina*, daily during acclimation, and two or three times each day during the test at a rate of approximately 150 brine shrimp/mysid/day.
 Test chamber: 20-liter glass aquaria (21 cm in width, 40 cm in length, and 26 cm in height) that contained up to 8 liters of test solution (test media depth ranged from 4 to 10 cm).
4. Environmental conditions (in-life period)
 Dissolved oxygen: 5.5 to 8.3 mg/L
 pH: 7.8 to 8.3
 Temperature: 23.4 to 26.0°C (mean, 24.5°C)
 Photoperiod: 16 hr photoperiod (45 footcandles) and 8 hr darkness which included 15 minutes of transitional light preceding and following the 16-hr light interval

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
 31-October-1996 to 30-November-1996
2. Experimental treatments
 The effects of DPX-MP062 on the growth and reproduction of *Mysidopsis bahia* (<24-hours old) were assessed in an unaerated, flow-through, 28-day test.

A range-finding test was conducted under static-renewal conditions with mysids that were less than 24-hours old. Nominal concentrations of DPX-MP062 were 0 mg DPX-MP062/L (control and solvent control), 0.010, 0.050, 0.10, 0.36, and 0.50 mg/L, with media renewal on Days 1 to 4, 7 to 11, and 14 to 17. After 18 days of exposure survival was at least 80% at 0 (control and solvent control), 0.010, 0.050, and 0.10 mg/L, and 0% at 0.36 and 0.50 mg/L. Gravid females were observed in test vessels containing 0 (control and solvent control), 0.010, 0.050, and 0.10 mg/L.

In the definitive test, treatments consisted of a dilution water control, a solvent control (0.1 mL/L dimethylformamide), and five nominal concentrations of 0.023, 0.047, 0.090, 0.18, and 0.36 mg DPX-MP062/L. The highest tested concentration was the reported solubility limit of the test substance in seawater. A total of two replicates, each containing thirty, <24-hour-old mysids, were

tested per concentration (60 mysids/concentration) and control. Test concentrations were maintained *via* flow-through conditions using an intermittent flow proportional diluter. When the sex of mysids could be determined (Day 14 of the exposure) mysids within each vessel were rearranged so that a single male and female pair was placed in each of the chambers. Up to 10 chambers were utilised. Extra, unpaired mysids were sexually differentiated and placed in Chambers 11 and 12.

3. Observations

The number of surviving organisms and the occurrence of sublethal effects on behaviour or appearance (loss of equilibrium, erratic swimming, loss of reflex, excitability, discoloration, or change in behaviour) were determined visually and recorded initially and at 24-hour intervals. Dead test organisms were removed when first observed. Beginning on Day 14 of the exposure the mysids were categorised by sex. Female mysids were defined as those mysids with visible brood pouches. Offspring were counted and removed every day after Day 15, the first day that young were present. At the termination of the test the total length of each surviving first generation mysid was determined.

Analytical determination of DPX-MP062 concentration in the test solutions was performed with samples collected from each replicate test vessel on Days 0, 7, 14, 21, and 28.

4. Statistics

Data that were statistically analyzed included: 1) the number of first generation mysids surviving 28 days of exposure, 2) the number of young per surviving female after 28 days of exposure (defined as the sum of the total number of young each day divided by the number of surviving females that day), 3) total length of surviving first generation mysids at the conclusion of the test, 4) blotted wet weight of surviving first generation mysids at the conclusion of the test, and 5) dry weight of surviving first generation mysids at the conclusion of the test. No sublethal effects (other than effects on growth and reproduction) were observed at any concentration at the test termination. Therefore, statistical analysis of sublethal effect data was not warranted.

Results of the toxicity test were analyzed, when warranted, by standard statistical techniques. Control and solvent control data were compared with a parametric "t" test and in all cases no differences were observed ($\alpha = 0.05$). Control and solvent control data were pooled prior to subsequent statistical analyses. The Shapiro-Wilk's test was used to determine that data were normally distributed, and Bartlett's test was used to determine that variances were homogeneous. A one-way analysis of variance (ANOVA) and Bonferroni's test was then used to compare treatment and pooled control means. Survival data were arc sine [square root(Y)] transformed prior to analysis. All calculations were performed using mean measured concentrations of the active ingredient.

The NOEC is the highest tested concentration at which no measured biological parameter is statistically different ($\alpha = 0.05$) from the control. The LOEC is the lowest tested concentration at which any measured biological parameter is statistically different ($\alpha = 0.05$) from the control. The MATC is calculated as the geometric mean of the NOEC and the LOEC.

II. RESULTS AND DISCUSSION

A. FINDINGS

Mean, measured concentrations of DPX-MP062 were 0.0184, 0.0407, 0.0847, 0.183, and 0.349 mg DPX-MP062/L, and ranged from 80 to 102% of the targeted nominal concentrations. All chemical and physical parameters for the 28-day study were within acceptable ranges. All validation criteria were met for the study.

A summary of percent adult survival, total young produced per surviving female and length and dry weight of surviving adults is shown in Table 106. Water control and solvent control survival at the end of the test was at least 87% in each replicate. Offspring production averaged 5.6 and 6.1 young per female in the water control and solvent control, respectively. Mysids exhibiting lethargy and erratic swimming were observed on Day 1 in test vessels with mean measured concentrations of 0.183 and 0.349 mg/L, and on Day 2 in test vessels with a mean measured concentration of 0.183 mg/L. These effects were not observed at any other time during the test. No other sublethal effects (other than effects on growth and reproduction)

were observed in any concentration during the test and statistical analysis of sublethal effect data was not warranted.

Exposure of mysids to DPX-MP062 resulted in a NOEC of 0.0184 mg/L when treatment data were compared to pooled water control and solvent control data (one-way ANOVA, Bonferroni's t-test, $p < 0.05$).

The most sensitive measure of toxicity determined by statistical analysis of survival, growth, and reproduction data was the survival of first generation mysids after 28 days of exposure.

Table 106
Summary of test endpoints following exposure of *Mysidopsis bahia* to DPX-MP062 for 28 days

Mean, measured concentrations of DPX-MP062 (mg/L)	Replicate	Percent survival at Day 28	Production of young/female by Day 28 ^a	Mean ^b total length (mm)	Mean weight (mg) ^b	
					Wet	Dry
ND ^c (water control)	1	93	5.4	8.2	4.3	0.84
	2	87	5.8	8.3	4.6	0.83
ND (solvent control)	1	90	5.3	8.0	3.6	0.73
	2	87	6.9	8.3	4.6	0.92
0.0184	1	90	6.2	8.3	4.2	0.86
	2	83	8.4	8.2	4.2	0.80
0.0407	1	70 ^d	6.0	8.0	4.4	0.85
	2	80 ^d	4.9	7.7	4.4	0.82
0.0847	1	20 ^d	2.0 ^d	7.8	4.5	0.65
	2	23 ^d	4.3 ^d	8.3	4.3	0.77
0.183	1	0.0 ^e	- ^e	- ^e	- ^e	- ^e
	2	0.0 ^e	- ^e	- ^e	- ^e	- ^e
0.349	1	0.0 ^e	- ^e	- ^e	- ^e	- ^e
	2	0.0 ^e	- ^e	- ^e	- ^e	- ^e

^a Young production is defined as the sum of the total number of young each day divided by the number of surviving females that day.

^b Total length and weight means are the means of the entire data set.

^c ND = none detected at or above the limit of quantitation of 0.0100 mg/L.

^d Means are significantly different than the pooled controls (one-way ANOVA, Bonferroni's t-test, $p < 0.05$).

^e Means assumed to be significantly different than the pooled controls.

III. CONCLUSION

The 28-day NOEC (no observable effect concentration), based on mean, measured concentrations and on survival of first generation mysids after 28 days of exposure, was 0.0184 mg DPX-MP062/L for *Mysidopsis bahia*. The LOEC (lowest observed effect concentration) and MATC (maximum acceptable toxicant concentration), were 0.047 and 0.0274 mg DPX-MP062/L, respectively, based on mean measured concentration and survival of first generation mysids after 28-days exposure.

(Boeri, R.L., Magazu, J.P., Ward, T.J., 1997)

RMS comment

This study was not conducted in accordance with the current guideline and was conducted with the old material DPX-MP062. It is however considered in the risk assessment as no other study is available for the active substance on this species. The 28-day NOEC based on mean measured concentrations was 0.0184 mg DPX-MP062/L for *Mysidopsis bahia*. This study is considered acceptable.

Development and emergence in Chironomus species (spiked water tests)

Report: Radford, K. (2000); DPX-MP062: To assess the toxicity to the sediments dwelling phase of the midge *Chironomus riparius*

DuPont Report No.: DuPont-4055

Guidelines: draft OECD 219 **Deviations:** None

Testing Facility: Huntingdon Life Sciences Ltd., Huntingdon, Cambridgeshire, England, UK

Testing Facility Report No.: DPT 520

GLP: Yes

Certifying Authority: Department of Health (U.K.)

I. MATERIALS AND METHODS**A. MATERIALS**

- | | | |
|----|---------------------------------|--|
| 1. | Non-radiolabeled test material: | DPX-MP062 technical |
| | Lot/Batch #: | MP062-219-A |
| | Purity: | 98.4% |
| | Description: | Solid (white powder) |
| | CAS#: | 144171-61-9 |
| 2. | Radiolabeled test material | [¹⁴ C-]DPX-JW062EL (enriched with DPX-KN128 to simulate the enantiomeric ratio of DPX-MP062) |
| | Lot/Batch #: | HOTC File No. 461 |
| | Radiochemical purity: | 98.4% |
| | Specific activity: | 33.500 µCi/mg |
| 3. | Controls: | Negative control and solvent control |
| | Test vehicle: | Solvent acetone (0.10 mL acetone/L water) |
| | Toxic reference: | None |
| 4. | Test organism: | Midge |
| | Species: | <i>Chironomus riparius</i> |
| | Age/life stage at dosing: | Less than 36 hours old (first instar) |
| | Initial population: | 10 larvae per test chamber |
| | Source: | Huntingdon Life Sciences Ltd. in-house culture |
| | Diet: | TetraMin [®] fish food suspended in dilution water |
| | Test chamber: | Glass beakers (8 cm diameter) |
| 5. | Environmental conditions: | Dissolved oxygen: 6.2-8.3 mg/L |
| | | pH: 5.5 to 7.3 |
| | Temperature: | 19-20°C in test chambers |
| | Photoperiod: | 16 hours light (800-900 lux) and 8 hours dark |

B. STUDY DESIGN AND METHODS

1. Experimental initiated/completed
24-October-2000 to 21-November-2000

2. Experimental treatments

A study was conducted to assess the toxicity of DPX-MP062 to the sediment dwelling phase of the non-biting midge, *Chironomus riparius*, using a static test system with application of the test substance to the aqueous phase. The test substance was applied in the radiolabeled form known as [^{14}C]DPX-JW062EL, a mixture of radiolabeled enantiomers with the same enantiomeric ratio as DPX-MP062. The study was initiated with first instar chironomid less than 36 hours old. Daily records were maintained for each culture, which included the general condition of the larvae and the female:male ratio of successfully emerged adults. The larvae were fed daily with Tetramin[®] fish food suspension dispersed in culture medium, at a rate of 0.5 mg/larva for Days -1 to 9 and 1.0 mg/larva from Day 10 to test end. Four replicates of (nominally) 10 chironomid larvae per concentration group were exposed for 28 days to nominal concentrations of 3.125, 6.25, 12.5, 25, 50, 100, 200, 400, and 800 μg [^{14}C]DPX-JW062EL/L dispersed in Elendt M4 culture medium. Environmental data (pH, dissolved oxygen, and temperature) were recorded at the start and end of the study, and once weekly throughout the study. The results of all chemical analyses are based on total radioactivity measurements expressed as concentrations of the active ingredient.

II. RESULTS AND DISCUSSION

A. FINDINGS

Analysis of the dose solutions showed that the applied doses were 99-109% of nominal. Based on the liquid scintillation counts of the overlying water, the concentrations of [^{14}C]DPX-JW062EL in the overlying water decreased from 26-48% on Day 0 to 13-23% of applied dose on Day 28 (Table 107). Samples of the pore water collected after the 28-day exposure period accounted for 0.21–0.46% of the total radiolabeled test substance added to each vessel (Table 108). Samples of the dry sediment on Day 28 contained 17-41% of the total radiolabeled test substance added to each vessel (Table 108).

Table 107
Concentrations of ^{14}C -DPX-JW062EL in overlying water

Nominal concentration ($\mu\text{g/L}$)	Actual applied concentration ($\mu\text{g/L}$)	Mean measured concentration ($\mu\text{g/L}$)			
		Day 0	Day 7	Day 14	Day 28
Control	Control	ND ^a	ND	ND	ND
Solvent control	Solvent control	ND	ND	ND	ND
3.125	3.252	1.57	0.619	0.726	0.632
6.25	6.80	3.19	1.42	1.60	1.41
12.5	13.3	5.55	2.48	3.04	2.73
25	26.2	9.49	4.75	5.55	5.25
50	52.8	20.0	11.6	13.7	11.3
100	102.1	26.6	12.9	14.8	13.4
200	198.3	66.9	41.0	50.0	43.4
400	405.0	119	78.8	92.2	76.5
800	817.5	252	165	214	190

^a not detected

Table 108
Distribution of [¹⁴C-] DPX-JW062EL in the test system on Day 28
(water, pore water, and sediment phases)

Actual applied concentration (µg JW062EL/L)	Measured concentration ^a at Day 28 (mean of replicate vessels)		
	Overlying water (µg/L)	Pore-water (µg/L)	Sediment (µg/kg)
Control	ND ^b	ND	ND
Solvent control	ND	ND	ND
3.252	0.632	<0.352	6.60
6.80	1.41	0.630	12.8
13.3	2.73	1.64	26.4
26.2	5.25	3.34	48.3
52.8	11.3	6.47	85.5
102.1	13.4	8.20	84.3
198.3	43.4	27.2	305
405.0	76.5	56.2	688
817.5	190	128	1276

^a Calculated using unrounded figures

^b not detected

Effects of IN-DPX-JW062EL on adult emergence and development: The percentage emergence rate for midges was reduced, compared to the combined controls, at and above an applied dose of 52.8 µg/L (Table 109). The development rate was reduced, compared to the combined controls, at and above an applied dose of 52.8 µg/L.

Table 109
Emergence data at Day 28

Actual applied concentration (µg JW062EL/L)	Mean % emergence	Mean development rate	% of emerging adults	
			Males	Females
Control	85.0	0.0622	35	65
Solvent control	86.3	0.0644	40	60
3.252	87.5	0.0661	60	40
6.80	92.5	0.0637	60	40
13.3	80.0	0.0620	40	60
26.2	77.5	0.0579	45	55
52.8	52.5 ^a	0.0486 ^a	35	65
102.1	60.0 ^a	0.0454 ^a	75	25
198.3	20.0 ^a	0.0294 ^a	35	65
405.0	0 ^a	0 ^a	0	0
817.5	0 ^a	0 ^a	0	0

^a p <0.01 for a two-tailed Williams test for monotonic trend compared to combined controls

III. CONCLUSION

The results are presented in terms of applied concentration. Based on the combined data from the solvent and non-solvent controls, the EC₅₀ estimate for emergence was 125 µg [¹⁴C]DPX-JW062EL/L. The NOEC for emergence was 26.2 µg [¹⁴C]DPX-JW062EL/L. The EC₅₀ estimate for development rate was 153 µg [¹⁴C]DPX-JW062EL/L. The NOEC for development rate in this study was 26.2 µg [¹⁴C]DPX-JW062EL/L.

(Radford, K., 2000)

RMS comment

The sediment dwelling organisms study DuPont-4055 was conducted under guideline draft OECD 219. The applicant indicates that it meets the current guideline (OECD 219).

This study was assessed for previous Annex I inclusion and was conducted with the old material DPX-MP062 (radiolabelled form). The active substance was applied via the overlying water. As new chronic toxicity study is available on the same species (*Chironomus riparius*) for the new material DPX-KN128, this study is not considered essential.

Report: Thomas, S.T., Martin, K.H., Gallagher, S.P., Bodle, E.S. (2014); ¹⁴C DPX-KN128: A prolonged sediment toxicity test with *Chironomus riparius* using spiked water

DuPont Report No.: DuPont-35832, Revision No. 1

Guidelines: OECD 219 (2004) **Deviations:** None

Testing Facility: Wildlife International Ltd. (USA), Easton, Maryland, USA

Testing Facility Report No.: 112A-430

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 effects of ¹⁴C-indoxacarb administered in spiked water on the midge (*Chironomus riparius*) were determined in a static, 28-day test. The test was conducted in accordance with the OECD Guideline for Testing of Chemicals 219. Treatments consisted of a negative control, solvent (acetone) control and five nominal indoxacarb (DPX-KN128) concentrations of 0.95, 3.1, 9.8, 31, and 100 µg a.s./L. The corresponding mean measured concentrations in overlying water were 0.82, 2.6, 8.5, 26, and 92 µg a.s./L, respectively, based on total radioactive residues (TRR). The 28-Day LC₅₀ value based on emergence and mortality of *Chironomus riparius* exposed to ¹⁴C-indoxacarb administered in spiked water was 28 µg a.s./L, with 95% confidence limits of 8.5 and 92 µg a.s./L. The 28-Day LOEC was 8.5 µg a.s./L and the NOEC was 2.6 µg a.s./L based on the development rates and development time, the most sensitive endpoints.

I. MATERIALS AND METHODS

A. MATERIALS

- | | | |
|----|----------------------------|---|
| 1. | Radiolabeled test material | [Indanone-1- ¹⁴ C]indoxacarb |
| | Lot/Batch #: | 1643850 |
| | Radiochemical purity | 98.8% |
| | Specific activity: | 49.50 µCi/mg |
| 2. | Controls: | Negative control and solvent control |
| | Test vehicle: | Solvent acetone (0.10 mL acetone/L water) |
| | Toxic reference: | None |
| 3. | Test organism: | Midge |
| | Species: | <i>Chironomus riparius</i> |
| | Age/life stage at dosing: | 1-4 days (first instar) |
| | Initial population: | 20 larvae per test chamber |
| | Source: | Environmental Consulting and Testing of Superior, Wisconsin |
| | Diet: | Rabbit food supplied by Hartz, Secaucus, New Jersey during holding and TetraMin [®] Flake food supplied by Doctors Foster and Smith, Blacksburg, Virginia during the test. |
| | Test chamber: | One quart glass jars |
| 4. | Environmental conditions: | Dissolved oxygen: ≥7.4 mg/L (≥82% of saturation) |
| | | pH: 7.9 to 8.5 |
| | Temperature: | 20 ± 2°C in test chambers; and measured continuously in a beaker of water adjacent to the test chambers, measured to the nearest 1°C. |
| | Photoperiod: | 16 hours light (516 lux at test initiation) and 8 hours dark including 30 min transitional period preceding and following the 16-hr light interval. |

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
20-March-2013 to 17-April-2013
2. Experimental treatments
The effects of [Indanone-1-¹⁴C]-indoxacarb administered in overlying water on the midge (*Chironomus riparius*) were determined in a static, 28-day test. Treatments consisted of a negative and solvent (acetone) control and five nominal indoxacarb concentrations of 0.95, 3.1, 9.8, 31, and 100 µg a.s./L (mean measured concentrations of 0.82, 2.6, 8.5, 26, and 92 µg a.s./L based on total radioactive residues). Each treatment group had four replicates used for biological observations. Each replicate was initiated with 20 first instar larvae. Four additional replicates were included in the test design for analytical measurements. Each analytical replicate contained 20 organisms except for those being sampled on Day 0. At each sampling interval, an entire analytical replicate from each treatment group was sacrificed for the measurement of indoxacarb in sediment, overlying water and pore water.
3. Observations
Observations were made daily of the survival and emergence of the midges.
4. Statistics
The 28-day LC₅₀ was determined by analyzing the number of organisms that failed to emerge during the study as well as the number of organisms that emerged and died using binomial probability with nonlinear interpolation. The NOEC and LOEC were determined by visual interpretation of the dose-response pattern and statistical analyses of the mean development times, emergence ratios and development rates. The data were analyzed to determine any statistical differences between the

negative and solvent control groups. Since there were no differences, the controls were pooled and the treatment groups were compared to the pooled control using a Dunnett's test.

II. RESULTS AND DISCUSSION

A. FINDINGS

Analytical verification of [Indanone-1-¹⁴C]-indoxacarb concentrations were made in sediment, overlying water and pore water sampled on Days 0, 7, and 28 of the test. Mean measured concentrations of indoxacarb in the overlying water were 0.82, 2.6, 8.5, 26, and 92 µg a.s./L, based on total radioactive residues. All chemical and physical parameters for the 28-day study were within acceptable ranges. There were measured amounts of indoxacarb in the solvent control pore water on Days 7 and 28. Since background radioactivity is subtracted from the final measured result, the measureable amount of indoxacarb may be a result of a small amount of contamination during processing of the samples. All validity criteria were met for the study.

A summary of the 28-Day LC₅₀, NOEC and LOEC are shown in Table 110.

Table 110
Summary of effects for indoxacarb to the midge (*Chironomus riparius*)

Endpoint	Mean measured concentration in sediment based on TRR	Effect
28-Day LC ₅₀	28 µg a.s./L, 95% confidence interval of 8.5 and 92 µg a.s./L	Based on number failing to emerge and number that emerged and died.
28-Day EC ₁₀	13.1 µg a.s./L, 95% confidence interval of 9.70 and 17.6 µg a.s./L	Based on emergence ratio
28-Day EC ₂₀	17.6 µg a.s./L, 95% confidence interval of 13.7 and 22.5 µg a.s./L	Based on emergence ratio
28-Day EC ₁₀	1.68 µg a.s./L, 95% confidence interval of 0.78 and 3.62 µg a.s./L	Based on development rate
28-Day EC ₂₀	6.04 µg a.s./L, 95% confidence interval of 3.55 and 10.3 µg a.s./L	Based on development rate
NOEC	2.6 µg a.s./L	Based on development rates and development time, most sensitive endpoints.
LOEC	8.5 µg a.s./L	Based on development rates and development time, most sensitive endpoints.

III. CONCLUSION

The 28-Day LC₅₀ value based on emergence and/or mortality of *Chironomus riparius* exposed to [Indanone-1-¹⁴C]-indoxacarb administered in overlying water was 28 µg a.s./L, with 95% confidence limits of 0.82 and 92 µg a.s./L. There was a treatment-related effect on mean emergence ratios between the pooled control and the 26 and 92 µg a.s./L treatment groups. There was a treatment-related effect on mean development times between the pooled control and the 8.5, 26 and 92 µg a.s./L treatment groups. There was a treatment-related effect on mean development rates between the pooled control and the 8.5, 26, and 92 µg a.s./L treatment groups. Therefore, the 28-Day LOEC was 8.5 µg a.s./L and the NOEC was 2.6 µg a.s./L. The EC₁₀, based on the emergence ratio of surviving midges exposed to ¹⁴C-indoxacarb administered in overlying water was 13.1 µg a.s./L, with 95% confidence limits of 9.70 and 17.6 µg a.s./L and the EC₂₀ was 17.6 µg a.s./L, with 95% confidence limits of 13.7 and 22.5 µg a.s./L. The EC₁₀, based on the development rate of surviving midges exposed to ¹⁴C-indoxacarb administered in overlying water was 1.68 µg a.s./L, with 95% confidence limits of 0.78 and 3.62 µg a.s./L and the EC₂₀ was 6.04 µg a.s./L, with 95% confidence limits of 3.55 and 10.3 µg a.s./L.

(Thomas, S.T., Martin, K.H., Gallagher, S.P., Bodle, E.S., 2014)

RMS comment

No table of results was provided in the original study summary. Mean values are reported below:

Mean measured concentration in overlying water (µg a.s./L)	Mean development time (days)	Mean development rate	Emergence ratio
Negative control	13.6	0.0766	0.96
Solvent control	13.3	0.0781	0.95
Pooled control	13.5	0.0774	0.96
0.82	13.7	0.0759	0.99
2.6	14.3	0.0731**	0.93
8.5	17.9*	0.0584*	0.94
26	22.8*	0.0453*	0.58*
92	25.8*	0.0397*	0.05*

*there was statistically significant difference (p<0.05) from the pooled control using Dunnett's t-test

**there was statistically significant difference (p<0.05) from the pooled control using Dunnett's t-test however the difference was not considered biologically significant.

This study is valid according to validity criteria.

This study was conducted in compliance with the current guideline OECD 219 however the artificial sediment uses alpha-cellulose as its source of organic matter instead of peat moss. As the study DuPont-35832 revision 1, was conducted with a non-OECD source of carbon in the sediment, a new study (DuPont-41246) was conducted with a OECD sediment (the applicant considers the endpoint issued from DuPont-41246 more relevant for the risk assessment). On this point, despite the fact that the sediment was not the one recommended in the guideline, RMS considers that alpha-cellulose might nevertheless be used in the case of indoxacarb (its Koc suggesting strong adsorption to the organic matter in the sediment and alpha-cellulose being an organic matter). The percentage of organic carbon in sediment was of 2.4%. RMS is of the opinion that the endpoint based on concentrations in overlying water, if worst-case, should be used in the risk assessment. Exposure via overlying water remained stable during this test. RMS also notes that indoxacarb did not partition so quickly to sediment in this test (measured concentration < LOQ at Day 7) although partitioning to sediment occurred before Day 7 in DuPont-41246.

Besides, a NOEC value of 2.6 µg DPX-KN128 /L was considered reliable by the study authors. However it is noted in the study report that the development rate of the midges is statistically significantly different from the control. RMS considers the EC10 of 1.68 µg DPX-KN128/L (95% confidence interval of 0.78 and 3.62 µg a.s./L) based on development rate more relevant.

Report: Thomas, S.T., Siddiqui, A.I., Gallagher, S.P., Krueger, H.O. (2015); ¹⁴C-Indoxacarb (DPX-KN128) technical: A prolonged sediment toxicity test with the midge (*Chironomus riparius*) using spiked water

DuPont Report No.: DuPont-41246

Guidelines: OECD 219 (2004) **Deviations:** None

Testing Facility: Wildlife International Ltd. (USA), Easton, Maryland, USA

Testing Facility Report No.: 112A-530

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:

The effects of water-incorporated indoxacarb on survival, development time, and both emergence and development rates of the midge (*Chironomus riparius*) were determined in an aerated, static, 28-day sediment toxicity test. The test was conducted in accordance with the OECD Guideline for Testing of Chemicals 219 (2004). Treatments consisted of a negative control, buffered control, solvent (methanol) control, and five nominal indoxacarb concentrations of 1.3, 2.5, 5.0, 10, and 20 µg/L. The corresponding mean, measured concentrations in overlying water were 0.91, 1.8, 3.8, 6.8, and 15 µg/L, respectively, based on total radioactive residues (TRR). The 28-day LC₅₀ value based on emergence and mortality of *Chironomus riparius* exposed to water-incorporated indoxacarb was >20 µg/L, the highest concentration tested. The 28-day LOEC (lowest observed effect concentration) was 5.0 µg/L and the NOEC (no observed effect concentration) was 2.5 µg/L for *Chironomus riparius* exposed to water-incorporated indoxacarb based on mean development rates.

I. MATERIALS AND METHODS

A. MATERIALS

- | | | |
|----|----------------------------|---|
| 1. | Radiolabeled test material | [¹⁴ C]Indoxacarb |
| | Lot/Batch #: | 1643850 ([Indanone-1- ¹⁴ C]) |
| | Radiochemical purity: | 98.8% |
| | Specific activity: | 49.50 µCi/mg |
| 2. | Controls: | Negative Control, Buffered Control and Solvent Control |
| | Test vehicle: | Solvent methanol (60 µL methanol/600 mL overlying water) |
| | Toxic reference: | None |
| 3. | Test organism: | Midge |
| | Species: | <i>Chironomus riparius</i> |
| | Age/life stage at dosing: | 1-4 days (first instar) |
| | Initial population: | 20 larvae per test chamber |
| | Source: | Environmental Consulting and Testing of Superior, Wisconsin |
| | Diet: | TetraMin® Flake food supplied by Doctors Foster and Smith, Blacksburg, Virginia during holding and during the test. |
| | Test chamber: | One quart glass jars containing approximately 2 cm of test batch sediment and 600 mL of overlying water covered with a loose plastic cover |
| 4. | Environmental conditions: | Dissolved oxygen: ≥8.1 mg/L (≥90% of saturation) |
| | | pH: 5.7–9.1 |
| | Temperature: | 20 ± 2°C in test chambers; and measured continuously in a beaker of water adjacent to the test chambers, measured to the nearest 0.01°C (18.33 to 20.32°C). |
| | Photoperiod: | 16 hours light (136 lux at test initiation) and 8 hours dark including 30 min transitional period preceding and following the 16-hr light interval. |
| 5. | Sediment: | Formulated sediment containing 74% sand, 21% clay and 5% peat moss. Percent organic carbon was 1.3%. The pH of the sediment was 7.6. |

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
30-October-2014 to 27-November-2014

2. Experimental treatments

The effects of water-incorporated [^{14}C]indoxacarb on the midge (*Chironomus riparius*) were determined in an aerated, static, 28-day sediment toxicity test.

Formulated sediment was used as the sediment in the test. The sediment was composed of 74% sand, 21% clay, and 5% peat moss. The dry ingredients were mixed together in a PK-Twinshell mixer and stored ambient when not in use. A sample of the sediment was sent to Agvise Laboratories, Northwood, ND to determine the sediment composition. The percent organic carbon content was found to be 1.3%.

Treatments consisted of a negative, buffered, and solvent (methanol) controls and five nominal indoxacarb concentrations of 1.3, 2.5, 5.0, 10, and 20 $\mu\text{g/L}$ (mean measured concentrations of 0.91, 1.8, 3.8, 6.8, and 15 $\mu\text{g/L}$ based on total radioactive residues). Each treatment group had four replicates used for biological observations. Each replicate was initiated with 20 first instar larvae. Six additional replicates were included in the test design for analytical measurements. Each analytical replicate contained 20 organisms except for those being sampled on Day 0. At each sampling interval, an entire analytical replicate from each treatment group was sacrificed for the measurement of indoxacarb in sediment, overlying water, and pore water.

3. Observations

The number of surviving midge larvae and any abnormal behaviour was recorded daily. The number of fully emerged males and females was recorded daily during the period of expected emergence.

4. Statistics

The data precluded the calculation of a 28-day LC_{50} since there was no treatment group with greater than 50% mortality observed. The NOEC and LOEC were determined by visual interpretation of the dose-response pattern and statistical analyses of the mean development times, emergence ratios, and development rates. The data were analysed using an f-test to determine any statistical differences between the negative, buffered and solvent control groups. Since there was a difference between the negative and solvent controls for emergence ratio and development rate, the treatment groups were compared to the pooled (negative and buffered) control using a Dunnett's test to identify those treatment groups that were statistically different. There was no difference between the controls for development time; therefore, the treatment groups were compared to the pooled (negative, buffered, and solvent) control using a Dunnett's test to identify those treatment groups that were significantly different.

An ANOVA procedure looking at the interaction between sexes was used to evaluate sensitivity between sexes for development rates. There were no significant interactions found between sex and treatments for development rate therefore the data for each sex were pooled for this variable. There was no feasible way to determine the sex of 1-4 day old larvae at the time organisms were added to test chambers. Evaluations of the sensitivity between sexes for emergence ratio were not possible because calculations require knowing how many individuals of each sex are initially exposed. Therefore, the emergence ratio data for both sexes were also pooled for analysis.

II. RESULTS AND DISCUSSION

A. FINDINGS

A summary of the 28-day LC_{50} , NOEC, and LOEC are shown in Table 111.

Analytical verification of [^{14}C]indoxacarb concentrations was made in sediment, overlying water, and pore water sampled on Days 0, 7, 14, 21, and 28 of the test (Table 112) and of indoxacarb (by fractionation) on Days 0, 7, 14, 21, and 28 (Table 113). Mean measured concentrations of [^{14}C]indoxacarb in the overlying water were 0.91, 1.8, 3.8, 6.8, and 15 $\mu\text{g/L}$, based on total radioactive residues.

The LOQ value for radiolabeled concentrations in sediment was 1.52 $\mu\text{g/kg}$. The LOQ value for radiolabeled concentrations in overlying water and pore water was 0.0455 $\mu\text{g/L}$.

For chromatographic methods, the LOQ in sediment was 1.12 µg/kg. The LOQ for overlying water and pore water was 0.0455 µg/L.

A mass balance for [¹⁴C]indoxacarb (based on total radioactive residue, TRR) was calculated on Days 0, 7, 14, 21, and 28 of the study in overlying water, pore water, and sediment. On Day 0, the total amount of [¹⁴C]indoxacarb (overlying water, pore water, and sediment) in the nominal 1.3, 2.5, 5.0, 10, and 20 µg/L treatment groups was 119, 109, 104, 97.2, and 105% of nominal, respectively. After 7 days, the total amount of [¹⁴C]indoxacarb was 76.8, 89.6, 139, 81.6, and 100% of nominal, respectively. After 14 days, the total amount of [¹⁴C]indoxacarb was 120, 107, 97.4, 113, and 99.4% of nominal, respectively. After 21 days, the total amount of [¹⁴C]indoxacarb was 78.0, 105, 94.7, 108, and 111% of nominal, respectively. At study termination (Day 28), the total amount of [¹⁴C]indoxacarb was 68.6, 87.0, 67.4, 88.8, and 93.4% of nominal, respectively. A summary of analytical recoveries (total radioactive residues) is shown in Table 112 and a summary of mass balance approximations is shown in Table 114.

Table 111
Summary of effects for indoxacarb to the midge (*Chironomus riparius*)

Endpoint	Nominal concentration in overlying water	Effect
28-Day LC ₅₀	>20 µg/L, the highest concentration tested	Based on number failing to emerge and number that emerged and died.
NOEC	2.5 µg/L	Based on development rate.
LOEC	5.0 µg/L	Based on development rate.

Table 112
Measured [¹⁴C]indoxacarb and analytical recoveries in sediment, overlying water, and pore water

Nominal indoxacarb concentration (µg/L)	Interval	Measured indoxacarb equivalents			
		Sediment ^a (µg/kg)	Overlying water ^b (µg/L)	Overlying water (% of nominal)	Pore water ^b (µg/L)
Negative control (0.0)	0	<LOQ	<LOQ	---	<LOQ
	7	<LOQ	0.0630	---	<LOQ
	14	<LOQ	<LOQ	---	<LOQ
	21	<LOQ	<LOQ	---	<LOQ
	28	<LOQ	<LOQ	---	<LOQ
Buffered control (0.0)	0	<LOQ	<LOQ	---	<LOQ
	7	<LOQ	<LOQ	---	<LOQ
	14	<LOQ	0.0466	---	<LOQ
	21	<LOQ	0.220	---	0.0754
	28	<LOQ	<LOQ	---	<LOQ
Solvent control (0.0)	0	<LOQ	<LOQ	---	<LOQ
	7	<LOQ	0.0523	---	<LOQ
	14	<LOQ	<LOQ	---	<LOQ
	21	<LOQ	0.166	---	0.0569
	28	<LOQ	<LOQ	---	0.0529
1.3	0	<LOQ	1.39	107	<LOQ
	7	<LOQ	0.821	63.2	0.445
	14	3.59	0.791	60.8	0.719
	21	<LOQ	0.837	64.4	0.699
	28	<LOQ	0.710	54.6	0.655
2.5	0	<LOQ	2.57	103	0.138
	7	2.43	1.71	68.6	1.04
	14	4.90	1.59	63.4	1.32
	21	4.35	1.74	69.7	1.58
	28	2.70	1.59	63.6	1.44
5.0	0	<LOQ	5.03	101	0.196
	7	12.8	4.19	83.9	1.09
	14	6.02	3.55	71.1	2.72
	21	6.42	3.30	66.0	2.89
	28	<LOQ	3.13	62.6	2.40
10	0	<LOQ	9.55	95.5	0.471
	7	17.7	4.35	43.5	3.59
	14	22.3	6.32	63.2	4.76
	21	16.0	7.08	70.8	5.50
	28	9.25	6.67	66.7	5.59
20	0	<LOQ	20.8	104	0.846
	7	31.5	12.7	63.6	6.27
	14	32.5	13.1	65.7	9.44
	21	37.2	14.2	71.1	12.9
	28	24.6	13.0	64.9	9.98

^a The LOQ value for radiolabeled concentrations in sediment was 1.52 µg/kg and radiolabeled concentrations in water was 0.0455 µg/L.

^b The LOQ value for radiolabeled concentrations in overlying water and pore water was 0.0455 µg/L.

Table 113
Measured concentrations of indoxacarb (by fractionation) and analytical recoveries in overlying water, pore water, and sediment

Nominal indoxacarb concentration (µg/L)	Interval	Measured indoxacarb concentration			
		Sediment ^a (µg/kg)	Overlying water ^b (µg/L)	Overlying water (% of nominal)	Pore water ^b (µg/L)
1.3	0	--	--	--	--
	7	--	--	--	--
	14	4.26	--	--	--
	21	--	--	--	--
	28	--	--	--	--
2.5	0	--	--	--	--
	7	3.76	0.965	38.6	--
	14	8.22	0.931	37.2	0.917
	21	4.50	1.05	42.0	1.36
	28	4.44	1.20	48.0	0.978
5.0	0	--	--	--	--
	7	4.46	2.04	40.8	--
	14	7.26	2.61	52.2	2.35
	21	8.08	2.14	42.8	1.99
	28	6.89	2.12	42.4	2.06
10	0	--	2.29	22.9	--
	7	27.1	2.77	27.7	2.57
	14	20.3	4.76	47.6	3.84
	21	9.14	5.71	57.1	4.52
	28	12.1	4.72	47.2	4.69
20	0	--	3.71	18.6	--
	7	27.7	7.09	35.5	4.33
	14	43.6	9.36	46.8	6.75
	21	41.3	10.4	52.0	10.5
	28	23.6	8.57	42.9	7.86

^a The limit of quantitation (LOQ) was 1.12 µg/kg.

^b The limit of quantitation (LOQ) was 0.0455 µg/L.

Table 114
Mass balance approximations for Day 0, 7, 14, 21, and 28

Study day	Nominal test conc. (µg/L)	Mass of sediment in test system (kg)	Volume of pore water in test system (L)	Nominal ¹⁴ C indoxacarb in test system (µg)	¹⁴ C indoxacarb in sediment ^a (µg)	¹⁴ C indoxacarb in overlying water ^b (µg)	¹⁴ C indoxacarb in pore water ^c (µg)	Total ¹⁴ C indoxacarb in test system (µg)	% of nominal ¹⁴ C indoxacarb in test system
0	1.3	0.122	0.0200	0.780	0.093	0.834	0.000	0.927	119
0	2.5	0.119	0.0190	1.50	0.091	1.54	0.003	1.64	109
0	5.0	0.133	0.0230	3.00	0.101	3.02	0.005	3.12	104
0	10	0.123	0.0220	6.00	0.093	5.73	0.010	5.83	97.2
0	20	0.133	0.0180	12.0	0.101	12.5	0.015	12.6	105
7	1.3	0.124	0.0270	0.780	0.094	0.493	0.012	0.599	76.8
7	2.5	0.121	0.0220	1.50	0.295	1.03	0.023	1.34	89.6
7	5.0	0.127	0.0230	3.00	1.62	2.51	0.025	4.16	139
7	10	0.124	0.0255	6.00	2.20	2.61	0.092	4.90	81.6
7	20	0.134	0.0270	12.0	4.21	7.62	0.169	12.0	100
14	1.3	0.124	0.0205	0.780	0.443	0.475	0.015	0.933	120
14	2.5	0.127	0.0200	1.50	0.623	0.954	0.026	1.60	107
14	5.0	0.122	0.0220	3.00	0.731	2.13	0.060	2.92	97.4
14	10	0.129	0.0240	6.00	2.88	3.79	0.114	6.79	113
14	20	0.118	0.0230	12.0	3.85	7.86	0.217	11.9	99.4

^a [¹⁴C]indoxacarb in sediment = concentration of [¹⁴C]indoxacarb in sediment multiplied by the dry weight of sediment in the test system.

^b [¹⁴C]indoxacarb in overlying water = concentration of [¹⁴C]indoxacarb in overlying water multiplied by the volume of water in the test system (0.6 L).

^c [¹⁴C]indoxacarb in pore water = concentration of [¹⁴C]indoxacarb in pore water multiplied by the volume of pore water in the test system.

Table 114
Mass balance approximations for Day 0, 7, 14, 21, and 28 (continued)

Study day	Nominal test conc. (µg/L)	Mass of sediment in test system (kg)	Volume of pore water in test system (L)	Nominal ¹⁴ C indoxacarb in test system (µg)	¹⁴ C indoxacarb in sediment ^a (µg)	¹⁴ C indoxacarb in overlying water ^b (µg)	¹⁴ C indoxacarb in pore water ^c (µg)	Total ¹⁴ C indoxacarb in test system (µg)	% of nominal ¹⁴ C indoxacarb in test system
21	1.3	0.120	0.0220	0.780	0.091	0.502	0.015	0.609	78.0
21	2.5	0.115	0.0200	1.50	0.499	1.04	0.032	1.57	105
21	5.0	0.123	0.0240	3.00	0.791	1.98	0.069	2.84	94.7
21	10	0.130	0.0260	6.00	2.08	4.25	0.143	6.47	108
21	20	0.120	0.0240	12.0	4.45	8.52	0.310	13.3	111
28	1.3	0.123	0.0240	0.780	0.093	0.426	0.016	0.535	68.6
28	2.5	0.118	0.0225	1.50	0.319	0.954	0.032	1.31	87.0
28	5.0	0.115	0.0240	3.00	0.087	1.88	0.058	2.02	67.4
28	10	0.128	0.0265	6.00	1.18	4.00	0.148	5.33	88.8
28	20	0.127	0.0300	12.0	3.11	7.80	0.299	11.2	93.4

^a [¹⁴C]indoxacarb in sediment = concentration of [¹⁴C]indoxacarb in sediment multiplied by the dry weight of sediment in the test system.

^b [¹⁴C]indoxacarb in overlying water = concentration of [¹⁴C]indoxacarb in overlying water multiplied by the volume of water in the test system (0.6 L).

^c [¹⁴C]indoxacarb in pore water = concentration of [¹⁴C]indoxacarb in pore water multiplied by the volume of pore water in the test system.

During the study, the mean percent [^{14}C]indoxacarb in the overlying water decreased from 102 to 62.5% of the nominal [^{14}C]indoxacarb in the test system. The mean percent [^{14}C]indoxacarb in the sediment increased from 4.7% on Day 0 to 16.3% on Day 28. The mean percent [^{14}C]indoxacarb in the pore water on Day 0 was 0.1% of the nominal [^{14}C]indoxacarb in the test systems and increased to 2.2% of the nominal [^{14}C]indoxacarb in the test systems by the end of the study. The results are shown in Table 115.

Table 115
Mean percent of indoxacarb equivalents in sediment, overlying water, and pore water

Study day	% in Sediment	% in Overlying water	% in Pore water	Total %
0	4.7	102	0.1	107
7	31.5	64.5	1.4	97.3
14	40.6	64.8	1.9	107
21	28.6	68.4	2.3	99.2
28	16.3	62.5	2.2	81.1

There was a significant difference between the negative and solvent control groups for development rate and emergence ratio; therefore, the treatment groups were compared to the pooled (negative and buffered) control group to determine if there were any significant reductions. There were no significant differences between the negative, buffered, and solvent controls for development time; therefore, the treatment groups were compared to the pooled (negative, buffered, and solvent) controls to determine if there were any significant reductions.

The LC_{50} based on the number of emerged, surviving midges exposed to sediment incorporated indoxacarb was $>20 \mu\text{g/L}$, the highest nominal concentration tested.

There were no statistically significant differences ($p>0.05$) for development time in any treatment group when compared to the pooled control group. There were statistically significant differences ($p<0.05$) for emergence ratio and development rate when compared to the pooled control group. Therefore, the NOEC for development rate (the most sensitive endpoint) was determined to be $2.5 \mu\text{g/L}$ and the LOEC for development rate was determined to be $5.0 \mu\text{g/L}$. The emergence ratios are reported in Table 116. The development times are reported in Table 117. The development rates are reported in Table 118.

Table 116
Summary of emergence ratios of *Chironomus riparius* exposed to [^{14}C]indoxacarb for 28 days in a prolonged sediment toxicity test using spiked water

Nominal [^{14}C]indoxacarb concentrations ($\mu\text{g/L}$)	Mean measured concentrations ($\mu\text{g/L}$) in overlying water	Emergence ratio ^a				
		A	B	C	D	Mean
Negative control (0.0)	<LOQ	1.00	0.95	1.00	1.00	0.99
Buffered control (0.0)	0.067	0.95	1.00	0.90	0.95	0.95
Solvent control (0.0)	0.057	0.75	1.00	0.90	0.85	0.88
Pooled control		-	-	-	-	0.97 (NC and BC)
1.3	0.91	1.00	0.85	0.95	0.95	0.94
2.5	1.8	0.95	0.95	0.95	0.90	0.94
5.0	3.8	0.80	0.95	0.45	0.85	0.76
10	6.8	1.00	0.90	0.00	0.95	0.71
20	15	0.40	0.70	0.80	0.55	0.61*

^a A–D represent replicate test chambers containing twenty midges each.

*indicates a statistically significant difference in comparison to the pooled control (negative + buffered control) ($p \leq 0.05$) using Dunnett's test.

Table 117
Summary of development time of *Chironomus riparius* exposed to [^{14}C]indoxacarb for 28 days in a prolonged sediment toxicity test using spiked water

Nominal [^{14}C]indoxacarb concentrations ($\mu\text{g/L}$)	Mean measured concentrations ($\mu\text{g/L}$) in overlying water	Development time ^a				
		A	B	C	D	Mean
Negative control (0.0)	<LOQ	14.5	16.7	16.0	14.7	15.5
Buffered control (0.0)	0.067	15.5	14.6	14.9	15.4	15.1
Solvent control (0.0)	0.057	13.6	14.2	14.0	13.6	13.8
Pooled control		-	-	-	-	14.8 (NC, BC and SC)
1.3	0.91	14.5	16.9	15.2	14.3	15.2
2.5	1.8	14.4	13.6	14.8	15.4	14.6
5.0	3.8	15.7	15.2	20.1	17.7	17.2
10	6.8	17.4	19.8	--	18.5	18.6
20	15	20.4	18.9	20.4	19.3	19.7

^a A-D represent replicate test chambers containing twenty midges each.

Table 118
Summary of development rate of *Chironomus riparius* exposed to [^{14}C]indoxacarb for 28 days in a prolonged sediment toxicity test using spiked water

Nominal [^{14}C]indoxacarb concentrations ($\mu\text{g/L}$)	Mean measured concentrations ($\mu\text{g/L}$) in overlying water	Development rate ^a				
		A	B	C	D	Mean
Negative control (0.0)	<LOQ	0.0724	0.0619	0.0651	0.0715	0.0677
Buffered control (0.0)	0.067	0.0673	0.0717	0.0703	0.0678	0.0693
Solvent control (0.0)	0.057	0.0766	0.0735	0.0749	0.0766	0.0754
Pooled control		-	-	-	-	0.0685 (NC and BC)
1.3	0.91	0.0722	0.0610	0.0692	0.0731	0.0689
2.5	1.8	0.0722	0.0763	0.0708	0.0684	0.0719
5.0	3.8	0.0667	0.0686	0.0514	0.0587	0.0614*
10	6.8	0.0597	0.0521	--	0.0560	0.0559*
20	15	0.0507	0.0553	0.0513	0.0542	0.0529*

^a A-D represent replicate test chambers containing twenty midges each.

*indicates a statistically significant difference in comparison to the pooled control (negative + buffered control) ($p \leq 0.05$) using Dunnett's test.

III. CONCLUSION

The 28-day LC₅₀ value based on emergence and/or mortality of *Chironomus riparius* exposed to water-incorporated [¹⁴C]indoxacarb was >20 µg/L, the highest concentration tested. There were treatment related effects observed on mean emergence ratios in the highest treatment group when compared to the pooled controls and for development rates in the 5.0, 10, and 20 µg/L treatment groups when compared to the pooled control group. Therefore, the 28-day LOEC was 5.0 µg/L and the 28-day NOEC was 2.5 µg/L.

(Thomas, S.T., Siddiqui, A.I., Martin, K.H., Gallagher, S.P., 2015)

RMS comment

This study was conducted in compliance with the current guideline. This study is acceptable.

The percentage of organic carbon in sediment was of 1.3%.

Concerning development rate

The NOEC value of 1.8 µg DPX-KN128/L based on mean measured concentration (for the effects on the development rate) is more relevant for the risk assessment than the value based on nominals. An EC10 value for development rate was calculated by RMS: EC10 = 4.37 µg DPX-KN128/L (95% confidence intervals: 1.76-8.74). This value is higher than the LOEC of 3.8 µg DPX-KN128/L at which effects on development rate were already slightly above 10%. RMS considers the NOEC value more relevant for the risk assessment.

Besides, due to the partition of indoxacarb to the sediment, TER based on PEC sediment should also be provided. Therefore another NOEC expressed in µg/kg sediment (based on the same study), should be determined using the geometric mean value of measured concentrations in sediment. The applicant provided a geometric mean value of 4.98 µg/kg sediment, however RMS does not find the same value. This value is obviously based on measured concentrations of indoxacarb by fractionation. The measured concentrations in sediment were <LOQ, 2.43, 4.90, 4.35, and 2.70 µg/kg at 0, 7, 14, 21, 28 days respectively. The geometric mean value calculated by RMS is 2.92 µg/kg sediment (assuming a LOQ value of 1.52 µg/kg sediment at day 0 in the calculation). RMS considers the geometric mean of 2.92 µg/kg sediment relevant for the risk assessment for effects on the development rate.

NOEC_{development rate} = 2.92 µg/kg sediment

Concerning emergence ratio

According to the study report, the NOEC for emergence ratio would be of 6.8 µg/L (based on mean measured) as statistically significant effects were observed only at the highest tested dose. However RMS notes that this parameter was quite variable at the tested concentrations of 5, 10 and 20 µg/L (nominals). EC10 was calculated by RMS. RMS did not exclude the replicate C at the concentration of 6.8 µg/L in the calculation. In this replicate, no emergence occurred (although a mean ratio of 0.95 was obtained for the three other replicates). In the absence of justification, this replicate is not considered as an outlier. EC10 value is of 1.83 µg/L (95% confidence intervals: 0.022-7.65). The NOEC value for this parameter is covered by the one set for the development rate as the emergence ratio in all replicates are similar to those of the control at the concentration of 1.8 µg/L.

Concerning development time

No statistically significant effect was found at all concentrations tested. EC10 value for development time was calculated by RMS: EC10 = 2.17 µg/L (95% confidence intervals: 0.91-3.58).

Report: Thomas, S.T., Kendall, T.Z., Martin, K.H., Gallagher, S.P., Krueger, H.O. (2014); IN-KT413: A prolonged sediment toxicity test with *Chironomus riparius* using spiked water

DuPont Report No.: DuPont-36116

Guidelines: OECD 219 (2004) **Deviations:** None

Testing Facility: Wildlife International Ltd. (USA), Easton, Maryland, USA

Testing Facility Report No.: 112A-434

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:

The effects of IN-KT413 administered in spiked water on the midge (*Chironomus riparius*) were determined in a static, 28-day test. The test was conducted in accordance with the OECD Guideline for Testing of Chemicals 219. Treatments consisted of a negative control, solvent (acetone) control and five nominal IN-KT413 concentrations of 0.010, 0.032, 0.10, 0.33, and 1.0 mg/L. The corresponding mean measured concentrations in overlying water were 0.0012, 0.0062, 0.024, 0.10, and 0.28 mg/L, respectively. The 28-day LC₅₀ value based on emergence and mortality of *Chironomus riparius* exposed to IN-KT413 administered in spiked water was >0.28 mg/L, the highest concentration tested. The 28-Day LOEC was 0.28 mg IN-KT413/L and the NOEC was 0.10 mg IN-KT413/L based on the development rate, the most sensitive endpoint.

I. MATERIALS AND METHODS

A. MATERIALS

- | | | |
|----|---------------------------|--|
| 1. | Test material: | IN-KT413 technical metabolite |
| | Lot/Batch #: | KT413-003 |
| | Purity: | 99.7% |
| | Description: | Solid |
| | CAS#: | Not given |
| 2. | Controls: | Negative control and solvent control |
| | Test vehicle: | Solvent acetone (0.10 mL acetone/L water) |
| | Toxic reference: | None |
| 3. | Test organism: | Midge |
| | Species: | <i>Chironomus riparius</i> |
| | Age/life stage at dosing: | 1-4 days (first instar) |
| | Initial population: | 20 larvae per test chamber |
| | Source: | Environmental Consulting and Testing of Superior, Wisconsin |
| | Diet: | Rabbit food supplied by Hartz, Secaucus, New Jersey during holding and TetraMin [®] Flake food supplied by Doctors Foster and Smith, Blacksburg, Virginia during the test. |
| | Test chamber: | One quart glass jars |
| 4. | Environmental conditions: | Dissolved oxygen: ≥ 7.6 mg/L ($\geq 84\%$ of saturation) |
| | | pH: 8.1 to 8.5 |
| | Temperature: | $20 \pm 2^{\circ}\text{C}$ in test chambers; and $19\text{--}21^{\circ}\text{C}$ measured continuously in a beaker of water adjacent to the test chambers, measured to the nearest 1°C . |
| | Photoperiod: | 16 hours light (556 lux at test initiation) and 8 hours dark including 30 min transitional period preceding and following the 16-hr light interval. |

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
01-May-2013 to 29-May-2013

2. Experimental treatments

The effects of IN-KT413 administered in overlying water on the midge (*Chironomus riparius*) were determined in a static, 28-day test. Treatments consisted of a negative and solvent (acetone) control and five nominal IN-KT413 concentrations of 0.010, 0.032, 0.10, 0.33 and 1.0 mg/L (mean measured concentrations of 0.0012, 0.0062, 0.024, 0.10 and 0.28 mg/L). Each treatment group had four replicates used for biological observations. Each replicate was initiated with 20 first instar larvae. Four additional replicates were included in the test design for analytical measurements. Each analytical replicate contained 20 organisms except for those being sampled on Day 0. At each sampling interval, an entire analytical replicate from each treatment group was sacrificed for the measurement of IN-KT413 in sediment, overlying water and pore water.

3. Observations

Observations were made daily of the survival and emergence of the midges.

4. Statistics

The survival and emergence data precluded the calculation of a 28-day LC_{50} and therefore, the 28-day LC_{50} was determined to be greater than the highest concentration tested. The NOEC and LOEC were determined by visual interpretation of the dose-response pattern and statistical analyses of the mean development times, emergence ratios and development rates. The data were analyzed to determine any statistical differences between the negative and solvent control groups. Since there were no differences, the controls were pooled and the treatment groups were compared to the pooled control using a Dunnett's test.

II. RESULTS AND DISCUSSION

A. FINDINGS

Analytical verification of IN-KT413 concentrations was made in sediment, overlying water and pore water sampled on Days 0, 7, and 28 of the test. Mean measured concentrations of IN-KT413 in the overlying water were 0.0012, 0.0062, 0.024, 0.10 and 0.28 mg/L. All chemical and physical parameters for the 28-day study were within acceptable ranges. All validity criteria were met for the study.

A summary of the 28-Day LC_{50} , NOEC and LOEC are shown Table 119.

Table 119
Summary of effects for IN-KT413 to the midge (*Chironomus riparius*)

Endpoint	Mean Measured Concentration in Overlying Water	Effect
28-Day LC_{50}	>0.28 mg/L the highest concentration tested	Based on number failing to emerge and number that emerged and died.
NOEC	0.10 mg/L	Based on development rate, most sensitive endpoint.
LOEC	0.28 mg/L	Based on development rate, most sensitive endpoint.

III. CONCLUSION

The 28-day LC₅₀ value based on emergence and/or mortality of *Chironomus riparius* exposed to IN-KT413 administered in overlying water was >0.28 mg/L, the highest concentration tested. There were treatment related effects observed on mean development time and mean emergence ratio in the 0.28 mg/L treatment group when compared to the pooled control. There were treatment related effects observed for development rate between the pooled control group and the 0.10 and 0.28 mg/L treatment groups. Therefore, the 28-Day LOEC was 0.28 mg IN-KT413/L and the NOEC was 0.10 mg IN-KT413/L.

(Thomas, S.T., Kendall, T.Z., Martin, K.H., Gallagher, S.P., Krueger, H.O., 2014)

RMS comment

No table of results was provided in the original study summary. Mean values are reported below:

Mean measured concentration in overlying water (mg /L)	Mean development time (days)	Mean development rate	Emergence ratio
Negative control	14.6	0.0718	0.98
Solvent control	14.4	0.727	0.99
Pooled control	14.5	0.0723	0.98
0.0016	14.6	0.0720	1.00
0.0062	14.5	0.0723	1.00
0.024	14.4	0.0730	0.95
0.10	14.9	0.0702*	1.00
0.28	16.3*	0.0637*	0.89*

*there was statistically significant difference (p<0.05) from the pooled control using Dunnett's t-test

This study was conducted in compliance with the current guideline.

Statistically significant effects on development rate were found at the concentration of 0.10 mg/L. The NOEC is therefore of 0.024 mg IN-KT413/L (mean measured).

The EC10 values were calculated by RMS:

EC10 = 0.245 mg IN-KT413/L (95% confidence intervals: 0.22-0.27) based on effects on the development rate.

EC10 = 0.28 mg/L (95% confidence intervals: 0.24-0.31) based on emergence ratio.

EC10 = 0.088 mg/L (95% confidence intervals: 0.081-0.17) based on development time.

This study is acceptable.

Report: Radford, K. (2001); IN-JT333: To assess the toxicity to the sediment dwelling phase of the midge *Chironomus riparius*

DuPont Report No.: DuPont-4054

Guidelines: draft OECD 218 **Deviations:** None

Testing Facility: Huntingdon Life Sciences Ltd., Huntingdon, Cambridgeshire, England, UK

Testing Facility Report No.: DPT 521

GLP: Yes

Certifying Authority: Department of Health (U.K.)

I. MATERIALS AND METHODS

A. MATERIALS

- | | | |
|----|---------------------------------|--|
| 1. | Non-radiolabeled test material: | IN-JT333 technical metabolite |
| | Lot/Batch #: | JT333-1461 |
| | Purity: | 97.5% |
| | Description: | Solid powder |
| | CAS#: | 144171-39-1 |
| 2. | Radiolabeled test material | [¹⁴ C]-IN-JT333 |
| | Lot/Batch #: | 2729-096 |
| | Radiochemical purity: | 99.0% |
| | Specific activity: | 42.640 µCi/mg |
| 3. | Controls: | Negative control and solvent control |
| | Test vehicle: | Solvent acetone (0.10 mL acetone/L water) |
| | Toxic reference: | None |
| 4. | Test organism: | Midge |
| | Species: | <i>Chironomus riparius</i> |
| | Age/life stage at dosing: | Less than 36 hours (first instar) |
| | Initial population: | 10 larvae per test chamber |
| | Source: | Huntingdon Life Sciences Ltd. in-house culture |
| | Diet: | TetraMin [®] fish food suspended in dilution water. |
| | Test chamber: | Glass beakers (8 cm in diameter) |
| 5. | Environmental conditions: | Dissolved oxygen: 6.8 to 7.9 mg/L (≥80% of saturation) |
| | | pH: 6.4 to 7.0 |
| | Temperature: | 19-20°C |
| | Photoperiod: | 16 hours light and 8 hours dark. |

B. STUDY DESIGN AND METHODS

1. Experimental initiated/completed
08-December-2000 to 05-January-2001

2. Experimental treatments

A study was conducted to assess the toxicity of IN-JT333 to the sediment dwelling phase of the non-biting midge, *Chironomus riparius*, using a static test system with application of the test substance to the sediment, following OECD Method 218. The study was initiated with first instar chironomid less than 36 hours old. Daily records were maintained for each culture which included the general condition of the larvae and the female:male ratio of successfully emerged adults. The larvae were fed daily with TetraMin[®] fish food suspension, dispersed in culture medium, at a rate of 0.5 mg/larva for Days 0 to 9 and 1.0 mg/larva from Day 10 to test end. Four replicates of (nominally) 10 chironomid larvae per concentration group were exposed for 28 days to nominal concentrations of 0.075, 0.15, 0.30, 0.60, 1.2, 2.4, 4.8, 9.6, 19, and 40 µg [¹⁴C]IN-JT333/g organic carbon. The artificial sediment used in this study contained 1.9% organic carbon based on dry weights of the components. Environmental data (pH, dissolved oxygen, and temperature) were recorded at the start and end of the study, and once weekly throughout the study. The results of all chemical analyses are based on total radioactivity measurements expressed as concentrations of the active ingredient.

II. RESULTS AND DISCUSSION

A. FINDINGS

Analysis of the dose solutions showed that the applied doses were 96-107% of nominal. The majority of the radiolabeled test substance remained in the sediment throughout the study. Samples of the dry sediment from Day 0 destructive vessels contained 75-97% of the total radiolabeled test substance added to each vessel, and samples on Day 28 contained 78-86% (Table 122). Concentrations of [^{14}C]IN-JT333 in samples of the pore water collected after the 28-day exposure period were below the limit of detection (Table 121). Samples of the overlying water on Day 28 contained <1% of the total radiolabeled test substance added to each vessel (Table 120).

Table 120
Concentrations of ^{14}C -IN-JT333 in overlying water

Nominal concentration ($\mu\text{g/g}$)	Actual applied concentration ($\mu\text{g/g}$ organic carbon)	Mean measured concentration ($\mu\text{g/g}$)			
		Day 0	Day 7	Day 14	Day 28
Control	Control	ND ^a	ND	ND	ND
Solvent control	Solvent control	ND	ND	ND	ND
0.075	0.072	ND	ND	ND	ND
0.15	0.160	ND	ND	ND	ND
0.30	0.311	ND	ND	ND	ND
0.60	0.611	ND	ND	ND	ND
1.2	1.27	ND	ND	ND	ND
2.4	2.49	ND	ND	ND	ND
4.8	5.05	ND	ND	ND	ND
9.6	9.84	<0.116	<0.116	0.135	0.174
19	19.6	0.137	0.148	0.196	0.309
40	41.0	0.354	0.383	0.483	0.823

^a Not Detected

Table 121
Concentrations of ^{14}C -IN-JT333 in sediment pore water

Actual applied concentration ($\mu\text{g/g}$ organic carbon)	Sediment pore water concentration ($\mu\text{g/L}$)	
	Day 7	Day 28
Control	ND ^a	— ^b
Solvent control	ND	—
0.072 (Day 0)	ND	—
0.072 (Day 7)	—	ND
0.160	ND	—
0.311	ND	—
0.611	ND	—
1.27	ND	—
2.49	ND	—
5.05	ND	—
9.84	0.003	—
19.6	0.005	—
41.0 (Day 0)	0.009	—
41.0 (Day 7)	—	ND

^a Not Detected

^b Not sampled

Table 122
Concentrations of ^{14}C -IN-JT333 in sediment

Actual applied concentration ($\mu\text{g/g}$ organic carbon)	Measured concentration ($\mu\text{g/g}$ organic carbon)		
	Day 0	Day 7	Day 28
Control	ND ^a	ND	ND
Solvent control	ND	ND	ND
0.072	0.070	0.062	0.062
0.160	0.141	— ^b	0.131
0.311	0.251	—	0.260
0.611	0.492	—	0.497
1.27	1.06	—	1.00
2.49	2.08	—	1.93
5.05	3.96	—	4.23
9.84	7.42	—	7.91
19.6	14.7	—	16.0
41.0	34.0	37.4	33.2

^a Not Detected at the limit of detection

^b Not analysed

Effects of IN-JT333 on adult emergence and development: The percentage emergence rate for midges was reduced compared to the combined controls at and above the applied concentration of 19.6 $\mu\text{g/g}$ organic compound. The development rate was reduced, compared to the combined controls, at and above the applied concentration of 9.84 $\mu\text{g/g}$ organic compound.

Table 123
Emergence data at Day 28

Actual applied concentration (µg/g organic carbon)	Mean measured concentrations (calculated by RMS)	Mean % emergence	Mean development rate	% of emerging adults	
				Males	Females
Control	-	70.0	0.0534	45	55
Solvent control	-	87.5	0.0535	40	60
0.072	0.065	80.0	0.0534	55	45
0.160	0.136	85.0	0.0536	40	60
0.311	0.255	72.5	0.0526	45	55
0.611	0.494	75.0	0.0553	55	45
1.27	1.03	70.0	0.0536	60	40
2.49	2.004	65.0	0.0511	40	60
5.05	4.093	65.0	0.0529	45	55
9.84	7.661	75.0	0.0505 ^a	40	60
19.6	15.34	47.5 ^a	0.0476 ^b	40	60
41.0	34.82	15.0 ^b	0.0221 ^c	60	40

^a p < 0.05 for a two-tailed Williams test for monotonic trend compared to combined controls

^b p < 0.01 for a two-tailed Williams test for monotonic trend compared to combined controls

^c Not included in the statistical analysis

III. CONCLUSION

The results are presented in terms of applied sediment concentration. Based on the combined data from the solvent and non-solvent controls, the EC₅₀ estimate for emergence was 24.7 µg [¹⁴C]IN-JT333/g organic carbon. The NOEC for emergence was 9.84 µg [¹⁴C]IN-JT333/g organic carbon. The EC₅₀ estimate for development rate was 37.0 µg [¹⁴C]IN-JT333/g organic carbon. The NOEC for development rate in this study was 5.05 µg [¹⁴C]IN-JT333/g organic carbon.

(Radford, K., 2001)

RMS comment

The sediment dwelling organisms study DuPont-4054 was conducted under guideline draft OECD 218. The applicant indicates that it meets the current guideline (OECD 218). This study was submitted in the original DAR (AD3). The NOEC for emergence was 9.84 µg [¹⁴C]IN-JT333/g organic carbon. The NOEC for development rate was 5.05 µg [¹⁴C]IN-JT333/g organic carbon.

EC10 values were calculated by RMS. These values are based on geometric mean values.

EC10 = 8.41 µg/g organic carbon (95% confidence intervals: 3.87-14.13) for effects on emergence.

EC10 = 13.52 µg/g organic carbon (95% confidence intervals: 9.47-18.85) for effects on development rate. The calculation of the EC10 based on development rate is based on mean values as no values are available for each replicate in the study report). RMS considers however that the uncertainty on the EC10 on development rate is covered by the EC10 calculated for emergence. No recalculation is therefore necessary.

The NOEC for development rate of 5.05 µg/g o.c. equivalent to 0.096 µg/g dry weight sediment, based on 1.9% o.c., is used for risk assessment.

This study is still considered acceptable.

Report: Thomas, S.T., Martin, K.H., Gallagher, S.P., Krueger, H.O. (2014); IN-KG433: A prolonged sediment toxicity test with *Chironomus riparius* using spiked sediment

DuPont Report No.: DuPont-35825

Guidelines: OECD 218 (2004) **Deviations:** None

Testing Facility: Wildlife International Ltd. (USA), Easton, Maryland, USA

Testing Facility Report No.: 112A-431

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:

The effects of sediment incorporated IN-KG433 on the midge (*Chironomus riparius*) were determined in an aerated, static, 28-day test. The test was conducted in accordance with the OECD Guideline 218. Treatments consisted of a negative control, solvent (acetone) control and five nominal IN-KG433 concentrations of 0.063, 0.13, 0.25, 0.50 and 1.0 mg/kg. The results of the test are based on the Day 0 measured concentrations in sediment: 0.0027, 0.089, 0.17, 0.32 and 0.64 mg/kg. The 28-Day LC₅₀ value based on emergence and mortality of *Chironomus riparius* exposed to sediment-incorporated IN-KG433 was 0.40 mg/kg, with 95% confidence limits of 0.32 and 0.64 mg/kg. The 28-Day LOEC was 0.32 mg IN-KG433/kg and the NOEC was 0.17 mg IN-KG433/kg based on emergence ratios, the most sensitive endpoint.

I. MATERIALS AND METHODS

A. MATERIALS

- | | | |
|----|---------------------------|---|
| 1. | Test material: | IN-KG433 technical metabolite |
| | Lot/Batch #: | KG433-004 |
| | Purity: | 99.9% |
| | Description: | Solid, Crystalline |
| | CAS#: | NA |
| 2. | Controls: | Negative Control and Solvent Control |
| | Test vehicle: | Solvent acetone
(10 mL acetone/kg sediment – allowed to evaporate) |
| | Toxic reference: | None |
| 3. | Test organism: | Midge |
| | Species: | <i>Chironomus riparius</i> |
| | Age/life stage at dosing: | 1-4 days (first instar) |
| | Initial population: | 20 larvae per test chamber |
| | Source: | Environmental Consulting and Testing of Superior, Wisconsin |
| | Diet: | Rabbit Food supplied by Hartz, Secaucus, New Jersey during holding and TetraMin® Flake food supplied by Doctors Foster and Smith, Blacksburg, Virginia during the test. |
| | Test chamber: | One quart glass jars |
| 4. | Environmental conditions: | Dissolved oxygen: ≥7.2 mg/L (≥80% of saturation)
pH: 8.0 to 8.6 |
| | Temperature: | 20 ± 2°C in test chambers; and measured continuously in a beaker of water adjacent to the test chambers, measured to the nearest 1°C. |
| | Photoperiod: | 16 hours light (367 lux at test initiation) and 8 hours dark including 30 min transitional period preceding and following the 16-hr light interval. |

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed

13-February-2013 to 13-March-2013

2. Experimental treatments

The effects of sediment incorporated IN-KG433 on the midge (*Chironomus riparius*) were determined in an aerated, static, 28-day test. Treatments consisted of a negative and solvent (acetone) control and five nominal IN-KG433 concentrations of 0.063, 0.13, 0.25, 0.50 and 1.0 mg/kg. Each treatment group had four replicates used for biological observations. Each replicate was initiated with 20 first instar larvae. Four additional replicates were included in the test design for analytical measurements. Each analytical replicate contained 20 organisms except for those being sampled on Day 0. At each sampling interval, an entire analytical replicate from each treatment group was sacrificed for the measurement of IN-KG433 in sediment, overlying water and pore water.

3. Observations

Observations were made daily of the survival and emergence of the midges.

4. Statistics

The 28-Day LC_{50} was determined by analyzing the number of organisms that failed to emerge during the study as well as the number of organisms that emerged and died using binomial probability with nonlinear interpolation. The NOEC and LOEC were determined by visual interpretation of the dose-response pattern and statistical analyses of the mean development times, emergence ratios and development rates. The data were analyzed to determine any statistical differences between the negative and solvent control groups. Since there were no differences, the controls were pooled and the treatment groups were compared to the pooled control using a Dunnett's test.

II. RESULTS AND DISCUSSION

A. FINDINGS

Analytical verification of IN-KG433 concentrations was made in sediment, overlying water and pore water sampled on Days 0, 7, and 28 of the test. All chemical and physical parameters for the 28-day study were within acceptable ranges. All validity criteria were met for the study.

A summary of the 28-Day LC_{50} , NOEC and LOEC are shown in Table 124.

Table 124
Summary of effects for IN-KG433 to the midge (*Chironomus riparius*)

Endpoint	Day 0 measured concentration in sediment	Effect
28-Day LC ₅₀	0.40 mg IN-KG433/kg, 95% confidence interval of 0.32 and 0.64 mg/kg	Based on number failing to emerge and number that emerged and died.
28-Day EC ₁₀	0.23 mg IN-KG433/kg, 95% confidence interval of 0.14 and 0.36 mg/kg	Based on emergence ratio
28-Day EC ₂₀	0.30 mg IN-KG433/kg, 95% confidence interval of 0.19 and 0.41 mg/kg	Based on emergence ratio
28-Day EC ₁₀	0.55 mg IN-KG433/kg, 95% confidence interval of 0.41 and 0.75 mg/kg	Based on development rate
28-Day EC ₂₀	0.76 mg IN-KG433/kg, 95% confidence interval of 0.54 and 1.1 mg/kg	Based on development rate
NOEC	0.17 mg IN-KG433/kg	Based on emergence ratios, most sensitive endpoint.
LOEC	0.32 mg IN-KG433/kg	Based on emergence ratios, most sensitive endpoint.

III. CONCLUSION

The 28-day LC₅₀ value based on emergence and/or mortality of *Chironomus riparius* exposed to sediment-incorporated IN-KG433 was 0.40 mg/kg, with 95% confidence limits of 0.32 and 0.64 mg/kg. There was a treatment related effect observed for mean development time and mean development rate between the pooled control group and the 0.64 mg IN-KG433/kg treatment group. There were treatment related effects observed for emergence ratios between the pooled control group and the 0.32 and 0.64 mg IN-KG433/kg treatment groups. Therefore, the 28-day LOEC was 0.32 mg IN-KG433/kg and the 28-day NOEC was 0.17 mg IN-KG433/kg. The EC₁₀, based on the emergence ratio of surviving midges exposed to IN-KG433 administered in sediment was 0.23 mg/kg, with 95% confidence limits of 0.14 and 0.36 mg/kg and the EC₂₀ was 0.30 mg/kg, with 95% confidence limits of 0.19 and 0.41 mg/kg. The EC₁₀, based on the development rate of surviving midges exposed to IN-KG433 administered in sediment was 0.55 mg/kg, with 95% confidence limits of 0.41 and 0.75 mg/kg and the EC₂₀ was 0.76 mg/kg, with 95% confidence limits of 0.54 and 1.1 mg/kg; however, these values are unreliable since they are outside the concentration range tested.

(Thomas, S.T., Martin, K.H., Gallagher, S.P., Krueger, H.O., 2014)

RMS comment

No table of results was provided in the original study summary. Mean values are reported below:

Day 0 measured concentration in sediment (mg /kg)	Mean development time (days)	Mean development rate	Emergence ratio
Negative control	14.0	0.0746	0.98
Solvent control	14.3	0.0737	0.98
Pooled control	14.1	0.0742	0.98
0.0027	14.0	0.0747	0.95
0.089	14.3	0.0736	0.94
0.17	13.7	0.0762	0.91
0.32	14.5	0.0726	0.69*
0.64	16.9*	0.0642*	0.19*

*there was statistically significant difference (p<0.05) from the pooled control using Dunnett's t-test

This study was conducted in compliance with the current guideline.

The 28-day NOEC was 0.17 mg IN-KG433/kg (measured concentration at Day 0).

The EC10 was 0.23 mg IN-KG433/kg (95% confidence interval of 0.14 and 0.36 mg/kg. This study is acceptable.

Report: Thomas, S.T., Kendall, T.Z., Martin, K.H., Gallagher, S.P., Krueger, H.O. (2013); IN-KT413: A prolonged sediment toxicity test with *Chironomus riparius* using spiked sediment

DuPont Report No.: DuPont-35824

Guidelines: OECD 218 (2004) **Deviations:** None

Testing Facility: Wildlife International Ltd. (USA), Easton, Maryland, USA

Testing Facility Report No.: 112A-433A

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:

The effects of sediment-incorporated IN-KT413 on the midge (*Chironomus riparius*) were determined in an aerated, static, 28-day test. The test was conducted in accordance with the OECD Guideline for Testing of Chemicals 218. Treatments consisted of a negative control, solvent (acetone) control and five nominal IN-KT413 concentrations of 6.3, 13, 25, 50, and 100 mg/kg. The corresponding mean, measured concentrations in sediment were 3.8, 7.5, 17, 34, and 65 mg IN-KT413/kg, respectively. The 28-day LC₅₀ value based on emergence and mortality of *Chironomus riparius* exposed to sediment-incorporated IN-KT413 was 46.1 mg/kg, with 95% confidence limits of 34 and 65 mg/kg. The 28-day LOEC was 17 mg IN-KT413/kg and the NOEC was 7.5 mg IN-KT413/kg based on development rate and development time, the most sensitive endpoints.

I. MATERIALS AND METHODS

A. MATERIALS

- | | | |
|----|---------------------------|--|
| 1. | Test material: | IN-KT413 technical metabolite |
| | Lot/Batch #: | KT413-003 |
| | Purity: | 99.7% |
| | Description: | Solid |
| | CAS#: | Not given |
| 2. | Controls: | Negative control and solvent control |
| | Test vehicle: | Solvent acetone (10 mL acetone/kg sediment) |
| | Toxic reference: | None |
| 3. | Test organism: | Midge |
| | Species: | <i>Chironomus riparius</i> |
| | Age/life stage at dosing: | 1-4 days (first instar) |
| | Initial population: | 20 larvae per test chamber |
| | Source: | Environmental Consulting and Testing of Superior, Wisconsin |
| | Diet: | Rabbit food supplied by Hartz, Secaucus, New Jersey during holding and TetraMin [®] Flake food supplied by Doctors Foster and Smith, Blacksburg, Virginia during the test. |
| | Test chamber: | One quart glass jars |
| 4. | Environmental conditions: | Dissolved oxygen: ≥ 6.5 mg/L ($\geq 72\%$ of saturation) |
| | | pH: 8.0 to 8.6 |
| | Temperature: | $20 \pm 2^{\circ}\text{C}$ in test chambers; and $20\text{--}21^{\circ}\text{C}$ measured continuously in a beaker of water adjacent to the test chambers, measured to the nearest 1°C . |
| | Photoperiod: | 16 hours light (393 lux at test initiation) and 8 hours dark including 30 min transitional period preceding and following the 16-hr light interval. |

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
15-July-2013 to 12-August-2013
2. Experimental treatments
The effects of sediment-incorporated IN-KT413 on the midge (*Chironomus riparius*) were determined in an aerated, static, 28-day test. Treatments consisted of a negative and solvent (acetone) control and five nominal IN-KT413 concentrations of 6.3, 13, 25, 50 and 100 mg/kg (mean measured concentrations of 3.8, 7.5, 17, 34 and 65 mg/kg). Each treatment group had four replicates used for biological observations. Each replicate was initiated with 20 first instar larvae. Four additional replicates were included in the test design for analytical measurements. Each analytical replicate contained 20 organisms except for those being sampled on Day 0. At each sampling interval, an entire analytical replicate from each treatment group was sacrificed for the measurement of IN-KT413 in sediment, overlying water and pore water.
3. Observations
Observations were made daily of the survival and emergence of the midges.
4. Statistics
The 28-day LC_{50} was determined using a binomial test. The NOEC and LOEC were determined by visual interpretation of the dose-response pattern and statistical analyses of the mean development times, emergence ratios and development rates. The data were analyzed to determine any statistical differences between the negative and solvent control groups. Since there were no differences, the controls were pooled and the

treatment groups were compared to the pooled control using a Dunnett's test. The EC₁₀ and EC₂₀ were calculated for the development rate and emergence ratio using a Versteeg model.

II. RESULTS AND DISCUSSION

A. FINDINGS

Analytical verification of IN-KT413 concentrations was made in sediment, overlying water and pore water sampled on Days 0, 7, and 28 of the test. Mean measured concentrations of IN-KT413 in the sediment were 3.8, 7.5, 17, 34 and 65 mg/kg. All validity criteria were met for the study.

A summary of the 28-day LC₅₀, 28-day EC₁₀ and EC₂₀, NOEC, and LOEC are shown in Table 125.

Table 125
Summary of effects for sediment-incorporated IN-KT413 to the midge (*Chironomus riparius*)

Endpoint	Mean Measured Concentration in Overlying Water	Effect
28-Day LC ₅₀	46.1 mg IN-KT413/kg 95% confidence limits of 34 and 65 mg IN-KT413/kg	Based on number failing to emerge and number that emerged and died.
28-Day EC ₁₀	17.3 mg IN-KT413/kg 95% confidence interval of 8.06 and 37.2 mg IN-KT413/kg	Based on emergence ratio
28-Day EC ₂₀	26.1 mg IN-KT413/kg 95% confidence interval of 15.3 and 44.5 mg IN-KT413/kg	Based on emergence ratio
28-Day EC ₁₀	30.2 mg IN-KT413/kg 95% confidence interval of 20.4 and 44.7 mg IN-KT413/kg	Based on development rate
28-Day EC ₂₀	75.9 mg IN-KT413/kg 95% confidence interval of 57.0 and 101 mg IN-KT413/kg	Based on development rate
NOEC	7.5 mg IN-KT413/kg	Based on development rate and development time, most sensitive endpoints.
LOEC	17 mg IN-KT413/kg	Based on development rate and development time, most sensitive endpoints.

III. CONCLUSION

The 28-day LC₅₀ value based on emergence and/or mortality of *Chironomus riparius* exposed to sediment-incorporated IN-KT413 was 46.1 mg/kg, with 95% confidence limits of 34 and 65 mg/kg. There were treatment related effects observed on mean emergence ratio in the 34 and 65 mg IN-KT413/kg treatment groups when compared to the pooled control. There were treatment related effects observed for development rate and development time between the pooled control group and the 17, 34, and 65 mg IN-KT413/kg treatment groups. Therefore, the 28-day LOEC was 17 mg IN-KT413/kg and the NOEC was 7.5 mg IN-KT413/kg. The EC₁₀ based on the emergence ratio of midges exposed to sediment spiked with IN-KT413 was 17.3 mg/kg with 95% confidence limits of 8.06 and 37.2 mg/kg and the EC₂₀ was 26.1 mg/kg, with 95% confidence limits of 15.3 and 44.5 mg/kg. The EC₁₀ based on the development rate of midges exposed to sediment spiked with IN-KT413 was 30.2 mg/kg, with 95% confidence limits of 20.4 and 44.7 mg/kg and the EC₂₀ was 75.9 mg/kg, with 95% confidence limits of 57.0 and 101 mg/kg.

(Thomas, S.T., Kendall, T.Z., Martin, K.H., Gallagher, S.P., Krueger, H.O., 2013)

RMS comment

No table of results was provided in the original study summary. Mean values are reported below:

Mean measured concentration in sediment (mg /kg)	Mean development time (days)	Mean development rate	Emergence ratio
Negative control	14.2	0.0740	0.94
Solvent control	13.9	0.0754	0.99
Pooled control	14.0	0.0747	0.96
3.8	14.6	0.0722	0.99
7.5	14.2	0.0743	0.95
17	15.1*	0.0695*	0.90
34	15.7*	0.0664*	0.68*
65	17.1*	0.0611*	0.44*

*there was statistically significant difference ($p < 0.05$) from the pooled control using Dunnett's t-test

This study was conducted in compliance with the current guideline.

The 28-day NOEC was 7.5 mg IN-KT413/kg based on mean measured concentrations. RMS also notes that measured concentration at the beginning of the test (8.61 mg/kg) would have been acceptable according to OECD 218. The 28-day EC10 was 17.3 mg IN-KT413/kg (95% confidence interval of 8.06 and 37.2 mg IN-KT413/kg) based on mean measured concentrations.

This study is acceptable.

Report: Aufderheide, J. (2004a); IN-MP819: Chronic toxicity test with midge larvae (*Chironomus riparius*) using spiked sediment

DuPont Report No.: DuPont-13231

Guidelines: draft OECD 218 **Deviations:** None

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

Testing Facility Report No.: 48369

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.

I. MATERIALS AND METHODS

A. MATERIALS

1. Non-radiolabeled test material: IN-MP819 technical metabolite
Lot/Batch #: MP819-004
Purity: 97.0%
Description: White powder
CAS#: Not available
2. Radiolabeled test material ¹⁴C-IN-MP819
Lot/Batch #: 48338-1-34A
Radiochemical purity: 90.1%
Specific activity: 50.5 µCi/mg
3. Controls: Negative control and solvent control
Test vehicle: Solvent acetonitrile and formic acid (0.10 mL acetonitrile/L water)
Toxic reference: None
4. Test organism: Midge
Species: *Chironomus riparius*
Age/life stage at dosing: Less than 1-2 days (first instar)
Initial population: 20 larvae per test chamber
Source: ABC Laboratories, Inc., in-house culture
Diet: Flake fish food suspension and concentrated green algae *Pseudokirchneriella subcapitata* during the test.
Test chamber: One-litre glass jars (approximately 9.5 x 17 cm) covered with an emergence trap consisting of a polyethylene jar with a screen mesh lid.
5. Environmental conditions: Dissolved oxygen: 5.0 - 8.6 mg/L
pH: 7.6 to 8.4
Temperature: 20.0 - 20.8°C
Photoperiod: 16 hours light (509 - 659 lux) and 8 hours dark including 30 min transitional period preceding and following the 16-hr light interval.

B. STUDY DESIGN AND METHODS

1. Study initiated/completed
04-December-2003 to 18-May-2004

2. Test system

A study was conducted to assess the toxicity of [¹⁴C]-IN-MP819 to the sediment dwelling phase of the non-biting midge, *Chironomus riparius*, using a static test system with application of the test substance to the sediment, following final draft OECD TG 218. Test organism cultures were maintained in laboratory freshwater under environmental conditions similar to those used for the definitive toxicity test.

Spiked sediments were prepared by adding control or test solution to fine silica sand and allowing the solvent matrix to evaporate overnight. The spiked sand was mixed with the remaining components of the artificial sediment to prepare the appropriate test substance concentrations. The nominal concentrations of IN-MP819 tested were 6.3, 13, 25, 50 and 100 mg/kg dry weight sediment. The study was initiated with first instar chironomids less than 48 hours old. A volume of 2.5-3.0 mL of a concentrated green algae (*Pseudokirchneriella subcapitata*) suspension was added to each test chamber from Day -3 to study initiation. In addition, the larvae were fed daily throughout the study with a 2 g/L flake fish food suspension, dispersed in culture medium, at a rate of 2.5-5 mL for Days 0 to 13 and 5-10 mL from Day 14 to test end. Eight replicate test chambers were prepared for each control and test substance concentration. Four replicates were used for biological observations and 2 replicates each were used for the Days 0 and 28 chemical analyses. Each chamber contained 200 gram sediment and 600 mL of laboratory freshwater. Twenty chironomid larvae were

randomly assigned to each replicate test chamber at test initiation. The artificial sediment used in this study contained 76% sand, 20% kaolinite clay, and 4% sphagnum peat; total organic carbon content of the sediment was 2.6% by analysis. Calcium carbonate was added to adjust the pH to approximately 7.0. Environmental data (pH, dissolved oxygen, and temperature) were recorded at the start and end of the study and once weekly during the study. Biological observations were recorded daily during the study, daily emergence observations were recorded from the time of first emergence until test termination. The results of all chemical analyses are based on total radioactivity measurements expressed as concentrations of the active substance.

II. RESULTS AND DISCUSSION

A. FINDINGS

The Day 0 measured sediment concentrations were <MQL (control), <MQL (solvent control), 5.96, 11.7, 23.3, 43.0, and 85.8 mg ¹⁴C-IN-MP819 equivalents per kilogram of dry sediment and ranged from 86–95% of nominal concentrations. Measured Day 28 sediment concentrations were <MQL, <MQL, 5.7, 11.1, 22.7, 42.3, and 86.5 mg ¹⁴C-IN-MP819 equivalents/kg dry sediment and ranged from 85–91% of nominal concentrations. The majority of the radiolabeled test substance remained in the sediment throughout the study.

Table 126
Measured concentrations of ¹⁴C-IN-MP819 in overlying water as TRR

Nominal sediment concentration (mg/kg dw)	Day 0 (mg/L)	Day 28 (mg/L)	Mean (mg/L)
Control	<MQL ^a	<MQL ^b	<MQL
Solvent control	<MQL ^a	<MQL ^b	<MQL
6.3	0.0121	0.0168	0.0145
13	0.0207	0.0288	0.0248
25	0.0416	0.0580	0.0498
50	0.0652	0.0958	0.0805
100	0.0844	0.126	0.105

^a MQL = 0.00475 mg/L as TRR

^b MQL = 0.00600 mg/L as TRR

Table 127
Measured concentrations of ¹⁴C-IN-MP819 in porewater as TRR

Nominal sediment concentration (mg/kg dw)	Day 0 (mg/L)	Day 28 (mg/L)	Mean (mg/L)
Control	<MQL ^a	<MQL ^b	<MQL
Solvent control	<MQL ^a	<MQL ^b	<MQL
6.3	0.0856	0.0271	0.0564
13	0.147	0.0552	0.101
25	0.295	0.105	0.200
50	0.538	0.178	0.358
100	0.934	0.263	0.599

^a MQL = 0.0106 mg/L as TRR

^b MQL = 0.00968 mg/L as TRR

Table 128
Measured concentrations of ¹⁴C-IN-MP819 in sediment as TRR (% of nominal)

Nominal sediment concentration (mg/kg dw)	Day 0 (mg/kg dwt)	Day 28 (mg/kg dwt)	Mean (mg/kg dwt)	Mean (mg/kg OC) ^c
Control	<MQL ^a	<MQL ^b	<MQL	<MQL
Solvent control	<MQL ^a	<MQL ^b	<MQL	<MQL
6.3	5.96 (95)	5.70 (90)	5.83 (93)	224
13	11.7 (90)	11.1 (85)	11.4 (88)	438
25	23.3 (93)	22.7 (91)	23.0 (92)	885
50	43.0 (86)	42.3 (85)	42.7 (85)	1640
100	85.8 (86)	86.5 (87)	86.2 (86)	3320

^a MQL = 0.152 mg/kg

^b MQL = 0.179 mg/kg

^c Mean measured concentrations normalised for organic carbon content of 2.6%

Effects of IN-MP819 on adult emergence and development: The NOEC and LOEC for gender ratio at emergence and developmental rate based on mean measured sediment concentrations were 86.2 and >86.2 mg/kg ¹⁴C-IN-MP819 (as TRR) on a dry weight sediment basis. The NOEC and LOEC were 3320 and >3320 mg/kg ¹⁴C-IN-MP819 (as TRR) on an organic carbon-normalised basis.

Table 129
Emergence and development rate data on Day 28

Nominal sediment concentration (mg/kg dw)	Mean % emergence	Gender ratio ^a	Mean development rate
Control	86	1.0	0.0503
Solvent control	93	1.6	0.0495
6.3	95	1.6	0.0511
13	76	1.1	0.0514
25	81	1.0	0.0505
50	70	1.1	0.0518
100	75	1.2	0.0515

^a Total emergent females divided by total emergent males

III. CONCLUSION

It was not possible to calculate EC₅₀ values for either emergence or development rate. Based on mean measured sediment concentrations of ¹⁴C-IN-MP819 (as TRR), the NOEC values for emergence and development rate were 86.2 mg/kg ¹⁴C-IN-MP819 on a dry weight sediment basis and 3320 mg/kg ¹⁴C-IN-MP819 on an organic carbon-normalised basis (2.6% OC), the highest concentration tested.

(Aufderheide, J., 2004a)

RMS comment

The sediment dwelling organisms study DuPont-13231 was conducted under guideline draft OECD 218. The applicant indicates that it meets the current guideline (OECD 218). This study was submitted in the original DAR (AD3).

Based on mean measured sediment concentrations of ^{14}C -IN-MP819 (as TRR), the NOEC values for emergence and development rate were 86.2 mg/kg ^{14}C -IN-MP819 on a dry weight sediment basis. No EC10 value was calculated as no treatment related effect was observed.

This study is still considered acceptable.

Report: Aufderheide, J. (2004b); IN-MS775: Chronic toxicity test with midge larvae (*Chironomus riparius*) using spiked sediment

DuPont Report No.: DuPont-13232

Guidelines: draft OECD 218 **Deviations:** None

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

Testing Facility Report No.: 48370

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.

I. MATERIALS AND METHODS

A. MATERIALS

1. Non-radiolabeled test material: IN-MS775 technical metabolite
Lot/Batch #: MS775-003
Purity: 99.5%
Description: Light brown solid
CAS#: Not available
2. Radiolabeled test material: ^{14}C -IN-MS775
Lot/Batch #: 48339-1-16-1
Radiochemical purity: 98.3%
Specific activity: 57.7 $\mu\text{Ci/mg}$
3. Controls: Negative control and solvent control
Test vehicle: Solvent acetone (6.67 mL acetone/kg sediment – allowed to evaporate)
Toxic reference: None
4. Test organism: Midge
Species: *Chironomus riparius*
Age/life stage at dosing: Less than 1-2 days (first instar)
Initial population: 20 larvae per test chamber
Source: ABC Laboratories, Inc., in-house culture
Diet: Flake fish food suspension and concentrated green algae *Pseudokirchneriella subcapitata* during the test.
Test chamber: One-litre glass jars (approximately 9.5 x 17 cm covered with an emergence trap consisting of a polyethylene jar with a screen mesh lid)
5. Environmental conditions: Dissolved oxygen: 3.6 - 8.9 mg/L
pH: 7.7 - 8.6
Temperature: 19.2 - 20.8°C
Photoperiod: 16 hours light (582 - 703 lux) and 8 hours dark including 30 min transitional period preceding and following the 16-hr light interval.

B. STUDY DESIGN AND METHODS

1. Study initiated/completed
04-December-2003 to 18-May-2004

2. Test system

A study was conducted to assess the toxicity of [^{14}C]-IN-MS775 to the sediment dwelling phase of the non-biting midge, *Chironomus riparius*, using a static test system with application of the test substance to the sediment, following final draft OECD TG 218. Test organism cultures were maintained in laboratory freshwater under environmental conditions similar to those used for the definitive toxicity test.

Spiked sediments were prepared by adding control or test solution to fine silica sand and allowing the solvent matrix to evaporate overnight. The spiked sand was mixed with the remaining components of the artificial sediment to prepare the appropriate test substance concentrations. The nominal concentrations of IN-MS775 tested were 0.63, 1.3, 2.5, 5.0, 10, and 25 mg/kg dry weight sediment. The study was initiated with first instar chironomids less than 72 hours old. A volume of 2.5-3.0 mL of a concentrated green algae (*Pseudokirchneriella subcapitata*) suspension was added to each test chamber from Day -3 to study initiation. In addition, the larvae were fed daily throughout the study with a 2 g/L flake fish food suspension, dispersed in culture medium, at a rate of 2.5-5 mL for Days 0 to 10 and 5-10 mL from Day 11 to test end. Eight replicate test chambers were prepared for each control and test substance concentration. Four replicates were used for biological observations and 2 replicates each were used for the Days 0 and 28 chemical analyses. Each chamber contained 200 gram sediment and 600 mL of laboratory freshwater. Twenty chironomid larvae were randomly assigned to each replicate test chamber at test initiation. The artificial sediment used in this study contained 76% sand, 20% kaolinite clay and 4% sphagnum peat. The total organic carbon content of the sediment was 2.6% by analysis. Calcium carbonate was added to adjust the pH to approximately 7.0. Environmental data (pH, dissolved oxygen, and temperature) were recorded at the start and end of the study and once weekly during the study. Biological observations were recorded daily during the study, daily emergence observations were recorded from the time of first emergence until test termination. The results of all chemical analyses were based on total radioactivity residue (TRR) measurements expressed as concentrations of the active ingredient.

II. RESULTS AND DISCUSSION

A. FINDINGS

The Day 0 measured sediment concentrations were <MQL (control), <MQL (solvent control), 0.519, 1.07, 2.07, 4.08, 8.15, and 19.6 mg ^{14}C -IN-MS775 equivalents per kilogram of dry sediment and ranged from 78-83% of nominal concentrations. Measured Day 28 sediment concentrations were <MQL, <MQL, 0.563, 1.09, 2.33, 4.26, 8.67, and 21.8 mg ^{14}C -IN-MS775 equivalents/kg dry sediment and ranged from 84-93% of nominal concentrations. The majority of the radiolabeled test substance remained in the sediment throughout the study.

Table 130
Measured concentrations of ^{14}C -IN-MS775 in overlying water as TRR

Nominal sediment concentration (mg/kg dw)	Day 0 (mg/L)	Day 28 (mg/L)	Mean (mg/L)
Control	<MQL ^a	<MQL ^b	<MQL
Solvent control	<MQL ^a	<MQL ^b	<MQL
0.63	<MQL ^a	<MQL ^b	<MQL
1.3	<MQL ^a	<MQL ^b	<MQL
2.5	<MQL ^a	<MQL ^b	<MQL
5.0	<MQL ^a	<MQL ^b	<MQL
10	0.00297	0.00300	0.00299
25	0.00738	0.00843	0.00791

^a MQL = 0.00184 mg/L as TRR

^b MQL = 0.00233 mg/L as TRR

Table 131
Measured concentrations of ^{14}C -IN-MS775 in pore water as TRR

Nominal sediment concentration (mg/kg dw)	Day 0 (mg/L)	Day 28 (mg/L)	Mean (mg/L)
Control	<MQL ^a	<MQL ^b	<MQL
Solvent control	<MQL ^a	<MQL ^b	<MQL
0.63	<MQL ^a	<MQL ^b	<MQL
1.3	<MQL ^a	<MQL ^b	<MQL
2.5	0.00371	<MQL ^b	0.00288 ^c
5.0	0.00724	0.00378	0.00551
10	0.0167	0.00789	0.0123
25	0.0430	0.0192	0.0311

^a MQL = 0.0106 mg/L as TRR

^b MQL = 0.00968 mg/L as TRR

^c ½ MQL on Day 28 used to calculate mean

Table 132
Measured concentrations of ^{14}C -IN-MS775 in sediment as TRR (% of nominal)

Nominal sediment concentration (mg/kg dw)	Day 0 (mg/kg dw)	Day 28 (mg/kg dw)	Mean (mg/kg dw)	Mean (mg/kg OC) ^c
Control	<MQL ^a	<MQL ^b	<MQL	<MQL
Solvent control	<MQL ^a	<MQL ^b	<MQL	<MQL
0.63	0.519 (82)	0.563 (89)	0.541 (86)	20.8
1.3	1.07 (82)	1.09 (84)	1.08 (83)	41.5
2.5	2.07 (83)	2.33 (93)	2.20 (88)	84.6
5.0	4.08 (82)	4.26 (85)	4.17 (83)	160
10	8.15 (82)	8.67 (87)	8.41 (84)	323
25	19.6 (78)	21.8 (87)	20.7 (83)	796

^a MQL = 0.0625 mg/kg

^b MQL = 0.0692 mg/kg

^c Mean, measured concentrations normalised for organic carbon content of 2.6%

Effects of IN-MS775 on adult emergence and development: The NOEC and LOEC for gender ratio for emergence based on mean measured sediment concentrations were 2.20 and 4.17 mg/kg ¹⁴C-IN-MS775 (as TRR) on a dry weight sediment basis. The NOEC and LOEC for development rate based on mean measured sediment concentrations were 4.17 and 8.41 mg/kg ¹⁴C-IN-MS775 (as TRR) on a dry weight sediment basis. The EC₅₀ values for emergence ratio and development rate were estimated as 4.81 mg/kg (95% C.I. = 4.25-5.36) and 8.04 mg/kg (95% C.I. = 4.93-11.1) ¹⁴C-IN-MS775 (as TRR), respectively, on a dry weight sediment basis.

The corresponding organic carbon-normalised NOEC and LOEC values for emergence ratio and development rate were 84.6, 160 (emergence), 160, and 323 (development) mg/kg ¹⁴C-IN-MS775 (as TRR), respectively. The EC₅₀ values for emergence ratio and development rate were estimated as 185 mg/kg (95% C.I. = 163-206) and 309 mg/kg (95% C.I. = 190-427) ¹⁴C-IN-MS775 (as TRR), respectively, on an organic carbon-normalised basis.

Table 133
Emergence and development rate data on Day 28

Nominal sediment concentration (mg/kg dw)	Mean % emergence	Gender ratio ^a	Mean development rate
Control	81	0.9	0.04523
Solvent control	90	1.5	0.04258
0.63	79	0.8	0.04548
1.3	80	1.2	0.04408
2.5	85	1.0	0.04403
5.0	53 ^c	0.8	0.04160
10	6 ^c	N/C ^b	0.01888 ^c
25	0 ^c	0	0 ^c

^a Total emergent females divided by total emergent males

^b No emergent females

^c Significantly different from control at p=0.05 based on one-tailed Dunnett's test

III. CONCLUSION

Based on mean measured sediment concentrations and emergence ratio, the lowest EC₅₀ value from the test was 4.81 mg/kg ¹⁴C-IN-MS775 (as TRR) on a dry weight sediment basis or 185 mg/kg OC ¹⁴C-IN-MS775 (as TRR) on an organic carbon-normalised basis. The NOEC and LOEC values for emergence ratio were 2.20 and 4.17 mg ¹⁴C-IN-MS775 (as TRR)/kg dry weight sediment or 84.6 and 160 mg/kg OC ¹⁴C-IN-MS775 (as TRR) on an organic carbon-normalised basis.

(Aufderheide, J., 2004b)

RMS comment

The sediment dwelling organisms study DuPont-13232 was conducted under the guideline draft OECD 218. The applicant indicates that it meets the current guideline (OECD 218). This study was submitted in the original DAR (AD3).

RMS notes that the oxygen concentration was less than 60 % of the air saturation value at the end of the test but was similar between all tested concentrations.

The NOEC value for emergence ratio was 2.20 mg ¹⁴C-IN-MS775 (as TRR)/kg dry weight sediment based on 2.6% organic carbon sediment (84.6 mg/kg OC ¹⁴C-IN-MS775 (as TRR) on an organic carbon-normalised basis).

EC10 values were calculated by RMS:

EC10 = 2.97 mg/kg ¹⁴C-IN-MS775 (95% confidence intervals: 2.30-3.77) for effects on emergence.
EC10 = 4.79 mg/kg ¹⁴C-IN-MS775 (95% confidence intervals: 2.89-7.59) for effects on development rate.

No effects were observed on the gender ratio. The value of 0 reported for the highest tested concentration indicates that neither males nor females emerged.

This study is still considered acceptable. The NOEC for emergence of 2.2 mg/kg dry weight sediment is used for risk assessment.

B.9.2.6. Effects on algal growth

Report: Sloman, T.L., Leva, S.E. (1997a); DPX-MP062 (consisting of 75% DPX-KN128 and 25% IN-KN127): Influence on growth and growth rate of the green alga *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*)

DuPont Report No.: AMR 4273-96

Guidelines: EEC 92/69 Method C3, U.S.EPA 123-2, 122-2 **Deviations:** None

Testing Facility: DuPont Stine-Haskell Research Center, Newark, Delaware, USA

Testing Facility Report No.: AMR 4273-96

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.

I. MATERIALS AND METHODS

A. MATERIALS

- | | |
|-----------------------------|---|
| 1. Test material: | DPX-MP062 technical |
| Lot/Batch #: | MP062-51 |
| Purity: | 94.51%, by analysis |
| Description: | Solid |
| CAS#: | None for DPX-MP062
DPX-KN128: 173584-44-6 |
| Stability of test compound: | Shown not to be stable in the test system by analysis |
| 2. Control: | AAP nutrient medium |
| Test vehicle: | AAP nutrient medium |
| Toxic reference: | None |

3. Test organism: Green alga
 Species: *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*)
 Initial population: Approximately 3000 cells/mL
 Source: Michael Ziegenfuss - The Academy of Natural Sciences of Philadelphia - Philadelphia, Pennsylvania
 Test chamber: 250-mL Erlenmeyer flask containing 50 mL of test solution and fitted with a sterilised foam stopper
 Growth medium: AAP nutrient medium
 pH: 7.25 to 7.44 at test initiation and 7.89 to 9.36 at test termination
4. Environmental conditions (in-life period)
 Temperature: 24.6 to 24.8°C (Environmental growth chamber and surrogate vessel)
 Photoperiod: 24 hour photoperiod (4413.3 lux)

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
 21-February-1997 to 26-February-1997

2. Test system

The effect of DPX-MP062 on the growth and reproduction of *Pseudokirchneriella subcapitata* (*Selenastrum capricornutum*) was tested in a limit test study design specified in EEC C3 for low solubility substances. Algae were exposed to a nominal concentration of 0.110 mg DPX-MP062/L, the approximate solubility limit in the test medium. The cultures were incubated without medium renewal for 120 hours. Samples were taken at 0, 24, 48, 72, 96, and 120 hours and cell densities calculated by on counting.

II. RESULTS AND DISCUSSION

A. FINDINGS

The effects of DPX-MP062 on cell density, area under the growth curve, and growth rate of *Pseudokirchneriella subcapitata* (*Selenastrum capricornutum*) are shown in Table 134.

Table 134
Summary of algal growth inhibition following exposure of *Pseudokirchneriella subcapitata* (*Selenastrum capricornutum*) to DPX-MP062 for 120 hours

Measured concentration at start (mg DPX-MP062/L)	Mean cell density (cells/mL)	% Inhibition		
		Cell density	Growth rate	Area under the growth curve
Control	5.02×10^6	—	—	—
0.110	5.99×10^6	-19.5 ^b	-2.5 ^b	-28.0 ^b

^a Represents pooled blank and solvent controls

^b Negative values indicate stimulation of cell growth

III. CONCLUSIONS

There were no detectable inhibitory effects on the cell density, growth, and growth rate of *Pseudokirchneriella subcapitata* (*Selenastrum capricornutum*) after 72 or 120 hours exposure to a measured concentration of 0.110 mg DPX-MP062/L, the solubility limit in the test medium.

(Sloman, T.L., Leva, S.E., 1997a)

RMS comment

This study was submitted in the original DAR (2000). It was conducted with test material DPX-MP062 technical, and was conducted under the guidelines EEC 92/69 Method C3, and U.S. EPA 123-2, 122-2. The applicant notes that this study partially meets the current guideline (OECD 201); deviations include only three replicates instead of six for a limit test. The summary above was revised by the applicant and endpoints for 72 and 96 hours were not reported however, as new toxicity study is available on the same species (*Pseudokirchneriella subcapitata*) for the new material DPX-KN128, this study is not considered essential.

Report: Aufderheide, J. (2014); Indoxacarb (DPX-KN128): Growth inhibition test with the unicellular green alga, *Pseudokirchneriella subcapitata*

DuPont Report No.: DuPont-38349

Guidelines: OECD 201, OCSPP 850.4500 (2012) **Deviations:** None

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

Testing Facility Report No.: 80403

GLP: Yes

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

Executive summary:

The acute toxicity of indoxacarb (DPX-KN128) to the unicellular green alga, *Pseudokirchneriella subcapitata*, was determined in a 96-hour growth inhibition test. The test was conducted in accordance with the U.S. Environmental Protection Agency, Ecological Effects Test Guidelines, OCSPP 850.4500 and OECD Guideline No. 201. Treatments consisted of an untreated control, a solvent control, an abiotic (stability) control, and five nominal concentrations of 0.013, 0.025, 0.050, 0.10 and 0.20 mg a.s./L. The corresponding 72-hour geometric mean measured concentrations of indoxacarb were 0.00485, 0.0128, 0.0232, 0.0423, and 0.0793 mg a.s./L. The corresponding 96-hour geometric mean measured concentrations of indoxacarb were 0.00485, 0.00702, 0.0179, 0.0309, and 0.0674 mg a.s./L. The 96-hour geometric mean measured concentration of indoxacarb in the nominal 0.20 mg a.s./L abiotic control was 0.0778 mg a.s./L. The 72- and 96-hour IC₅₀ and NOEC for *Pseudokirchneriella subcapitata* were based on 72- and 96-hour geometric mean measured concentrations of indoxacarb and area under the growth curve, growth rate, and yield. The 72-hour I_bC₅₀, I_rC₅₀, and I_yC₅₀ values based on area, growth rate, and yield were >0.0793 mg a.s./L, respectively. The 72-hour NOEC value based on area, growth rate, and yield was 0.0793 mg a.s./L (72-hour geometric mean measured concentrations). The 96-hour I_bC₅₀, I_rC₅₀, and I_yC₅₀ values based on area, growth rate, and yield were >0.0674 mg a.s./L, respectively. The 96-hour NOEC value based on area, growth rate, and yield was 0.0674 mg a.s./L (96-hour geometric mean, measured concentrations).

I. MATERIALS AND METHODS

A. MATERIALS

- | | | |
|----|---|--|
| 1. | Test material: | Indoxacarb technical |
| | Lot/Batch #: | KN128-374 |
| | Purity: | 98.8% |
| | Description: | Solid |
| | CAS#: | 173584-44-6 |
| | Stability of test compound: | Determined to be unstable in the test system |
| 2. | Control: | AAP nutrient medium |
| | Test vehicle: | Dimethylformamide (DMF) |
| | Toxic reference: | None |
| 3. | Test organism: | Unicellular green alga |
| | Species: | <i>Pseudokirchneriella subcapitata</i> |
| | Initial population: | 10000 cells/mL |
| | Source: | ABC Laboratories, Inc., Columbia, Missouri In house culture, parent culture from University of Texas |
| | Test chamber: | 250-mL Erlenmeyer flask with a foam stopper, containing 100 mL of test solution. |
| | Growth medium: | AAP nutrient medium |
| | pH | 7.6 at test initiation and 8.7 to 9.1 at test termination |
| 4. | Environmental conditions (in-life period) | |
| | Temperature: | 23.3 to 25.4°C |
| | Photoperiod: | 24-hour light photoperiod (4198 to 4622 lux) |

B. STUDY DESIGN AND METHODS

1. Experimental start/completion
28-January-2014 to 07-February-2014

2. Experimental treatments

The effect of indoxacarb to the green alga *Pseudokirchneriella subcapitata* was determined in a static, acute 96-hour test. The algae were exposed to an untreated control, a solvent control, and five nominal concentrations of 0.013, 0.025, 0.050, 0.10 and 0.20 mg a.s./L (72-hour geometric mean measured concentrations of 0.00485, 0.0128, 0.0232, 0.0423, and 0.0793 mg a.s./L, and 96-hour geometric mean measured concentrations of 0.00485, 0.00702, 0.0179, 0.0309, and 0.0674 mg a.s./L) in an AAP nutrient medium for 96 hours, without test medium renewal. An abiotic (stability) control was included in the test to determine the stability of indoxacarb in AAP nutrient medium under the same environmental conditions without the algae. The untreated control and solvent control were tested with six replicates, and each test concentration was tested as four replicates. The abiotic control was tested as a single test unit. The initial cell density was 10000 cells/mL. Test units were incubated in an environmental chamber for 96 hours.

3. Observations

Test concentrations for indoxacarb were measured on Days 0, 3(72 hours) and 4 (96 hours) to verify target test concentrations and stability of the test item.

Biomass, based on cell count, was determined approximately 24, 48, 72, and 96 hours after test initiation. Yield was determined by subtracting the initial cell count from the test end cell count.

Area under the growth curve and the growth rate were determined for each day of the exposure and were based on cell count.

Area, yield, and growth rate, all based on cell count, were recorded and expressed as percent inhibition relative to the untreated control following exposure to indoxacarb for 96 hours.

4. Statistics

All statistical analyses were performed with SAS software version 9.3. Prior to the IC and NOEC calculations, the control and solvent control groups were evaluated for statistical differences by comparing the means of cell density and cell density yield at the 72- and 96-hour time points and area under the growth curve and specific growth rate over 0-72 and 0-96 hours. The planned comparison, or Least Significant Difference (LSD) test, was performed by inspecting the p value for the t-test between control means and showed no statistically significant difference between the control groups. All further statistical analyses were conducted using the solvent control.

The LOEC and NOEC values, based on area under the growth curve, growth rate, and yield, were estimated using a one-way analysis of variance (ANOVA) procedure and a one-tailed Dunnett's test ($p = 0.05$) where the alternate hypothesis was that the mean for the growth parameter was reduced in comparison to the control. Prior to the Dunnett's test, a Shapiro-Wilk's test and a Levene's test were conducted to test for normality and homogeneity of variance, respectively, over treatments at each time point. If the results from the Shapiro-Wilk's and Levene's tests indicated normality and insignificant heterogeneity (*i.e.*, $p > 0.01$), the analysis was performed on the non-transformed raw data. In instances of non-normality or heterogeneity (*i.e.*, $p < 0.01$), a square root transformation was performed. If both the non-transformed raw data and the transformed data exhibited non-normality or inequality of variance, a non-parametric analysis of variance was performed on the ranks of the raw data values. Parametric analyses were performed on all area under the growth curve, specific growth rate, and yield data at the 24-, 48-, 72-, and 96-hour time points.

II. RESULTS AND DISCUSSION

A. FINDINGS

The 72-hour geometric mean measured concentrations of indoxacarb were 0.00485, 0.0128, 0.0232, 0.0423, and 0.0793 mg a.s./L, ranging from 37 to 51% of the nominal. The 96-hour geometric mean measured concentrations of indoxacarb were 0.00485, 0.00702, 0.0179, 0.0309, and 0.0674 mg a.s./L, ranging from 28 to 37% of the nominal. The 96-hour geometric mean, measured concentration of the 0.20 mg a.s./L abiotic control was 0.0778 mg a.s./L representing 39% of nominal. The untreated control and solvent control solutions contained no detectable concentrations of indoxacarb on Days 0, 3, and 4. Indoxacarb was determined to be unstable over the course of the test without the presence of algae. All validation criteria were met for the study.

A summary of algal growth inhibition following exposure of *Pseudokirchneriella subcapitata* to indoxacarb for 72 and 96 hours is presented in the tables that follow.

Table 135

Summary of algal growth inhibition following exposure of *Pseudokirchneriella subcapitata* to indoxacarb for 72 hours

72-Hour geometric mean, measured indoxacarb concentration (mg a.s./L)	% Inhibition relative to the solvent control ^a		
	Area	Growth rate	Yield
Untreated control (0.0)	—	—	—
Solvent control (0.0)	—	—	—
0.00485	8	3	11
0.0128	4	1	5
0.0232	2	1	4
0.0423	14	4	15
0.0793	13	5	17

^a Positive values indicate inhibition

Table 136

Summary of algal growth inhibition following exposure of *Pseudokirchneriella subcapitata* to indoxacarb for 96 hours

96-Hour geometric mean, measured indoxacarb concentration (mg a.s./L)	% Inhibition relative to the solvent control ^a		
	Area	Growth Rate	Yield
Untreated control (0.0)	—	—	—
Solvent control (0.0)	—	—	—
0.00485	9	2	8
0.00702	4	1	5
0.0179	-0.2	-0.4	-3
0.0309	9	1	5
0.0674	9	1	4

^a Positive values indicate inhibition

III. CONCLUSION

Growth inhibition values based on geometric mean measured indoxacarb concentrations on *Pseudokirchneriella subcapitata* were as follows:

Area:	72-hr I_bC_{50} = >0.0793 mg indoxacarb/L
	72-hr NOEC = 0.0793mg indoxacarb/L
	96-hr I_bC_{50} = >0.0674 mg indoxacarb/L
	96-hr NOEC = 0.0674 mg indoxacarb/L
Growth Rate:	72-hr I_rC_{50} = >0.0793 mg indoxacarb/L
	72-hr NOEC = 0.0793 mg indoxacarb/L
	96-hr I_rC_{50} = >0.0674 mg indoxacarb/L
	96-hr NOEC = 0.0674 mg indoxacarb/L
Yield:	72-hr I_yC_{50} = >0.0793 mg indoxacarb/L
	72-hr NOEC = 0.0793 mg indoxacarb/L
	96-hr I_yC_{50} = >0.0674 mg indoxacarb/L
	96-hr NOEC = 0.0674 mg indoxacarb/L

(Aufderheide, J., 2014)

RMS comment

This study was conducted in compliance with the current guideline.

The numbers of algal cells in the blank and solvent controls after 72 hours were greater than 16 times the initial cell density (62 and 63.7 respectively). The coefficients of variation for daily growth rates in the blank and solvent controls after 72 hours were less than 35% (29.87 and 29.45% respectively). The coefficients of variation of average specific growth rates in the blank and solvent controls after 72 hours were less than 7% (2.53 and 1.84 % respectively). The validity criteria of the guideline are therefore fulfilled.

Remark concerning the calculation of the coefficients of variation of average specific growth rates: the coefficients of variation for daily growth rates were calculated for each individual control replicate first (CV calculated for average specific growth rates at the different times) and averaged among replicates only after.

This study is acceptable.

RMS notes that the values of cell density, area under the growth curve, growth rate and yield, were more or less higher for solvent control than for blank control. The percentages of inhibition, based on solvent control, are therefore conservative.

RMS calculated the percentages of inhibition using the results at 24 and 48 hours to highlight the transient effects.

72-Hour geometric mean, measured indoxacarb concentration (mg a.s./L)	96-Hour geometric mean, measured indoxacarb concentration (mg a.s./L)	% Inhibition relative to the solvent control											
		Area				Growth rate				Yield			
		0- 24 h	0- 48 h	0- 72 h	0- 96 h	0- 24 h	0- 48 h	0- 72 h	0- 96 h	24 h	48 h	72 h	96 h
solvent control (0.0)	solvent control (0.0)			—	—			—	—			—	—
0.00485	0.00485	22	5	8	8	12	-2	3	1	22	-7	11	8
0.0128	0.00702	10	3	4	4	6	0	1	1	10	-2	4	5
0.0232	0.0179	25*	4	2	0	14	-3	1	-1	25*	-11	4	-3
0.0423	0.0309	48*	18*	14	9	32*	-1	4	1	48*	-3	15	5
0.0793	0.0674	51*	14	13	9	34*	-4	5	1	51*	-12	17	4

^a Positive values indicate inhibition

* Significant reduction as compared to the solvent control (Dunnet's test, $p = 0.05$)

RMS notes that significant effects were observed at 24 and 48 h for the highest tested concentrations but no longer observed thereafter.

The following endpoints are considered relevant for the risk assessment:

Area: 72-hr I_bC_{50} = >0.0793 mg indoxacarb/L
72-hr NOEC = 0.0793mg indoxacarb/L

Growth Rate: 72-hr I_rC_{50} = >0.0793 mg indoxacarb/L
72-hr NOEC = 0.0793 mg indoxacarb/L

Yield: 72-hr I_yC_{50} = >0.0793 mg indoxacarb/L
72-hr NOEC = 0.0793 mg indoxacarb/L

Report: Sloman, T.L., Leva, S.E. (1997b); IN-JT333 (metabolite of DPX-MP062): Influence on growth and growth rate of the green alga *Pseudokirchneriella subcapitata* (formerly called *Selenastrum capricornutum*)

DuPont Report No.: AMR 4259-96

Guidelines: EEC 92/69 Method C3, USEPA 123-2, 122-2 **Deviations:** None

Testing Facility: DuPont Stine-Haskell Research Center, Newark, Delaware, USA

Testing Facility Report No.: AMR 4259-96

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.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: IN-JT333 technical metabolite
 Lot/Batch #: JT333-20
 Purity: 98.7%
 Description: Solid
 CAS#: 144171-39-1
 Stability of test compound: Shown to be not stable in the test system by analysis
2. Control: AAP nutrient medium
 Test vehicle: AAP nutrient medium
 Toxic reference: None
3. Test organism: Green alga
 Species: *Pseudokirchneriella subcapitata* (formerly called *Selenastrum capricornutum*)
 Initial population: Approximately 3000 cells/mL
 Source: Michael Ziegenfuss - The Academy of Natural Sciences of Philadelphia - Philadelphia, Pennsylvania
 Test chamber: 250-mL Erlenmeyer flask containing 50 mL of test solution and fitted with a sterilised foam stopper
 Growth medium: AAP nutrient medium
 pH: 7.36 to 7.45 at test initiation and 7.72 to 8.76 at test termination
4. Environmental conditions (in-life period)
 Temperature: 24.6 to 25.1°C (Environmental growth chamber and surrogate vessel)
 Photoperiod: 24 hour photoperiod (4230 to 5540 lux)

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
 07-February-1997 to 12-February-1997
2. Test system
 The effect of IN-JT333 on the growth and growth rate of *Pseudokirchneriella subcapitata* (*Selenastrum capricornutum*) was tested in limit test study design specified in EEC C3 for low solubility substances. Algae were exposed to 0.017 mg IN-JT333/L measured at test start, which was determined to be the maximum solubility in the test medium. Cultures were incubated without medium renewal for 120 hours. Samples were taken at 0, 24, 48, 72, 96, and 120 hours and cell densities estimated based on counting.

II. RESULTS AND DISCUSSION

A. FINDINGS

The effects of IN-JT333 on the cell density, area under the growth curve, and growth rate of *Pseudokirchneriella subcapitata* (*Selenastrum capricornutum*) are shown in Table 137.

Table 137
Summary of algal growth inhibition following exposure of *Pseudokirchneriella subcapitata* (*Selenastrum capricornutum*) to IN-JT333 for 120 hours

Measured concentration at start (mg IN-JT333/L)	Mean cell density (cells/mL)	% Inhibition		
		Cell density	Growth rate	Area under growth curve
Control ^a	5.13×10^6	—	—	—
0.017	5.58×10^6	-8.4 ^b	-1.0 ^b	-7.8 ^b

^a Represents pooled blank and solvent controls

^b Negative values indicate stimulation of cell growth

III. CONCLUSIONS

There was no effect on the cell density, area under the growth curve, and growth rate of *Pseudokirchneriella subcapitata* (*Selenastrum capricornutum*) after 72 or 120 hours exposure to a measured concentration of 0.017 mg IN-JT333/L, the maximum solubility in the test medium.

(Sloman, T.L., Leva, S.E., 1997b)

RMS comment

This study was submitted in the original DAR (2000). It was conducted, under guidelines EEC 92/69 Method C3, and U.S. EPA 123-2, 122-2. The applicant notes that this study partially meets the current guideline (OECD 201); deviations include only three replicates instead of six for a limit test. However, reconducting the study is unlikely to yield a significantly different result because the concentration tested was the limit of solubility and there were no significant effects on any parameter measured in the study at any of the time points (72, 96, and 120 hours) when biological measurements were taken. Therefore, this study is relied upon. The summary above was revised by the applicant and endpoints for 72 and 96 hours were not reported. RMS reported these missing values below:

	% Inhibition		
	Cell density	Growth rate	Area under growth curve
72 hour	-41	-6.5	-21.3
96 hour	-2.0	-0.4	-11.1

The cell concentration in the control cultures increased by a factor of at least 16 within three days. The study is therefore valid according to validity criteria of OECD 201 (1984). RMS notes that the deviation from the nominal or measured initial concentration is not within the range of ± 20 %, therefore analysis of the results should be based on geometric mean concentration during exposure. Based on measured concentrations at day 0 and day 5, the geometric mean is 7.5 µg/L. This study is still considered acceptable.

Report: Rebstock, M. (2014); IN-JU873: Growth inhibition test with the unicellular green alga, *Pseudokirchneriella subcapitata*

DuPont Report No.: DuPont-35820

Guidelines: OECD 201 **Deviations:** None

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

Testing Facility Report No.: 69135

GLP: Yes

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

Executive summary:

The acute toxicity of IN-JU873 to the unicellular green alga, *Pseudokirchneriella subcapitata*, was determined in a 72-hour growth inhibition test. The test was conducted in accordance with OECD Guideline No. 201. Treatments consisted of an untreated control, solvent control, an abiotic (stability) control, and five nominal concentrations of 0.031, 0.063, 0.13, 0.25, and 0.50 mg IN-JU873/L. The corresponding geometric mean measured concentrations of IN-JU873 were 0.00281, 0.0101, 0.0332, 0.131, and 0.265 mg/L. The geometric mean measured concentration of IN-JU873 in the nominal 0.50 mg/L abiotic control was 0.354 mg/L. The 72-hour EC₅₀ and NOEC for *Pseudokirchneriella subcapitata* were based on geometric mean measured concentrations of IN-JU873 and cell density (biomass), growth rate, and yield. The 72-hour EbC₅₀ (biomass), ErC₅₀ (growth rate), and EyC₅₀ (yield) values based on geometric mean measured concentrations and biomass, growth rate, and yield were uniformly >0.265 mg IN-JU873/L, the highest concentration tested. The 72-hour NOEC values based on geometric mean measured concentrations and biomass, growth rate, and yield were uniformly 0.0332 mg IN-JU873/L.

I. MATERIALS AND METHODS

A. MATERIALS

- | | | |
|----|---|--|
| 1. | Test material: | IN-JU873 technical metabolite |
| | Lot/Batch #: | JU873-005 |
| | Purity: | 99.1% |
| | Description: | Solid, crystalline |
| | CAS#: | 144172-25-8 |
| | Stability of test compound: | Determined to not be stable in the test system |
| 2. | Control: | AAP nutrient medium |
| | Test vehicle: | DMF |
| | Toxic reference: | None |
| 3. | Test organism: | Unicellular green alga |
| | Species: | <i>Pseudokirchneriella subcapitata</i> |
| | Initial population: | 5000 cells/mL |
| | Source: | ABC Laboratories, Inc., Columbia, Missouri In house culture, parent culture from University of Texas |
| | Test chamber: | 250-mL Erlenmeyer flask with a foam stopper, containing 100 mL of test solution. |
| | Growth medium: | AAP nutrient medium |
| | pH | 7.4 to 7.6 at test initiation and 8.4 to 8.6 at test termination |
| 4. | Environmental conditions (in-life period) | |
| | Temperature: | 22.6 to 23.5°C |
| | Photoperiod: | 24-hour photoperiod (8516 to 8591 lux) |

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
22-October-2013 to 25-October-2013
2. Experimental treatments
The effect of IN-JU873 to the green alga *Pseudokirchneriella subcapitata* was determined in a static, acute 72-hour test. The algae were exposed to an untreated control, solvent control, and five nominal concentrations of

0.031, 0.063, 0.13, 0.25, and 0.50 mg IN-JU873/L in an AAP nutrient medium for 72 hours, without test medium renewal. An abiotic (stability) control was included in the test to determine the stability of IN-JU873 in AAP nutrient medium under the same environmental conditions without the algae. The untreated control and solvent control were tested as 6 replicates and each test concentration was tested as 3 replicates. The abiotic control was tested as a single test unit. The initial cell density was 5000 cells/mL. Test units were incubated in an environmental chamber for 72 hours.

3. Observations

Test concentrations for IN-JU873 were measured on Day 0 and Day 3 (72 hours) to verify target test concentrations and stability of the test item.

Biomass, based on cell count, was determined approximately 24, 48, and 72 hours after test initiation. Yield was determined by subtracting the initial cell count from the test end cell count.

Growth rate was determined on Day 3 and was based on cell count.

Biomass, yield, and growth rate, all based on cell count, were recorded and expressed as percent inhibition relative to the untreated control following exposure to IN-JU873 for 72 hours.

4. Statistics

All statistical analyses were performed with SAS software, version 9.3. Prior to the EC and NOEC calculations, the control and solvent control groups were evaluated for statistical differences by comparing the means of cell density and cell density yield at the 72-hour time points and specific growth rate over 0-72 hours. The planned comparison, or Least Significant Difference (LSD) test, was performed by inspecting the p value for the t-test between control means and showed no statistically significant difference between the control groups. All further statistical analyses were conducted using the pooled control treatments.

The LOEC and NOEC values, based on cell density, growth rate, and yield, were estimated using a one-way analysis of variance (ANOVA) procedure and a one-tailed Dunnett's test ($p = 0.05$) where the alternate hypothesis was that the mean for the growth parameter was reduced or enhanced in comparison to the control. Prior to the Dunnett's test, a Shapiro-Wilk's test and a Levene's test were conducted to test for normality and homogeneity of variance, respectively, over treatments at each time point. If the results from the Shapiro-Wilk's and Levene's tests indicated normality and insignificant heterogeneity (*i.e.*, $p > 0.01$), the analysis was performed on the non-transformed raw data. In instances of non-normality or heterogeneity (*i.e.*, $p < 0.01$), a square root transformation was performed. If both the non-transformed raw data and the transformed data exhibited non-normality or inequality of variance, a non-parametric analysis of variance was performed on the ranks of the raw data values. Non-parametric analyses were performed on all specific growth rate, cell density, and yield data.

II. RESULTS AND DISCUSSION

A. FINDINGS

The geometric mean measured concentrations of IN-JU873 were 0.00281, 0.0101, 0.0332, 0.131, and 0.265 mg IN-JU873/L, ranging from 9 to 53% of the nominal. The geometric mean measured concentration of the 0.50 mg IN-JU873/L abiotic control was 0.354 mg IN-JU873/L, representing 71% of nominal. The untreated control and solvent control solutions contained no detectable concentrations of IN-JU873 on both Day 0 and Day 3. IN-JU873 was determined to not be stable over the course of the test. All validation criteria were met for the study.

A summary of algal growth inhibition following exposure of *Pseudokirchneriella subcapitata* to IN-JU873 for 72 hours is presented in the table that follows.

Table 138
Summary of algal growth inhibition following exposure of *Pseudokirchneriella subcapitata* to IN-JU873 for 72 hours

Nominal IN-JU873 concentration (mg/L)	Geometric mean measured IN-JU873 concentration (mg/L)	% Inhibition relative to the pooled control ^a		
		Biomass	Growth Rate	Yield
Untreated control (0.0)	Untreated control (0.0)	—	—	—
Solvent control (0.0)	Solvent control (0.0)	—	—	—
0.031	0.00281	2	0	2
0.063	0.0101	0	0	0
0.13	0.0332	0	0	0
0.25	0.131	18*	4*	18*
0.50	0.265	37*	9*	37*

^a Positive values indicate inhibition

* Significantly different from the pooled control (Dunnett's, $\alpha = 0.05$)

III. CONCLUSION

Growth inhibition values based on geometric mean measured IN-JU873 concentrations on *Pseudokirchneriella subcapitata* were as follows:

Biomass:	72-hr E_bC_{50} = >0.265 mg IN-JU873/L 72-hr NOEC = 0.0332 mg IN-JU873/L
Growth Rate:	72-hr E_rC_{50} = >0.265 mg IN-JU873/L 72-hr NOEC = 0.0332 mg IN-JU873/L
Yield:	72-hr E_yC_{50} = >0.265 mg IN-JU873/L 72-hr NOEC = 0.0332 mg IN-JU873/L

(Rebstock M., 2014)

RMS comment

This study was conducted in compliance with the current guideline.

The numbers of algal cells in the blank and solvent controls after 72 hours were greater than 16 times the initial cell density (143 in both blank and solvent controls). The coefficients of variation for daily growth rates in the blank and solvent controls after 72 hours were less than 35% (5.0 and 5.18% respectively). The coefficients of variation of average specific growth rates in the blank and solvent controls after 72 hours were less than 7% (0.81 and 0.57 % respectively). The validity criteria of the guideline are therefore fulfilled.

Remark concerning the calculation of the coefficients of variation of average specific growth rates: the coefficients of variation for daily growth rates were calculated for each individual control replicate first (CV calculated for average specific growth rates at the different times) and averaged among replicates only after.

This study is acceptable.

RMS notes that the values of cell density, area under the growth curve, growth rate and yield, were very similar between blank and solvent controls. The percentages of inhibition are therefore based on pooled data.

RMS calculated the percentages of inhibition using the results at 24 and 48 hours to highlight the transient effects.

72-Hour geometric mean, measured indoxacarb concentration (mg a.s./L)	% Inhibition relative to the pooled control								
	biomass			Growth rate			Yield		
	0-24 h	0-48 h	0-72 h	0-24 h	0-48 h	0-72 h	24 h	48 h	72 h
Pooled control (0.0)	-	-	-	-	-	-	-	-	-
0.00281	-3	-1	2	-2	0	0	-3	-1	2
0.0101	-3	2	0	-2	0	0	-3	2	0
0.0332	6	13*	0	4	4*	0	7	13*	0
0.131	3	14*	18*	2	5*	4*	4	15*	18*
0.265	10*	22*	37*	7*	7*	9*	13*	23*	37*

* Significant reduction as compared to the solvent control (Dunnet's test, $p = 0.05$)

RMS notes that significant effects were observed at 24 and 48 h for the 3 highest tested concentrations but only for the 2 highest tested concentrations thereafter.

The following endpoints are considered relevant for the risk assessment:

Biomass:	72-hr E_bC_{50} = >0.265 mg IN-JU873/L 72-hr NOEC = 0.0332 mg IN-JU873/L
Growth Rate:	72-hr E_rC_{50} = >0.265 mg IN-JU873/L 72-hr NOEC = 0.0332 mg IN-JU873/L
Yield:	72-hr E_yC_{50} = >0.265 mg IN-JU873/L 72-hr NOEC = 0.0332 mg IN-JU873/L

Report: Gaertner, K. (2013); IN-KB687: Growth inhibition test with the unicellular green alga, *Pseudokirchneriella subcapitata*

DuPont Report No.: DuPont-35822

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

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

Testing Facility Report No.: 69141

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 IN-KB687 to the unicellular green alga, *Pseudokirchneriella subcapitata*, was determined in a 72-hour growth inhibition test. The test was conducted in accordance with the OECD Guideline No. 201. Treatments consisted of an untreated control, an abiotic (stability) control, and five nominal concentrations of 0.024, 0.076, 0.24, 0.76, and 2.4 mg IN-KB687/L. The corresponding mean measured concentrations of IN-KB687 were 0.0227, 0.0679, 0.212, 0.633, and 2.26 mg/L. The mean measured concentration of IN-KB687 in the nominal 2.4 mg/L abiotic control

was 2.33 mg/L. The 72-hour EC₅₀, LOEC, and NOEC for *Pseudokirchneriella subcapitata* were based on mean measured concentrations of IN-KB687 and cell density (biomass), growth rate, and yield. The 72-hour E_bC₅₀, E_rC₅₀, and E_yC₅₀ values based on biomass, growth rate, and yield were 1.41, >2.26, and 1.39 mg IN-KB687/L, respectively. NOEC values based on biomass, growth rate, and yield were 0.0227, 0.0679, and 0.0227 mg IN-KB687/L, respectively. The 72-hour LOEC values based on biomass, growth rate, and yield were 0.0679, 0.212, and 0.0679 mg IN-KB687/L, respectively.

I. MATERIALS AND METHODS

A. MATERIALS

- | | |
|--|---|
| 1. Test material: | IN-KB687 technical metabolite |
| Lot/Batch#: | KB687-002 |
| Purity: | 99.8% |
| Description: | Solid, powder |
| CAS#: | 177905-10-1 |
| Stability of test compound: | Stable up to 96 Hours |
| 2. Control: | FWAM nutrient medium |
| Test vehicle: | FWAM nutrient medium |
| Toxic reference: | None |
| 3. Test organism: | Unicellular green alga |
| Species: | <i>Pseudokirchneriella subcapitata</i> |
| Initial population: | 5000 cells/mL |
| Source: | ABC Laboratories, Inc., Columbia, Missouri In house culture, parent culture from University of Texas - Austin |
| Test chamber: | 250-mL Erlenmeyer flask with a foam stopper, containing 100 mL of test solution. |
| Growth medium: | FWAM nutrient medium |
| pH | 7.5 at test initiation and 7.9 to 8.1 at test termination |
| 4. Environmental conditions (in-life period) | |
| Temperature: | 23.2 to 24.0°C |
| Photoperiod: | 24-hour photoperiod (8079 to 8123 lux) |

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
26-March-2013 to 29-March-2013
2. Experimental treatments
The effect of IN-KB687 to the green alga *Pseudokirchneriella subcapitata* was determined in a static, acute 72-hour test. The algae were exposed to an untreated control and five nominal concentrations of 0.024, 0.076, 0.24, 0.76, and 2.4 mg IN-KB687/L in FWAM nutrient medium for 72 hours, without test medium renewal. An abiotic (stability) control was included in the test to determine the stability of IN-KB687 in FWAM nutrient medium under the same environmental conditions without the algae. The untreated control was tested as 6 replicates and each test concentration was tested as 3 replicates. The abiotic control was tested as a single test unit. The initial cell density was 5000 cells/mL. Test units were incubated in an environmental chamber for 72 hours.
3. Observations
Test concentrations for IN-KB687 were measured on Day 0 and Day 3 (72 hours) to verify target test concentrations and stability of the test item.

Biomass, based on cell count, was determined approximately 24, 48, and 72 hours after test initiation. Yield was determined by subtracting the initial cell count from the test end cell count.

Growth rate was determined on Day 3 and was based on cell count.

Biomass, yield, and growth rate, all based on cell count, were recorded and expressed as percent inhibition relative to the untreated control following exposure to IN-KB687 for 72 hours.

4. Statistics

All statistical analyses were performed with SAS software, version 9.3. Prior to all statistical evaluations, a one-way analysis of variance was performed to identify statistical outliers within each data set. The LOEC and NOEC values, based on cell density, growth rate, and yield, were estimated using a one-way analysis of variance (ANOVA) procedure and a one-tailed Dunnett's test ($p = 0.05$) where the alternate hypothesis was that the mean for the growth parameter was reduced or enhanced in comparison to the control. Prior to the Dunnett's test, a Shapiro-Wilk's test and a Levene's test were conducted to test for normality and homogeneity of variance, respectively, over treatments at each time point. If the results from the Shapiro-Wilk's and Levene's tests indicated normality and insignificant heterogeneity (*i.e.*, $p > 0.01$), the analysis was performed on the non-transformed raw data. In instances of non-normality or heterogeneity (*i.e.*, $p < 0.01$), a square root transformation was performed. If both the non-transformed raw data and the transformed data exhibited non-normality or inequality of variance, a non-parametric analysis of variance was performed on the ranks of the raw data values. Non-parametric analyses were performed on specific growth rate data from 0-24 and 0-48 hours, cell density and yield at 24- and 48 hour time points. Parametric analyses were performed on specific growth rate data from 0-72 hours, and cell density and yield at the 72 hour time point.

II. RESULTS AND DISCUSSION

A. FINDINGS

The mean measured concentrations of IN-KB687 were 0.0277, 0.0679, 0.212, 0.633, and 2.26 mg/L, ranging from 83 to 95% of the nominal, indicating accuracy of the test concentration solutions. The mean measured concentration of the 2.4 mg IN-KB687/L abiotic control after 72 hours was 2.33 mg IN-KB687/L representing 97% of the nominal. The untreated control solution contained no detectable concentrations of IN-KB687 on both Day 0 and Day 3. IN-KB687 was determined to be stable over the course of the test. All validation criteria were met for the study.

A summary of algal growth inhibition following exposure of *Pseudokirchneriella subcapitata* to IN-KB687 for 72 hours is presented in the table that follows:

Table 139
Summary of algal growth inhibition following exposure of *Pseudokirchneriella subcapitata* to IN-KB687 for 72 hours

Nominal IN-KB687 concentration (mg/L)	Mean measured IN-KB687 concentration (mg/L)	% Inhibition relative to the control ^a		
		Biomass	Growth rate	Yield
Untreated control (0.0)	Untreated control (0.0)	—	—	—
0.024	0.0227	-3	-1	-3
0.076	0.0679	8*	2	8*
0.24	0.212	14*	3*	14*
0.76	0.633	20*	5*	20*
2.4	2.26	69*	24*	69*

^a Positive values indicate inhibition

* Significantly different from the control (Dunnett's, $\alpha = 0.05$)

III. CONCLUSION

Growth inhibition values based on mean measured IN-KB687 concentrations obtained on *Pseudokirchneriella subcapitata* were as follows:

Biomass:	72-hr E_bC_{50} = 1.41 mg IN-KB687/L (95% CI: 1.30–1.52) 72-hr NOEC = 0.0227mg IN-KB687/L
Growth Rate:	72-hr E_rC_{50} = >2.26 mg IN-KB687/L 72-hr NOEC = 0.0679 mg IN-KB687/L
Yield:	72-hr E_yC_{50} = 1.39 mg IN-KB687/L (95% CI: 1.28–1.50) 72-hr NOEC = 0.0227 mg IN-KB687/L

(Gaertner, K., 2013)

RMS comment

This study was conducted in compliance with the current guideline.

The number of algal cells in the control after 72 hours was greater than 16 times the initial cell density (117). The coefficient of variation for daily growth rates in control after 72 hours was less than 35% (7.13%). The coefficients of variation of average specific growth rates in the control after 72 hours were less than 7% (0.65%). The validity criteria of the guideline are therefore fulfilled.

Remark concerning the calculation of the coefficient of variation of average specific growth rates: the coefficients of variation for daily growth rates were calculated for each individual control replicate first (CV calculated for average specific growth rates at the different times) and averaged among replicates only after.

This study is acceptable.

The following endpoints are considered relevant for the risk assessment:

Biomass:	72-hr E_bC_{50} = 1.41 mg IN-KB687/L (95% CI: 1.30–1.52) 72-hr NOEC = 0.0227mg IN-KB687/L
Growth Rate:	72-hr E_rC_{50} = >2.26 mg IN-KB687/L 72-hr NOEC = 0.0679 mg IN-KB687/L
Yield:	72-hr E_yC_{50} = 1.39 mg IN-KB687/L (95% CI: 1.28–1.50) 72-hr NOEC = 0.0227 mg IN-KB687/L

Report: Sloman, T.L., Leva, S.E. (1997); IN-KG433 (metabolite of DPX-MP062): Influence on growth and growth rate of the green alga *Selenastrum capricornutum*

DuPont Report No.: AMR 4280-97

Guidelines: EEC Method C.3. (1992), USEPA 122-2 (1986), USEPA 123-2 (1986) **Deviations:** None

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

Testing Facility Report No.: AMR 4280-97

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 IN-KG433 technical on the green alga *Selenastrum capricornutum* was determined in a 120-hour limit test without test medium renewal. The test was conducted in accordance with (i) EU Commission Directive 92/69/EEC, Method C3, and (ii) Non-Target Aquatic Plant Studies, U.S. Pesticide Assessment Guidelines, Subdivision J, 122-2, 123-2, following the study design specified in EEC C3 for substances with low solubility.

Treatments consisted of an untreated control, a solvent control (0.1 mL/L acetone), an abiotic (stability) control and one nominal concentration of 0.300 mg IN-KG433/L nutrient medium. The corresponding mean measured concentration of the tested concentration was 0.114 mg IN-KG433/L. This concentration was determined to be the maximum solubility in the test medium.

IN-KG433 had no detectable inhibitory effect on the growth and growth rate of *Selenastrum capricornutum* when exposed to a measured concentration of 0.114 mg IN-KG433/L of nutrient medium for up to 120 hours.

The 72-, 96-, and 120-hour EC₅₀ and NOEC values for *Selenastrum capricornutum* based on a mean, measured concentrations and cell count (density), area under the curve and growth rate were all >0.114 mg IN-KG433/L.

I. MATERIALS AND METHODS

A. MATERIALS

- | | |
|--|---|
| 1. Test material: | IN-KG433 technical |
| Lot/Batch #: | KG433-3 |
| Purity: | 98.0% |
| Description: | Off-white solid |
| CAS#: | 526224-31-7 |
| Stability of test compound: | IN-KG433 was not stable in the test medium over the course of the definitive test, as evidenced by the analytical recoveries obtained from the 0-hour (day 0) and the 120-hour (day 5) abiotic control test solution. |
| 2. Control: | Culture medium control: AAP nutrient medium |
| Solvent control: | 0.1 ml HPLC grade acetone/L AAP nutrient medium |
| | Abiotic control: 0.300 mg/L IN-KG433 with AAP nutrient medium |
| Test vehicle: | Stock solutions of IN-KG433 were prepared in acetone and then diluted to test concentration in AAP nutrient medium |
| Toxic reference: | None |
| 3. Test organism: | Green alga |
| Species: | <i>Selenastrum capricornutum</i> |
| Initial population: | Approximately 3000 cells/mL |
| Source: | Michael Ziegenfuss, The Academy of Natural Sciences of Philadelphia, Philadelphia, Pennsylvania, USA |
| Test chamber: | 250-mL Erlenmeyer flask containing 50 mL of test solution and fitted with a sterilised foam stopper |
| Growth medium: | AAP nutrient medium |
| pH | 7.42 to 7.50 at test initiation and 8.24 to 9.52 at test termination |
| 4. Environmental conditions (in-life period) | |
| Shaking speed: | 95 or 100 rpm |
| Temperature: | 24.7 to 24.8°C (Environmental growth chamber) |
| Photoperiod: | Continuous photoperiod (4100 to 5470, mean 4666.7 lux) |

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed

21-March-1997 to 26-March-1997

2. Experimental treatments

The effect of IN-KG433 technical on the green alga *Selenastrum capricornutum* was determined in a 120-hour limit test without test medium renewal. Treatments consisted of an untreated (blank) control of AAP nutrient medium, a solvent control of 0.1 mL/L acetone in nutrient medium, and one nominal concentration of 0.300 mg IN-KG433/L nutrient medium. In addition, an abiotic control (0.300 mg IN-KG433/L) was included in the test to determine the stability of IN-KG433 in AAP nutrient medium under the same environmental conditions without the algae. The test concentration and the controls were tested as three replicates each. The initial cell density was 3000 cells/mL. Test units were incubated in an environmental chamber for up to 120 hours.

3. Observations

Test solutions were measured on Day 0 and Day 5 (120 hours) to verify stability of the test item.

Cell counts were recorded approximately 24, 48, 72, 96, and 120 hours after test initiation.

Growth rate was determined at approximately 24, 48, 72, 96, and 120 hours after test initiation and was based on cell count.

Cell count (cell density), area under the growth curve, and growth rate were expressed as percent inhibition relative to the untreated control (data for pooled blank and/or solvent control) following exposure to IN-KG433 for 72, 96, and 120 hours.

4. Statistics

The F-test statistic was used to determine if the solvent control and blank control could be pooled for use in the Welch's t-test. A Welch's t-test was used to determine the levels of inhibition relative to either the solvent control or the pooled blank and solvent controls.

II. RESULTS AND DISCUSSION

A. FINDINGS

IN-KG433 was not stable for the duration of the test, based on recoveries from the 0- and 120-hour abiotic control test solutions. The mean measured concentration of IN-KG433 in the 0-hour (Day 0) test solution was 0.114 mg/L. After 120 hours, IN-KG433 was not detected in the abiotic control or test rate solutions. All validation criteria were met for the study.

The effects of IN-KG433 on the cell density, area under the growth curve, and growth rate of *Selenastrum capricornutum* are shown in Table 140.

Table 140
Summary of algal growth inhibition following exposure of *Selenastrum capricornutum*
to IN-KG433 for up to 120 hours

Measured concentration at start (mg IN-KG433/L)	Mean cell density (cells/mL)	% Inhibition ^{a,b}		
		Cell density	Growth rate	Area under growth curve
72-Hours				
Control ^c	3.7×10^5	–	–	–
0.114	4.1×10^5	-11.3	-2.3	-5.9
96-Hours				
Control ^d	1.6×10^6	–	–	–
0.114	2.0×10^6	-27.0	-2.7	-15.1
120-Hours				
Control ^c	4.6×10^6	–	–	–
0.114	4.9×10^6	-4.7	-0.6	-10.3

^a Negative values indicate stimulation of cell growth.

^b The Welch's t-test indicated no significant inhibition at the measured 0.114 mg/L rate.

^c Represents pooled data for blank and solvent controls.

^d The mean healthy cell count at the 96-hour interval for the test concentration was expressed relative to the solvent control and the mean area under the growth curve and growth rate relative to the pooled blank and solvent controls.

III. CONCLUSIONS

The 72-, 96-, and 120-hour EC₅₀ and NOEC values for *S. capricornutum* based on the mean measured concentrations for all parameters (cell count, area under the curve and growth rate) were >0.114 mg IN-KG433/L.

(Sloman, T.L., Leva, S.E., 1997)

RMS comment

This study was not conducted according to the current guideline OECD 201. RMS notes that no cell was counted at 24-hours in replicates 2 and 3 (table 3 of the study report). It is also noted that an initial measured concentration was used for the determination of the endpoint. As the substance was not stable throughout the study, a geometric mean measured concentration should be used.

The applicant was asked to provide this calculation and replied that it is not possible to calculate a geometric mean measured concentration as levels of the test item were below LOQ at the end of the study. IN-KG433 is extremely light sensitive and is rather instable in aquatic systems. Static-renewal or flow-through designs are required to maintain concentration. As these designs are not possible with algae studies, it is instead proposed to use the parent endpoint with a 10x safety factor in the risk assessment.

Besides RMS notes that the coefficient of variation for daily growth rates in control after 72 hours were high (> 35% according to validity criteria of OECD 201).

RMS considers this study not reliable.

Report: Mays, C. (2015); IN-KN124: Growth inhibition test with the unicellular green alga, *Pseudokirchneriella subcapitata*

DuPont Report No.: DuPont-43112

Guidelines: OECD 201, OCSPP 850.4500 (2012) **Deviations:** None

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

Testing Facility Report No.: 82057

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 IN-KN124 to the unicellular green alga, *Pseudokirchneriella subcapitata*, was determined in a 96-hour growth inhibition test. The test was conducted in accordance with the U.S. Environmental Protection Agency, Ecological Effects Test Guidelines, OCSPP 850.4500 and OECD Guideline No. 201. Treatments consisted of an untreated control, a vehicle control, an abiotic (stability) control, and five nominal concentrations of 0.013, 0.025, 0.050, 0.10, and 0.20 mg IN-KN124/L. The corresponding 72-hour geometric mean measured concentrations of IN-KN124 were 0.00799, 0.0138, 0.0183, 0.0270, and 0.0478 mg IN-KN124/L. The corresponding 96-hour geometric mean measured concentrations of IN-KN124 were 0.00517, 0.0104, 0.0125, 0.0176, and 0.0296 mg IN-KN124/L. The 96-hour measured concentration of IN-KN124 in the nominal 0.20 mg IN-KN124/L abiotic control was 0.0395 mg IN-KN124/L. The 72- and 96-hour EC₅₀ and NOEC for *Pseudokirchneriella subcapitata* were based on 72- and 96-hour geometric mean, measured concentrations, respectively, of IN-KN124 and area under the growth curve, growth rate, and yield. The 72-hour E_bC₅₀, E_rC₅₀, and E_yC₅₀ values based on area, growth rate, and yield were >0.0478 mg IN-KN124/L, respectively. The 72-hour NOEC value based on area, growth rate, and yield was 0.0478 mg IN-KN124/L (72-hour geometric mean, measured concentrations), respectively. The 96-hour E_bC₅₀, E_rC₅₀, and E_yC₅₀ values based on area, growth rate, and yield were >0.0296 mg IN-KN124/L, respectively. The 96-hour NOEC value based on area, growth rate, and yield was 0.0296 mg IN-KN124/L (96-hour geometric mean measured concentrations), respectively.

I. MATERIALS AND METHODS

A. MATERIALS

- | | |
|--|--|
| 1. Test material: | IN-KN124 technical metabolite |
| Lot/Batch#: | KN124-001 |
| Purity: | 99.8% |
| Description: | Solid |
| CAS#: | 200568-73-6 |
| Stability of test compound: | Determined to be unstable in the test system |
| 2. Control: | FWAM nutrient medium |
| Test vehicle: | Dimethylformamide (DMF) |
| Toxic reference: | None |
| 3. Test organism: | Unicellular green alga |
| Species: | <i>Pseudokirchneriella subcapitata</i> |
| Initial population: | 10000 cells/mL |
| Source: | ABC Laboratories, Inc., Columbia, Missouri In-house culture, parent culture from University of Texas |
| Test chamber: | 250-mL Erlenmeyer flask with a foam stopper, containing 100 mL of test solution. |
| Growth medium: | FWAM nutrient medium |
| pH | 7.3 to 7.5 at test initiation and 7.6 to 7.8 at test termination |
| 4. Environmental conditions (in-life period) | |
| Temperature: | 23.6 to 24.8°C |
| Photoperiod: | 24-hour light photoperiod (3405 to 4354 lux) |

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed

20 July 2015 to 28 July 2015

2. Experimental treatments

The effect of IN-KN124 to the green alga *Pseudokirchneriella subcapitata* was determined in a static, acute 96-hour test. The algae were exposed to an untreated control, vehicle control, and five nominal concentrations of 0.013, 0.025, 0.050, 0.10, and 0.20 mg IN-KN124/L (72-hour geometric mean measured concentrations of 0.00799, 0.0138, 0.0183, 0.0270, and 0.0478 mg IN-KN124/L, and 96-hour geometric mean measured concentrations of 0.00517, 0.0104, 0.0125, 0.0176, and 0.0296 mg IN-KN124/L) in FWAM nutrient medium for 96 hours, without test medium renewal. An abiotic (stability) control was included in the test to determine the stability of IN-KN124 in FWAM nutrient medium under the same environmental conditions without the algae. The untreated control, vehicle control, and each test concentration were tested as four replicates. The abiotic control was tested as a single test unit. The initial cell density was 10000 cells/mL. Test units were incubated in an environmental chamber for 96 hours.

3. Observations

Test concentrations for IN-KN124 were measured on Days 0, 3 (72 hours) and 4 (96 hours) to verify target test concentrations and stability of the test item.

Biomass, based on cell count, was determined approximately 24, 48, 72, and 96 hours after test initiation. Yield was determined by subtracting the initial cell count from the test end cell count.

Area under the growth curve and growth rate were determined for each day of the exposure and were based on cell count.

Area, yield, and growth rate, all based on cell count, were recorded and expressed as percent inhibition relative to the untreated control following exposure to IN-KN124 for 96 hours.

4. Statistics

Due to insufficient inhibition in all test replicates, the EC₅₀ values for yield, area under the growth curve, and growth rate were reported as greater than the maximum geometric mean measured concentrations. Additionally the NOEC for yield, area under the growth curve, and growth rate were reported as the maximum geometric mean measured concentrations.

II. RESULTS AND DISCUSSION

A. FINDINGS

The 72-hour geometric mean measured concentrations of IN-KN124 were 0.00799, 0.0138, 0.0183, 0.0270, and 0.0478 mg IN-KN124/L, ranging from 24 to 61% of the nominal. The 96-hour mean measured concentrations of IN-KN124 were 0.00517, 0.0104, 0.0125, 0.0176, and 0.0296 mg IN-KN124/L, ranging from 15 to 42% of the nominal. The 96-hour measured concentration of the 0.20 mg IN-KN124/L abiotic control was 0.0395 mg IN-KN124/L representing 20% of nominal. The untreated control and vehicle control solutions contained no detectable concentrations of IN-KN124 on Days 0, 3, and 4. IN-KN124 was determined to be unstable over the course of the test without the presence of algae. All validation criteria were met for the study.

A summary of algal growth inhibition following exposure of *Pseudokirchneriella subcapitata* to IN-KN124 for 72 and 96 hours is presented in the tables that follow.

Table 141

Summary of algal growth inhibition following exposure of *Pseudokirchneriella subcapitata* to IN-KN124 for 72 hours

72-Hour Geomean, measured concentration (mg IN-KN124/L)	% Inhibition relative to the solvent control ^a		
	Area	Growth Rate	Yield
Blank control (0.0)	—	—	—
Vehicle control (0.0)	-1	-1	-3
0.00799	-11	-2	-8
0.0138	-16	-3	-14
0.0183	-7	-2	-7
0.0270	-7	-1	-4
0.0478	-6	-1	-4

^a positive values indicate inhibition

Table 142

Summary of algal growth inhibition following exposure of *Pseudokirchneriella subcapitata* to IN-KN124 for 96 hours

96-Hour Geomean, measured concentration (mg IN-KN124/L)	% Inhibition relative to the solvent control ^a		
	Area	Growth Rate	Yield
Blank control (0.0)	—	—	—
Vehicle control (0.0)	-2	0	-2
0.00517	-12	-2	-13
0.0104	-11	-2	-9
0.0125	-7	-1	-7
0.0176	-6	-1	-5
0.0296	-6	-1	-6

^a positive values indicate inhibition

III. CONCLUSION

Growth inhibition values based on 72-hour or 96-hour geometric mean measured IN-KN124 concentrations on *Pseudokirchneriella subcapitata* were as follows:

Area:	72-hr E_bC_{50} = >0.0478 mg IN-KN124/L 72-hr NOEC = 0.0478 mg IN-KN124/L 96-hr E_bC_{50} = >0.0296 mg IN-KN124/L 96-hr NOEC = 0.0296 mg IN-KN124/L
Growth Rate:	72-hr ErC_{50} = >0.0478 mg IN-KN124/L 72-hr NOEC = 0.0478 mg IN-KN124/L 96-hr ErC_{50} = >0.0296 mg IN-KN124/L 96-hr NOEC = 0.0296 mg IN-KN124/L
Yield:	72-hr EyC_{50} = >0.0478 mg IN-KN124/L 72-hr NOEC = 0.0478 mg IN-KN124/L 96-hr EyC_{50} = >0.0296 mg IN-KN124/L 96-hr NOEC = 0.0296 mg IN-KN124/L

Mays, C., 2015

RMS comment

This study was conducted in compliance with the current guideline.

Four replicates were available for both blank and vehicle controls. The numbers of algal cells in the blank and vehicle controls after 72 hours were greater than 16 times the initial cell density (51 and 52 respectively). The coefficients of variation for daily growth rates in the blank and vehicle controls after 72 hours were less than 35% (16.9 and 17.50% respectively). The coefficient of variation of average specific growth rates in the vehicle control after 72 hours was less than 7% (6.52 %) fulfilling the validity criteria, but not after 96 hours (10.20%). The coefficient of variation of average specific growth rates in the blank control after 72 hours was of 7.32 % i.e. slightly above the validity criteria (<7%) and 4.97 after 96 hours. The validity criteria of the guideline are not completely fulfilled. RMS considers the endpoints at 72 hours reliable for the risk assessment but not at 96 hours.

Remark concerning the calculation of the coefficients of variation of average specific growth rates: the coefficients of variation for daily growth rates were calculated for each individual control replicate first (CV calculated for average specific growth rates at the different times) and averaged among replicates only after. This study is acceptable.

RMS notes that the values of cell density, area under the growth curve, growth rate and yield, were very similar between blank and vehicle controls. The percentages of inhibition should therefore be based on pooled data. However, as no inhibition was seen at all tested rates, recalculation was not necessary.

The following endpoints are considered relevant for the risk assessment:

Area:	72-hr E_bC_{50} = >0.0478 mg IN-KN124/L 72-hr NOEC = 0.0478 mg IN-KN124/L 96-hr E_bC_{50} = not acceptable 96-hr NOEC = not acceptable
Growth Rate:	72-hr ErC_{50} = >0.0478 mg IN-KN124/L 72-hr NOEC = 0.0478 mg IN-KN124/L 96-hr ErC_{50} = not acceptable 96-hr NOEC = not acceptable
Yield:	72-hr EyC_{50} = >0.0478 mg IN-KN124/L 72-hr NOEC = 0.0478 mg IN-KN124/L 96-hr EyC_{50} = not acceptable 96-hr NOEC = not acceptable

Report: Amoroso, T. (2015); IN-KN125: Growth inhibition test with the unicellular green alga, *Pseudokirchneriella subcapitata*

DuPont Report No.: DuPont-43103, Revision No. 1

Guidelines: OECD 201, OCSPP 850.4500 (2012) **Deviations:** None

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

Testing Facility Report No.: 82060

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 IN-KN125 to the unicellular green alga, *Pseudokirchneriella subcapitata*, was determined in a 96-hour growth inhibition test. The test was conducted in accordance with the U.S. Environmental Protection Agency, Ecological Effects Test Guidelines, OCSPP 850.4500 and OECD Guideline No. 201. Treatments consisted of an untreated control, a vehicle control, an abiotic (stability) control, and five nominal concentrations of 0.013, 0.025, 0.050, 0.10, and 0.20 mg IN-KN125/L. The corresponding 72-hour geometric mean measured concentrations of IN-KN125 were 0.00710, 0.0109, 0.0256, 0.0400, and 0.0508 mg IN-KN125/L. The corresponding 96-hour geometric mean measured concentrations of IN-KN125 were 0.00566, 0.00956, 0.0163, 0.0256, and 0.0370 mg IN-KN125/L. The 96-hour measured concentration of IN-KN125 in the nominal 7.5 mg/L abiotic control was 0.0194 mg IN-KN125/L. The 72- and 96-hour EC_{50} and NOEC for *Pseudokirchneriella subcapitata* were based on 72- and 96-hour geometric mean, measured concentrations, respectively, of IN-KN125 and area under the growth curve, growth rate, and yield. The 72-hour E_bC_{50} , ErC_{50} , and EyC_{50} values based on area, growth rate, and yield were all >0.0508 mg IN-KN125/L, the highest concentration tested. The 72-hour NOEC value based on area, growth rate, and yield was 0.0508 mg IN-KN125/L (72-hour geometric mean, measured concentration). The 96-hour E_bC_{50} , ErC_{50} , and EyC_{50} values based on area, growth rate, and yield were all >0.0370 mg IN-KN125/L, the highest concentration tested. The 96-hour NOEC value based on area, growth rate, and yield was 0.0370 mg IN-KN125/L (96-hour geometric mean measured concentration).

I. MATERIALS AND METHODS

A. MATERIALS

- | | |
|--|--|
| 1. Test material: | IN-KN125 technical metabolite |
| Lot/Batch #: | KN125-005 |
| Purity: | 99.4% |
| Description: | Solid |
| CAS#: | 200568-74-7 |
| Stability of test compound: | Determined to be unstable in the test system |
| 2. Control: | FWAM nutrient medium |
| Test vehicle: | Dimethylformamide (DMF) |
| Toxic reference: | None |
| 3. Test organism: | Unicellular green alga |
| Species: | <i>Pseudokirchneriella subcapitata</i> |
| Initial population: | 10000 cells/mL |
| Source: | ABC Laboratories, Inc., Columbia, Missouri In-house culture, parent culture from University of Texas |
| Test chamber: | 250-mL Erlenmeyer flask with a foam stopper, containing 100 mL of test solution. |
| Growth medium: | FWAM nutrient medium |
| pH | 7.5 to 7.6 at test initiation and 8.0 to 8.2 at test termination |
| 4. Environmental conditions (in-life period) | |
| Temperature: | 23.3 to 24.7°C |
| Photoperiod: | 24-hour light photoperiod (4121 to 4206 lux) |

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
07 July 2015 to 11 July 2015 (completion of sample analysis)
2. Experimental treatments
The effect of IN-KN125 to the green alga *Pseudokirchneriella subcapitata* was determined in a static, acute 96-hour test. The algae were exposed to an untreated control, vehicle control, and five nominal concentrations of 0.013, 0.025, 0.050, 0.10, and 0.20 mg IN-KN125/L (72-hour geometric mean measured concentrations of 0.00710, 0.0109, 0.0256, 0.0400, and 0.0508 mg IN-KN125/L, and 96-hour geometric mean measured concentrations of 0.00566, 0.00956, 0.0163, 0.0256, and 0.0370 mg IN-KN125/L) in FWAM nutrient medium for 96 hours, without test medium renewal. An abiotic (stability) control was included in the test to determine the stability of IN-KN125 in FWAM nutrient medium under the same environmental conditions without the algae. The untreated control, vehicle control, and each test concentration were tested as four replicates. The abiotic control was tested as a single test unit. The initial cell density was 10000 cells/mL. Test units were incubated in an environmental chamber for 96 hours.
3. Observations
Test concentrations for IN-KN125 were measured on days 0, 3 (72 hours) and 4 (96 hours) to verify target test concentrations and stability of the test item.

Biomass, based on cell count, was determined approximately 24, 48, 72, and 96 hours after test initiation. Yield was determined by subtracting the initial cell count from the test end cell count.

Area under the growth curve and growth rate were determined for each day of the exposure and were based on cell count.

Area, yield, and growth rate, all based on cell count, were recorded and expressed as percent inhibition relative to the untreated control following exposure to IN-KN125 for 96 hours.

4. Statistics

All statistical analyses were performed with SAS software and Ecotats.

The LOEC and NOEC values, based on area under the growth curve, growth rate, and yield, were estimated using a one-way analysis of variance (ANOVA) procedure and a one-tailed Dunnett's test and/or Jonckheere Trend test ($p = 0.05$) where the alternate hypothesis was that the mean for the growth parameter was reduced in comparison to the control. Prior to the Dunnett's test and Jonckheere Trend test, a Shapiro-Wilk's test and a Levene's test were conducted to test for normality and homogeneity of variance, respectively, over treatments at each time point. If the results from the Shapiro-Wilk's and Levene's tests indicated normality and insignificant heterogeneity (i.e., $p > 0.01$), the analysis was performed on the non-transformed raw data. In instances of non-normality or heterogeneity (i.e., $p < 0.01$), a square root transformation was performed. If both the non-transformed raw data and the transformed data exhibited non-normality or inequality of variance, a non-parametric analysis of variance was performed on the ranks of the raw data values. Parametric analyses were performed on 72 and 96 hour cell density, growth rate, and yield data, and the 48 and 96 hour area under the curve data. Non-parametric analyses were performed for the 24 and 48 hour cell density growth rate, and yield data, and the 24 and 72 hour area under the growth curve data.

II. RESULTS AND DISCUSSION

A. FINDINGS

The 72-hour geometric mean measured concentrations of IN-KN125 were 0.00710, 0.0109, 0.0256, 0.0400, and 0.0508 mg IN-KN125/L, ranging from 25 to 55% of the nominal. The 96-hour mean measured concentrations of IN-KN125 were 0.00566, 0.00956, 0.0163, 0.0256, and 0.0370 mg IN-KN125/L, ranging from 19 to 44% of the nominal. The 96-hour measured concentration of the 0.20 mg IN-KN125/L abiotic control was 0.0194 mg IN-KN125/L representing 10% of nominal. The untreated control and vehicle control solutions contained no detectable concentrations of IN-KN125 on Days 0, 3, and 4. IN-KN125 was determined to be unstable over the course of the test without the presence of algae. All validation criteria were met for the study.

A summary of algal growth inhibition following exposure of *Pseudokirchneriella subcapitata* to IN-KN125 for 72 and 96 hours is presented in the tables that follow.

Table 143
Summary of algal growth inhibition following exposure of *Pseudokirchneriella subcapitata* to IN-KN125 for 72 hours

72-Hour Geometric Mean, measured concentration (mg IN-KN125/L)	% Inhibition relative to the control ^a		
	Area	Growth Rate	Yield
Blank control (0.0)	—	—	—
Vehicle control (0.0)	---	---	---
0.00710	1	0	0
0.0109	0	0	-1
0.0256	0	-1	-3
0.0400	1	0	0
0.0508	-1	0	-1

^a positive values indicate inhibition

Table 144
Summary of algal growth inhibition following exposure of *Pseudokirchneriella subcapitata* to IN-KN125 for 96 hours

96-Hour Geometric Mean, measured concentration (mg IN-KN125/L)	% Inhibition relative to the control ^a		
	Area	Growth Rate	Yield
Blank control (0.0)	—	—	—
Vehicle control (0.0)	---	---	---
0.00566	0	0	1
0.00956	0	0	0
0.0163	-1	0	-1
0.0256	1	0	2
0.0370	2	1	3

^a positive values indicate inhibition

III. CONCLUSION

Growth inhibition values based on 72-hour or 96-hour geometric mean measured IN-KN125 concentrations on *Pseudokirchneriella subcapitata* were as follows:

Area:	72-hr EbC ₅₀ = >0.0508 mg IN-KN125/L 72-hr NOEC = 0.0508 mg IN-KN125/L 96-hr EbC ₅₀ = >0.0370 mg IN-KN125/L 96-hr NOEC = 0.0370 mg IN-KN125/L
Growth Rate:	72-hr EbC ₅₀ = >0.0508 mg IN-KN125/L 72-hr NOEC = 0.0508 mg IN-KN125/L 96-hr EbC ₅₀ = >0.0370 mg IN-KN125/L 96-hr NOEC = 0.0370 mg IN-KN125/L
Yield:	72-hr EyC ₅₀ = >0.0508 mg IN-KN125/L 72-hr NOEC = 0.0508 mg IN-KN125/L 96-hr EyC ₅₀ = >0.0370 mg IN-KN125/L 96-hr NOEC = 0.0370 mg IN-KN125/L

(Amoroso, T., 2015)

RMS comment

This study was conducted in compliance with the current guideline.

Four replicates were available for both blank and vehicle controls. The numbers of algal cells in the blank and vehicle controls after 72 hours were greater than 16 times the initial cell density (47.2 and 49.4 respectively). The coefficients of variation for daily growth rates in the blank and vehicle controls after 72 hours were less than 35% (5.7 and 11.16 % respectively). The coefficient of variation of average specific growth rates in the vehicle control after 72 hours was slightly above the validity criteria of 7% (8.78 %), but not after 96 hours (3.05%). The coefficient of variation of average specific growth rates in the blank control after 72 hours was of 1.85% (<7%) and 2.45 % after 96 hours. Considering the obvious absence of toxicity at all tested rates, the deviation to validity criteria in vehicle control at 72 h is considered minor and the endpoints reported below are valid.

Remark concerning the calculation of the coefficients of variation of average specific growth rates: the coefficients of variation for daily growth rates were calculated for each individual control replicate first (CV calculated for average specific growth rates at the different times) and averaged among replicates only after.

This study is acceptable.

RMS notes that the values of cell density, area under the growth curve, growth rate and yield, were very similar between blank and vehicle controls. The percentages of inhibition should therefore be based on pooled data. However, as no inhibition was seen at all tested rates, recalculation was not necessary.

The following endpoints are considered relevant for the risk assessment:

Area:	72-hr EbC ₅₀ = >0.0508 mg IN-KN125/L
	72-hr NOEC = 0.0508 mg IN-KN125/L
	96-hr EbC ₅₀ = >0.0370 mg IN-KN125/L
	96-hr NOEC = 0.0370 mg IN-KN125/L
Growth Rate:	72-hr EbC ₅₀ = >0.0508 mg IN-KN125/L
	72-hr NOEC = 0.0508 mg IN-KN125/L
	96-hr EbC ₅₀ = >0.0370 mg IN-KN125/L
	96-hr NOEC = 0.0370 mg IN-KN125/L
Yield:	72-hr EyC ₅₀ = >0.0508 mg IN-KN125/L
	72-hr NOEC = 0.0508 mg IN-KN125/L
	96-hr EyC ₅₀ = >0.0370 mg IN-KN125/L
	96-hr NOEC = 0.0370 mg IN-KN125/L

Report: Boeri, R.L., Ward, T.J. (2000); IN-KT413: Influence on growth and growth rate of the alga, *Selenastrum capricornutum*

DuPont Report No.: DuPont-3935

Guidelines: OECD 201 (1984), U.S. EPA 123-2 (1986) **Deviations:** None

Testing Facility: T.R. Wilbury Laboratories, Inc., Marblehead, Massachusetts, USA

Testing Facility Report No.: 1990-DU

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.

I. MATERIALS AND METHODS

A. MATERIALS

- | | |
|--|---|
| 1. Test material: | IN-KT413 technical metabolite |
| Lot/Batch #: | KT413-3 |
| Purity: | 97.0%, by analysis |
| Description: | White powder |
| CAS#: | Not available |
| Stability of test compound: | Shown to be stable in the test system by analysis |
| 2. Control: | Deionised water |
| Test vehicle: | Deionised water |
| Toxic reference: | None |
| 3. Test organism: | Green alga |
| Species: | <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 |
| Test chamber: | 250-mL Erlenmeyer flask containing 100 mL of test solution and loosely capped with inverted glass beakers |
| Growth medium: | AAP nutrient medium |
| pH | 7.4 to 7.5 at test initiation and 9.2 to 10.2 at test termination |
| 4. Environmental conditions (in-life period) | |
| Temperature: | 23.2 to 24.1°C (Environmental growth chamber and surrogate vessel) |
| Photoperiod: | 24 hour photoperiod (7700 to 7800 lux) |

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
16-April-2000 to 01-August-2000

2. Experimental treatments

The effects of IN-KT413 on the growth and growth rate of *Selenastrum capricornutum* were measured under static conditions. The test was performed under static conditions with five concentrations of test substance and a water control at $24 \pm 2^\circ\text{C}$. Nominal concentrations of IN-KT413 were 0 mg/L (control), 9.0, 17, 33, 65, and 130 mg/L. Initial measured concentrations of IN-KT413 were <LOQ (limit of quantitation of 0.109 mg/L; test medium control), 8.10, 15.4, 30.3, 58.5, and 108 mg/L. Because not all 72 hour measured concentrations were greater than 70% of nominal concentrations, initial measured concentrations were used for all calculations. No visible insoluble material was noted during the toxicity test. However aggregations of cells were observed in all non-control test vessels at 72 hours.

II. RESULTS AND DISCUSSION

A. FINDINGS

The effects of IN-KT413 on the growth and growth rate of *Selenastrum capricornutum* are shown in Table 145.

Table 145
Summary of algal growth inhibition following exposure of *Selenastrum capricornutum* to IN-KT413 for 72 hours

Nominal dose (initial measured dose) mg/L	Mean cell density (cells/mL)	Percent of control		
		Cell density	Area under the growth curve	Growth rate
Water control	2.792×10^6	—	—	—
9 (8.10)	1.840×10^6	66	73	92
17 (15.4)	1.713×10^6	61	66	91
33 (30.3)	1.433×10^6	51	55	88
65 (58.5)	1.487×10^6	53	58	88
130 (108)	0.925×10^6	33	40	81

III. CONCLUSIONS

Growth inhibition values obtained with IN-KT413 on *Selenastrum capricornutum* were as follows:

Cell density:	72-hour EC ₅₀ = 39.7 mg/L 72-hour NOEC <8.1 mg/L calculated 72-hr NOAEC = 1.8 mg/L
Area under the growth curve:	72-hour EC ₅₀ = 61.7 mg/L 72-hour NOEC <8.1 mg/L
Growth rate:	72-hour EC ₅₀ >108 mg/L 72-hour NOEC <8.1 mg/L

(Boeri, R.L., Ward, T.J., 2000)

RMS comment

This study was submitted in the original DAR (AD3, 2005). The biomass in the control cultures have increased by a factor > 16 (279). The mean coefficient of variation for section-by-section specific growth rates in the control cultures did not exceed 35% (10.81). The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures did not exceed 7% (4.33). The study is therefore valid according to validity criteria of the most recent version of OECD 201. RMS notes that the deviation from the nominal of measured concentration at 72 h is not within the range of $\pm 20\%$, therefore analysis of the results should be based on geometric mean concentration during exposure. Based on measured concentrations at day 0 and day 3 at the highest dose tested at which less than 50% effect on growth rate were observed, the geometric mean is > 105 mg/L. EC50_{growth rate} is therefore > 105 mg IN-KT413/L. This study is still considered acceptable.

Report: Brugger, K.E. (2001); IN-KT413: Extrapolation of a NOAEC from toxicity data with *Selenastrum capricornutum*

DuPont Report No.: DuPont-6620

Guidelines: Not applicable **Deviations:** None

Testing Facility: DuPont Stine-Haskell Research Center, Newark, Delaware, USA

Testing Facility Report No.: DuPont-6620

GLP: Not applicable

Certifying Authority: Not applicable.

The effect of IN-KT413 on *Selenastrum capricornutum* was determined using algal cultures with sterile synthetic medium. Three replicates at 8.1, 15.4, 30.3, 58.5, and 108.0 mg/L IN-KT413, measured at test initiation, were incubated for 72 hours, and cell counts were taken at 24-hour intervals. A 48-hour recovery phase was added for the highest test concentration to evaluate the potential for regrowth of cells.

The predicted No Observed Adverse Effects Concentration (NOAEC) was calculated using USEPA benchmarking methods. Cell count data were fit to four models (linear, polynomial, power, and Hill), and a best fit was selected based on chi-square analysis. The benchmark reference (= NOAEC) was defined as the percentage effect of concern, most often set at 10% effect or EC₁₀. A benchmark dose was then established with the best-fit model and corresponds to the exposure concentration that is predicted to elicit the EC_x.

II. RESULTS AND DISCUSSION

A. FINDINGS

The effects of IN-KT413 on the growth of *Selenastrum capricornutum* are shown in Table 146. The 72-hr EC₅₀s for IN-KT413, based on initial measured concentrations, were 39.7 (cells per mL), >108 (growth rate), and 61.7 mg/L (area under the curve). The 72-hr NOEC for each endpoint was less than the lowest test concentration (<8.10 mg/L).

The Hill model fit the data better than the power model (chi-square value = 0.88 vs 0.65) to enable prediction of a NOAEC. The predicted NOAEC was 1.80 mg/L.

Table 146
Summary of algal growth inhibition following exposure of *Selenastrum capricornutum* to IN-KT413 technical for 72 hours

Mean measured concentrations at test start (mg/L)	Mean cell density (cells/mL)			
	0 hrs	24 hrs	48 hrs	72 hrs
Water control	10000	55000	418000	2792000
8.1	10000	51000	399000	1840000
15.4	10000	43000	349000	1713000
30.3	10000	40000	275000	1433000
58.5	10000	45000	312000	1487000
108.0	10000	46000	248000	925000

III. CONCLUSIONS

The most sensitive endpoint for growth inhibition (cell density) data obtained with IN-KT413 on *Selenastrum capricornutum* were as follows:

Cell density:	72-hour EC_{50} = 39.7 mg/L 72-hour NOAEC = 1.80 mg/L
Area under the growth curve:	72-hour EC_{50} = 61.7 mg/L
Growth rate:	72-hour EC_{50} >108 mg/L

(Brugger, K.E., 2001)

RMS comment

This position paper was assessed for previous Annex I inclusion and considered supplemental to DuPont-3935 (original DAR, AD3, 2005). Because the lowest concentration tested still shows significant adverse effect, an additional report is provided (Brugger, 2001). In this report a benchmarking method is used to calculate an EC_{10} , that is supposed to be equivalent to NOAEC. This is not considered essential for the risk assessment.

Report: Holou, M. (2013); IN-MK638: Growth inhibition test with the unicellular green alga, *Pseudokirchneriella subcapitata*

DuPont Report No.: DuPont-35821

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

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

Testing Facility Report No.: 69138

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 IN-MK638 to the unicellular green alga, *Pseudokirchneriella subcapitata*, was determined in a 72-hour growth inhibition test. The test was conducted in accordance with the OECD Guideline No. 201. Treatments consisted of an untreated control, an abiotic (stability) control, and five nominal concentrations of 0.63, 1.9, 5.7, 17, and 50 mg IN-MK638/L. The corresponding mean measured concentrations of IN-MK638 were 0.641, 1.90, 5.43, 15.9, and 52.6 mg/L. The mean measured concentration of IN-MK638 in the nominal 50 mg/L abiotic control was 51.6 mg/L. The 72-hour EC_{50} and NOEC for *Pseudokirchneriella subcapitata* were based on mean measured concentrations of IN-MK638 and cell density (biomass), growth rate, and yield. The 72-hour E_bC_{50} , E_rC_{50} , and E_yC_{50} values based on biomass, growth rate, and yield were 7.55, 37.2, and 7.45 mg IN-MK638/L, respectively. The 72-hour NOEC values based on biomass, growth rate, and yield were uniformly 0.641 mg IN-MK638/L.

I. MATERIALS AND METHODS

A. MATERIALS

- | | | |
|----|---|--|
| 1. | Test material: | IN-MK638 technical metabolite |
| | Lot/Batch #: | MK638-002 |
| | Purity: | 99.9% |
| | Description: | Solid, Crystalline |
| | CAS#: | 82971-90-2 |
| | Stability of test compound: | Stable up to 96 Hours |
| 2. | Control: | AAP nutrient medium |
| | Test vehicle: | AAP nutrient medium |
| | Toxic reference: | None |
| 3. | Test organism: | Unicellular green alga |
| | Species: | <i>Pseudokirchneriella subcapitata</i> |
| | Initial population: | 5000 cells/mL |
| | Source: | ABC Laboratories, Inc., Columbia, Missouri In house culture, parent culture from University of Texas |
| | Test chamber: | 250-mL Erlenmeyer flask with a foam stopper, containing 100 mL of test solution. |
| | Growth medium: | AAP nutrient medium |
| | pH | 7.4 to 7.5 at test initiation and 7.8 to 8.1 at test termination |
| 4. | Environmental conditions (in-life period) | |
| | Temperature: | 22.4 to 24.3°C |
| | Photoperiod: | 24-hour photoperiod (8127 to 8276 lux) |

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
26-February-2013 to 01-March-2013
2. Experimental treatments
The effect of IN-MK638 to the green alga *Pseudokirchneriella subcapitata* was determined in a static, acute 72-hour test. The algae were exposed to an untreated control and five nominal concentrations of 0.63, 1.9, 5.7, 17, and 50 mg IN-MK638/L in an AAP nutrient medium for 72 hours, without test medium renewal. An abiotic (stability) control was included in the test to determine the stability of IN-MK638 in an AAP nutrient medium under the same environmental conditions without the algae. The untreated control was tested as 6 replicates and each test concentration was tested as 3 replicates. The abiotic control was tested as a single test unit. The initial cell density was 5000 cells/mL. Test units were incubated in an environmental chamber for 72 hours.
3. Observations
Test concentrations for IN-MK638 were measured on Day 0 and Day 3 (72 hours) to verify target test concentrations and stability of the test item.

Biomass, based on cell count, was determined approximately 24, 48, and 72 hours after test initiation. Yield was determined by subtracting the initial cell count from the test end cell count.

Growth rate was determined on Day 3 and was based on cell count.

Biomass, yield, and growth rate, all based on cell count, were recorded and expressed as percent inhibition relative to the untreated control following exposure to IN-MK638 for 72 hours.

4. Statistics

All statistical analyses were performed with SAS software, version 9.3. Prior to all statistical evaluations, a one-way analysis of variance was performed to identify statistical outliers within each data set. The LOEC and NOEC values, based on cell density, growth rate, and yield, were estimated using a one-way analysis of variance (ANOVA) procedure and a one-tailed Dunnett's test ($p = 0.05$) where the alternate hypothesis was that the mean for the growth parameter was reduced or enhanced in comparison to the control. Prior to the Dunnett's test, a Shapiro-Wilk's test and a Levene's test were conducted to test for normality and homogeneity of variance, respectively, over treatments at each time point. If the results from the Shapiro-Wilk's and Levene's tests indicated normality and insignificant heterogeneity (*i.e.*, $p > 0.01$), the analysis was performed on the non-transformed raw data. In instances of non-normality or heterogeneity (*i.e.*, $p < 0.01$), a square root transformation was performed. If both the non-transformed raw data and the transformed data exhibited non-normality or inequality of variance, a non-parametric analysis of variance was performed on the ranks of the raw data values. Non-parametric analyses were performed on specific growth rate data from 0-24 and 0-48 hours, adjacent growth rate data from 0-24 hours, and on cell density and yield data at the 24- and 48-hour time points. Parametric analyses were performed on specific growth rate data from 0-72 hours, adjacent growth rate data from 24-48 and 48-72 hours and cell density and yield data at the 72-hour time point.

II. RESULTS AND DISCUSSION

A. FINDINGS

The mean measured concentrations of IN-MK638 were 0.641, 1.90, 5.43, 15.9, and 52.6 mg/L, ranging from 94 to 105% of the nominal, indicating accuracy of the test concentration solutions. The mean measured concentration of IN-MK638 in the 50 mg/L abiotic control after 72 hours was 51.6 mg/L representing 103% of the nominal. The untreated control solution contained no detectable concentrations of IN-MK638 on both Day 0 and Day 3. IN-MK638 was determined to be stable over the course of the test. All validation criteria were met for the study.

A summary of algal growth inhibition following exposure of *Pseudokirchneriella subcapitata* to IN-MK638 for 72 hours is presented in the table that follows.

Table 147
Summary of algal growth inhibition following exposure of *Pseudokirchneriella subcapitata* to IN-MK638 for 72 hours

Nominal IN-MK638 concentration (mg/L)	Mean measured IN-MK638 concentration (mg/L)	% Inhibition relative to the control ^a		
		Biomass	Growth Rate	Yield
Untreated control (0.0)	Untreated control (0.0)	—	—	—
0.63	0.641	1	0	1
1.9	1.90	31*	7*	31*
5.7	5.43	42*	11*	42*
17	15.9	62*	18*	62*
50	52.6	97*	64*	97*

^a Positive values indicate inhibition

* Significantly different from the control (Dunnett's, $\alpha = 0.05$)

III. CONCLUSION

Growth inhibition values based on mean measured IN-MK638 concentrations obtained with IN-MK638 on *Pseudokirchneriella subcapitata* were as follows:

Biomass:	72-hr E_bC_{50} = 7.55 mg IN-MK638/L (95% CI: 5.55–9.54) 72-hr NOEC = 0.641 mg IN-MK638/L
Growth Rate:	72-hr E_rC_{50} = 37.2 mg IN-MK638/L (95% CI: 34.8–39.6) 72-hr NOEC = 0.641 mg IN-MK638/L
Yield:	72-hr E_yC_{50} = 7.45 mg IN-MK638/L (95% CI: 5.45–9.45) 72-hr NOEC = 0.641 mg IN-MK638/L

(Holou, M., 2013)

RMS comment

This study was conducted in compliance with the current guideline.

The number of algal cells in the control after 72 hours was greater than 16 times the initial cell density (185). The coefficient of variation for daily growth rates in control after 72 hours was less than 35% (15.74%). The coefficients of variation of average specific growth rates in the control after 72 hours were less than 7% (1.60 %). The validity criteria of the guideline are therefore fulfilled.

Remark concerning the calculation of the coefficient of variation of average specific growth rates: the coefficients of variation for daily growth rates were calculated for each individual control replicate first (CV calculated for average specific growth rates at the different times) and averaged among replicates only after.

This study is acceptable.

The following endpoints are considered relevant for the risk assessment:

Biomass:	72-hr E_bC_{50} = 7.55 mg IN-MK638/L (95% CI: 5.55–9.54) 72-hr NOEC = 0.641 mg IN-MK638/L
Growth Rate:	72-hr E_rC_{50} = 37.2 mg IN-MK638/L (95% CI: 34.8–39.6) 72-hr NOEC = 0.641 mg IN-MK638/L
Yield:	72-hr E_yC_{50} = 7.45 mg IN-MK638/L (95% CI: 5.45–9.45) 72-hr NOEC = 0.641 mg IN-MK638/L

Report: Bergfield, A. (2013); IN-MK643: Growth inhibition test with the unicellular green alga, *Pseudokirchneriella subcapitata*

DuPont Report No.: DuPont-36161

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

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

Testing Facility Report No.: 69277

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:

The acute toxicity of IN-MK643 to the unicellular green alga, *Pseudokirchneriella subcapitata*, was determined in a 72-hour growth inhibition test. The test was conducted in accordance with OECD Guideline No. 201. Treatments consisted of an untreated control, an abiotic (stability) control, and five nominal concentrations of 1.2, 3.7, 11, 33, and 100 mg IN-MK643/L. The corresponding mean measured concentrations of IN-MK643 were 1.23, 3.40, 10.4, 32.0, and 102 mg/L. The mean measured concentration of IN-MK643 in the nominal 100 mg/L abiotic control was 102 mg/L. The 72-hour EC₅₀ and NOEC for *Pseudokirchneriella subcapitata* were based on mean measured concentrations of IN-MK643 and cell density (biomass), growth rate, and yield. The 72-hour EbC₅₀, ErC₅₀, and EyC₅₀ values based on mean measured concentrations and biomass, growth rate, and yield were 31.8, 59.7, and 31.8 mg IN-MK643/L, respectively. The 72-hour NOEC values based on biomass, growth rate, and yield were uniformly 3.40 mg IN-MK643/L, based on mean measured concentrations.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:	IN-MK643 technical metabolite
Lot/Batch #:	MK643-002
Purity:	96.7%
Description:	Solid, powder
CAS#:	877681-12-4
Stability of test compound:	Determined to be stable in the test system
2. Control:	AAP nutrient medium
Test vehicle:	AAP nutrient medium
Toxic reference:	None
3. Test organism:	Unicellular green alga
Species:	<i>Pseudokirchneriella subcapitata</i>
Initial population:	5000 cells/mL
Source:	ABC Laboratories, Inc., Columbia, Missouri In house culture, parent culture from University of Texas
Test chamber:	250-mL Erlenmeyer flask with a foam stopper, containing 100 mL of test solution.
Growth medium:	AAP nutrient medium
pH	7.4 to 7.5 at test initiation and 7.7 to 8.1 at test termination
4. Environmental conditions (in-life period)	
Temperature:	23.2 to 23.9°C
Photoperiod:	24-hour photoperiod (7905 to 8204 lux)

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
12-March-2013 to 15-March-2013

2. Experimental treatments

The effect of IN-MK643 to the green alga *Pseudokirchneriella subcapitata* was determined in a static, acute 72-hour test. The algae were exposed to an untreated control and five nominal concentrations of 1.2, 3.7, 11, 33, and 100 mg IN-MK643/L in an AAP nutrient medium for 72 hours, without test medium renewal. An abiotic (stability) control was included in the test to determine the stability of IN-MK643 in an AAP nutrient medium under the same environmental conditions without the algae. The untreated control was tested as 6 replicates and each test concentration was tested as 3 replicates. The abiotic control was tested as a single test

unit. The initial cell density was 5000 cells/mL. Test units were incubated in an environmental chamber for 72 hours.

3. Observations

Test concentrations for IN-MK643 were measured on Day 0 and Day 3 (72 hours) to verify target test concentrations and stability of the test item.

Biomass, based on cell count, was determined approximately 24, 48, and 72 hours after test initiation. Yield was determined by subtracting the initial cell count from the test end cell count.

Growth rate was determined on Day 3 and was based on cell count.

Biomass, yield, and growth rate, all based on cell count, were recorded and expressed as percent inhibition relative to the untreated control following exposure to IN-MK643 for 72 hours.

4. Statistics

All statistical analyses were performed with SAS software, version 9.3. Prior to all statistical evaluations, a one-way analysis of variance was performed to identify statistical outliers within each data set. The LOEC and NOEC values, based on cell density, growth rate, and yield, were estimated using a one-way analysis of variance (ANOVA) procedure and a one-tailed Dunnett's test ($p = 0.05$) where the alternate hypothesis was that the mean for the growth parameter was reduced or enhanced in comparison to the control. Prior to the Dunnett's test, a Shapiro-Wilk's test and a Levene's test were conducted to test for normality and homogeneity of variance, respectively, over treatments at each time point. If the results from the Shapiro-Wilk's and Levene's tests indicated normality and insignificant heterogeneity (*i.e.*, $p > 0.01$), the analysis was performed on the non-transformed raw data. In instances of non-normality or heterogeneity (*i.e.*, $p < 0.01$), a square root transformation was performed. If both the non-transformed raw data and the transformed data exhibited non-normality or inequality of variance, a non-parametric analysis of variance was performed on the ranks of the raw data values. Non-parametric analyses were performed on all specific growth rate data, adjacent growth rate data from 0-24 hours, and on cell density and yield data at the 24-, 48-, and 72-hour time points. Parametric analyses were performed on adjacent growth rate data from the 24-48 and 48-72 hour time points.

II. RESULTS AND DISCUSSION

A. FINDINGS

The mean measured concentrations of IN-MK643 were 1.23, 3.40, 10.4, 32.0, and 102 mg/L, ranging from 92 to 103% of the nominal, indicating accuracy of the test concentration solutions. The mean measured concentration of the 100 mg IN-MK643/L abiotic control was 102 mg IN-MK643/L representing 102% of nominal. The untreated control solution contained no detectable concentrations of IN-MK643 on both Day 0 and Day 3. IN-MK643 was determined to be stable over the course of the test. All validation criteria were met for the study.

A summary of algal growth inhibition following exposure of *Pseudokirchneriella subcapitata* to IN-MK643 for 96 hours is presented in the Table 148.

Table 148
Summary of algal growth inhibition following exposure of *Pseudokirchneriella subcapitata* to IN-MK643 for 72 hours

Nominal IN-MK643 concentration (mg/L)	Mean measured IN-MK643 concentration (mg/L)	% Inhibition relative to the control ^a		
		Biomass	Growth Rate	Yield
Untreated control (0.0)	Untreated control (0.0)	—	—	—
1.2	1.23	0	0	0
3.7	3.40	-3	0	-3
11	10.4	27*	6*	27*
33	32.0	50*	14*	51*
100	102	98*	83*	99*

^a Positive values indicate inhibition

* Significantly different from the control (Dunnett's, $\alpha = 0.05$)

III. CONCLUSION

Growth inhibition values based on mean measured IN-MK643 concentrations obtained with IN-MK643 on *Pseudokirchneriella subcapitata* were as follows:

Biomass: 72-hr E_bC_{50} = 31.8 mg IN-MK643/L
72-hr NOEC = 3.40 mg IN-MK643/L

Growth Rate: 72-hr E_rC_{50} = 59.7 mg IN-MK643/L
72-hr NOEC = 3.40 mg IN-MK643/L

Yield: 72-hr E_bC_{50} = 31.8 mg IN-MK643/L
72-hr NOEC = 3.40 mg IN-MK643/L

(Bergfield, A., 2013)

RMS comment

This study was conducted in compliance with the current guideline.

The number of algal cells in the control after 72 hours was greater than 16 times the initial cell density (135). The coefficient of variation for daily growth rates in control after 72 hours was less than 35% (8.29%). The coefficients of variation of average specific growth rates in the control after 72 hours were less than 7% (1.0 %). The validity criteria of the guideline are therefore fulfilled.

Remark concerning the calculation of the coefficient of variation of average specific growth rates: the coefficients of variation for daily growth rates were calculated for each individual control replicate first (CV calculated for average specific growth rates at the different times) and averaged among replicates only after.

This study is acceptable.

The following endpoints are considered relevant for the risk assessment:

Biomass:	72-hr E_bC_{50} = 31.8 mg IN-MK643/L 72-hr NOEC = 3.40 mg IN-MK643/L
Growth Rate:	72-hr E_rC_{50} = 59.7 mg IN-MK643/L 72-hr NOEC = 3.40 mg IN-MK643/L
Yield:	72-hr E_bC_{50} = 31.8 mg IN-MK643/L 72-hr NOEC = 3.40 mg IN-MK643/L

Report: Sloman, T.L. (2003a); IN-MP819: Influence on growth and growth rate of the green alga *Selenastrum capricornutum*

DuPont Report No.: DuPont-11493

Guidelines: OECD 201, EEC Method C3 **Deviations:** None

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

Testing Facility Report No.: DuPont-11493

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.

I. MATERIALS AND METHODS

A. MATERIALS

- | | |
|--|--|
| 1. Test material: | IN-MP819 technical metabolite |
| Lot/Batch #: | MP819-002 |
| Purity: | 98.6% |
| Description: | Light yellow solid |
| CAS#: | Not available |
| Stability of test compound: | Shown not to be stable in the test system by analysis |
| 2. Control: | AAP nutrient medium |
| Test vehicle: | AAP nutrient medium |
| Toxic reference: | None |
| 3. Test organism: | Green alga |
| Species: | <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 |
| Test chamber: | 250-mL Erlenmeyer flask containing 50 mL of test solution and fitted with a sterilised foam stopper |
| Growth medium: | AAP nutrient medium |
| pH | 7.40 to 7.42 at test initiation and 7.23 to 7.55 at test termination |
| 4. Environmental conditions (in-life period) | |
| Temperature: | 24.7oC (Environmental growth chamber and surrogate vessel) |
| Photoperiod: | 24 hour photoperiod (6220 to 7900 lux) |

B. STUDY DESIGN AND METHODS

1. Study initiated/completed
14-March-2003 to 26-June-2003

2. Experimental treatments

The effect of IN-MP819 on *Selenastrum capricornutum* was determined using algal cultures with AAP nutrient medium, incubated in an incubator at $24 \pm 2^\circ\text{C}$ for 72 hours. Because of the insensitivity of *Selenastrum capricornutum* to IN-MP819, three replicates at one concentration, 634 µg/L, measured at test initiation, were tested. Cell counts were taken at 24-hour intervals.

II. RESULTS AND DISCUSSION

A. FINDINGS

The effects of IN-MP819 on the growth and growth rate of *Selenastrum capricornutum* are shown in Table 149.

Table 149
Summary of algal growth inhibition following exposure of *Selenastrum capricornutum* to IN-MP819 for 72 hours

Concentration µg IN-MP819/L	Mean cell density (cells/mL)	% Inhibition relative to control		
		Cell density	Area under the growth curve	Growth rate
Blank control	1.8×10^6	—	—	—
634	1.7×10^6	5.77	9.71	1.20

III. CONCLUSIONS

Growth inhibition values obtained with IN-MP819 on *Selenastrum capricornutum* were as follows:

Cell density:	72-hour EC_{50} : >634 µg/L 72-hour NOEC: 634 µg/L
Area under the growth curve:	72-hour EC_{50} : >634 µg/L 72-hour NOEC: 634 µg/L
Growth rate:	72-hour EC_{50} : >634 µg/L 72-hour NOEC: 634 µg/L

(Sloman, T.L., 2003a)

RMS comment

This study was submitted in the original DAR (AD3, 2005). It was conducted, under guideline OECD 201, and EEC Method C3. Deviations include only three replicates instead of six for a limit test. However, reconducting the study is unlikely to yield a significantly different result because there were no significant effects on any parameter measured in the study. Therefore, this study is relied upon. The cell concentration in the control cultures increased by a factor of at least 16 within three days. The study is therefore valid according to validity criteria of the older version of OECD 201 (1984). RMS notes that the deviation from the nominal of measured concentration at 72 h is not within the range of $\pm 20\%$, therefore analysis of the results should be based on geometric mean concentration during exposure. Based on measured concentrations at day 0 and day 3, the geometric mean is 358 µg/L. This study is still considered acceptable.

Report: Sloman, T.L. (2003b); IN-MS775: Influence on growth and growth rate of the green alga *Selenastrum capricornutum*

DuPont Report No.: DuPont-12092

Guidelines: OECD 201, EEC Method C3 **Deviations:** None

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

Testing Facility Report No.: DuPont-12092

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.

I. MATERIALS AND METHODS

A. MATERIALS

- | | |
|--|--|
| 1. Test material: | IN-MS775 technical metabolite |
| Lot/Batch #: | MS775-002 |
| Purity: | 99.7%, by analysis |
| Description: | Light yellow solid |
| CAS#: | Not available |
| Stability of test compound: | Shown not to be stable in the test system by analysis |
| 2. Control: | AAP nutrient medium |
| Test vehicle: | AAP nutrient medium |
| Toxic reference: | None |
| 3. Test organism: | Green alga |
| Species: | <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 |
| Test chamber: | 250-mL Erlenmeyer flask containing 50 mL of test solution and fitted with a sterilised foam stopper |
| Growth medium: | AAP nutrient medium |
| pH | 7.42 to 7.72 at test initiation and 7.95 to 8.88 at test termination |
| 4. Environmental conditions (in-life period) | |
| Temperature: | 24.7 to 24.8°C (Environmental growth chamber and surrogate vessel) |
| Photoperiod: | 24 hour photoperiod (6810 to 8660 lux) |

B. STUDY DESIGN AND METHODS

1. Study initiated/completed
30-April-2003 to 07-August-2003
2. Experimental treatments
The effect of IN-MS775 on *Selenastrum capricornutum* was determined using algal cultures with AAP nutrient medium, incubated in an incubator at $24 \pm 2^\circ\text{C}$ for 72 hours. Because of the insensitivity of

Selenastrum capricornutum to IN-MS775 as determined in a pretest, three replicates at one concentration, 100 µg IN-MS775/L of nutrient medium, measured at test initiation, were tested. Cell counts were taken at 24-hour intervals.

II. RESULTS AND DISCUSSION

A. FINDINGS

The effects of IN-MS775 on the growth and growth rate of *Selenastrum capricornutum* are shown in Table 150.

Table 150
Summary of algal growth inhibition following exposure of *Selenastrum capricornutum* to IN-MS775 for 72 hours

µg/L	Mean cell density (cells/mL)	% Inhibition relative to control		
		Cell density	Area under the growth curve	Growth rate
Blank control	4.9×10^6	—	—	—
Solvent control	5.0×10^6	-2.10	-6.60	-0.35
100	5.1×10^6	-2.1	0.63	-0.37

III. CONCLUSIONS

Growth inhibition values obtained with IN-MS775 on *Selenastrum capricornutum* were as follows:

Cell density:	72-hour EC ₅₀ >100 µg/L 72-hour NOEC >100 µg/L
Area under the growth curve:	72-hour EC ₅₀ >100 µg/L 72-hour NOEC >100 µg/L
Growth rate:	72-hour EC ₅₀ >100 µg/L 72-hour NOEC >100 µg/L

(Sloman, T.L., 2003b)

RMS comment

This study was submitted in the original DAR (AD3, 2005). It was conducted, under guidelines OECD 201, and EEC Method C3. Deviations include only three replicates instead of six for a limit test. However, reconducting the study is unlikely to yield a significantly different result because there were no significant effects on any parameter measured in the study. Therefore, this study is relied upon. The cell concentration in the control cultures increased by a factor of at least 16 within three days. The study is therefore valid according to validity criteria of the older version of OECD 201 (1984). RMS notes that the deviation from the nominal of measured concentration at 72 h is not within the range of ± 20 %, therefore analysis of the results should be based on geometric mean concentration during exposure. Based on measured concentrations at day 0 and day 3, the geometric mean is 52 µg/L. This study is still considered acceptable.

Report: Goudie, O.J. (2015b); IN-U8E24: Growth inhibition test with the unicellular green alga, *Pseudokirchneriella subcapitata*

DuPont Report No.: DuPont-43484

Guidelines: OECD 201 (2006, corrected 2011), OCSPP 850.4500 (2012) **Deviations:** None

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

Testing Facility Report No.: 82063

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:

The acute toxicity of IN-U8E24 to the unicellular green alga, *Pseudokirchneriella subcapitata*, was determined in a 96-hour growth inhibition test. The test was conducted in accordance with the U.S. Environmental Protection Agency, Ecological Effects Test Guidelines, OCSPP 850.4500 and OECD Guideline No. 201. Treatments consisted of an untreated control, an abiotic (stability) control, and five nominal concentrations of 7.5, 15, 30, 60, and 120 mg IN-U8E24/L. The corresponding 72-hour geometric mean measured concentrations of IN-U8E24 were 6.10, 12.9, 31.8, 52.8, and 103 mg IN-U8E24/L. The corresponding 96-hour geometric mean measured concentrations of IN-U8E24 were 5.38, 11.7, 27.3, 47.7, and 92.8 mg IN-U8E24/L. The 96-hour measured concentration of IN-U8E24 in the nominal 7.5 mg/L abiotic control was 4.60 mg IN-U8E24/L. The 72- and 96-hour EC₅₀ and NOEC for *Pseudokirchneriella subcapitata* were based on 72- and 96-hour geometric mean, measured concentrations, respectively, of IN-U8E24 and area under the growth curve, growth rate, and yield. The 72-hour E_bC₅₀, E_rC₅₀, and E_yC₅₀ values based on area, growth rate, and yield were 31.3, 55.2, and 32.6 mg IN-U8E24/L, respectively. The 72-hour NOEC value based on area, growth rate, and yield was 6.10, 6.10, and 6.10 mg IN-U8E24/L (72-hour geometric mean, measured concentrations), respectively. The 96-hour E_bC₅₀, E_rC₅₀, and E_yC₅₀ values based on area, growth rate, and yield were 23.3, 58.3, and 21.3 mg IN-U8E24/L, respectively. The 96-hour NOEC value based on area, growth rate, and yield was 5.38, 11.7, and 5.38 mg IN-U8E24/L (96-hour geometric mean measured concentrations), respectively.

I. MATERIALS AND METHODS

A. MATERIALS

- | | |
|--|--|
| 1. Test material: | IN-U8E24 technical metabolite |
| Batch/Lot#: | U8E24-000 |
| Purity: | 90.7% |
| Description: | Solid |
| CAS#: | Not available |
| Stability of test compound: | Determined to be unstable in the test system |
| 2. Control: | FWAM nutrient medium |
| Test vehicle: | None |
| Toxic reference: | None |
| 3. Test organism: | Unicellular green alga |
| Species: | <i>Pseudokirchneriella subcapitata</i> |
| Initial population: | 10000 cells/mL |
| Source: | ABC Laboratories, Inc., Columbia, Missouri In-house culture, parent culture from University of Texas |
| Test chamber: | 250-mL Erlenmeyer flask with a foam stopper, containing 100 mL of test solution. |
| Growth medium: | FWAM nutrient medium |
| pH | 7.4 at test initiation and 7.5 to 7.9 at test termination |
| 4. Environmental conditions (in-life period) | |
| Temperature: | 23.2 to 25.0°C |
| Photoperiod: | 24-hour light photoperiod (4197 to 4247 lux) |

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed

06 July 2015 to 10 July 2015

2. Experimental treatments

The effect of IN-U8E24 to the green alga *Pseudokirchneriella subcapitata* was determined in a static, acute 96-hour test. The algae were exposed to an untreated control and five nominal concentrations of 7.5, 15, 30, 60, and 120 mg IN-U8E24/L (72-hour geometric mean measured concentrations of 6.10, 12.9, 31.8, 52.8, and 103 mg IN-U8E24/L, and 96-hour geometric mean measured concentrations of 5.38, 11.7, 27.3, 47.7, and 92.8 mg IN-U8E24/L) in FWAM nutrient medium for 96 hours, without test medium renewal. An abiotic (stability) control was included in the test to determine the stability of IN-U8E24 in FWAM nutrient medium under the same environmental conditions without the algae. The untreated control and each test concentration were tested as four replicates. The abiotic control was tested as a single test unit. The initial cell density was 10000 cells/mL. Test units were incubated in an environmental chamber for 96 hours.

3. Observations

Test concentrations for IN-U8E24 were measured on days 0, 3 (72 hours) and 4 (96 hours) to verify target test concentrations and stability of the test item.

Biomass, based on cell count, was determined approximately 24, 48, 72, and 96 hours after test initiation. Yield was determined by subtracting the initial cell count from the test end cell count.

Area under the growth curve and growth rate were determined for each day of the exposure and were based on cell count.

Area, yield, and growth rate, all based on cell count, were recorded and expressed as percent inhibition relative to the untreated control following exposure to IN-U8E24 for 96 hours.

4. Statistics

All statistical analyses were performed with SAS software and Ecotats.

The LOEC and NOEC values, based on area under the growth curve, growth rate, and yield, were estimated using a one-way analysis of variance (ANOVA) procedure and a one-tailed Dunnett's test and/or Jonckheere Trend test ($p = 0.05$) where the alternate hypothesis was that the mean for the growth parameter was reduced in comparison to the control. Prior to the Dunnett's test and Jonckheere Trend test, a Shapiro-Wilk's test and a Levene's test were conducted to test for normality and homogeneity of variance, respectively, over treatments at each time point. If the results from the Shapiro-Wilk's and Levene's tests indicated normality and insignificant heterogeneity (i.e., $p > 0.01$), the analysis was performed on the non-transformed raw data. In instances of non-normality or heterogeneity (i.e., $p < 0.01$), a square root transformation was performed. If both the non-transformed raw data and the transformed data exhibited non-normality or inequality of variance, a non-parametric analysis of variance was performed on the ranks of the raw data values. Parametric analyses were performed on the 24, 48, and 96 hour cell density data, 24, 48, and 96 hour area under the curve data, 24 hour growth rate data, and 24, 48, and 96 hour yield data. Non-parametric analyses were performed on the 72 hour cell density data, 72 hour area under the growth curve data, 48, 72, and 96 hour growth rate data, and 72 hour yield data.

II. RESULTS AND DISCUSSION

A. FINDINGS

The 72-hour geometric mean measured concentrations of IN-U8E24 were 6.10, 12.9, 31.8, 52.8, and 103 mg IN-U8E24/L, ranging from 81 to 106% of the nominal. The 96-hour mean measured concentrations of IN-U8E24 were 5.38, 11.7, 27.3, 47.7, and 92.8 mg IN-U8E24/L, ranging from 72 to 91% of the nominal. The 96-hour measured

concentration of the 7.5 mg IN-U8E24/L abiotic control was 4.60 mg IN-U8E24/L representing 61% of nominal. The untreated control solution contained no detectable concentrations of IN-U8E24 on days 0, 3, and 4. IN-U8E24 was determined to be unstable over the course of the test without the presence of algae. All validation criteria were met for the study.

A summary of algal growth inhibition following exposure of *Pseudokirchneriella subcapitata* to IN-U8E24 for 72 and 96 hours is presented in the tables that follow.

Table 151
Summary of algal growth inhibition following exposure of *Pseudokirchneriella subcapitata* to IN-U8E24 for 72 hours

72-Hour Geometric Mean, measured concentration (mg IN-U8E24/L)	% Inhibition relative to the blank control ^a		
	Area	Growth Rate	Yield
Blank control (0.0)	—	—	—
6.10	0	0	1
12.9	24 *	6 *	22 *
31.8	45 *	15	45 *
52.8	78 *	48	86 *
103	94 *	84	98 *

^a positive values indicate inhibition

* significantly different from the blank control (Jonckheere-Terpstra, alpha = 0.05)

Table 152
Summary of algal growth inhibition following exposure of *Pseudokirchneriella subcapitata* to IN-U8E24 for 96 hours

96-Hour Geometric Mean, measured concentration (mg IN-U8E24/L)	% Inhibition relative to the blank control ^a		
	Area	Growth Rate	Yield
Blank control (0.0)	—	—	—
5.38	-1	0	-1
11.7	22 *	5	22 *
27.3	55 *	18 *	62 *
47.7	85 *	40 *	89 *
92.8	97 *	74 *	99 *

^a positive values indicate inhibition

* significantly different from the blank control (Jonckheere-Terpstra, alpha = 0.05)

III. CONCLUSION

Growth inhibition values based on 72-hour or 96-hour geometric mean measured IN-U8E24 concentrations on *Pseudokirchneriella subcapitata* were as follows:

Area:	72-hr EbC ₅₀ = 31.3 mg IN-U8E24/L 72-hr NOEC = 6.10 mg IN-U8E24/L 96-hr EbC ₅₀ = 23.3 mg IN-U8E24/L 96-hr NOEC = 5.38 mg IN-U8E24/L
Growth Rate:	72-hr ErC ₅₀ = 55.2 mg IN-U8E24/L 72-hr NOEC = 6.10 mg IN-U8E24/L 96-hr ErC ₅₀ = 58.3 mg IN-U8E24/L 96-hr NOEC = 11.7 mg IN-U8E24/L
Yield:	72-hr EyC ₅₀ = 32.6 mg IN-U8E24/L 72-hr NOEC = 6.10 mg IN-U8E24/L 96-hr EyC ₅₀ = 21.3 mg IN-U8E24/L 96-hr NOEC = 5.38 mg IN-U8E24/L

Recovery was assessed for the 27.3, 47.7, and 92.8 mg IN-U8E24/L treatments utilizing the same growth chamber as the definitive test. Based on 371-, 840-, and 263-fold increases in healthy cell count in five days, the effects upon growth of *P. subcapitata* were found to be algistatic at the 96-hr geometric mean measured IN-U8E24 concentrations of 27.3, 47.7, and 92.8 mg IN-U8E24/L, respectively.

(Goudie, O.J., 2015b)

RMS comment

This study was conducted in compliance with the current guideline.

Four replicates were available for control. The number of algal cells in the control after 72 hours was greater than 16 times the initial cell density (52.5). The coefficient of variation for daily growth rates in control after 72 hours was less than 35% (10.55%). The coefficients of variation of average specific growth rates in the control after 72 and 96 hours were less than 7% (1.52 and 1.58% respectively). The validity criteria of the guideline are therefore fulfilled.

Remark concerning the calculation of the coefficient of variation of average specific growth rates: the coefficients of variation for daily growth rates were calculated for each individual control replicate first (CV calculated for average specific growth rates at the different times) and averaged among replicates only after.

This study is acceptable.

The following endpoints are considered relevant for the risk assessment:

Area:	72-hr EbC ₅₀ = 31.3 mg IN-U8E24/L 72-hr NOEC = 6.10 mg IN-U8E24/L 96-hr EbC ₅₀ = 23.3 mg IN-U8E24/L 96-hr NOEC = 5.38 mg IN-U8E24/L
Growth Rate:	72-hr ErC ₅₀ = 55.2 mg IN-U8E24/L 72-hr NOEC = 6.10 mg IN-U8E24/L 96-hr ErC ₅₀ = 58.3 mg IN-U8E24/L 96-hr NOEC = 11.7 mg IN-U8E24/L
Yield:	72-hr EyC ₅₀ = 32.6 mg IN-U8E24/L 72-hr NOEC = 6.10 mg IN-U8E24/L 96-hr EyC ₅₀ = 21.3 mg IN-U8E24/L 96-hr NOEC = 5.38 mg IN-U8E24/L

Report: Goudie, O.J. (2015a); IN-UYG24: Growth inhibition test with the unicellular green alga, *Pseudokirchneriella subcapitata*

DuPont Report No.: DuPont-43421

Guidelines: OECD 201 (2006, corrected 2011), OCSPP 850.4500 (2012) **Deviations:** None

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

Testing Facility Report No.: 82066

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:

The acute toxicity of IN-UYG24 to the unicellular green alga, *Pseudokirchneriella subcapitata*, was determined in a 96-hour growth inhibition test. The test was conducted in accordance with the U.S. Environmental Protection Agency, Ecological Effects Test Guidelines, OCSPP 850.4500 and OECD Guideline No. 201. Treatments consisted of an untreated control, an abiotic (stability) control, and five nominal concentrations of 7.5, 15, 30, 60, and 120 mg IN-UYG24/L. The corresponding 72-hour geometric mean measured concentrations of IN-UYG24 were 6.64, 13.2, 27.4, 53.0, and 106 mg IN-UYG24/L. The corresponding 96-hour geometric mean measured concentrations of IN-UYG24 were 6.26, 12.5, 26.5, 51.5, and 104 mg IN-UYG24/L. The 96-hour measured concentration of IN-UYG24 in the nominal 120 mg IN-UYG24/L abiotic control was 95.2 mg IN-UYG24/L. The 72- and 96-hour EC₅₀ and NOEC for *Pseudokirchneriella subcapitata* were based on 72- and 96-hour geometric mean, measured concentrations, respectively, of IN-UYG24 and area under the growth curve, growth rate, and yield. The 72-hour EbC₅₀, ErC₅₀, and EyC₅₀ values based on area, growth rate, and yield were 74.5, >106, and 73.0 mg IN-UYG24/L, respectively. The 72-hour NOEC value based on area, growth rate, and yield was 6.64, 27.4, and 6.64 mg IN-UYG24/L (72-hour geometric mean, measured concentrations), respectively. The 96-hour EbC₅₀, ErC₅₀, and EyC₅₀ values based on area, growth rate, and yield were 77.2, >104, and 78.7 mg IN-UYG24/L, respectively. The 96-hour NOEC value based on area, growth rate, and yield was 12.5, 51.5, and 26.5 mg IN-UYG24/L (96-hour geometric mean measured concentrations), respectively.

I. MATERIALS AND METHODS

A. MATERIALS

- | | |
|--|--|
| 1. Test material: | IN-UYG24 technical metabolite |
| Batch/Lot#: | UYG24-001 |
| Purity: | 96.1% |
| Description: | Solid |
| CAS#: | Not available |
| Stability of test compound: | Determined to be stable in the test system |
| 2. Control: | FWAM nutrient medium |
| Test vehicle: | None |
| Toxic reference: | None |
| 3. Test organism: | Unicellular green alga |
| Species: | <i>Pseudokirchneriella subcapitata</i> |
| Initial population: | 10000 cells/mL |
| Source: | ABC Laboratories, Inc., Columbia, Missouri In-house culture, parent culture from University of Texas |
| Test chamber: | 250-mL Erlenmeyer flask with a foam stopper, containing 100 mL of test solution. |
| Growth medium: | FWAM nutrient medium |
| pH | 7.5 at test initiation and 7.6 to 7.8 at test termination |
| 4. Environmental conditions (in-life period) | |
| Temperature: | 22.8 to 24.8°C |
| Photoperiod: | 24-hour light photoperiod (4572 to 4762 lux) |

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
27 July 2015 to 03 August 2015 (completion of recovery test)
2. Experimental treatments
The effect of IN-UYG24 to the green alga *Pseudokirchneriella subcapitata* was determined in a static, acute 96-hour test. The algae were exposed to an untreated control and five nominal concentrations of 7.5, 15, 30, 60, and 120 mg IN-UYG24/L (72-hour geometric mean measured concentrations of 6.64, 13.2, 27.4, 53.0, and 106 mg IN-UYG24/L, and 96-hour geometric mean measured concentrations of 6.26, 12.5, 26.5, 51.5, and 104 mg IN-UYG24/L) in FWAM nutrient medium for 96 hours, without test medium renewal. An abiotic (stability) control was included in the test to determine the stability of IN-UYG24 in FWAM nutrient medium under the same environmental conditions without the algae. The untreated control and each test concentration were tested as four replicates. The abiotic control was tested as a single test unit. The initial cell density was 10000 cells/mL. Test units were incubated in an environmental chamber for 96 hours.
3. Observations
Test concentrations for IN-UYG24 were measured on Days 0, 3 (72 hours) and 4 (96 hours) to verify target test concentrations and stability of the test item.

Biomass, based on cell count, was determined approximately 24, 48, 72, and 96 hours after test initiation. Yield was determined by subtracting the initial cell count from the test end cell count.

Area under the growth curve and growth rate were determined for each day of the exposure and were based on cell count.

Area, yield, and growth rate, all based on cell count, were recorded and expressed as percent inhibition relative to the untreated control following exposure to IN-UYG24 for 96 hours.

4. Statistics

All statistical analyses were performed with SAS software and Ecostats.

The LOEC and NOEC values, based on area under the growth curve, growth rate, and yield, were estimated using a one-way analysis of variance (ANOVA) procedure and a one-tailed Dunnett's test and/or Jonckheere Trend test ($p = 0.05$) where the alternate hypothesis was that the mean for the growth parameter was reduced in comparison to the control. Prior to the Dunnett's test and Jonckheere Trend test, a Shapiro-Wilk's test and a Levene's test were conducted to test for normality and homogeneity of variance, respectively, over treatments at each time point. If the results from the Shapiro-Wilk's and Levene's tests indicated normality and insignificant heterogeneity (i.e., $p > 0.01$), the analysis was performed on the non-transformed raw data. In instances of non-normality or heterogeneity (i.e., $p < 0.01$), a square root transformation was performed. If both the non-transformed raw data and the transformed data exhibited non-normality or inequality of variance, a non-parametric analysis of variance was performed on the ranks of the raw data values. Parametric analyses were performed on all cell density and growth rate data, and on the 48, 72, and 96 hour area under the growth curve and yield data. Non-parametric analyses were performed for the 24 hour area under the growth curve and yield data.

II. RESULTS AND DISCUSSION

A. FINDINGS

The 72-hour geometric mean measured concentrations of IN-UYG24 were 6.64, 13.2, 27.4, 53.0, and 106 mg IN-UYG24/L, ranging from 88 to 91% of the nominal. The 96-hour mean measured concentrations of IN-UYG24 were 6.26, 12.5, 26.5, 51.5, and 104 mg IN-UYG24/L, ranging from 83 to 88% of the nominal. The 96-hour measured concentration of the 120 mg IN-UYG24/L abiotic control was 95.2 mg IN-UYG24/L representing 79% of nominal. The untreated control solution contained no detectable concentrations of IN-UYG24 on days 0, 3, and 4. IN-UYG24 was determined to be stable over the course of the test without the presence of algae. All validation criteria were met for the study.

A summary of algal growth inhibition following exposure of *Pseudokirchneriella subcapitata* to IN-UYG24 for 72 and 96 hours is presented in the tables that follow.

Table 153
Summary of algal growth inhibition following exposure of *Pseudokirchneriella subcapitata* to IN-UYG24 for 72 hours

72-Hour Geometric Mean, measured concentration (mg IN-UYG24/L)	% Inhibition relative to the blank control ^a		
	Area	Growth Rate	Yield
Blank control (0.0)	—	—	—
6.64	1	0	-1
13.2	13 *	4 ^b	17 *
27.4	22 *	6 ^b	23 *
53.0	43 *	13 *	42 *
106	57 *	20 *	58 *

^a positive values indicate inhibition

^b significantly different from the blank control (Jonckheere-Terpstra, $\alpha = 0.05$), but not considered biologically significant

* significantly different from the blank control (Jonckheere-Terpstra, $\alpha = 0.05$)

Table 154
Summary of algal growth inhibition following exposure of *Pseudokirchneriella subcapitata* to IN-UYG24 for 96 hours

96-Hour Geometric Mean, measured concentration (mg IN-UYG24/L)	% Inhibition relative to the blank control ^a		
	Area	Growth Rate	Yield
Blank control (0.0)	—	—	—
6.26	0	0	-1
12.5	9 ^b	1 ^b	5 ^b
26.5	13 *	1 ^b	6 ^b
51.5	40 *	9 ^b	38 *
104	59 *	16 *	59 *

^a positive values indicate inhibition

^b significantly different from the blank control (Jonckheere-Terpstra, alpha = 0.05), but not considered biologically significant.

* significantly different from the blank control (Jonckheere-Terpstra, alpha = 0.05)

III. CONCLUSION

Growth inhibition values based on 72-hour or 96-hour geometric mean measured IN-UYG24 concentrations on *Pseudokirchneriella subcapitata* were as follows:

Area:	72-hr EbC ₅₀ = 74.5 mg IN-UYG24/L 72-hr NOEC = 6.64 mg IN-UYG24/L 96-hr EbC ₅₀ = 77.2 mg IN-UYG24/L 96-hr NOEC = 12.5 mg IN-UYG24/L
Growth Rate:	72-hr ErC ₅₀ = >106 mg IN-UYG24/L 72-hr NOEC = 27.4 mg IN-UYG24/L 96-hr ErC ₅₀ = >104 mg IN-UYG24/L 96-hr NOEC = 51.5 mg IN-UYG24/L
Yield:	72-hr EyC ₅₀ = 73.0 mg IN-UYG24/L 72-hr NOEC = 6.64 mg IN-UYG24/L 96-hr EyC ₅₀ = 78.7 mg IN-UYG24/L 96-hr NOEC = 26.5 mg IN-UYG24/L

Recovery was assessed for the 104 mg IN-UYG24/L 96-hour geometric mean measured treatment utilizing the same growth chamber as the definitive test. Based on 78-fold increase in healthy cell count in three days, the effect upon growth of *P. subcapitata* were found to be algistatic at the 96-hr geometric mean measured IN-UYG24 concentration of 104 mg IN-UYG24/L, respectively.

(Goudie, O.J., 2015a)

RMS comment

This study was conducted in compliance with the current guideline.

Four replicates were available for control. The number of algal cells in the control after 72 hours was greater than 16 times the initial cell density (67.6). The coefficient of variation for daily growth rates in control after 72 hours was less than 35% (7.11%). The coefficients of variation of average specific growth rates in the control after 72

and 96 hours were less than 7% (2.85 and 3.45% respectively). The validity criteria of the guideline are therefore fulfilled.

Remark concerning the calculation of the coefficient of variation of average specific growth rates: the coefficients of variation for daily growth rates were calculated for each individual control replicate first (CV calculated for average specific growth rates at the different times) and averaged among replicates only after.

This study is acceptable.

The following endpoints are considered relevant for the risk assessment:

Area:	72-hr EbC ₅₀ = 74.5 mg IN-UYG24/L
	72-hr NOEC = 6.64 mg IN-UYG24/L
	96-hr EbC ₅₀ = 77.2 mg IN-UYG24/L
	96-hr NOEC = 12.5 mg IN-UYG24/L
Growth Rate:	72-hr ErC ₅₀ = >106 mg IN-UYG24/L
	72-hr NOEC = 27.4 mg IN-UYG24/L
	96-hr ErC ₅₀ = >104 mg IN-UYG24/L
	96-hr NOEC = 51.5 mg IN-UYG24/L
Yield:	72-hr EyC ₅₀ = 73.0 mg IN-UYG24/L
	72-hr NOEC = 6.64 mg IN-UYG24/L
	96-hr EyC ₅₀ = 78.7 mg IN-UYG24/L
	96-hr NOEC = 26.5 mg IN-UYG24/L

B.9.2.7. Effects on aquatic macrophytes

Report: Sloman, T.L., Leva, S.E. (1997); DPX-MP062 (consisting of 75% DPX-KN128 and 25% DPX-KN127): Influence on growth and reproduction of *Lemna gibba* G3

DuPont Report No.: AMR 3602-95, Revision No. 1

Guidelines: USEPA 122-2 **Deviations:** None

Testing Facility: DuPont Stine-Haskell Research Center, Newark, Delaware, USA

Testing Facility Report No.: AMR 3602-95, Revision No. 1

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 DPX-MP062 to the floating fresh water vascular plant *Lemna gibba* G3 was determined in a static 14-day test. The test was conducted in accordance with Tier 1 Non-Target Aquatic Plants Studies, Pesticide Assessment Guidelines, and Subdivision J, 122-2. Treatments consisted of an untreated control, an abiotic (stability) control, a solvent control (0.1 mL acetone) and one nominal concentration of 100 µg DPX-MP062/L in a 20X AAP nutrient medium. The corresponding mean measured concentration of the test concentration was 84.3 µg DPX-MP062/L. DPX-MP062 had no significant inhibitory effect on the growth and reproduction of *Lemna gibba* G3. The 14-day EC₅₀ and NOEC values were based on mean measured concentrations of DPX-MP062 and frond count and biomass. The 14-day EC₅₀ based on frond count was >84.3 µg DPX-MP062/L and the NOEC was 84.3 µg DPX-MP062/L. The 14-day EC₅₀ based on biomass was also >84.3 µg DPX-MP062/L and the NOEC was 84.3 µg DPX-MP062/L.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: DPX-MP062 technical
Lot/Batch #: MP062-51
Purity: 94.51%
Description: Off-white solid
CAS#: 144171-61-9
Stability of test compound: Not stable in test system
2. Control: 20X AAP nutrient medium
Solvent Control: 0.1 mL HPLC grade acetone/L 20X AAP nutrient medium
Test vehicle: 20X AAP nutrient medium
Toxic reference: None
3. Test organism: Duckweed
Species: *Lemna gibba* G3
Initial population: 5 plants with 3 fronds each
Source: Dr. Janet P. Sloven, Ph.D. - USDA/ARS - Horticulture Crop Quality Lab, Beltsville, Maryland, USA
Test chamber: 350 ml Pyrex glass jars with loose fitting glass lids
Growth media: 20X AAP nutrient medium
pH: 7.06 to 7.72 at test initiation and 8.60 to 9.22 at test termination
4. Environmental conditions (in-life period)
Temperature: 23.9-25.0°C (Environmental growth chamber)
Photoperiod: 24 hours, continuous light, 4630 to 4720 lux

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
12-June-1996 to 28-June 1996
2. Experimental treatments
The effect of DPX-MP062 to the floating fresh water vascular plant *Lemna gibba* G3 was determined in a static, 14-day test. The plants were exposed to an untreated control, a solvent control (0.1 mL HPLC grade acetone) and one nominal concentration of 100 µg DPX-MP062/L in a 20X AAP medium for 14 days. An abiotic control (100 µg DPX-MP062/L, nominal) was included in the test to determine the stability of DPX-MP062 in a 20X AAP nutrient medium under the same environmental conditions without the plants. Each test concentration and the untreated control were tested as 3 replicates. Five plants with three fronds each were used per replicate. Test units were incubated in an environmental chamber for 14 days.
3. Observations
Test solutions were measured on Day 0 and Day 14 to verify stability of the test item. Frond counts were made on days 0, 2, 5, 9, 12, and 14. Biomass was determined at the completion of the 14-day test. Frond count and biomass were expressed as percent inhibition relative to the untreated control following exposure to DPX-MP062 for 14 days.
4. Statistics
The F-test statistic was used to determine if the solvent control and blank control could be pooled for use in the Welch's t-test. The F-test statistic showed that the solvent control and blank control variances for each of the frond number and biomass were not significantly different and, therefore, were pooled to use in the Welch's t-test. Welch's t-test was used to determine significance of percent inhibition of frond count and biomass.

II. RESULTS AND DISCUSSION

A. FINDINGS

The mean measured concentration of DPX-MP062 in the Day 0 nominal 100 µg/L test solution was 84.3 µg/L, or approximately 84% of the expected value. DPX-MP062 was determined not to be stable over the course of the definitive test, as evidenced by the analytical recoveries obtained from the Day 0 and the Day 14 test and abiotic control test solutions. After 14 days, DPX-MP062 was not detected in the abiotic control and nominal 100 µg/L test solutions.

Data on frond count and biomass following exposure of *Lemna gibba* G3 to DPX-MP062 for 14 days are summarised in Table 155.

Table 155
Effect of DPX-MP062 on the growth and reproduction of *Lemna gibba*

Mean measured concentration (µg/L)	14-Day mean frond number	% Inhibition relative to control ^a	14-Day mean biomass (mg)	% Inhibition relative to control ^a
0 (control) ^b	732	—	103.2	—
84.3	725	1.0	118.2	-14.5%

^a Not different from control by the Welch's t-test, $p \geq 0.05$.

^b Data from pooled blank and solvent controls.

III. CONCLUSIONS

DPX-MP062 had little or no inhibitory effect on the growth and reproduction of *Lemna gibba* G3 exposed to a mean, measured concentration of 84.3 µg DPX-MP062/L, the maximum solubility level in algal growth medium. Growth inhibition endpoints with DPX MP062 on *Lemna gibba* were as follows:

Frond number:	14-day EC ₅₀ >84.3 µg DPX-MP062/L 14-day NOEC = 84.3 µg DPX-MP062/L
Frond biomass:	14-day EC ₅₀ >84.3 µg DPX-MP062/L 14-day NOEC = 84.3 µg DPX-MP062/L

(Sloman, T.L., Leva, S.E., 1997)

RMS comment

This study was not conducted according to the current guideline OECD 221. This study was conducted with the old material DPX-MP062. RMS notes that an initial measured concentration was used for the determination of the endpoint. As the substance was not stable throughout the study, a geometric mean measured concentration should be used. A geometric mean measured concentration was calculated by the applicant (0.01668 mg/L). However the measured concentration being below the limit of detection at the end of the test, RMS cannot ascertain correct exposure during the test. RMS considers this study not reliable but also not compulsory according to the Regulation 283-2013.

B.9.2.8. Further testing on aquatic organisms

No further testing available.

B.9.3. EFFECTS ON ARTHROPODS**B.9.3.1. Effects on bees**

Report: Palmer, S.J., Beavers, J.B. (1994); DPX-JW062-47: A dietary LC₅₀ toxicity study with the honey bee

DuPont Report No.: HLO 276-94

Guidelines: U.S. EPA 141-1

- | | |
|-------------------|---------------------|
| 1. Test material: | DPX-JW062 technical |
| Lot/Batch #: | JW062-47 |
| Purity: | 95.5% |

The acute oral toxicity study HLO 276-94, originally submitted under EU Rev8 Point IIA 8.3.1.1 and conducted with test material DPX-JW062 technical, was conducted under guideline U.S. EPA 141-1. A review of this study indicates that it does not meet the current guideline (OECD 213) and has been superseded with DuPont-36500. Therefore this study is not relied upon.

RMS comment

Study summarised in Indoxacarb DAR, Volume 3, B9, 2000. No summary was provided for this Annex I renewal.

Report: Nengel, S., (1996); DPX-JW062 (racemic mixture of DPX-KN128 and DPX-KN127) 60WG formulation: Acute oral and contact toxicity to the honey bee, *Apis mellifera*

DuPont Report No.: AMR 3541-95

Guidelines: EPPO 170 (1992)

- | | |
|-------------------|----------------|
| 1. Test material: | DPX-JW062 60WG |
| Lot/Batch #: | JW062-135 |
| Purity: | 300 g a.s./kg |

The acute oral toxicity study AMR 3541-95, originally submitted under EU Rev8 Point IIA 8.3.1.1 and conducted with test material DPX-JW062 60WG, was conducted under guideline EPPO 170 (1992). A review of this study indicates that it does not meet the current guideline (OECD 213) and has been superseded with DuPont-36500.

RMS comment

Study summarised in Indoxacarb DAR, Volume 3, B9, 2000. No summary was provided for this Annex I renewal.

Report: Kling, A. (2000); DPX-MP062: Acute oral and contact toxicity to the honeybee, *Apis mellifera* L.

DuPont Report No.: DuPont-3995

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

Testing Facility: GAB Biotechnologie, GmbH, Neifern-Oschelbronn, Germany

Testing Facility Report No.: 20001112/01-BLEU

GLP: Yes

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

I. MATERIALS AND METHODS

A. MATERIALS

- | | |
|-----------------------------|---|
| 1. Test material: | DPX-MP062 technical |
| Lot/Batch #: | MP062-219A |
| Purity: | 74.66% |
| Description: | White dry powder |
| CAS#: | DPX-MP062: 144171-61-9
DPX-KN128: 173584-44-6 |
| Stability of test compound: | Not determined in the test system |
| 2. Control: | Oral test: Either an aqueous sucrose solution with the final concentration of 500 g/L (50% w/v) or an aqueous sucrose solution mixed with the maximum concentration of acetone which was found in the highest test item solution (2.56 mL acetone diluted to total volume of 20 mL with 50% sugar solution which corresponds to 12.8%)
Contact Test: One control group was treated with acetone and a second control group treated only with tap water |
| Test vehicle: | Oral test: Acetone and sugar solution
Contact Test: Acetone |
| Toxic reference: | Dimethoate a.s. |
| 3. Test organism: | Honey bees |
| Species: | <i>Apis mellifera carnica</i> |
| Age at dosing: | 22 to 32 days |
| Source: | Bee hives located in Welzheim, Germany |
| Diet: | 50% sucrose solution |
| Water: | See diet |
| Test chamber: | High-grade steel cage (10 cm wide × 5.5 cm long × 8.5 cm high) |
| Wetting agent | Palmolive® (contact test) |
| 4. Environmental conditions | |
| (In-life period) | |
| Temperature: | 24.0 to 25.0°C |
| Relative humidity: | 52 to 62% |
| Photoperiod: | Continuous dark |

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
01-August-2000 to 04-August-2000

2. Test system

Acute oral and contact toxicity of DPX-MP062 to honey bees (*Apis mellifera* L.) was tested in a laboratory study conducted according to OECD Guideline Nos. 213 and 214. The bees were exposed to the test substance by feeding and topical application. These tests were conducted with five test substance treatment rates plus a control and four toxic standard treatment rates; five replicates per treatment; and 10 honey bees per replicate. In the contact toxicity test, 2 µL of the test substance solution was applied ventrally to the thorax of each bee. In the oral toxicity test a quantity of 250 µL of treated sucrose solution was offered to each cage of 10 bees for 5 hours after a starvation period of 2 hours. Bees were observed at 4, 24, 48, and 72 hours after treatment for mortality and behavioural effects.

II. RESULTS AND DISCUSSION

A. FINDINGS

The LD₅₀ for DPX-MP062 in the acute oral toxicity test was calculated based on the actual intake. It was determined to be 0.194 µg DPX-KN128/bee with a 95% confidence limit of 0.172 to 0.219 µg DPX-KN128/bee after 72 hours. The LD₅₀ for DPX-MP062 in the acute contact toxicity test was calculated to be 0.070 µg DPX-KN128/bee after 72 hours. The 95% confidence limit for the contact LD₅₀ could not be determined. This corresponds to an acute oral toxicity 72-hour LD₅₀ of 0.260 µg DPX-MP062/bee (95% CI = 0.230 to 0.293 µg DPX-MP062/bee) and an acute contact toxicity 72-hour LD₅₀ of 0.094 µg DPX-MP062/bee.

The results of the oral and contact toxicity of dimethoate to honey bees in these tests were in the expected range, indicating the validity of these tests.

Table 156
Acute oral toxicity of DPX-MP062 to honey bees

Treatment ^a	Test substance intake ^b	Mean mortality [%] ^c		
		24 h	48 h	72 h
Control ^d	—	4.0	4.0	4.0
Control ^e	—	0.0	2.0	2.0
0.079	0.095	8.0	12.2	14.3
0.119	0.139	16.0	30.6	38.8
0.178	0.197	24.0	36.7	38.8
0.267	0.297	60.0	79.6	79.6
0.400	0.420	72.0	85.7	85.7

^a Treatments are specified as intended uptake, in mean µg DPX-KN128/bee per treatment

^b Test substance intake is based on actual uptake, in mean µg DPX-KN128/bee per treatment

^c Test mortality for treatments is corrected for control mortality
(mortality at 0 µg DPX-KN128/bee; 50% aqueous sucrose solution mixed with acetone)

^d Control: 50% aqueous sucrose solution

^e Control: 50% aqueous sucrose solution mixed with acetone

Table 157
Acute contact toxicity of DPX-MP062 to honey bees

Treatment ^a	Mean mortality[%]		
µg DPX-KN128 per bee	24 h	48 h	72 h
Control (water)	0.0	0.0	0.0
Control (acetone)	0.0	0.0	0.0
0.0376	2.0	10.0	12.0
0.064	12.0	24.0	24.0
0.108	74.0	92.0	94.0
0.184	94.0	100.0	100.0
0.313	92.0	98.0	100.0

^a Treatments are specified as mean µg DPX-KN128/bee per treatment rate, applied ventrally.

III. CONCLUSIONS

The 72 h acute oral LD₅₀ of DPX-MP062 was calculated based on actual intake and was 0.194 µg DPX-KN128/bee. The oral LOEL was estimated as 0.095 µg DPX-KN128 per bee. These are equivalent to 0.26 and 0.13 µg DPX-MP062/bee.

The 72 h acute contact LD₅₀ of DPX-MP062 was determined by this test to be 0.070 µg DPX-KN128/bee. The contact LOEL was determined to be 0.0376 µg DPX-KN128 per bee. These are equivalent to 0.09 and 0.05 µg DPX-MP062/bee.

The determination of the NOEL values of DPX-MP062 for the acute contact and the oral toxicity tests were not applicable.

(Kling, A., 2000)

RMS comment

Study summarised in Indoxacarb DAR, Volume 3, B9, AD1 2005. A more detailed summary was provided for this Annex I renewal.

This study was assessed for previous Annex I inclusion and was conducted with the old material DPX-MP062. As new acute toxicity study is available on the bee for the new material DPX-KN128, this study is not considered essential.

Report: Kling, A., (2014); Indoxacarb (DPX-KN128) technical: Acute oral and contact toxicity to the honeybee, *Apis mellifera* L. under laboratory conditions

DuPont Report No.: DuPont-36500

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

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

Testing Facility Report No.: S13-01455

GLP: Yes

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

Executive summary:

Acute 48-hour oral and contact toxicity tests on honey bees (*Apis mellifera* L.) were conducted with indoxacarb (DPX-KN128) in the laboratory under OECD Guidelines 213 and 214 (1998).

The oral toxicity test treatments consisted of four toxic reference treatment rates, two controls (aqueous sucrose solution and aqueous sucrose solution containing 10% acetone) and five nominal concentrations of 48.0, 86.0, 154, 278, and 500 ng a.s./bee. The doses for the oral test based on measured consumption of application solution (actual consumption) were 53.2, 96.7, 163, 300, and 538 ng a.s./bee. The contact toxicity treatments consisted of four toxic reference treatment rates, two controls (tap water and acetone) and five nominal concentrations of 30.0, 48.0, 78.0, 126, and 200 ng a.s./bee. The 48-hour LD₅₀ for honey bees based on mortality could be determined as 232 ng a.s./bee in the oral toxicity test and 68.2 ng a.s./bee in the contact toxicity test. The NOEL (No Observed Effect Level) could be determined to be 163 ng a.s./bee in the oral toxicity test and 48 ng a.s./bee in the contact toxicity test. Some behavioural differences between test item treatments with indoxacarb and the controls were observed in the oral and contact toxicity test.

I. MATERIALS AND METHODS

A. MATERIALS

- | | |
|---|---|
| 1. Test material: | Indoxacarb technical |
| Lot/Batch #: | KN128-098 |
| Purity: | 95.8% |
| Description: | Solid, Powder |
| CAS #: | 173584-44-6 |
| Stability of test compound: | Sufficient for test purpose |
| 2. Control: | Oral test: 50% (w/v) aqueous sucrose solution
50% (w/v) aqueous sucrose solution + 10% acetone
Contact test: tap water
acetone |
| Test vehicle: | Oral test: 50% (w/v) aqueous sucrose solution + 10% acetone
Contact test: acetone |
| Toxic reference: | Perfekthion (dimethoate a.s.) |
| 3. Test organism: | Honey bees |
| Species: | <i>Apis mellifera</i> |
| Age at dosing: | adult worker bees |
| Source: | Beekeeper Mr. Wolters. Im Bannen 38-54.
56727 Mayen. Germany |
| Diet: | 50% (w/v) aqueous sucrose solution |
| Water: | See diet |
| Test chamber: | Stainless steel cages (base: 8 × 4 cm, height: 6 cm) |
| Wetting agent: | Denk mit® (contact test) |
| 4. Environmental conditions (In-life phase) | |
| Temperature: | 25.4 to 26.5°C |
| Relative humidity: | 50.6 to 60.2% |
| Photoperiod: | Continuous dark. except during the assessments |

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
17-July-2013 to 19-July-2013

2. Experimental treatments

The acute 48-hour oral and contact toxicity of indoxacarb was determined in honey bees (*Apis mellifera* L.). The oral toxicity test treatments consisted of four toxic reference treatment rates, two controls (50% aqueous sucrose solution and 50% aqueous sucrose solution containing 10% acetone) and five nominal concentrations of 48.0, 86.0, 154, 278, and 500 ng a.s./bee. The contact toxicity treatments consisted of four toxic reference treatment rates, two controls (tap water and acetone) and five nominal test item concentrations of 30.0, 48.0, 78.0, 126, and 200 ng a.s./bee. Four replicates per treatment and 10 honey bees per replicate (total 40 bees per treatment) were used for the test item concentration, controls and toxic reference. Perfekthion (dimethoate) was used as the toxic reference in these tests. In the oral test, bees were offered the test solutions in 50% (w/v) aqueous sucrose solution containing 10% acetone. In the contact test, bees were dosed with indoxacarb by topical application with a 2-µL droplet applied to the dorsal thorax of each bee.

3. Observations

Assessments for mortalities and sublethal effects were carried out 4, 24, and 48 hours after treatment in the oral and contact tests.

4. Statistics

The LD₅₀ values of the reference item treatment with 95% confidence intervals were calculated by means of a probit analysis using the statistic program SAS V 9.2, Proprietary Software. (Ed. 2002-2008). Fisher's Exact Test (Bonferroni-Holms corrected, one-sided, $\alpha = 0.05$) 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 NOEL.

II. RESULTS AND DISCUSSION

A. FINDINGS

Control mortality in the oral and contact test after 48 hours was <10.0%. The oral and contact toxicity of the toxic reference, dimethoate, to honey bees in these tests fell within the accepted range. All validation criteria were within acceptable limits indicating the validity of these tests.

Actual test item intake in the oral tests and mortality results for the oral and contact tests at 24 and 48 hours are given in Table 158 and Table 159.

Table 158
Mortality in the oral test with indoxacarb

Indoxacarb ^a (ng a.s./bee)	Mortality [%]		Corrected mortality [%] ^b	
	24 h	48 h	24 h	48 h
Control (50% (w/v) aqueous sucrose solution)	0.0	0.0	-	-
Control (50% (w/v) aqueous sucrose solution+10% acetone)	2.5	2.5	-	-
53.2	5.0	5.0	2.6	2.6
96.7	7.5	7.5	5.1	5.1
163	12.5	15.0	10.3	12.8
300	80.0	82.5	79.5	82.1
538	97.5	100.0	97.4	100.0

^a Doses based on actual consumption of the test item

^b Mortality corrected for corresponding control mortality

Table 159
Mortality in the contact test with indoxacarb

Indoxacarb (ng a.s./bee)	Mortality [%]	
	24 h	48 h
0 (Control - tap water)	2.5	2.5
0 (Control – acetone)	0.0	0.0
30.0	0.0	0.0
48.0	0.0	7.5
78.0	62.5	70.0
126	97.5	100.0
200	100.0	100.0

In the oral toxicity test behavioural differences between test item treatments with indoxacarb and the controls were mainly observed at the assessment 4 hours after application, especially in the two highest treatment groups. In the following assessments few affected bees were observed, which do not clearly correlate with increasing dose levels.

In the contact toxicity test some behavioural differences between test item treatments with indoxacarb and the controls occurred at the assessment 24 hours after application at the treatment levels of 48 and 78 ng a.s./bee. Only few bees were still recorded as affected at these treatment groups on the next day.

III. CONCLUSIONS

The effects of indoxacarb were assessed in an acute oral and contact honey bee toxicity test conducted in the laboratory.

The 48-hour oral LD₅₀ value for honey bees based on the mean actual intake of indoxacarb was 232 ng a.s./bee.

The 48-hour contact LD₅₀ value for honey bees based on nominal concentrations of indoxacarb was 68.2 ng a.s./bee.

The NOEL (No Observed Effect Level) could be determined to be 163 ng a.s./bee in the oral toxicity test. The NOEL in the contact toxicity test was determined to be 48 ng a.s./bee.

(Kling, A., 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. The 48-hour oral LD₅₀ value for honey bees based on the mean actual intake of indoxacarb was 232 ng a.s./bee. The 48-hour contact LD₅₀ value for honey bees based on nominal concentrations of indoxacarb was 68.2 ng a.s./bee. This study is acceptable.

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

DuPont Report No.: DuPont-38350

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

Testing Facility: IBACON, Rossdorf, Germany

Testing Facility Report No.: 86951105

GLP: Yes

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

Executive summary:

The aim of this study was to determine the acute contact and oral toxicity of indoxacarb to the bumblebee (*Bombus terrestris* L.) in a laboratory study. A contact test with 0.8, 0.4, 0.2, 0.1 and 0.05 µg indoxacarb/bee and an oral test with 0.33, 0.17, 0.08, 0.04 and 0.02 µg indoxacarb/bee were conducted according to van der Steen (2001), OECD 213/214 (1998), with modifications and adaptations, and current recommendations of the non-Apis ring test group (2014). The contact LD₅₀ (96 h) based on measured concentration was 0.25 µg a.s./bee. The oral LD₅₀ (96 h) based on measured concentration was 0.07 µg a.s./bee.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Indoxacarb technical
Lot Number: KN128-374
Purity: 98.8%
Description: White solid
CAS#: 173584-44-6
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) containing 1% acetone;
Contact test: Acetone
2. Control Vehicle without test item and without solvent
Solvent 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.
Acclimatization: Contact Test: 16 hours 35 minutes
Oral Test: 24 hours 35 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: Acclimatization: 23–27°C
Exposure: 23–27°C
Relative humidity: Acclimatization: 55–66%
Exposure: 55–66%

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
13-May-2014 to 21-June-2014
2. Experimental treatments
Application in the oral test:

The test item was dissolved in acetone and mixed with 50% w/v sucrose solution in order to achieve a treated feeding solution containing 1% acetone.

The reference item was 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 solvent control acetone was added to 50% w/v sucrose solution in order to achieve an untreated feeding solution containing 5% acetone.

The treated food was offered in syringes, which were weighed before and after introduction into the cages (duration of uptake ranged from 1 hour 5 minutes up to 2 hours 15 minutes for the test item treatments). After a maximum of 2 hours 15 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 in an appropriate carrier (acetone) 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. A solvent control was treated with pure acetone. The reference item was applied in 5 µL tap water (dimethoate made up in tap water containing 0.5% Tween 80).

Note: Tween 80 was used to improve the adhesion of the droplet on the bee body. Tween 80 is non-toxic to bumblebees

3. Statistics

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 Weibull Analysis using linear maximum likelihood regression.

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

A. FINDINGS

All study validity criteria were achieved.

In the contact test, 30 worker bees per treatment group (30 replicates) were exposed to doses of 0.8, 0.4, 0.2, 0.1, 0.05 and 0.025 µg indoxacarb/bee by dorsal application of a 5 µL droplet (test item in acetone) on the bumblebee thorax. At test end (96 hours after application) there was 100% mortality in the highest dosing group (0.8 µg a.s./bee) and 93.3% in the 0.4 µg a.s./bee group. At 0.2 µg a.s./bee, 43.3% mortality occurred. The 0.1 and 0.05 µg a.s./bee dose level group showed 3.3% mortality. At a dose level of 0.025 µg a.s./bee, 6.7% mortality occurred. In the control group treated with tap water containing 0.5% Tween 80, 10.0% mortality occurred. In the solvent control group treated with acetone, 3.3% mortality occurred. Behavioural abnormalities correlated with dose and increased with time. In the higher dosing groups (0.8, 0.4 and 0.2 µg a.s./bee) nearly all surviving bees were affected or moribund. In the lower dosing groups (0.1, 0.05 and 0.025 µg a.s./bee) bees were only affected. The number of affected bees decreased with dose.

In the oral test, the target dose levels of 0.32, 0.16, 0.08, 0.04 and 0.02 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 0.33, 0.17, 0.08, 0.04 and 0.02 µg a.s./bee. At test end (96 hours after application) there was 100% mortality in the highest dosing groups (0.33 and 0.17 µg a.s./bee). At a dose level of 0.08 µg a.s./bee 53.3% mortality occurred. At 0.04 µg a.s./bee 13.3% of the treatment group died. At 0.02 µg a.s./bee 10.0% mortality occurred. There was no mortality in the control group (50% w/v sucrose solution). In the solvent control group (50% w/v sucrose solution containing 5% acetone) 3.3% mortality occurred. Behavioural abnormalities (affected or moribund) correlated with dose and decreased with time. Most of the surviving bees in the highest treatment groups (0.33 µg a.s./bee and 0.17 µg a.s./bee) were affected from 24 hours onwards. In the lower dosing groups (0.08 and 0.04 µg a.s./bee) behavioural abnormalities (affected, moribund) correlated with dose from 48 hours onwards, decreasing with time. No behavioural abnormalities were observed at the 0.02 µg a.s./bee dose level.

Mortality of the bumblebees treated with reference item (dimethoate, 400 g/L EC) was 96.7% in the contact test (12 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 summarised in the table that follows.

Table 160
Toxicity of Indoxacarb to bumblebees (*Bombus terrestris* L.) in an acute contact and oral toxicity test

Test item	Indoxacarb	
Test object	<i>Bombus terrestris</i> L.	
Exposure	contact (acetone)	oral (50% w/v sucrose solution containing 1% acetone)
LD ₅₀ [µg a.s./bee]	0.25 (96 h)	0.07 (96 h)

III. CONCLUSIONS

The effects of indoxacarb 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 0.25 µg a.s./bee. The oral LD₅₀ (96 h) based on measured concentration was 0.07 µg a.s./bee.

(Haupt, S., 2014)

RMS comment

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

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 0.25 µg a.s./ bumble bee. The oral LD₅₀ (96 h) based on measured concentration was 0.07 µg a.s./bumble bee. This study is acceptable.

Report: Palmer, S.J., Beavers, J.B. (1994); DPX-JW062-47: An acute contact toxicity study with the honey bee

DuPont Report No.: HLO 277-94

Guidelines: USEPA 141-1

-
- | | |
|-------------------|---------------------|
| 1. Test material: | DPX-JW062 technical |
| Lot/Batch #: | JW062-47 |
| Purity: | 95.5% |

The acute contact toxicity study HLO 277-94, originally submitted under EU Rev8 Point IIA 8.3.1.1., and conducted with test material DPX-JW062 technical, was conducted under guideline USEPA 141-1. A review of this study indicates that it does not meet the current guideline (OECD 214), is not relied upon and has been superseded with DuPont-36500.

RMS comment

Study summarised in Indoxacarb DAR, Volume 3, B9, 2000. No summary was provided for this Annex I renewal.

Report: Kling, A., (2014); Indoxacarb (DPX-KN128) technical: Assessment of chronic effects to the honeybee, *Apis mellifera* L., in a 10 days continuous laboratory feeding test

DuPont Report No.: DuPont-36490

Guidelines: Not applicable **Deviations:** None

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

Testing Facility Report No.: S13-01456

GLP: Yes

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

Executive summary:

Chronic effects of the test item indoxacarb (DPX-KN128) 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 1% acetone and indoxacarb at the concentration levels of 286, 514, 926, 1667 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 application feeding solution, also containing 1% acetone. 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 a mortality of 0.0, 2.5, 17.5, 67.5 and 97.5% was observed at the test item concentration levels of 286, 514, 926, 1667 and 3000 µg a.s./kg, respectively, at the end of the test. The concentration level of 514 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 926 µg a.s./kg was therefore determined to be the LOEC (Lowest Observed Effect Concentration).

There were some sub-lethal effects recorded at all treatment levels. The effects increased with increasing concentration level tested and increasing duration after application. The largest share of affected bees was observed at the highest treatment group of 3000 µg a.s./kg, where all surviving bees from assessment E6 on were recorded as affected.

The daily mean food consumption of aqueous sucrose application feeding solution in the test item groups was not statistically significantly lower compared to the control group (day-by-day comparison) in all test item groups throughout

the whole 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 cage 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 1383 µg a.s./kg (with the 95% confidence limits of 1213 to 1561 µg a.s./kg).

The 10-day LD₅₀, based on the test item consumption per bee per day, was determined to be 64.9 ng a.s./bee/day (with the 95% confidence limits of 56.0 to 75.6 ng a.s./bee/day).

I. MATERIALS AND METHODS

A. MATERIALS

- | | |
|---|--|
| 1. Test material: | Indoxacarb technical |
| Batch/Lot Number: | KN128-098 |
| Purity: | 95.8% |
| Description: | Solid, Powder |
| CAS Registry Number: | 173584-44-6 |
| Stability in Solution: | Sufficient for test purpose |
| 2. Control: | 50% (w/v) aqueous sucrose (feeding) solution containing 1% acetone |
| Test vehicle | 50% (w/v) aqueous sucrose (feeding) solution containing 1% acetone |
| 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 × 4 cm, height: 6 cm |
| 4. Environmental conditions (In-life phase) | |
| Temperature: | 26.3–33.3°C |
| Relative humidity: | 29.5–70.1% |
| Photoperiod: | Continuous dark, except during the assessments |

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
30-July-2013 to 26-August-2013
2. Experimental treatments
One control and five test item concentrations of 286, 514, 926, 1667 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 application 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, one-sided, $\alpha = 0.05$) 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 Bonferroni-U Exact test (left sided; $\alpha = 0.05$).

II. RESULTS AND DISCUSSION

A. FINDINGS

In the control group the mortality was 2.0% at the final assessment after 10 days.

In the test item group a mortality of 0.0, 2.5, 20.0, 67.5 and 97.5% was observed at the test item concentration levels of 286, 514, 926, 1667 and 3000 µg a.s./kg at the end of the test. The concentration level of 514 µg a.s./kg was determined to be the NOEC based on mortality. The concentration level of 926 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 Table 161.

Table 161
Summary of chronic effects of indoxacarb technical after continuous feeding on honey bees

Treatment	Control	Indoxacarb technical [µg a.s./kg]				
		286	514	926	1667	3000
Cumulative mortality [%]	2.0	0.0	2.5	17.5 ^d	67.5 ^d	97.5 ^d
Corrected cumulative mortality [%]	-	-2.0	0.5	15.8	66.8	97.4
Overall mean daily consumption of aqueous sucrose feeding solution per replicate [mg/bee] ^a	42.8	42.9	41.6	46.3	44.9	67.8
Mean intake accumulated over test days [ng a.s./bee]	-	122.99	214.87	430.58	751.83	2019.00
Mean intake per bee per day [ng a.s./bee/day]	-	12.3	21.5	43.1	75.2	202
LC ₅₀ (95% confidence limits)	1383 µg a.s./kg (1213 to 1561 µg a.s./kg)					
LD ₅₀ (95% confidence limits)	64.9 ng a.s./bee/day (56.0 to 75.6 ng a.s./bee/day)					
NOEC ^b	514 µg a.s./kg					
LOEC ^c	926 µg a.s./kg					

^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; one side; $\alpha = 0.05$))

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

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

III. CONCLUSIONS

The effects of indoxacarb technical were assessed in a 10-day oral laboratory toxicity test.

The 10-day LC₅₀ was determined to be 1383 µg a.s./kg (with the 95% confidence limits of 1213 to 1561 µg a.s./kg).

The 10-day LD₅₀ based on the test item consumption per bee per day was determined to be 64.9 ng a.s./bee/day (with the 95% confidence limits of 56.0 to 75.6 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 ≤ 15% at the end of the test fulfilling the validity criteria. The 10-day LC₅₀ was determined to be 1383 µg a.s./kg. The 10-day LD₅₀ based on the test item consumption per bee per day was determined to be 64.9 ng a.s./bee/day.

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

DuPont Report No.: DuPont-36493

Guidelines: Oomen *et al.* (1992) and OECD guidance document No. 75 **Deviations:** None

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

Testing Facility Report No.: S13-00881

GLP: Yes

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

Executive summary:

The effects of the test item indoxacarb (DPX-KN128) technical 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 recommendations by EFSA (2012). This study was conducted in Niefern-Öschelbronn in Southern Germany (region: Baden-Württemberg) from July to August 2013 and included a total of three treatment groups:

- Indoxacarb technical treatment group T with the daily application of a feeding solution (200 mL 50% sucrose solution plus <1% acetone as a solvent 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 application of a feeding solution (200 mL 50% sucrose solution per hive per day) at a 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 200 mL 50% sucrose solution (plus <1% acetone, added for comparison with T) per hive and day over a period of 9 days.

Feeding was done over a period of 9 days from 11 July 2013 (0DAF; DAF = days after first feeding) to 19 July 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) later 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 applications of indoxacarb technical 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 (≥180 cells containing eggs, ≥200 cells containing young larvae and ≥178 cells containing old larvae at the first assessment (BFD0), shortly before the first feeding),
- Behaviour of the honey bees in front of the hives.

Daily feeding of indoxacarb technical in a 50% (w/w) sucrose solution (plus <1% acetone as a solvent) (200 mL per day) at a concentration of 100 µg indoxacarb/kg over a period of 9 days had transient effects during the feeding period on subsequent honey bee mortality (1DAF to 9DAF) and on honey bee behaviour (1DAF to 9DAF). These effects diminished quickly and completely after the end of feeding.

There was no effect of the indoxacarb technical treatments on the colony size, amount of brood, amount of food, and colony condition.

The effects of the indoxacarb technical treatments on brood of different age were as follows:

- Development of eggs: the brood index was lower and the termination rate was higher than the control on all assessments from BFD+5 to BFD+21. There was no effect on the compensation index.
- Development of young larvae and old larvae: there was no effect of the indoxacarb technical treatments on the brood index, compensation index and termination rate of young larvae and old larvae.

I. MATERIAL AND METHODS

A. MATERIALS:

- | | |
|--|---|
| 1. Test material: | Indoxacarb technical |
| Lot/Batch: | KN128-098 |
| Purity: | 95.8% |
| Description: | Solid |
| CAS#: | 173584-44-6 |
| Stability of test compound: | Not available |
| Reference item: | Insegar 25WG |
| Batch: | SMO2K433 |
| Content of a.s., nominal: | 25.0 % (w/w) |
| Description: | Insegar 25WG (Fenoxycarb) |
| CAS#: | 72490-01-8 |
| Stability in solution: | Sufficient for test purpose (at least 1 hour) |
| 2. Vehicle and/or control: | 50% (w/w) sucrose solution, plus <1% acetone in T and C |
| 3. Test organism | |
| Species: | <i>Apis mellifera</i> L. |
| Age at dosing: | Direct exposure of adult honey bees trough feeding;
indirect exposure of all stages of development |
| Source: | LAVES Institut für Bienenkunde, Celle, Germany |
| Diet: | Honey bees are freely flying, feeding of test item,
reference item and control as a 50% sucrose solution |
| 4. Environmental conditions during the exposure period | |
| Temperature (min/max): | 9.2–35.8°C |
| Relative humidity: | 29.3–100% |
| Photoperiod (exposure): | natural light conditions |

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
06-July-2013 to 08-August-2013
2. Experimental treatments
Indoxacarb technical treatment group T with the daily application of a feeding solution (200 mL sucrose 50% solution plus <1% acetone as a solvent 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 application of a feeding solution (200 mL 50% sucrose solution per hive per day) at a 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 200 mL 50% sucrose solution (plus <1% acetone, added for comparison with T) per hive and day over a period of 9 days.

3. Observations

The influence of the 9 test item applications of indoxacarb technical 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 (≥ 180 cells containing eggs, ≥ 200 cells containing young larvae and ≥ 178 cells containing old larvae at the first assessment (BFD0), shortly before the first feeding),
- Behaviour of the honey bees in front of the hives.

II. RESULTS AND DISCUSSION

A. MORTALITY

Mortality after installation of the colonies at the test location until the first feeding of the sucrose test solution (4DBF to 0DBF; DBF = Days before first application of the feeding solution) was similar in all three treatment groups. Mean daily mortality was in the range from 1.3 to 10.8 dead honey bees/day in the control, from 1.5 to 5.0 dead honey bees/day in T and 0.8 to 17.8 dead honey bees/day in R before application of the feeding solution. Mean daily mortality over the whole period (4DBF to 0DBF) was 3.7 dead honey bees/day in the control, 3.4 dead honey bees/day in T and 5.8 dead honey bees in R. There were no statistically significant differences between the three treatment groups in the period before the first application of the feeding solution (Tukey's Studentised Range test, $p \leq 0.05$).

During the period of daily feeding of the sucrose test solution (0DAF to 8DAF in the evening, corresponding to data from mortality assessments on 1DAF to 9DAF; DAF = days after first application of the feeding solution) the daily mortality values were in the range from 1.0 to 31.8 dead honey bees/day in the control, from 34.0 to 262.5 dead honey bees/day in T and from 1.5 to 75.0 dead honey bees/day in R. The daily mortality from 1DAF to 9DAF in T and the daily mortality on 1DAF and on 9DAF in R were significantly different from the control (pooled t-Test, $p \leq 0.05$).

The mean daily mortality during this period (1DAF to 9DAF) was 7.0 dead honey bees/day in the control, 113.9 dead honey bees/day in T and 14.7 dead honey bees/day in R. These values were statistically significantly higher in the treatment group T and in R compared to the control (pooled t-Test, $p \leq 0.05$).

During further observation of the colonies after the end for the feeding period (10DAF to 28DAF), daily mortality was in the range from 0.3 to 113.5 dead honey bees/day in the control, from 0.8 to 107.0 dead honey bees/day in T and from 1.3 to 327.0 dead honey bees/day in R. In the test item treatment T, mortality on 17DAF was significantly different from the control (pooled t-Test, $p \leq 0.05$) but this slight difference (13.0 dead honey bees in T compared to 4.3 dead honey bees in C) is well within the range of natural variability observed during this study and it is of no biological relevance.

Mortality in the reference item treatment R was statistically significantly higher than the control on 10DAF, 12DAF, 14DAF to 17DAF, 19DAF, 23DAF and 28DAF (pooled t-Test, $p \leq 0.05$). Increased mortality in R during this

period resulted mainly from large numbers of pupae that were removed from the combs and hives, an effect that could be expected from this reference item.

The mean daily mortality during the entire period after the applications of the feeding solution (10DAF to 28DAF) was 18.9 dead honey bees/day in the control, 19.6 dead honey bees/day in T (not statistically significant; pooled t-Test, $p \leq 0.05$) and 94.2 dead honey bees/day in R (statistically significant; pooled t-test, $p \leq 0.05$).

Overall, there was a transient effect of the indoxacarb technical treatment T on honey bee mortality during the period of daily feeding (1DAF to 9DAF). This effect diminished quickly and completely after the feeding of the test solution had ended.

There was no effect of the test item treatments on the number of dead young bees, pupae and malformed bees and pupae found in the dead bee trap. Before the start of feeding (4DBF to 0DBF), there was a total of 1.5 dead young or malformed bees and pupae in the control, 1.0 dead bees and pupae in T and 1.8 dead bees and pupae in R (mean values per replicate on all 5 days).

Table 162
Honey bee mean mortality - all life stages

Date	Timing	Mean Mortality (mean number of dead honey bees, larvae and pupae) per day and treatment group		
		C	T Indoxacarb technical	R (Insegar/Fenoxycarb)
07 Jul 2013	4DBF	10.8	5.0	17.8
08 Jul 2013	3DBF	3.0	4.3	2.3
09 Jul 2013	2DBF	1.3	4.5	4.5
10 Jul 2013	1DBF	2.3	1.5	0.8
11 Jul 2013 ^a	0DBF ^a	1.3	1.5	3.5
Mean (pre-application period, 4DBF to 0DBF)		3.7	3.4	5.8
STD		3.4	0.8	3.5
12 Jul 2013	1DAF ^b	31.8	59.8*	75.0*
13 Jul 2013	2DAF	9.0	252.3*	7.0
14 Jul 2013	3DAF	2.0	262.5*	1.5
15 Jul 2013	4DAF	1.5	83.3*	2.3
16 Jul 2013	5DAF	1.0	34.0*	1.8
17 Jul 2013	6DAF ^b	5.0	124.8*	7.5
18 Jul 2013	7DAF	6.5	90.8*	5.3
19 Jul 2013	8DAF ^c	4.3	36.0*	2.8
20 Jul 2013	9DAF	1.8	81.5*	28.8*
Mean (application period, 1DAF to 9DAF)		7.0	113.9*	14.7*
STD		3.2	48.7	5.7
21 Jul 2013	10DAF	1.3	8.3	11.3*
22 Jul 2013	11DAF	5.3	2.3	34.8
23 Jul 2013	12DAF ^b	7.5	10.0	31.0*
24 Jul 2013	13DAF	28.0	2.3	70.8
25 Jul 2013	14DAF	4.3	4.0	130.5*
26 Jul 2013	15DAF ^d	85.5 ^d	107.0 ⁴⁾	327.0* ⁴⁾
27 Jul 2013	16DAF ^b	12.8	20.5	87.8*
28 Jul 2013	17DAF	4.3	13.0*	86.0*
29 Jul 2013	18DAF	82.5	28.5	117.8
30 Jul 2013	19DAF	4.8	7.3	186.5*
31 Jul 2013	20DAF	38.3	54.3	150.3
01 Aug 2013	21DAF	9.5	10.5	42.3
02 Aug 2013	22DAF ^b	113.5	82.8	479.0
03 Aug 2013	23DAF	3.5	8.5	14.8*
04 Aug 2013	24DAF	1.5	2.8	2.8
05 Aug 2013	25DAF	2.0	4.0	1.3

Table 162
Honey bee mean mortality - all life stages (continued)

Date	Timing	Mean Mortality (mean number of dead honey bees, larvae and pupae) per day and treatment group		
		C	T Indoxacarb technical	R (Insegar/Fenoxycarb)
06 Aug 2013	26DAF	1.5	3.5	3.8
07 Aug 2013	27DAF	3.0	2.3	3.8
08 Aug 2013	28DAF	0.3	0.8	7.8*
Mean (post-application, (10DAF to 28DAF))		18.9	19.6	94.2*
STD		5.4	6.7	50.1

DBF/DAF = Days before/after first application of feeding solution

STD = Standard deviation

1DAF to 9DAF: Dead bees from dead bee traps during exposure to the feeding solution

10DAF to 28DAF: Dead bees from dead bee traps after the exposure to feeding solution

^a Start of the period of daily feeding in the evening of 11 Jul 2013, after the mortality assessment on that day (= 0DAF)

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

^c Period of daily feeding ended in the evening of 19 Jul 2013 (8DAF), after the mortality assessment on that day

^d High mortality on 15DAF (26 Jul 2013) resulted from a storm in the afternoon of 25 Jul 2013 that uncovered all hives and exposed the bees to wind and rain

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

During the feeding period, subsequent mortality (1DAF to 9DAF) was 0.8 dead bees and pupae in C, 1.3 dead bees and pupae in T and 3.5 dead bees and pupae in R.

After the feeding period until the end of the observation period (10DAF to 28DAF), there were 1.5 dead bees and pupae in C and 4.3 dead bees and pupae in T. There was a clear impact in R on the number of dead and malformed bees and pupae, with 891.8 dead bees and pupae.

Overall, there was no effect of the indoxacarb technical treatment T on the mortality of young bees or pupae and on malformations of the bees or pupae.

B BEHAVIOUR OF THE HONEY BEES

Before the application, normal behaviour was recorded in all treatments except one cramping bee in hive Ta (1DBF) and one cramping bee in Rb (1DBF) and Rd (0DBF). These observations are not related to any treatment since feeding of the test solution had not yet started, but they may result e.g. from guard bees stinging foreign bees trying to enter the hive.

After the application, no change in behaviour was observed in the control. Few inactive (motionless) honey bees (up to 3 honey bees per hive on 1DAF, 2DAF and 4DAF) and in one case trembling (4DAF) or locomotion problems (6DAF) were observed but these observations are considered of no biological relevance, and they may result from natural reasons as described above.

In the test item treatment T, inactive bees (up to 35 honey bees per hive and assessment date) and bees with locomotion problems (up to 32 honey bees per hive and assessment date) were noted during the 9-day period of application of the test solution (0DAF to 8DAF in the evening, related data corresponds to the assessment period from 1DAF to 9DAF). Cramping bees were observed mainly on 1DAF. During further observation of the colonies (10DAF to 28DAF), there were only occasional sightings of a very small number of individual bees in few hives that showed unusual behaviour. These observations are not considered to be of any biological relevance.

Small clusters of honey bees outside the hive were observed in T on 16DAF and 17DAF. Similar behaviour was observed in the control on 17DAF, therefore it is not considered treatment related but rather due to external conditions, *e.g.* high temperatures recorded on these days.

In the reference item treatment R, honey bees with locomotion problems and inactive bees were observed mainly during the period from 1DAF to 8DAF, confirming that the study design was appropriate to ensure exposure of the honey bees to the feeding solution. Few cramping bees were observed during the feeding period and few bees with symptoms were observed after the feeding period (10DAF to 28DAF). Cluster formation of numerous bees at the hive entrance was observed in R mainly from 11DAF to 18DAF.

Overall, there was an effect of the indoxacarb technical treatment T on honey bee behaviour during the 9 day period of daily feeding.

C BROOD DEVELOPMENT AND COLONY CONDITION

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 hive Tc where no significant amounts of pollen were found. Mean colony sizes (number of bees per hive) were similar in all three treatments: there were 7383 honey bees/hive in C, 8888 honey bees/hive in T and 9422 honey bees/hive in R on 0DBF.

During the study, the development of the colony sizes in C and T were similar and there was no effect of the test item. After a slight decline of the colony sizes that was observed in both treatments (C and T) at the following two assessments (5DAF and 11DAF), the maximum colony size was recorded on 15DAF in C (9113 honey bees/hive) and T (9943 honey bees/hive). In the reference item treatment R, 8930 honey bees/hive were recorded on 15DAF. At the next assessment (21DAF) a noticeable decline of the colony size was observed in all treatments (4430 honey bees/hive in C, 5105 honey bees/hive in T and 4050 honey bees/hive in R). Since this prominent decline was observed in all treatment groups including the control, it is not related to any treatment but rather to external conditions. At the last assessment (28DAF) all hives had recovered and bigger colony sizes were recorded in all treatments (9028 honey bees/hive in C, 7946 honey bees/hive in T and 6961 honey bees/hive in R).

Overall, there was no negative effect of the indoxacarb technical treatment on the size of the colonies (mean number of honey bees per hive).

Brood of all stages (eggs, larvae, capped brood) was present in all colonies at all assessments during the study except the lack of eggs in Rd at the last assessment. At the first assessment on BFD0/0DBF, the mean total amount of brood cells (brood of all stages) was similar in the three treatments (25875 brood cells in C, 24615 brood cells in T and 23310 brood cells in R). In C and R, the further development of the amount of brood until the last assessment was similar, showing a slight decrease to 16155 brood cells in C and to 14310 brood cells in T at the last assessment on 28DAF. There was no effect of the test item treatment on the amount of brood compared to the control.

In the reference item treatment R, the number of brood cells decreased strongly, reaching a minimum mean value of 3555 brood cells/hive on 21DAF and recovering only slightly until 28DAF (5085 brood cells/hive). The number of larvae decreased during the feeding period and was very small on 11DAF and 15DAF. This could be expected from this kind of reference item, confirming that the study design was appropriate to ensure exposure of the honey bees and their brood to the feeding solution. Due to hatching of the older brood that was already in pupal stage at the start of feeding and due to the high termination rate and low number of larvae on 11DAF and 15DAF, the number of capped cells containing pupae also declined, namely on 15DAF, 21DAF and 28DAF.

Overall, there was no effect of the indoxacarb technical treatment T on the amount of honey bee brood whereas there was a clear impact in the reference item treatment R.

All colonies had sufficient amount of nectar and pollen stores throughout the study.

D. DETAILED ASSESSMENT OF BROOD CELL CONTENTS

Development of the honey bee eggs 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.

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 3.58 and the mean compensation index was 3.73 on BFD+21. The termination rates on BFD+21 were between 11.87 and 48.02 with a mean value of 28.52.

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: 1.39; compensation index: 1.45) indicate that a large proportion of the observed eggs was removed shortly after the first application. On BFD+5, BFD+11, BFD+15 and BFD+21, the brood index was significantly different from the control (one-sided pooled t-test, $p \leq 0.05$). There were no significant differences in the compensation index of T compared to the control. The termination rate was significantly higher than the control on BFD+5, BFD+11, BFD+15 and BFD+21, with values between 32.76 to 80.61 and a mean value of 58.51 on BFD+21.

In the treatment group R, the effect of the reference item was detectable. At the assessment after application (BFD+5), the mean brood index was 2.01 and the mean compensation index was 2.02. Both indices are displaying a strong decrease on BFD+15 to 1.89 (brood index) and 1.90 and increased to 2.33 and 2.38 at the end of the observation period (BFD+21). The brood index and compensation index were significantly different from the control at BFD+15 and BFD+21. Consequently, high termination rates could be detected on BFD+15 (52.69) and BFD+21 (53.38). Both values are significantly different from the control (one-sided pooled t-test, $p \leq 0.05$).

Table 163
Honey bee brood/compensation indices

Treatment Group	Brood/Compensation Indices at 0, +5, +11, +15 and +21 days after brood area fixing day (BFD0) for marked cells (mean values of 4 replicates per treatment)				
	BFD0	BFD+5	BFD+11	BFD+15	BFD+21
Marked cells containing eggs (index value = 1) at the first assessment (BFD0):					
Mean C	1.00/1.00	2.12/2.15	2.94/3.01	2.87 /2.95	3.58/3.73
Mean T	1.00/1.00	1.39*/1.45	1.72*/1.84	1.70*/1.88	2.08*/2.59
Mean R	1.00/1.00	2.01/2.02	2.42/2.42	1.89*/1.90*	2.33*/2.38*
Marked cells containing young larvae (index value = 2) at the first assessment (BFD0):					
Mean C	2.00/2.00	3.03/3.04	2.85/2.85	2.89/2.97	3.56/3.66
Mean T	2.00/2.00	2.58/2.64	2.45/2.49	2.63/2.70	3.06/2.45*
Mean R	2.00/2.00	3.76/3.76	3.12/3.12	3.59/3.60	3.88/1.51*
Marked cells containing old larvae (index value = 3) at the first assessment (BFD0):					
Mean C	3.00/3.00	3.54/3.55	3.30/3.31	4.09/4.22	n.a.
Mean T	3.00/3.00	3.52/3.56	3.45/3.46	4.31/4.33	n.a.
Mean R	3.00/3.00	3.71/3.71	2.50*/2.50*	2.74*/3.06*	n.a.

BFD0 = Brood area fixing day

STD = standard deviation

* 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+21)

The termination rate of C reached a maximum value of 28.52 on BFD+21. The termination rate of T is increasing to 48.77 on BFD+5, 57.00 on BFD+11, 57.64 on BFD+15 and 58.51 on BFD+21. The values of BFD+5 to BFD+21 are statistically significantly higher than the respective values of C. The termination rate of R on BFD+15 (52.69) and BFD+21 (53.38) is statistically significantly higher than the control.

Overall, there was an effect of the indoxacarb technical treatment T on the brood index and termination rate of the eggs. There was also a clear impact in the reference item treatment R.

E. DEVELOPMENT OF THE YOUNG HONEY BEE LARVAE IN INDIVIDUAL CELLS

According to the development time of a worker honey bee from a young larvae to imago (adult bee) which normally averages approximately 15-17 days, it can be assumed that all young bees hatch until the assessment date BFD+21. Therefore, the study period covered one complete developmental cycle of the observed brood cells.

All treatment groups showed a successful development with rising brood index values from BFD0 to BFD+5, reaching values of 3.03 in C, 2.58 in T and 3.76 in R. The brood indices remained on a similar level during BFD+11 and BFD+15, which is not unusual because a normal developing bee is expected to be in the same stage (pupa) at all three assessments. On BFD+21, the brood index reached a similar level in all treatment groups, with 3.56 for C, 3.06 for T and 3.88 for R. There were no significant differences between the treatment groups.

The compensation index of the treatment groups developed in a similar way from BFD+5 to BFD+15. During this time, the values were in a range from 2.85 to 3.04 in C, from 2.49 to 2.70 in T and from 3.12 to 3.76 in R. There were no statistically significant differences during that period. The compensation index for young larvae in T and R on BFD+21 was significantly different from the control. To some extent this may be due to natural reasons, *i.e.*

early hatching of some of the brood before this assessment date. Therefore, this observation is not considered to be biologically relevant.

The termination rates of all treatment groups are on a similar level. The maximum termination rates are 28.83 for the control, 38.89 for T and 22.39 for R (BFD+21). There were no statistically significant differences between the treatment groups T and R compared to the control.

Overall, there was no significant effect of the indoxacarb technical treatment T on the brood index, compensation index and termination rate of young larvae while there was a clear impact in the reference item treatment R.

Table 164
Honey bee termination rates

Treatment Group	Termination rates at +5, +11, +15 and +21 days after brood area fixing day (BFD0) for marked cells (mean values of 4 replicates per treatment)			
	BFD+5	BFD+11	BFD+15	BFD+21
Marked cells containing eggs at the first assessment:				
Mean C	15.79	26.52	28.32	28.52
Mean T	48.77*	57.00*	57.64*	58.51*
Mean R	27.22	39.55	52.69*	53.38*
Marked cells containing young larvae at the first assessment:				
Mean C	23.15	28.83	28.83	28.83
Mean T	35.44	38.89	38.89	38.89
Mean R	5.93	22.02	22.21	22.39
Marked cells containing old larvae at the first assessment:				
Mean C	11.49	17.49	18.33	n.a.
Mean T	12.05	13.87	13.87	n.a.
Mean R	7.25	37.50*	45.18*	n.a.

BFD0= Brood area fixing day

STD = Standard deviation

* Significant 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+21)

F. DEVELOPMENT OF THE OLD HONEY BEE LARVAE IN INDIVIDUAL CELLS

According to the development time of a worker honey bee from an old larvae to imago (adult bee) which normally averages approximately 12-14 days, it can be assumed that almost all young bees already hatch until the assessment date BFD+15. Therefore, the study period covered one complete developmental cycle of the observed brood cells and no evaluation of the assessment on BFD+21 is 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 4.31 and 4.33 in T compared to 4.09 and 4.22 in the control. The termination rate at the end of development cycle of old larvae (BFD+15) was 13.87% in T compared to 18.33% 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+11 and BFD+15. At the end of the development cycle (BFD+15), the termination rate in R was 45.18% compared to 18.33% in the control.

Overall, there was no effect of the indoxacarb technical treatment T on the brood index, compensation index and termination rate of old larvae.

III. CONCLUSION

Daily feeding of indoxacarb technical in a 50% (w/w) sucrose solution (plus <1% acetone as a solvent) (200 mL per day) at a concentration of 100 µg indoxacarb/kg over a period of 9 days had transient effects during the feeding period on subsequent honey bee mortality (1DAF to 9DAF) and on honey bee behaviour (1DAF to 9DAF). These effects diminished quickly and completely after the end of feeding.

There was no effect of the indoxacarb technical treatments on the colony size, amount of brood, amount of food and colony condition.

The effects of the indoxacarb technical treatments on brood of different age were as follows:

Development of eggs: the brood index was lower and the termination rate was higher than the control on all assessments from BFD+5 to BFD+21. There was no effect on the compensation index.

Development of young larvae and old larvae: there was no effect of the indoxacarb technical treatments on the brood index, compensation index and termination rate of young larvae and old larvae.

(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).

There were four bee colonies per treatment group.

In this study with the technical active substance on bees (forager bees and brood), the following effects were identified:

- acute effects (mortality) on forager adult bees, which lasted more than 9 days
- effects on the brood (eggs and young larvae), which lasts for the duration of the study (21 days).

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

RMS partially agrees with the conclusions above. RMS considers that, for eggs and 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 the young larvae according to the study report). The brood / compensation indices for young larvae seem low even if not significantly different. 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).

Development of eggs: the brood index was lower and the termination rate was higher than the control on all assessments from BFD+5 to BFD+21.

Report: Berg, C. (2015); Indoxacarb (DPX-KN128) technical: A feeding study to evaluate effects on the brood of honey bees (*Apis Mellifera*, Hymenoptera, Apidae) in Germany 2015

DuPont Report No.: DuPont-43111

Guidelines: OEPP/EPPO Bulletin No. 22 (1992), OECD No. 75, (2007), EFSA (2013) **Deviations:** None

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

Testing Facility Report No.: S15-02382

GLP: Yes

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

Executive summary:

The effects of the test item Indoxacarb technical 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 recommendations by EFSA (2012). This study was conducted in Niefern-Öschelbronn in Southern Germany (region: Baden-Württemberg) from May to June 2015 and included a total of three treatment groups:

Indoxacarb technical treatment group T with the daily application of a feeding solution (200 mL 50% sucrose solution containing 0.5% acetone as a solvent 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 application of a feeding solution (200 mL 50% sucrose solution per hive per day) at a 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 200 mL 50% sucrose solution (containing 5% of acetone for comparison with T) per hive and day over a period of 9 days.

Feeding was done over a period of 9 days from 26-May-2015 (0DAF; DAF = days after first feeding) to 03-June-2015 (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) later 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 or in parallel by different personnel. 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 technical 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 (except one replicate in R with no young larvae present) 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 technical |
| Lot/Batch: | KN128-374 |
| Purity | 98.8% |

Description:	Solid
CAS#:	173584-44-6
Stability of test compound:	Not available
Reference item:	Insegar 25WG
Lot/Batch:	SMO1A411
Purity:	25.0% (w/w)
Description:	Insegar 25WG (Fenoxycarb)
CAS#:	72490-01-8
Stability in solution:	Sufficient for test purpose (at least 1 hour)
2. Vehicle and/or control:	50% (w/w) sucrose solution, plus <1% acetone in T and C
3. Test organism	
Species:	<i>Apis mellifera</i> L.
Age at dosing:	Direct exposure of adult honey bees trough feeding; indirect exposure of all stages of development
Source:	Eurofins Agrosience Services EcoChem GmbH, Niefern- Öschelbronn, Germany
Diet:	Honey bees are freely flying, feeding of test item, reference item and control as a 50% sucrose solution
4. Environmental conditions during the field phase (22 May 2015 – 23 June 2015):	
Temperature (min/max):	3.0–32.2°C
Relative Humidity:	35.1–100.0%
Photoperiod (exposure):	Natural light conditions

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed

21-May-2015 to 18-June-2015

2. Experimental treatments

Indoxacarb technical treatment group T with the daily application of a feeding solution (200 mL 50% sucrose solution containing 0.5% acetone as a solvent 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 application of a feeding solution (200 mL 50% sucrose solution per hive per day) at a 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 200 mL 50% sucrose solution (containing 5% of acetone for comparison with T) per hive and day over a period of 9 days.

3. Observations

The influence of the 9 test item applications of indoxacarb technical 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 (except one replicate in R with no young larvae present) 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.

II. RESULTS AND DISCUSSION

A. MORTALITY

Mortality after installation of the colonies at the test location until the first feeding of the sucrose test solution (4DBF to 0DBFev; DBF = days before first application of the feeding solution; ev = evening, shortly before the first feeding) was similar in all three treatment groups. Mean daily mortality was in the range from 8.5 to 27.5 dead honey bees/day in the control, from 5.0 to 21.8 dead honey bees/day in T and from 10.8 to 24.8 dead honey bees/day in R. Mean daily mortality over the whole period (4DBF to 0DBFev) was 16.8 dead honey bees/day in the control, 14.0 dead honey bees/day in T and 19.4 dead honey bees in R. There were no statistically significant differences between the three treatment groups in the period before the first application of the feeding solution (Tukey's Studentized Range test, $p \leq 0.05$).

During the period of daily feeding of the sucrose test solution (0DAF to 8DAF in the evening, corresponding to data from mortality assessments on 1DAF to 9DAF; DAF = days after first application of the feeding solution) the daily mortality values were in the range from 13.0 to 41.5 dead honey bees/day in the control, from 10.0 to 40.3 dead honey bees/day in T and from 13.8 to 42.8 dead honey bees/day in R. The mean daily mortality during this period (1DAF to 9DAF) was 28.0 dead honey bees/day in the control, 18.5 dead honey bees/day in T and 30.0 dead honey bees/day in R. There were no statistically significant differences between the test item treatment group T or the reference item group R compared to the control (pooled t-Test, $p \leq 0.05$).

During further observation of the colonies after the end for the feeding period (10DAF to 28DAF), daily mortality was in the range from 15.8 to 49.3 dead honey bees/day in the control, from 8.5 to 40.3 dead honey bees/day in T and from 24.5 to 359.8 dead honey bees/day in R. There were no statistically significant differences between the test item treatment group T compared to the control (pooled t-Test or Mann Whitney Exact test, $p \leq 0.05$). Mortality in the reference item treatment R was statistically significantly higher than the control on every day in the period from 13DAF to 28DAF (pooled t-Test, $p \leq 0.05$). Increased mortality in R during this period resulted mainly from large numbers of pupae that were removed from the combs and hives, an effect that could be expected from this reference item.

The mean daily mortality during the entire period after the applications of the feeding solution (10DAF to 28DAF) was 30.7 dead honey bees/day in the control, 21.4 dead honey bees/day in T (not statistically significant; (pooled t-Test) and 191.3 dead honey bees/day in R (statistically significant; pooled t-test, $p \leq 0.05$).

Overall, there was no effect of the Indoxacarb technical treatment T on mortality of adult honey bees, pupae or malformed bees or pupae during the period of daily feeding (1DAF to 9DAF) or afterwards (10DAF to 28DAF).

There was no effect of the test item treatments on the number of dead pupae and malformed bees and pupae found in the dead bee trap. Before the start of feeding (4DBF to 0DBFev), the mean total numbers were 1.3 dead pupae per hive in the control, 3.8 dead pupae in T and 6.0 dead pupae in R.

During the feeding period (1DAF to 9DAF), the mean total numbers of dead pupae and malformed bees and pupae per hive were 1.0 dead bees and pupae in C, 4.8 dead bees and pupae in T and 4.0 dead bees and pupae in R.

After the feeding period until the end of the observation period (10DAF to 28DAF), the mean total numbers of dead pupae and malformed bees and pupae per hive were 9.0 dead bees and pupae in C and 9.0 dead bees and pupae in T. There was a clear impact in R on the number of dead and malformed bees and pupae, with 3086.3 dead bees and pupae.

Overall, there was no effect of the indoxacarb technical treatment T on the mortality pupae and on malformations of the bees or pupae.

Table 165
Honey bee mean mortality - all life stages

Date	Timing	Mean Mortality (mean number of dead honey bees, larvae and pupae) per day and treatment group		
		C	T	R
22 May 2015	4DBF	8.5	10.3	10.8
23 May 2015	3DBF	27.5	20.0	24.8
24 May 2015	2DBF	17.5	13.0	21.0
25 May 2015	1DBF	9.0	5.0	13.8
26 May 2015	0DBF	17.0	13.8	22.0
26 May 2015	0DBFev	21.3	21.8	24.5
Mean 4DBF to 0DBF		16.8	14.0	19.4
STD		9.3	5.6	13.8
27 May 2015	1DAF	13.0	11.5	13.8
28 May 2015	2DAF	24.3	17.3	24.0
29 May 2015	3DAF	25.8	13.3	31.3
30 May 2015	4DAF	24.8	16.0	24.3
31 May 2015	5DAF	28.8	14.8	32.0
01 Jun 2015	6DAF	25.0	10.0	35.8
02 Jun 2015	7DAF	35.5	40.3	42.8
03 Jun 2015	8DAF	33.3	15.5	28.3
04 Jun 2015	9DAF	41.5	28.0	38.0
Mean 1DAF to 9DAF		28.0	18.5	30.0
STD		21.6	5.9	23.7
05 Jun 2015	10DAF	31.0	19.5	24.5
06 Jun 2015	11DAF	19.8	11.8	25.3
07 Jun 2015	12DAF	30.5	24.8	37.3
08 Jun 2015	13DAF	21.3	18.3	94.8*
09 Jun 2015	14DAF	49.3	40.3	248.3*
10 Jun 2015	15DAF	32.5	15.0	225.3*
11 Jun 2015	16DAF	45.3	26.5	223.5*
12 Jun 2015	17DAF	24.3	23.3	173.3*
13 Jun 2015	18DAF	23.3	24.5	228.8*
14 Jun 2015	19DAF	25.0	8.5	359.8*
15 Jun 2015	20DAF	18.8	8.5	227.0*
16 Jun 2015	21DAF	29.8	9.0	267.8*
17 Jun 2015	22DAF	15.8	13.8	234.3*
18 Jun 2015	23DAF	41.3	31.3	213.0*
19 Jun 2015	24DAF	48.8	39.0	233.5*
20 Jun 2015	25DAF	36.3	20.5	195.8*
21 Jun 2015	26DAF	37.0	20.8	208.3*
22 Jun 2015	27DAF	25.0	19.5	212.3*
23 Jun 2015	28DAF	28.3	32.3	202.0*
Mean 10DAF to 28DAF		30.7	21.4	191.3*
STD		12.9	4.2	49.1

DBF/DAF = Days before/after first feeding of sucrose test solution

ev = evening assessment shortly before the 1st application of the feeding solution

STD = Standard deviation

1DAF to 9DAF: Exposure to the feeding solution

10DAF to 28DAF: After exposure to the feeding solution

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

B. BEHAVIOUR OF THE HONEY BEES

In the period before the first feeding of the sugar solution, normal behaviour with very few exceptions were recorded in all treatments. There were 8 bees showing locomotion problems, 9 inactive bees, 2 cramping and 2 trembling bees in the control. In T, there were 4 bees with locomotion problems, 2 inactive, 5 cramping and 2 trembling bees. In R, 11 bees showing locomotion problems, 2 inactive and 3 cramping bees as well as one trembling bee could be observed. These observations are not related to any treatment since feeding of the test solution had not yet started, but they may result e.g. from guard bees stinging foreign bees trying to enter the hive.

In the period from 1DAF to 28DAF, no relevant changes in behaviour could be observed in the control, the test item treatment group T or in the reference item group R. There were 6 bees showing locomotion problems and 18 cramping bees in the control. In T, there were 13 bees with locomotion problems and 40 cramping bees. In R, there were 17 bees showing locomotion problems and 50 cramping bees. Considering that these numbers correspond to 4 replicates and 28 days, the numbers of bees showing unusual behaviour are very small and of no biological relevance, and they may result from natural reasons as described above.

Overall, there was no effect of the Indoxacarb technical treatment or the reference item group on honey bee behaviour during the 9-day period of daily feeding or afterwards up to 28 days after the first feeding.

C. BROOD DEVELOPMENT AND COLONY CONDITION

At the first assessment on 4DBF, all colonies were in a good and healthy condition with brood of all stages and food (nectar and pollen) present in all hives. Mean colony sizes (number of bees per hive) were similar in all three treatments: There were 10368 honey bees/hive in C, 12188 honey bees/hive in T and 9376 honey bees/hive in R.

During the study, the development of the colony sizes in C and T were similar and there was no effect of the test item. A slight decline of the colony sizes was observed in the treatment T at the next assessment on 0DBF (= BFD0), shortly before the first feeding of the sugar solution. In C and R, the colony size stayed on the same level. There were 10270 honey bees/hive in C, 10173 honey bees/hive in T and 9815 honey bees/hive in R.

On 6DAF, the colony size increased to 11716 honey bees/hive in C, 11846 honey bees/hive in T and 11928 honey bees/hive in R. On 11DAF, there was only a marginal increase in the colony size in all treatment groups. There were 12025 honey bees/hive in C, 12415 honey bees/hive in T and 12545 honey bees/hive in R. On 16DAF, the control group C and the treatment group T showed an increase in colony size whereas the colony size in R did not increase. There were 14755 honey bees/hive in C, 14999 honey bees/hive in T and 12529 honey bees/hive in R. On 22DAF, the colony size increased once again in C and T, whereas there was a decline in the reference item group R. The colony sizes were 16835 honey bees/hive in C, 17128 honey bees/hive in T and 10156 honey bees/hive in R. At the last assessment on 28DAF, the colony size in C reached 18054 honey bees/hive in C and 18460 in T. In R, the final value was 9929, indicating a clear impact of the reference item.

Overall, there was no negative effect of the indoxacarb technical treatment on the size of the colonies (mean number of honey bees per hive) whereas there was a clear effect of the reference item.

Brood of all stages (eggs, larvae, capped brood) was present in all colonies at all assessments during the study except the lack of eggs in Rb, in which no eggs were detected on 11DAF and 16DAF and no larvae were detected on 16DAF. At the first assessment on 4DBF, the mean total amount of brood cells (brood of all stages) was similar in the three treatment groups (28850 brood cells in C, 29000 brood cells in T and 26700 brood cells in R). In C and T, the further development of the amount of brood until the last assessment was similar, showing a slight decrease to 26550 brood cells in C and to 26350 brood cells in T at the 2nd assessment on 0DBF and increasing numbers of brood cells up to 34750 in C and 33600 in T on the last assessment on 28DAF. There was no effect of the test item treatment on the amount of brood compared to the control.

In the reference item treatment R, the number of brood cells decreased after 6DAF, reaching a minimum mean value of 14850 brood cells/hive on 16DAF and recovering only slightly until 28DAF (16000 brood cells/hive). This could be expected from this kind of reference item, confirming that the study design was appropriate to ensure exposure of the honey bees and their brood to the feeding solution.

Overall, there was no effect of the indoxacarb technical treatment on the amount of honey bee brood whereas there was a clear impact in the reference item treatment.

All colonies had sufficient amount of nectar and pollen stores throughout the study, except the replicates Cb, Cd and Tc which had no pollen on 0DBF.

D. 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 imago (adult bee) which normally averages approximately 21 days it can be assumed that almost all young bees hatch until the assessment date BFD+22. Therefore, the study covered one complete development cycle of the observed brood cells.

The colonies in the control as well as in the treatment group T showed a successful development with rising brood index values over the entire assessment period. The brood indices on BFD+16 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 4.11 and the mean compensation index was 4.40 on BFD+22. The termination rates on BFD+22 in were between 2.37 and 54.95 with a mean value of 17.78 in the control and between 1.84 and 9.82 in the treatment group T with a mean value of 4.58. There was no negative effect of the test item treatment on the development of the eggs.

In the treatment group R, the effect of the reference item was detectable. Whereas the brood indices were similar compared to the control up to BFD+11, the brood index decreased on BFD+16 to 2.47 and reached a final value of 1.63 on BFD+22. The compensation index for R was 2.49 on BFD+16 and 3.02 on BFD+22. The brood index was statistically significantly different from the control at BFD+22 (pooled t-test, ≤ 0.05). The final termination rate on BFD+22 was 67.50, which is statistically significantly higher compared to the control (pooled t-test, ≤ 0.05).

Overall, there was no effect of the indoxacarb technical treatment on the brood index and termination rate of the eggs. There was a clear impact in the reference item treatment.

Table 166
Honey bee brood/compensation indices

Treatment Group/Replicate	Brood/Compensation Indices at 0, +6, +11, +16 and +22 days after brood area fixing day (BFD0) for marked cells containing eggs at the first assessment				
	BFD0	BFD+6	BFD+11	BFD+16	BFD+22
Ca	1.00/1.00	3.07/3.09	3.66/3.71	3.66/3.72	4.52/4.68
Cb	1.00/1.00	3.15/3.15	3.85/3.85	3.83/3.83	4.79/4.83
Cc	1.00/1.00	1.90/1.95	1.95/2.03	1.80/2.14	2.25/3.16
Cd	1.00/1.00	3.22/3.22	3.92/3.92	3.92/3.92	4.88/4.93
Mean C	1.00/1.00	2.84/2.85	3.35/3.38	3.30/3.40	4.11/4.40
STD	0.00/0.00	0.63/0.60	0.94/0.90	1.01/0.85	1.25/0.83
Ta	1.00/1.00	3.34/3.34	3.93/3.93	3.93/3.93	4.91/4.93
Tb	1.00/1.00	3.54/3.54	3.87/3.87	3.85/3.87	4.82/4.90
Tc	1.00/1.00	3.50/3.50	3.88/3.88	3.88/3.89	4.85/4.93
Td	1.00/1.00	2.89/2.89	3.63/3.63	3.61/3.62	4.51/4.63
Mean T	1.00/1.00	3.32/3.32	3.83/3.83	3.82/3.83	4.77/4.85
STD	0.00/0.00	0.30/0.30	0.13/0.13	0.14/0.14	0.18/0.15
Ra	1.00/1.00	2.47/2.48	2.19/2.20	1.81/1.84	2.05/2.32
Rb	1.00/1.00	2.32/2.34	2.78/2.79	2.51/2.51	1.82/2.96
Rc	1.00/1.00	3.14/3.14	3.93/3.93	3.57/3.57	1.24/3.81
Rd	1.00/1.00	2.93/2.93	3.44/3.44	2.00/2.05	1.39/2.97
Mean R	1.00/1.00	2.72/2.72	3.09/3.09	2.47/2.49	1.63*/3.02*
STD	0.00/0.00	0.38/0.38	0.76/0.76	0.79/0.77	0.38/0.61

BFD0 = Brood area fixing day

STD = Standard deviation

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

Development of Young Honey Bee Larvae in Individual Cells

According to the development time of a worker honey bee from a young larvae to imago (adult bee) which normally averages approximately 15-17 days, it can be assumed that all young bees hatch until the assessment date BFD+22. Therefore, the study period covered one complete developmental cycle of the observed brood cells. The Compensation Index for young larvae at BFD+22 should be interpreted with care, since low values can occur if the development cycle has already been completed successfully at BFD+16.

All treatment groups showed a successful development with rising brood index values from BFD0 to BFD+6, reaching values of 3.77 in C, 3.89 in T and 3.65 in R. The brood indices remained on a similar level during BFD+11 and BFD+16, which is not unusual because a normal developing bee is expected to be in the same stage (pupa) at all three assessments. On BFD+22, the brood index reached a similar level in the treatment groups C and T, with 4.45 for C and 4.79 for T. There were no significant differences between the treatment groups C and T. The brood index of R reached a final value of 3.77. There were no statistically significant differences compared to the control (pooled t-test or Satterthwaite's test, ≤ 0.05).

The compensation index of the treatment groups developed in a similar way from BFD+6 to BFD+16. On BFD+16, the compensation indices were 4.21 in C, 4.48 in T and 3.77. On BFD+22, the compensation indices were 2.84 in C, 3.06 in T and 3.06 in R. The lower values indicate that some of the marked cells have already completed a development cycle at BFD+16. There were no statistically significant differences between the treatment groups (pooled t-test or Satterthwaite's test, ≤ 0.05).

The termination rates of all treatment groups were on a similar level. The mean termination rates were 11.09 for the control, 4.30 for T and 24.61 for R (BFD+22). There were no statistically significant differences between the treatment groups T and R compared to the control.

Overall, there was no significant effect of the indoxacarb technical treatment on the brood index, compensation index and termination rate of young larvae. There was no statistically significant effect on the development of young larvae in the reference item group.

Table 167
Honey bee termination rates for young larvae

Treatment Group/Replicate	Brood/Compensation Indices at +6, +11, +16 and +22 days after brood area fixing day (BFD0) for marked cells containing young larvae at the first assessment				
	BFD0	BFD+6	BFD+11	BFD+16	BFD+22
Ca	2.00/2.00	3.98/3.99	3.96/3.98	4.46/4.50	4.91/3.09
Cb	2.00/2.00	3.95/3.95	3.85/3.85	4.66/4.69	4.73/1.95
Cc	2.00/2.00	3.34/3.38	2.88/2.94	3.17/3.40	3.56/3.05
Cd	2.00/2.00	3.82/3.84	3.69/3.73	4.15/4.24	4.59/3.28
Mean C	2.00/2.00	3.77/3.79	3.60/3.63	4.11/4.21	4.45/2.84
STD	0.00/0.00	0.30/0.28	0.49/0.47	0.66/0.57	0.61/0.60
Ta	2.00/2.00	3.98/3.98	3.98/3.98	4.74/4.74	4.96/2.46
Tb	2.00/2.00	3.89/3.89	3.77/3.77	4.40/4.43	4.71/3.42
Tc	2.00/2.00	3.71/3.76	3.69/3.79	3.99/4.10	4.60/3.94
Td	2.00/2.00	3.97/3.97	3.91/3.91	4.63/4.64	4.87/2.43
Mean T	2.00/2.00	3.89/3.90	3.84/3.86	4.44/4.48	4.79/3.06
STD	0.00/0.00	0.13/0.10	0.13/0.10	0.33/0.28	0.16/0.74
Ra	2.00/2.00	3.88/3.88	3.64/3.65	3.98/4.08	4.18/2.99
Rb	2.00/2.00	3.20/3.23	2.96/3.00	3.23/3.26	3.45/2.45
Rc	2.00/2.00	3.86/3.86	3.80/3.80	3.96/3.96	3.68/3.75
Rd	---/---	---/---	---/---	---/---	---/---
Mean R	2.00/2.00	3.65/3.66	3.47/3.48	3.72/3.77	3.77/3.06
STD	0.00/0.00	0.39/0.37	0.45/0.43	0.43/0.44	0.37/0.65

BFD0 = Brood area fixing day

STD = Standard deviation

Development of Old Honey Bee Larvae in Individual Cells

According to the development time of a worker honey bee from an old larvae to imago (adult bee) which normally averages approximately 12-14 days, it can be assumed that almost all young bees already hatch until the assessment date BFD+16. Therefore, the study period covered one complete developmental cycle of the observed brood cells and no evaluation of the assessment on BFD+22 is 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+16 were 4.01 and 4.34 in T compared to 4.47 and 4.55 in the control. The termination rate at the end of development cycle of old larvae (BFD+16) was 19.90% in T compared to 10.67% in the control. None of these differences was statistically significant (pooled t-test, ≤ 0.05).

In the reference item treatment R, the brood index on BFD+16 was 3.39, which is significantly lower compared to the control. The compensation index on BFD+16 was 4.46 and not statistically significantly different compared to the control (pooled t-test, ≤ 0.05). The termination rate was 32.12% and statistically significantly higher compared to the control (pooled t-test, ≤ 0.05).

Overall, there was no effect of the indoxacarb technical treatment on the brood index, compensation index and termination rate of old larvae.

There was an impact in the reference item treatment.

Table 168
Honey bee termination rates for old larvae

Treatment Group/Replicate	Brood/Compensation Indices at +6, +11, +16 and +22 days after brood area fixing day (BFD0) for marked cells ¹) containing old larvae at the first assessment				
	BFD0	BFD+6	BFD+11	BFD+16	BFD+22
Ca	3.00/3.00	3.96/3.97	3.95/3.95	4.93/4.94	n.a.
Cb	3.00/3.00	3.93/3.93	3.91/3.92	4.89/4.91	n.a.
Cc	3.00/3.00	3.53/3.55	3.18/3.18	3.95/4.12	n.a.
Cd	3.00/3.00	3.29/3.29	3.27/3.27	4.09/4.24	n.a.
Mean C	3.00/3.00	3.68/3.69	3.58/3.58	4.47/4.55	n.a.
STD	0.00/0.00	0.32/0.32	0.41/0.41	0.52/0.43	n.a.
Ta	3.00/3.00	3.97/3.97	3.97/3.97	4.96/4.96	n.a.
Tb	3.00/3.00	3.65/3.70	3.59/3.65	4.49/4.62	n.a.
Tc	3.00/3.00	1.33/1.66	1.33/2.02	1.67/2.87	n.a.
Td	3.00/3.00	3.94/3.94	3.92/3.92	4.90/4.90	n.a.
Mean T	3.00/3.00	3.22/3.32	3.20/3.39	4.01/4.34	n.a.
STD	0.00/0.00	1.27/1.11	1.26/0.92	1.57/0.99	n.a.
Ra	3.00/3.00	3.85/3.85	3.71/3.74	4.17/4.59	n.a.
Rb	3.00/3.00	3.78/3.80	3.49/3.49	3.40/4.17	n.a.
Rc	3.00/3.00	3.97/3.97	3.92/3.92	2.80/4.48	n.a.
Rd	3.00/3.00	3.94/3.94	3.94/3.94	3.20/4.58	n.a.
Mean R	3.00/3.00	3.89/3.89	3.77/3.77	3.39/4.46	n.a.
STD	0.00/0.00	0.09/0.08	0.21/0.21	0.58/0.20	n.a.

BFD0 = Brood area fixing day

STD = Standard deviation

n.a. = not applicable (development cycle of old larvae completed before BFD+22)

III. CONCLUSION

Daily feeding of indoxacarb technical in a 50% (w/w) sucrose solution (200 mL per day, containing 0.5% acetone as a solvent) at a concentration of 100 µg indoxacarb/kg over a period of 9 days had had no effect on honeybee mortality.

There was no effect on the behaviour of the honey bees during or after the feeding of indoxacarb technical.

There was no effect on the colony size, the amount of brood cells or the colony condition following the feeding of indoxacarb technical.

Daily feeding of indoxacarb technical had no effect on the development in the individually marked cells of eggs, young larvae or old larvae.

Overall, Daily feeding of indoxacarb technical in a 50% (w/w) sucrose solution (200 mL per day, containing 0.5% acetone as a solvent) at a concentration of 100 µg indoxacarb/kg over a period of 9 days had caused no relevant effect on honey bee colonies.

(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). 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.

B.9.3.2. Effects on non-target arthropods other than bees

Non target arthropod data were conducted with the representative formulation, Indoxacarb 150 g/L EC to fulfil the requirements of this data point. Based on the SETAC ESCORT (2001) guidance and the Barrett *et al.*, 1994 SETAC “Guidance Document on Regulatory Testing Procedures for Pesticides and Non-Target Arthropods,” the formulated product is the most relevant consideration for exposure of non-target arthropods under field conditions. Please refer to the RAR Volume_3-CP for the study summaries.

Report: Mead-Briggs, M., Vinall, S. (1997a); DPX-MP062 150 SC and 30 WG: A laboratory study comparing the effects of two formulations over a range of doses on the parasitoid *Aphidius colemani*

DuPont Report No.: AMR 4681-97

Guidelines: SETAC-ESCORT (1994)

- | | |
|-------------------|--------------------------------------|
| 1. Test material: | DPX-MP062 150 g/L SC, DPX-MP062 30WG |
| Lot/Batch #: | MP062-141, MP062-39 |
| Purity: | 150 g a.s./L, 300 g a.s./kg |

The effects on *Aphidius colemani* study AMR 4681-97, conducted with test material DPX-MP062 150 g/L SC and DPX-MP062 30WG, was conducted under guideline SETAC-ESCORT (1994). A review of this study indicates that it fully meets the current guideline (M.P. Candolfi, S. Blümel, R. Forster *et al.* (2000): *Guidelines to evaluate side-effects of plant protection products to non-target arthropods. IOBC, BART and EPPO Joint Initiative*. ISBN: 92-9067-129-7.). However, since the study is conducted with test material DPX-MP062 150 g/L SC and DPX-MP062 30WG the study is

not relevant for the EU renewal of indoxacarb as data is available on the representative formulation, Indoxacarb 150 g/L EC.

RMS comment

Study summarised in Indoxacarb DAR, Volume 3, B9, 2000. Since the study is conducted with test material DPX-MP062 150 g/L SC and DPX-MP062 30WG the study is not relevant for the EU renewal of indoxacarb as data is available on the representative formulation, Indoxacarb 150 g/L EC. No summary was provided for this Annex I renewal.

Report: Mead-Briggs, M., Vinall, S. (1997b); DPX-MP062 150 SC and 30 WG: An extended laboratory study comparing the effects of two formulations on the parasitoid *Aphidius colemani*

DuPont Report No.: AMR 4683-97

Guidelines: SETAC-ESCORT (1994)

- | | |
|-------------------|--------------------------------------|
| 1. Test material: | DPX-MP062 150 g/L SC, DPX-MP062 30WG |
| Lot/Batch #: | MP062-141, MP062-39 |
| Purity: | 150 g a.s./L, 300 g a.s./kg |

The effects on *Aphidius colemani* study AMR 4683-97, conducted with test material DPX-MP062 150 g/L SC and DPX-MP062 30WG, was conducted under guideline SETAC-ESCORT (1994). A review of this study indicates that it fully meets the current guideline M.P. Candolfi, S. Blümel, R. Forster et al. (2000): *Guidelines to evaluate side-effects of plant protection products to non-target arthropods. IOBC, BART and EPPO Joint Initiative*. ISBN: 92-9067-129-7. However, since the study is conducted with test material DPX-MP062 150 g/L SC and DPX-MP062 30WG the study is not relevant for the EU renewal of indoxacarb as there is data available on the representative formulation, DPX-KN128 150 g/L EC, and therefore not relied upon.

RMS comment

Study summarised in Indoxacarb DAR, Volume 3, B9, 2000. Since the study is conducted with test material DPX-MP062 150 g/L SC and DPX-MP062 30WG the study is not relevant for the EU renewal of indoxacarb as data is available on the representative formulation, Indoxacarb 150 g/L EC. No summary was provided for this Annex I renewal.

Report: Vinall, S. (1997a); DPX-MP062 150 SC and 30 WG: A laboratory study comparing the effects of two formulations over a range of doses on the predatory mite *Typhlodromus pyri*

DuPont Report No.: AMR 4680-97

Guidelines: SETAC-ESCORT (1994), OVERMEER (1988)

- | | |
|-------------------|--------------------------------------|
| 1. Test material: | DPX-MP062 150 g/L SC, DPX-MP062 30WG |
| Lot/Batch #: | MP062-141, MP062-39 |
| Purity: | 150 g a.s./L, 300 g a.s./kg |

The effects on *Typhlodromus pyri* study AMR 4680-97, conducted with test material DPX-MP062 150 g/L SC and DPX-MP062 30WG, was conducted under guidelines SETAC-ESCORT (1994) and OVERMEER (1988). A review of this

study indicates that it fully meets the current guideline M.P. Candolfi, S. Blümel, R. Forster et al. (2000): *Guidelines to evaluate side-effects of plant protection products to non-target arthropods. IOBC, BART and EPPO Joint Initiative*. ISBN: 92-9067-129-7. However, since the study is conducted with test material DPX-MP062 150 g/L SC and DPX-MP062 30WG the study is not relevant for the EU renewal of indoxacarb as there is data available on the representative formulation, DPX-KN128 150 g/L EC, and therefore not relied upon.

RMS comment

Study summarised in Indoxacarb DAR, Volume 3, B9, 2000. Since the study is conducted with test material DPX-MP062 150 g/L SC and DPX-MP062 30WG the study is not relevant for the EU renewal of indoxacarb as data is available on the representative formulation, Indoxacarb 150 g/L EC. No summary was provided for this Annex I renewal.

Report: Vinall, S. (1997b); DPX-MP062 150 SC and 30 WG: An extended laboratory study comparing the effects of two formulations on the predatory mite *Typhlodromus pyri*

DuPont Report No.: AMR 4682-97

Guidelines: SETAC-ESCORT (1994)

- | | |
|-------------------|--------------------------------------|
| 1. Test material: | DPX-MP062 150 g/L SC, DPX-MP062 30WG |
| Lot/Batch #: | MP062-141, MP062-39 |
| Purity: | 150 g a.s./L, 300 g a.s./kg |

The effects on *Typhlodromus pyri* study AMR 4682-97, conducted with test material DPX-MP062 150 g/L SC and DPX-MP062 30WG, was conducted under guideline SETAC-ESCORT (1994). A review of this study indicates that it partially meets the current guideline M.P. Candolfi, S. Blümel, R. Forster et al. (2000): *Guidelines to evaluate side-effects of plant protection products to non-target arthropods. IOBC, BART and EPPO Joint Initiative*. ISBN: 92-9067-129-7; deviations include reproduction was not assessed, only mortality. However, reconducting the study is unlikely to yield a significantly different result because of no effect on reproduction seen at tier 1 in the previous study. However, since the study is conducted with test material DPX-MP062 150 g/L SC and DPX-MP062 30WG the study is not relevant for the renewal of indoxacarb as there is data available on the representative formulation, DPX-KN128 150 g/L EC, and therefore not relied upon.

RMS comment

Study summarised in Indoxacarb DAR, Volume 3, B9, 2000. Since the study is conducted with test material DPX-MP062 150 g/L SC and DPX-MP062 30WG the study is not relevant for the EU renewal of indoxacarb as data is available on the representative formulation, Indoxacarb 150 g/L EC. No summary was provided for this Annex I renewal.

B.9.4. EFFECTS ON NON-TARGET SOIL MESO- AND MACROFAUNA

B.9.4.1. Earthworm – sub-lethal effects

Report: Wachter, S. (1996b); Acute toxicity of DPX-MP062 technical on earthworms, *Eisenia foetida* using an artificial soil test

DuPont Report No.: AMR 3968-96

Guidelines: OECD 207, 87/302/EEC (1987) **Deviations:** None

Testing Facility: GAB Biotechnologie, GmbH, Neifern-Oschelbronn, Germany

Testing Facility Report No.: 96193/01-NLEf

GLP: Yes

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

I. MATERIALS AND METHODS

A. MATERIALS

- | | |
|--|--|
| 1. Test material: | DPX-MP062 technical |
| Lot/Batch #: | MP062-51A |
| Purity: | 94.54% |
| Description: | Off-white powder |
| CAS#: | DPX-MP062: 144171-61-9
DPX-KN128: 173584-44-6 |
| Stability of test compound: | Not determined in the test system |
| 2. Control: | Untreated (and moistened with deionised water) |
| Test vehicle: | Fine quartz sand |
| Toxic reference: | 2-Chloroacetamide |
| 3. Test organism: | Earthworm |
| Species: | <i>Eisenia foetida</i> |
| Age at dosing: | 2 months; life stage: adult with clitellum |
| Weight at dosing: | 300 to 600 mg |
| Test chamber: | 1-L bottling jars, loosely covered by glass lids, filled with approximately 500 g (dry weight equivalent) artificial soil. |
| Test medium: | Artificial soil prepared according to OECD 207 |
| Diet: | Unfed during test |
| Water content of soil: | Test initiation: 33.7% to 37.4%
Test termination: 33.5 to 38.5% |
| Soil pH: | Test initiation: 6.5; Test termination: 6.2–6.3 |
| 4. Environmental conditions (in-life period) | |
| Temperature: | 20 ± 2°C |
| Photoperiod: | 24-hour photoperiod (400 to 800 lux) |

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
08-August-1996 to 25-September-1996
2. Test system
The acute toxicity of DPX-MP062 to earthworms, *Eisenia foetida*, was determined during a 14-day exposure period in a laboratory study. Four replicates of 10 clitellated adult earthworms were exposed in approximately 642 gram wet weight artificial soil (69% sand, 20% clay, 10% peat moss, approximately 1% calcium carbonate) treated with DPX-MP062. Nominal concentrations were 0, 125, 223, 395, 703, and 1250 mg DPX-MP062/kg dry weight of soil, and were added to the soil as a water solution. Control soil had an equivalent amount of water added to it and was replicated four times with 10 earthworms per replicate.

During the test, soil temperature was $20 \pm 2^\circ\text{C}$, soil moisture ranged between 34.0–38.5%, and soil pH was 6.2 to 6.5 under continuous light. Worms were assessed for mortality and behavioural effects after 7 and 14 days. Each batch of 10 earthworms was weighed at the beginning and end of the test to determine any treatment-related effect on body weight.

II. RESULTS AND DISCUSSION

A. FINDINGS

Cumulative mortality, mean body weight, and average weight change values are provided in Table 169. The NOEC was determined with a Dunnett's test.

Table 169
Summary of mortality and body weight changes in earthworms exposed to DPX-MP062 for 14 days following application to soil

Concentration mg DPX-MP062/kg	Cumulative mortality (%)		Mean body weight/earthworm (mg)		Average weight change (%)
	Day 0	Day 14	Day 0	Day 14	
0 (water control)	0	0	38	32	-16
125	0	0	38	32	-16
223	0	0	38	30	-21
395	0	0	38	32	-16
703	0	0	38	31	-18
1250	0	0	39	31	-21 ^a

^a Significant at $p < 0.05$, Dunnett's test

III. CONCLUSION

The 14-day LC_{50} was >1250 mg DPX-MP062/kg dry soil. The NOEC = 703 mg DPX-MP062/kg dry soil based on body weight.

(Wachter, S., 1996b)

RMS comment

This study was submitted in the original DAR (mistakenly listed as study number AMR 3698-96 in the Indoxacarb DAR, Volume 3, B9, 2000). This study was conducted in compliance with the current guideline. The 14-day LC_{50} was >1250 mg DPX-MP062/kg dry soil. This study is still considered acceptable but only informative as acute toxicity studies are not required anymore.

Report: Noack, M. (2001); DPX-MP062 30 WG: Effects on reproduction and growth of the earthworm, *Eisenia foetida* (Savigny, 1826) in artificial soil

DuPont Report No.: DuPont-3864

Guidelines: ISO 11268-3 (1998), BBA VI 2-2 (1994) **Deviations:** None

- Test material: DPX-MP062 30WG
Lot/Batch #: MP062-171
Purity: 300 g a.s./kg

The earthworm – sub-lethal effects study DuPont-3864 was conducted with test material DPX-MP062 30WG, under guidelines ISO 11268-3 (1998) and BBA VI 202 (1994). Since the study was conducted with DPX-MP062 30WG, which is not the representative formulation for this renewal dossier, the study is not relevant for the EU renewal of indoxacarb and therefore not relied upon.

RMS comment

Study summarised in Indoxacarb DAR, Volume 3, B9, AD3 2005. Since the study was conducted with DPX-MP062 30WG, which is not the representative formulation for this renewal dossier, the study is not relevant for the EU renewal of indoxacarb. No summary was provided for this Annex I renewal.

Report: Pavic, B. (2013); Indoxacarb (DPX-KN128) technical: Effects on reproduction and growth of the earthworm, *Eisenia fetida*, in artificial soil with 5% peat

DuPont Report No.: DuPont-36101

Guidelines: OECD 222 (2004), ISO 11268-2 (1998) **Deviations:** None

Testing Facility: IBACON, Rossdorf, Germany

Testing Facility Report No.: 75243022

GLP: Yes

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

Executive summary:

The sublethal toxicity of indoxacarb (DPX-KN128) to earthworms, *Eisenia fetida*, was determined in a 56-day soil exposure laboratory study according to OECD 222, 2004 and ISO 11268-2, 1998. Adult earthworms were exposed to artificial soil (prepared according to OECD 222 but with reduced organic matter) treated with indoxacarb to obtain the nominal concentrations of 9.40, 16.91, 30.48, 54.80, and 98.64 mg a.s./kg dry artificial soil (corresponding to 9.0, 16.2, 29.2, 52.5, and 94.5 mg a.s./kg dry artificial soil, adjusted for purity) and to an untreated control (acetone treated quartz sand moistened with deionised water only). Mortality and growth (body weight) of the earthworm 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 estimated to be greater than 94.5 mg a.s./kg soil dry weight. The overall NOEC (No-Observable-Effect Concentration) for earthworms was determined to be the nominal concentration of 29.2 mg a.s./kg soil dry weight.

I. MATERIALS AND METHODS

A. MATERIALS

- | | |
|--|---|
| 1. Test material | Indoxacarb technical |
| Lot/Batch #: | DPX-KN128-098 |
| Purity: | 95.8% |
| Description: | Solid |
| CAS #: | 173584-44-6 |
| Stability of test compound: | Not determined in the test system |
| 2. Control: | Untreated (using the same amount of acetone treated sand as in the test item groups and moistened with deionised water) |
| Test vehicle: | Acetone |
| Toxic reference: | Carbendazim |
| 3. Test organism | Earthworm |
| Species: | <i>Eisenia fetida</i> |
| Age at dosing: | 11 to 12 months |
| Weight at dosing: | 301 to 591 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 <i>ca.</i> 500 g artificial soil dry weight |
| Test medium: | Artificial soil prepared according to OECD 222 but with reduced content of peat (5%), maximum water holding capacity of the artificial soil, as measured: 41% |
| Diet: | Finely ground cattle manure |
| Water (deionised) content of soil: | Initiation: 21.2 to 21.7% (equivalent to 51.8 to 53.0% of the maximum water holding capacity)
Termination: 21.8 to 23.6% (equivalent to 53.2 to 57.6% of the maximum water holding capacity) |
| Soil pH: | 6.4 at test start and 6.5 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-2012 to 25-January-2013

2. Experimental treatments

The sublethal toxicity of indoxacarb 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 but with reduced organic matter) treated with indoxacarb to obtain the nominal concentrations of 9.40, 16.91, 30.48, 54.80, and 98.64 mg a.s./kg dry artificial soil (corresponding to 9.0, 16.2, 29.2, 52.5, and 94.5 mg a.s./kg dry artificial soil, adjusted for purity) and to an untreated control (acetone treated quartz sand moistened with deionised 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 2012 to October 2012.

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 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 or Kolmogorov-Smirnov test and Levene's test ($\alpha = 0.05$). Further statistical evaluation of the NOEC for weight changes and reproduction was performed using Williams t-test (multiple comparison, $\alpha = 0.05$, two-sided for weight changes, one-sided smaller for reproduction). The LC_{50} after 28 days was not determined by statistical analysis as no mortality was observed.

II. RESULTS AND DISCUSSION

A. FINDINGS

No mortality was observed in any treatment group. The LC_{50} after 28 days was estimated to be greater than 94.5 mg a.s./kg soil dry weight. The food consumption of earthworms exposed to the test rates of indoxacarb was comparable to the control. No adverse behavioural effects were observed after 28 days 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. No statistically significant differences in reproduction (56-day assessment) of earthworms compared to the control were observed up to and including the test concentration of 29.2 mg a.s./kg dry soil. Cumulative mortality and weight change (of adults) at 28 days, and reproduction at 56 days are reported in Table 170.

Table 170
Sublethal toxicity of indoxacarb technical to earthworms *Eisenia fetida*

Indoxacarb concentration (mg a.s./kg soil dry weight adjusted for purity)	28-day mortality (%) mean	28-day weight change (%) mean ^a	56-day reproduction (# of juveniles) mean ^b
Control (0.0)	0	23.9	248
9.0	0	32.1 n.s.	249 n.s.
16.2	0	24.3 n.s.	262 n.s.
29.2	0	29.8 n.s.	213 n.s.
52.5	0	30.3 n.s.	180 *
94.5	0	24.5 n.s.	165 *

* Significant different from the control

n.s. Not significantly different from the control

^a Williams t-test, two sided, $\alpha = 0.05$

^b Williams test, one-sided smaller, $\alpha = 0.05$

III. CONCLUSIONS

Indoxacarb technical had no lethal effects or significant effects on growth and feeding activity of the earthworm *Eisenia fetida* when exposed to concentrations up to and including 94.5 mg a.s./kg artificial soil dry weight. Indoxacarb technical had no significant effects on reproduction of the earthworm *Eisenia fetida* when exposed to concentrations up to and including 29.2 mg a.s./kg artificial soil dry weight.

The overall NOEC (No-Observable-Effect Concentration) for indoxacarb technical was determined to be 29.2 mg a.s./kg dry artificial soil and the LOEC (Lowest-Observable-Effect Concentration) was determined to be 52.5 mg a.s./kg dry artificial soil.

The LC₅₀ for indoxacarb technical after 28 days was estimated to be greater than 94.5 mg a.s./kg soil dry weight, the highest concentration tested.

(Pavić, B., 2013)

RMS comment

This study is valid.

Indoxacarb technical had no lethal effects or significant effects on growth and feeding activity of the earthworm *Eisenia fetida* when exposed to concentrations up to and including 94.5 mg a.s./kg artificial soil dry weight. Indoxacarb technical had no significant effects on reproduction of the earthworm *Eisenia fetida* when exposed to concentrations up to and including 29.2 mg a.s./kg artificial soil dry weight.

The overall NOEC (No-Observable-Effect Concentration) for indoxacarb technical was determined to be 29.2 mg a.s./kg dry artificial soil and the LOEC (Lowest-Observable-Effect Concentration) was determined to be 52.5 mg a.s./kg dry artificial soil.

RMS calculated an EC10 value of 23.95 mg a.s./kg dry artificial soil (95% confidence intervals: 3.78-66.64) based on effects on reproduction. The EC10 is used in the risk assessment.

Report: Wachter, S. (1996a); Acute toxicity of IN-JT333 technical on earthworms, *Eisenia foetida* using an artificial soil test

DuPont Report No.: AMR 3826-96

Guidelines: OECD 207, 87/302/EEC (1987) **Deviations:** None

Testing Facility: GAB Biotechnologie, GmbH, Neifern-Oschelbronn, Germany

Testing Facility Report No.: 96192/01-NLEf

GLP: Yes

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: IN-JT333 technical metabolite>
Lot/Batch #: JT333-20
Purity: 98.7% by analysis
Description: White solid
CAS#: Not given
Stability of test compound: Stable at normal temperatures and storage conditions
2. Control: Deionised water
Test vehicle: Fine quartz sand
Toxic reference: 2-chloroacetamide
3. Test organism: Earthworm
Species: *Eisenia foetida*
Age at dosing: Approximately 2 month old adults with clitellum
Weight at dosing: 301 to 598 mg
Source: Laboratory, in-house culture (Laboratory: Arbeitsgemeinschaft, GAB Biotechnologie GmbH & IFU Umweltanalytik GmbH, Niefern-Öschelbronn, Germany)
Test chamber: 1-L glass vessels, loosely covered by glass-lids, filled with approximately 500 g (dry weight equivalent) artificial soil.
Test medium: Artificial soil prepared according to OECD 207
Diet: Unfed during test
Water content of soil: Test initiation: 35.1% to 37.1%
Test termination: 32.1 to 33.7%
Soil pH: Test initiation: 6.5; Test termination: 6.5
4. Environmental conditions (in-life period)
Temperature: 18 to 22°C
Photoperiod: 24 hour photoperiod (400 to 800 lux)

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
08-August-1996 to 11-September-1996

2. Experimental treatments

The acute toxicity of IN-JT333 to earthworms, *Eisenia foetida*, was determined during a 14-day exposure period in a laboratory study. Four replicates of 10 clitellated adult earthworms were exposed in approximately 642 gram wet weight artificial soil (69% sand, 20% clay, 10% peat moss, approximately 1% calcium carbonate) treated with IN-JT333. Nominal concentrations were 0, 100, 178, 316, 562, and 1000 mg IN-JT333/kg dry weight of soil and were added to the soil as a water solution. Control soil had an equivalent amount of water added to it. During the test, soil temperature was $20 \pm 2^\circ\text{C}$, soil moisture ranged between 32.1-37.1%, and soil pH was 6.5 to 6.6 under continuous light. Worms were assessed for mortality and behavioural effects after 7 and 14 days. Each batch of ten earthworms was weighed at the beginning and end of the test to determine any treatment-related effect on body weight.

II. RESULTS AND DISCUSSION

A. FINDINGS

Cumulative mortality, mean body weight, and average weight change values are provided in Table 171. The NOEC was determined with a Dunnett's test.

Table 171
Summary of mortality and body weight changes in earthworms exposed to IN-JT333 for 14 days following application to soil

Concentration (mg/kg)	Cumulative mortality (%)		Mean body weight/earthworm (mg)		Average weight change (%)
	Day 0	Day 14	Day 0	Day 14	
0 (water control)	0	0	40	34	-15
100	0	0	39	32	-18
178	0	0	38	31	-18
316	0	0	39	32	-18
562	0	0	39	31	-21*
1000	0	0	39	31	-21*

* significant at $p < 0.05$, Dunnett's test

III. CONCLUSION

The 14-day LC_{50} was >1000 mg IN-JT333/kg dry soil. The NOEC = 316 mg IN-JT333/kg dry soil based on body weight.

(Wachter, S., 1996a)

RMS comment

This study was submitted in the original DAR. This study was conducted in compliance with the current guideline. The 14-day LC_{50} was >1000 mg IN-JT333/kg dry soil. This study is still considered acceptable but only informative as acute toxicity studies are not required anymore.

Report: Lührs, U. (2013c); IN-JT333: Effects on reproduction and growth of the earthworm, *Eisenia fetida*, in artificial soil with 5% peat

DuPont Report No.: DuPont-36494

Guidelines: OECD 222 (2004), ISO 11268-2 (1998) **Deviations:** None

Testing Facility: IBACON, Rossdorf, Germany

Testing Facility Report No.: 75253022

GLP: Yes

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

Executive summary:

The sublethal toxicity of IN-JT333 to earthworms, *Eisenia fetida*, was determined in a 56-day soil exposure laboratory study according to OECD 222, 2004 and ISO 11268-2, 1998. Adult earthworms were exposed to artificial soil (prepared according to OECD 222 but with reduced organic matter) treated with the test item to obtain the nominal concentrations of 6.33, 12.65, 25.30, 50.61, and 101.2 mg IN-JT333/kg dry artificial soil (corresponding to 6.25, 12.5, 25.0, 50.0, and 100 mg IN-JT333/kg dry artificial soil, adjusted for purity) and to an untreated control (using the same amount of

acetone and quartz sand as in the test item treated groups moistened with deionised water). Mortality and growth (body weight) of the earthworm were assessed after 28 days and the effect on reproduction (number of juveniles produced) was assessed after 56 days. The LC_{50} after 28 days was determined to be greater than 100 mg IN-JT333/kg dry artificial soil. The NOAEC (No-Observable-Adverse-Effect Concentration) for earthworms based on mortality, reproduction, growth and nominal concentrations was 100 mg IN-JT333/kg dry artificial soil, the highest concentration tested.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material	IN-JT333 technical metabolite
Lot/Batch #:	JT333-017
Purity:	98.8%
CAS #:	144171-39-1
Stability of test compound:	Not determined in the test system
2. Control:	Untreated (using the same amount of acetone and sand as in the test item groups and moistened with deionised water)
Test vehicle:	Acetone
3. Test organism	Earthworm
Species:	<i>Eisenia fetida</i>
Age at dosing:	Approximately 8 months
Weight at dosing:	315 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 <i>ca.</i> 500 g artificial soil dry weight
Test medium:	Artificial soil prepared according to OECD 222 but with reduced content of peat (5%), maximum water holding capacity of the artificial soil, as measured: 40%
Diet:	Finely ground cattle manure
Water content of soil:	Initiation: 20.8 to 21.5% (equivalent to 52.0 to 53.7% of the maximum water holding capacity) Termination: 23.2 to 29.7% (equivalent to 58.1 to 74.3% of the maximum water holding capacity)
Soil pH:	6.1 to 6.2 at test start and 6.0 to 6.3 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
23-May-2013 to 25-October-2013
2. Experimental treatments
The sublethal toxicity of IN-JT333 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 but with reduced organic matter) treated with the test item to obtain the nominal concentrations of 6.33, 12.65, 25.30, 50.61, and 101.2 mg IN-JT333/kg dry artificial soil (corresponding to 6.25, 12.5, 25.0, 50.0, and 100 mg IN-JT333/kg dry artificial soil, adjusted for purity) and to an untreated control (using the same amount of acetone and quartz sand as in the test item treated groups moistened with deionised water). 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 2012 to October 2012.

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 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 and reproduction was performed using Williams t-test (multiple comparison, $\alpha = 0.05$, two-sided for weight changes, one-sided smaller for reproduction).

The LC₅₀ after 28 days was not determined by statistical analysis as no mortality was observed.

II. RESULTS AND DISCUSSION

A. FINDINGS

The EC₅₀ for reproduction of the reference item in the most recent test was 1.7 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 LC₅₀ after 28 days was determined to be greater than 100 mg IN-JT333/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 exposure in any of the treatment groups.

There was a statistically significant body weight increase compared to the control in all IN-JT333 treated groups, however, without any dose relation. A weight increase is not considered to be an adverse effect.

No statistically significant differences in reproduction (56-day assessment) of earthworms compared to the control were observed. Cumulative mortality and weight change (of adults) at 28 days, and reproduction at 56 days are reported in Table 172.

Table 172
Sublethal toxicity of IN-JT333 to earthworms

Nominal IN-JT333 concentration (mg/kg dry soil)	28-day mortality (%) mean	28-day weight change (%) mean	56-day reproduction (# of juveniles) mean
Control (0.0)	0	27.1	203
6.25	0	44.5 *	224 ^{n.s.}
12.5	0	40.7 *	223 ^{n.s.}
25.0	0	40.5 *	197 ^{n.s.}
50.0	0	43.9 *	193 ^{n.s.}
100	0	40.3 *	164 ^{n.s.}

n.s. not significantly different from the control

* Significantly different from the control but not considered to be adverse

Weight change: Williams t-test, two sided, $\alpha = 0.05$

Reproduction: Williams t-test, one-sided smaller, $\alpha = 0.05$

III. CONCLUSIONS

The overall NOAEC (No-Observable-Adverse-Effect Concentration) based on mortality, reproduction and growth and nominal concentrations was 100 mg IN-JT333/kg artificial soil dry weight, the highest concentration tested.

The LC₅₀ after 28 days was determined to be greater than 100 mg IN-JT333/kg soil dry weight.

The Lowest-Observable-Effect Concentration (LOEC) for IN-JT333 was estimated to be >100 mg IN-JT333/kg artificial soil dry weight, the highest concentration tested.

(Lührs, U., 2013c)

RMS comment

This study is valid.

RMS notes that a weight increase was observed in all treatment groups (but not dose related). This is not considered to be an adverse effect.

The overall NOAEC (No-Observable-Adverse-Effect Concentration) based on mortality, reproduction and growth and nominal concentrations was 100 mg IN-JT333/kg artificial soil dry weight, the highest concentration tested.

The Lowest-Observable-Effect Concentration (LOEC) for IN-JT333 was estimated to be >100 mg IN-JT333/kg artificial soil dry weight, the highest concentration tested.

RMS calculated an EC10 value of 54.86 mg IN-JT333/kg dry artificial soil (95% confidence intervals: 2.41-112.46) based on effects on reproduction. The EC10 is used in the risk assessment.

Report: Lührs, U. (2014b); IN-JT333: Effects on reproduction and growth of the earthworm, *Eisenia fetida*, in artificial soil, 2014

DuPont Report No.: DuPont-39714

Guidelines: OECD 222 (2004), ISO 11268-2 (2012) **Deviations:** None

Testing Facility: IBACON, Rossdorf, Germany

Testing Facility Report No.: 75254022

GLP: Yes

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

Executive summary:

The sublethal toxicity of IN-JT333 to earthworms, *Eisenia fetida*, was 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 0.316, 0.633, 1.265, 2.530 and 5.061 mg IN-JT333/kg dry artificial soil (corresponding to 0.313, 0.625, 1.25, 2.50 and 5.00 mg IN-JT333/kg dry artificial soil, adjusted for purity) and to an untreated control (using the same amount of acetone and quartz sand as in the test item treated groups moistened with deionised water). 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 estimated to be greater than 5.00 mg IN-JT333/kg dry artificial soil. The NOEC (No-

Observed-Effect Concentration) for earthworms based on mortality, reproduction, growth and nominal concentrations was 2.50 mg test item/kg dry artificial soil.

I. MATERIALS AND METHODS

A. MATERIALS

- | | |
|--|---|
| 1. Test material | IN-JT333 technical metabolite |
| Lot/Batch #: | JT333-017 |
| Purity: | 98.8% |
| CAS #: | 144171-39-1 |
| Stability of test compound: | Not determined in the test system |
| 2. Control: | Untreated (of acetone and fine quartz sand as in the test item treated groups and moistened with deionised water) |
| Test vehicle: | Acetone |
| 3. Test organism | Earthworm |
| Species: | <i>Eisenia fetida</i> |
| Age at dosing: | 9 to 10 months |
| Weight at dosing: | 304 to 599 mg |
| Source: | In-house laboratory culture (Laboratory: IBACON, Rossdorf, Germany) |
| Acclimatization period: | 1 day |
| Test chamber: | Plastic boxes with perforated transparent lids (volume: 1 L), filled with <i>ca.</i> 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: 57% |
| Diet: | Finely ground cattle manure |
| Water content of soil: | Initiation: 32.0 to 32.6% (equivalent to 56.2 to 57.1% of the maximum water holding capacity)
Termination: 33.5 to 36.6% (equivalent to 58.7 to 64.3% of the maximum water holding capacity) |
| Soil pH: | 5.7 to 6.0 at test start and 6.1 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
18-February-2014 to 16-April-2014

2. Experimental treatments

The sublethal toxicity of IN-JT333 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 0.316, 0.633, 1.265, 2.530 and 5.061 mg IN-JT333/kg dry artificial soil (corresponding to 0.313, 0.625, 1.25, 2.50 and 5.00 mg IN-JT333/kg dry artificial soil, adjusted for purity) and to an untreated control (using the same amount of acetone and quartz sand as in the test item treated groups moistened with deionised water). 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 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 and reproduction was performed using Williams t-test (multiple comparison, $\alpha = 0.05$, two-sided for weight changes, one-sided smaller for reproduction).

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 estimated to be greater than 5.00 mg IN-JT333/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 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. No statistically significant differences in reproduction (56-day assessment) were observed up to and including the concentration of 2.50 mg IN-JT333/kg dry artificial soil. At 5.00 mg IN-JT333/kg dry artificial soil reproduction was statistically significantly reduced. Cumulative mortality and weight change (of adults) at 28 days, and reproduction at 56 days are reported in Table 173.

Table 173
Sublethal toxicity of IN-JT333 to earthworms

Nominal IN-JT333 concentration (mg test item/kg dry soil)	28-day mortality (%) mean	28-day weight change (%) mean	56-day reproduction (# of juveniles) mean
Control (0.0)	0	32.3	238
0.313	0	31.6 n.s.	268 n.s.
0.625	0	33.2 n.s.	272 n.s.
1.25	0	33.4 n.s.	283 n.s.
2.50	0	35.3 n.s.	222 n.s.
5.00	0	39.1 n.s.	193*

* Significantly different from the control

Weight change: Williams t-test, two-sided, $\alpha = 0.05$

Reproduction: Williams t-test, one-sided smaller, $\alpha = 0.05$)

III. CONCLUSIONS

IN-JT333 had no significant lethal effects or effects on growth or feeding activity of the earthworm *Eisenia fetida* when exposed to concentrations up to and including 5.00 mg/kg dry artificial soil. Reproduction was statistically significantly reduced at 5.00 mg/kg dry artificial soil.

The overall NOEC (No-Observed-Effect Concentration) was determined to be 2.50 mg IN-JT333/kg dry artificial soil and the LOEC (Lowest-Observed-Effect Concentration) was determined to be 5.00 mg IN-JT333/kg dry artificial soil.

The LC₅₀ after 28 days was estimated to be greater than 5.00 mg IN-JT333/kg dry artificial soil, the highest concentration tested.

(Lührs, U., 2014b)

RMS comment

This study is valid.

IN-JT333 had no significant lethal effects or effects on growth or feeding activity of the earthworm *Eisenia fetida* when exposed to concentrations up to and including 5.00 mg/kg dry artificial soil. Reproduction was statistically significantly reduced at 5.00 mg/kg dry artificial soil.

The overall NOEC (No-Observed-Effect Concentration) was determined to be 2.50 mg IN-JT333/kg dry artificial soil and the LOEC (Lowest-Observed-Effect Concentration) was determined to be 5.00 mg IN-JT333/kg dry artificial soil.

No relevant EC₁₀ value could be calculated for IN-JT333, RMS proposes to use the NOEC value for the risk assessment.

Report: Lührs, U. (2002b); IN-JU873: Acute toxicity to the earthworm, *Eisenia fetida* in artificial soil

DuPont Report No.: DuPont-10068

Guidelines: OECD 207 (1984), ISO 11268-1 (1993) **Deviations:** None

Testing Facility: IBACON, Rossdorf, Germany

Testing Facility Report No.: 13371021

GLP: Yes

Certifying Authority: Hessisches Ministerium für Umwelt, Landwirtschaft und Forsten

I. MATERIALS AND METHODS

A. MATERIALS

- | | |
|--|---|
| 1. Test material: | IN-JU873 technical metabolite |
| Lot/Batch #: | JU873-005 |
| Purity: | 99.06% |
| Description: | White solid |
| CAS#: | Not available |
| Stability of test compound: | Test item is considered stable under test conditions |
| 2. Control: | Untreated (and moistened with deionised water) |
| Test vehicle: | Fine quartz sand |
| Toxic reference: | 2-Chloroacetamide |
| 3. Test organism: | Earthworm |
| Species: | <i>Eisenia fetida</i> |
| Age at dosing: | 9 months; life stage: adult with clitellum |
| Weight at dosing: | 333.8 to 429.3 mg |
| Source: | Laboratory, in-house culture (IBACON, Rossdorf, Germany) |
| Test chamber: | 1-L bottling jars, loosely covered by glass-lids, filled with approximately 500 g (dry weight equivalent) artificial soil. |
| Test medium: | Artificial soil prepared according to OECD 207 |
| Diet: | Unfed during test |
| Water content of soil: | Test initiation: 29.9% to 31.5% (equivalent to 48.7% to 51.3% of the maximum water holding capacity)
Test termination: 31.9% to 35.2% (equivalent to 52.0% to 57.4% of the maximum water holding capacity) |
| Soil pH: | Test initiation: 5.7–5.8; Test termination: 5.5 |
| 4. Environmental conditions (in-life period) | |
| Temperature: | 19 to 21°C; mean = 20°C |
| Photoperiod: | 24-hour photoperiod (400 to 752 lux) |

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
21-June-2002 to 10-July-2002
2. Experimental treatments
Acute toxicity of IN-JU873 to earthworms, *Eisenia fetida* (Savigny), was determined in a 14-day soil exposure laboratory study. Four replicates of 10 clitellated adult earthworms were each exposed to nominal concentrations of 10, 32, 100, 316, and 1000 mg IN-JU873/kg dry soil weight. Controls were replicated four times with 10 earthworms in each replicate. The toxic reference standard, 2-chloroacetamide, is tested at least once a year at five rates. Worms were assessed for mortality and sublethal effects after 7 and 14 days of exposure and earthworm body weights were assessed at Days 0 and 14.

II. RESULTS AND DISCUSSION

A. FINDINGS

Cumulative mortality results at 7 and 14 days and weight loss at 14 days are reported in the summary table below. No mortality was observed in any treatment group. Body weight changes were not significantly different compared to the control up to and including the concentration of 1000 mg/kg dry weight soil. No significant sublethal behavioural effects were observed.

The LD₅₀ for the toxic reference standard was 30.4 mg/kg dry soil, which is within accepted limits, indicating the validity of this test.

Table 174
Acute toxicity of IN-JU873 to earthworms

Treatment (mg IN-JU873/kg dry soil)	Cumulative mortality (%)		Cumulative weight change (%)
	7 days	14 days	14 days
0	0	0	1.1
10	0	0	-3.0
32	0	0	-1.6
100	0	0	-3.7
316	0	0	-2.9
1000	0	0	-0.3

III. CONCLUSION

The 14-day LC₅₀ was >1000 mg IN-JU873/kg dry soil. The NOEC = 1000 mg IN-JU873/kg dry soil.

(Luhrs, U., 2002b)

RMS comment

This study was submitted in the original DAR, AD3 2005. This study was conducted in compliance with the current guideline. The 14-day LC₅₀ was >1000 mg IN-JU873/kg dry soil. This study is still considered acceptable but only informative as acute toxicity studies are not required anymore.

Report: Lührs, U. (2013b); IN-JU873: Effects on reproduction and growth of the earthworm, *Eisenia fetida*, in artificial soil with 5% peat

DuPont Report No.: DuPont-36497

Guidelines: OECD 222 (2004), ISO 11268-2 (2012) **Deviations:** None

Testing Facility: IBACON, Rossdorf, Germany

Testing Facility Report No.: 82991022

GLP: Yes

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

Executive summary:

The sublethal toxicity of IN-JU873 to earthworms, *Eisenia fetida*, was 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 but with reduced organic matter) treated with the test item to obtain the nominal concentrations of 6.30, 12.6, 25.2, 50.4 and 100.8 mg IN-JU873/kg dry artificial soil (corresponding to 6.25, 12.5, 25.0, 50.0, and 100 mg IN-JU873/kg dry artificial soil, adjusted for purity) and to an untreated control (using the same amount of acetone and quartz sand as in the test item treated groups moistened with deionised water). 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 LC_{50} after 28 days was determined to be greater than 100 mg IN-JU873/kg dry artificial soil. The NOEC (No-Observable-Effect Concentration) for earthworms based on mortality, reproduction, growth and nominal concentrations was 50.0 mg IN-JU873/kg dry artificial soil.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material	IN-JU873 technical metabolite
Lot/Batch #:	JU873-006
Purity:	99.2%
CAS #:	144172-25-8
Stability of test compound:	Not determined in the test system
2. Control:	Untreated (using the same amount of acetone and sand as in the test item groups and moistened with deionised water)
Test vehicle:	Acetone
3. Test organism	Earthworm
Species:	<i>Eisenia fetida</i>
Age at dosing:	Approximately 9 months
Weight at dosing:	323 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 <i>ca.</i> 500 g artificial soil dry weight
Test medium:	Artificial soil prepared according to OECD 222 but with reduced content of peat (5%), maximum water holding capacity of the artificial soil, as measured: 40%
Diet:	Finely ground cattle manure
Water content of soil:	Initiation: 20.6% to 21.7% (equivalent to 51.4% to 54.4% of the maximum water holding capacity) Termination: 23.5% to 27.3% (equivalent to 58.7% to 68.4% of the maximum water holding capacity)
Soil pH:	6.3 to 6.4 at test start and 6.1 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
12-September-2013 to 08-November-2013

2. Experimental treatments

The sublethal toxicity of IN-JU873 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 but with reduced organic matter) treated with the test item to obtain the nominal concentrations of 6.30, 12.6, 25.2, 50.4 and 100.8 mg IN-JU873/kg dry artificial soil (corresponding to 6.25, 12.5, 25.0, 50.0, and 100 mg IN-JU873/kg dry artificial soil, adjusted for purity) and to an untreated control (using the same amount of acetone and quartz sand as in the test item treated groups moistened with deionised water). 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 2012 to October 2012.

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 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 Bonferroni-Welch t-test (multiple comparison, $\alpha = 0.05$, two-sided). The statistical evaluation of the NOEC for reproduction was performed using Williams t-test (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 1.7 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 100 mg IN-JU873/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 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.

The reproduction (56-day assessment) was not statistically significantly different compared to the control up to and including the concentration of 50.0 mg IN-JU873/kg dry artificial soil and was statistically significantly reduced at 100 mg IN-JU873/kg dry artificial soil.

Cumulative mortality and weight change (of adults) at 28 days, and reproduction at 56 days are reported in Table 175.

Table 175
Sublethal toxicity of IN-JU873 to earthworms

Nominal IN-JU873 concentration (mg/kg dry soil)	28-day mortality (%) mean	28-day weight change (%) mean	56-day reproduction (# of juveniles) mean
Control (0.0)	0	7.2	247
6.25	0	9.2 ^{n.s.}	208 ^{n.s.}
12.5	0	5.9 ^{n.s.}	204 ^{n.s.}
25.0	0	10.9 ^{n.s.}	266 ^{n.s.}
50.0	0	11.8 ^{n.s.}	234 ^{n.s.}
100	0	9.0 ^{n.s.}	179 [*]

n.s. not significantly different from the control

* Significant different from the control

Weight change: Bonferroni-Welch t-test, two sided, $\alpha = 0.05$; Reproduction: Williams t-test, one-sided smaller, $\alpha = 0.05$)

III. CONCLUSIONS

The LC₅₀ after 28 days was determined to be greater than 100 mg IN-JU873/kg soil dry weight.

The overall Lowest-Observable-Effect Concentration (LOEC) for IN-JU873 was determined to be 100 mg/kg artificial soil dry weight. The overall No-Observable-Effect Concentration (NOEC) was determined to be 50.0 mg IN-JU873/kg artificial soil dry weight.

(Lührs, U., 2013b)

RMS comment

This study is valid.

The overall Lowest-Observable-Effect Concentration (LOEC) for IN-JU873 was determined to be 100 mg/kg artificial soil dry weight. The overall No-Observable-Effect Concentration (NOEC) was determined to be 50.0 mg IN-JU873/kg artificial soil dry weight.

RMS calculated an EC10 value of 89.12 mg IN-JU873/kg dry artificial soil (95% confidence intervals: 17.66-104.40) based on effects on reproduction.

Report: Shanmugasundaram, R. (2011); IN-KB687: Effects on reproduction and growth of the earthworm, *Eisenia fetida*, in artificial soil with 5% peat

DuPont Report No.: DuPont-31720

Guidelines: OECD 222 (2004), ISO 11268-2 (1998) **Deviations:** None

Testing Facility: International Institute of Biotechnology and Toxicology (IIBAT), Kancheepuram District, Tamil Nadu, India

Testing Facility Report No.: 10803

GLP: Yes

Certifying Authority: National GLP Compliance Monitoring Authority (India)

Executive summary:

A study was conducted to determine the effect of IN-KB687 on mortality and growth of the earthworm *Eisenia fetida* after 4 weeks, and the effects on reproduction by determining the number of offsprings after 8 weeks according to OECD 222, 2004 and ISO 11268-2, 1998.

The earthworms were exposed for 28 days to artificial soil (prepared according to OECD 207, but with reduced content of peat) treated with five nominal concentrations of 6.25, 12.5, 25.0, 50.0, and 100 mg IN-KB687/kg soil dry weight and an untreated control. Mortality and growth (body weight) of the earthworms were assessed after 28 days and the effects on reproduction (number of juveniles produced) were assessed after 56 days. The LC₅₀ (50% Lethal Concentration) for earthworms based on mortality at 28 days and on nominal concentrations of IN-KB687 was >100 mg/kg soil dry weight, the highest concentration tested. The EC₅₀ (50% Effect Concentration) for earthworms based on growth and reproduction and on nominal concentrations of IN-KB687 was >100 mg/kg soil dry weight the highest concentration tested. The NOEC (No-Observed-Effect Concentration) for earthworms based on reproduction and growth and on nominal concentrations of IN-KB687 were 50 and 100 mg/kg soil dry weight, respectively.

I. MATERIALS AND METHODS

A. MATERIALS

- | | | |
|----|--|--|
| 1. | Test material: | IN-KB687 technical metabolite |
| | Lot/Batch #: | KB687-002 |
| | Purity: | 99.8% |
| | Description: | Solid, powder |
| | CAS #: | 177905-10-1 |
| | Stability of test compound: | Not determined in the test system |
| 2. | Control: | Untreated artificial soil, fine quartz sand |
| | Test vehicle: | Finely ground quartz sand |
| | Toxic reference: | Carbendazim (tested once per year) |
| 3. | Test organism: | Earthworm |
| | Species: | <i>Eisenia fetida</i> , Savigny |
| | Age at dosing: | 3 - 4 months (approximately) |
| | Weight at dosing: | 271 to 471 mg, including gut contents |
| | Source: | In-house laboratory culture |
| | Acclimation period: | 1 day |
| | Test chamber: | Glass beakers (2 L), filled with 500 g (dry weight equivalent) artificial soil and covered with perforated plastic lids to enable exchange of air and to minimise evaporation from the artificial soil. The height of the soil layer in the containers was approximately 6 cm. |
| | Test medium: | Artificial soil prepared according to OECD 207 test guideline but with reduced content of peat (5%). |
| | Feeding: | Finely ground cow manure |
| | Maximum water holding capacity of the OECD 207 artificial soil, as measured: | 48.51% |
| | Water content of soil: | Initiation: 24.46 to 26.09% (50.42 to 53.78% of the total water holding capacity)
Termination: 28.89 to 30.24% (59.55 to 62.34% of the total water holding capacity). |
| 4. | Environmental conditions | |
| | Temperature: | 18.7 to 22.0°C |
| | pH: | 6.70 to 7.50 at test start and 7.89 to 9.03 at test termination |
| | Photoperiod: | 16 hour light, 8 hour dark, photoperiod (406 to 600 Lux) |

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed

08-March-2011 to 03-May-2011

2. Experimental treatments

A study was conducted to determine the effect of IN-KB687 on mortality and growth of the earthworm *Eisenia fetida* after 4 weeks, and the effects on reproduction by determining the number of offsprings after 8 weeks according to OECD 222, 2004 and ISO 11268-2, 1998. Four replicates of ten earthworms for the test item groups and 8 replicates for the control (quartz sand) group (total of 40 and 80 individuals per treatment group, respectively) were each exposed to nominal concentrations of 6.25, 12.5, 25.0, 50.0, and 100 mg IN-KB687/kg soil dry weight and an untreated control (finely ground quartz sand moistened with deionised water). A toxic reference (carbendazim) is tested once per year to ensure sensitivity of the test system. The most recent test to this study was conducted from July to August, 2010.

3. Observations

Mortality, growth, and feeding activity of the earthworm *Eisenia fetida* were determined after 4 weeks (28 days) and the effects on reproduction were determined after 8 weeks (56 days).

4. Statistics

Data on body weight changes, reproduction, and food addition were tested for normal distribution and homoscedasticity using various tests. The data on food addition was not normally distributed and homogeneous and therefore, analysed using the Wilcoxon's Rank sum test (multiple comparison, $\alpha = 0.05$, one-sided) to compare with the untreated control. Since the data on body weight change and reproduction (number of juveniles produced) were normally distributed (Kolmogorov test) and homogeneous (Bartlett's test), Bonferroni t-test was used (multiple comparison, one sided, $\alpha = 0.05$). All the above mentioned statistical analyses were performed using TOXSTAT version 3.5.

II. RESULTS AND DISCUSSION

A. FINDINGS

Cumulative mortality and weight change (of adults) at 28 days, and reproduction at 56 days are reported in the summary table below. All validation criteria were met. The EC₅₀ of the toxic reference in the most recent test was 0.95 mg carbendazim/kg dry artificial soil weight.

No mortality was observed up to and including 100 mg IN-KB687/kg soil dry weight and the control (quartz sand).

No behavioural effects were observed in any treatments including the control during the course of the experiment.

The mean biomass change (percent weight change from initial weight) of the worms exposed to IN-KB687 ranged from +34.18% (6.25 mg/kg soil dry weight) to +45.28% (50 mg/kg dry soil). The mean biomass change of earthworms in the control was +34.37%. The mean biomass changes in any of the treatment groups were not significantly different, when compared to the control (Bonferroni t-test, $\alpha = 0.05$).

There were no significant effects on reproduction up to and including the concentration of 50 mg IN-KB687/kg soil dry weight, when compared to the control. However, there was a significant effect on reproduction in the maximum concentration, 100 mg IN-KB687/kg soil dry weight, when compared to the control. The mean number of juveniles produced per treatment group ranged from 86 (100 mg IN-KB687/kg soil dry weight) to 130.5 (6.25 mg IN-KB687/kg soil dry weight). The reduction in reproduction ranged from 1.23% (6.25 mg IN-KB687/kg soil dry weight) to 34.91% (100 mg IN-KB687/kg soil dry weight).

There was no significant difference in food consumption in treatments up to and including 50 mg IN-KB687/kg soil dry weight when compared to the control. However, there was a significant difference in food consumption at the

maximum concentration, 100 mg IN-KB687/kg soil dry weight when compared to the control. The mean amount of food added to each treatment group and the control during the first four weeks ranged from 45.35 g (100 mg IN-KB687/kg soil dry weight) to 48.22 g (6.25 mg IN-KB687/kg soil dry weight).

Table 176
Summary of the effects of IN-KB687 on earthworm mortality, growth and reproduction

Nominal concentration (mg/kg soil dry weight)	28-day Cumulative mortality (%)	28-day Cumulative weight change (%) mean	56-day Reproduction (# of juveniles) mean
Control (0.0)	0	+34.37	132.1
6.25	0	+34.18 ^{n.s.}	130.5 ^{n.s.}
12.5	0	+43.19 ^{n.s.}	117.3 ^{n.s.}
25	0	+44.49 ^{n.s.}	104.3 ^{n.s.}
50	0	+45.28 ^{n.s.}	104.3 ^{n.s.}
100	0	+45.24 ^{n.s.}	86.0 [*]

n.s. Not significantly different from the control

* Significant different from the control (Bonferroni t-test, one sided, $\alpha = 0.05$)

III. CONCLUSIONS

The LC₅₀ (50% Lethal Concentration) at 28 days was determined to be >100 mg IN-KB687/kg soil dry weight.

The EC₅₀ (50% Effect Concentration) based on reproduction and growth in terms of biomass was determined to be >100 mg IN-KB687/kg soil dry weight.

The NOEC (No-Observed-Effect Concentration) with respect to reproduction and growth in terms of biomass was determined to be 50 and 100 mg IN-KB687/kg soil dry weight, respectively.

(Shanmugasundaram, R., 2011)

RMS comment

This study is valid.

The NOEC (No-Observed-Effect Concentration) with respect to reproduction and growth in terms of biomass was determined to be 50 and 100 mg IN-KB687/kg soil dry weight, respectively.

RMS calculated an EC10 value of 11.41 mg IN-KB687/kg dry artificial soil (95% confidence intervals: 0.38-52.94) based on effects on reproduction. This EC10 is not considered reliable (large confidence interval) and is not used for the risk assessment. The NOEC of 50 mg IN-KB687/kg soil dry weight is used for the risk assessment.

Report: Luhrs, U. (2002a); IN-KG433: Acute toxicity to the earthworm, *Eisenia fetida* in artificial soil

DuPont Report No.: DuPont-10067

Guidelines: OECD 207 (1984), ISO 11268-1 (1993) **Deviations:** None

Testing Facility: IBACON, Rossdorf, Germany

Testing Facility Report No.: 13361021

GLP: Yes

Certifying Authority: Hessisches Ministerium für Umwelt, Landwirtschaft und Forsten

I. MATERIALS AND METHODS

A. MATERIALS

- | | |
|--|---|
| 1. Test material: | IN-KG433 technical metabolite |
| Lot/Batch #: | KG433-1 |
| Purity: | 81.5%, by analysis |
| Description: | White solid |
| CAS#: | Not available |
| Stability of test compound: | Test item is considered stable under test conditions |
| 2. Control: | Untreated (and moistened with deionised water) |
| Test vehicle: | Fine quartz sand |
| Toxic reference: | 2-Chloroacetamide |
| 3. Test organism: | Earthworm |
| Species: | <i>Eisenia fetida</i> |
| Age at dosing: | 9 months; life stage: adult with clitellum |
| Weight at dosing: | 315.7 to 415.9 mg |
| Source: | Laboratory, in-house culture (IBACON, Rossdorf, Germany) |
| Test chamber: | 1-L bottling jars, loosely covered by glass-lids, filled with approximately 500 g (dry weight equivalent) artificial soil. |
| Test medium: | Artificial soil prepared according to OECD 207 |
| Diet: | Unfed during test |
| Water content of soil: | Test initiation: 28.8% to 30.6% (equivalent to 46.9% to 49.9% of the maximum water holding capacity)
Test termination: 31.2% to 34.3% (equivalent to 50.9% to 55.9% of the maximum water holding capacity) |
| Soil pH: | Test initiation: 5.8; Test termination: 5.5–5.8 |
| 4. Environmental conditions (in-life period) | |
| Temperature: | 19 to 21°C; mean = 20°C |
| Photoperiod: | 24-hour photoperiod (462 to 740 lux) |

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
21-June-2002 to 10-July-2002
2. Experimental treatments
Acute toxicity of IN-KG433 to earthworms, *Eisenia fetida* (Savigny), was determined in a 14-day soil exposure laboratory study. Four replicates of 10 clitellated adult earthworms were each exposed to nominal concentrations of 10, 32, 100, 316, and 1000 mg IN-KG433/kg dry soil weight. Controls were replicated four times with 10 earthworms in each replicate. The toxic reference standard, 2-chloroacetamide, is tested at least once a year at five rates. Worms were assessed for mortality and sublethal effects after 7 and 14 days of exposure, and earthworm body weights were assessed at Days 0 and 14.

II. RESULTS AND DISCUSSION

A. FINDINGS

Cumulative mortality results at 7 and 14 days and weight loss at 14 days are reported in the summary table below. No mortality was observed in any treatment group. Body weight changes were not significantly different compared

to the control up to and including the concentration of 32 mg/kg dry weight soil, but decreased significantly from 100 to 1000 mg/kg dry weight soil (Dunnett's test, $p < 0.05$). No significant sublethal behavioural effects were observed.

The LD₅₀ for the toxic reference standard was 30.4 mg/kg dry soil, which is within accepted limits, indicating the validity of this test.

Table 177
Acute toxicity of IN-KG433 to earthworms

Treatment (mg IN-KG433/kg dry soil)	Cumulative mortality (%)		Cumulative weight change (%)
	7 days	14 days	14 days
0	0	0	1.1
10	0	0	-3.6
32	0	0	-0.4
100	0	0	-15.3 ^a
316	0	0	-23.7 ^a
1000	0	0	-30.0 ^a

^a Significant at $p < 0.05$, Dunnett's test

III. CONCLUSION

The 14-day LC₅₀ was >1000 mg IN-KG433/kg dry soil. The NOEC was 32 mg IN-KG433/kg dry soil based on body weight.

(Luhrs, U., 2002a)

(Luhrs, U., 2002b)

RMS comment

This study was submitted in the original DAR, AD3 2005. This study was conducted in compliance with the current guideline. The 14-day LC₅₀ was >1000 mg IN-KG433/kg dry soil. This study is still considered acceptable but only informative as acute toxicity studies are not required anymore.

Report: Lührs, U. (2013a); IN-KG433: Effects on reproduction and growth of the earthworm, *Eisenia fetida*, in artificial soil with 5% peat

DuPont Report No.: DuPont-36496

Guidelines: ISO 11268-2 (1998), OECD 222 (2004) **Deviations:** None

Testing Facility: IBACON, Rossdorf, Germany

Testing Facility Report No.: 75263022

GLP: Yes

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

Executive summary:

The sublethal toxicity of IN-KG433 to earthworms, *Eisenia fetida*, was determined in a 56-day soil exposure laboratory study according to OECD 222, 2004 and ISO 11268-2, 1998. Adult earthworms were exposed to artificial soil (prepared according to OECD 222 but with reduced organic matter) treated with the test item to obtain the nominal concentrations of 6.256, 12.51, 25.03, 50.05, and 100.1 mg IN-KG433/kg dry artificial soil (corresponding to 6.25, 12.5, 25.0, 50.0, and 100 mg IN-KG433/kg dry artificial soil, adjusted for purity) and to an untreated control (using the same amount of acetone and fine quartz sand as in the test item treated groups and moistened with deionised water). 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 100 mg IN-KG433/kg soil dry weight. The NOEC (No-Observable-Effect Concentration) for earthworms based on mortality, reproduction, growth and nominal concentrations was 50.0 mg IN-KG433/kg soil dry weight.

I. MATERIALS AND METHODS**A. MATERIALS**

1. Test material	IN-KG433 technical metabolite
Lot/Batch #:	KG433-004
Purity:	99.9%, by analysis
CAS #:	Not assigned
Stability of test compound:	Not determined in the test system
2. Control:	Untreated (using the same amount of fine quartz sand as in the test item treated groups and moistened with deionised water)
3. Test organism	Earthworm
Species:	<i>Eisenia fetida</i>
Age at dosing:	8 to 9 months
Weight at dosing:	301 to 596 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 <i>ca.</i> 500 g artificial soil dry weight
Test medium:	Artificial soil prepared according to OECD 222 but with reduced content of peat (5%)
Diet:	Finely ground cattle manure
Water content of soil:	Initiation: 19.4 to 20.5% (equivalent to 50.9 to 54.0% of the maximum water holding capacity) Termination: 22.3 to 25.0% (equivalent to 58.7 to 65.7% of the maximum water holding capacity)
Soil pH:	6.1 to 6.2 at test start and 5.9 to 6.1 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
27-June-2013 to 23-August-2013

2. Experimental treatments

The sublethal toxicity of IN-KG433 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 but with

reduced organic matter) treated with the test item to obtain the nominal concentrations of 6.256, 12.51, 25.03, 50.05, and 100.1 mg IN-KG433/kg dry artificial soil (corresponding to 6.25, 12.5, 25.0, 50.0, and 100 mg IN-KG433/kg dry artificial soil, adjusted for purity) and to an untreated control (using the same amount of acetone and fine quartz sand as in the test item treated groups and moistened with deionised water). 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 2012 to October 2012.

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 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 and reproduction was performed using Williams t-test (multiple comparison, $\alpha = 0.05$, two-sided for weight changes, one-sided smaller for reproduction).

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 1.7 mg carbendazim/kg artificial soil dry weight. 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 100 mg IN-KG433/kg soil dry weight. The food consumption of earthworms exposed to the test rates of the test item was comparable to the control up to and including the concentration of 50.0 mg IN-KG433/kg dry artificial soil and appeared to be reduced at the concentration of 100 mg IN-KG433/kg dry artificial soil. No adverse behavioural effects were observed after 28 days 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 not significantly different compared to the control up to and including the concentration of 50.0 mg IN-KG433/kg dry artificial soil. At the concentration of 100 mg IN-KG433/kg dry artificial soil reproduction was statistically significantly reduced compared to the control. Cumulative mortality and weight change (of adults) at 28 days, and reproduction at 56 days are reported in the summary table below.

Table 178
Sublethal toxicity of IN-KG433 to earthworms

Nominal IN-KG433 concentration (mg/kg dry soil)	28-day mortality (%) mean	28-day weight change (%) mean	56-day reproduction (# of juveniles) mean
Control (0.0)	0	15.4	220
6.25	0	19.9 ^{n.s.}	203 ^{n.s.}
12.5	0	18.8 ^{n.s.}	281 ^{n.s.}
25.0	0	23.8 ^{n.s.}	261 ^{n.s.}
50.0	0	23.0 ^{n.s.}	207 ^{n.s.}
100	0	15.0 ^{n.s.}	90 [*]

* Significant different from the control

n.s. Not significantly different from the control

Weight change: Williams t-test, two sided, $\alpha = 0.05$; Reproduction: Williams t-test, one-sided smaller, $\alpha = 0.05$

III. CONCLUSIONS

The LC_{50} after 28 days was determined to be greater than 100 mg IN-KG433/kg soil dry weight. The Lowest-Observable-Effect Concentration (LOEC) for IN-KG433 was determined to be 100 mg IN-KG433/kg artificial soil dry weight. The overall No-Observable-Effect Concentration (NOEC) was determined to be 50.0 mg IN-KG433/kg artificial soil dry weight. The EC_{10} value for reproduction was 55.3 mg IN-KG433/kg dry artificial soil and the EC_{20} was determined to be 65.7 mg IN-KG433/kg dry artificial soil.

(Lührs, U., 2013a)

RMS comment

This study is valid.

The Lowest-Observable-Effect Concentration (LOEC) for IN-KG433 was determined to be 100 mg IN-KG433/kg artificial soil dry weight. The overall No-Observable-Effect Concentration (NOEC) was determined to be 50.0 mg IN-KG433/kg artificial soil dry weight. The EC_{10} value for reproduction was 55.3 mg IN-KG433/kg dry artificial soil (95% confidence intervals: 13.8-70.9).

Report: Lührs, U. (2002e); IN-KT413: Acute toxicity to the earthworm, *Eisenia fetida* in artificial soil

DuPont Report No.: DuPont-11050

Guidelines: OECD 207 (1984), ISO 11268-1 (1993) **Deviations:** None

Testing Facility: IBACON, Rossdorf, Germany

Testing Facility Report No.: 14721021

GLP: Yes

Certifying Authority: Hessisches Ministerium für Umwelt, Landwirtschaft und Forsten

I. MATERIALS AND METHODS

A. MATERIALS

- | | |
|--|---|
| 1. Test material: | IN-KT413 technical metabolite |
| Lot/Batch #: | KT413-3 |
| Purity: | 97% |
| Description: | White solid |
| CAS#: | Not available |
| Stability of test compound: | Test item is considered stable under test conditions |
| 2. Control: | Untreated (and moistened with deionised water) |
| Test vehicle: | Fine quartz sand |
| Toxic reference: | 2-Chloroacetamide |
| 3. Test organism: | Earthworm |
| Species: | <i>Eisenia fetida</i> |
| Age at dosing: | 11 months; life stage: adult with clitellum |
| Weight at dosing: | 323.7 to 374.5 mg |
| Source: | Laboratory, in-house culture (IBACON, Rossdorf, Germany) |
| Test chamber: | 1-L bottling jars, loosely covered by glass-lids, filled with approximately 500 g (dry weight equivalent) artificial soil. |
| Test medium: | Artificial soil prepared according to OECD 207 |
| Diet: | Unfed during test |
| Water content of soil: | Test initiation: 31.8% to 33.4% (equivalent to 49.7% to 52.2% of the maximum water holding capacity)
Test termination: 32.1% to 36.2% (equivalent to 50.1% to 56.5% of the maximum water holding capacity) |
| Soil pH: | Test initiation: 5.5–5.6; Test termination: 5.6–6.0 |
| 4. Environmental conditions (in-life period) | |
| Temperature: | 19 to 21°C; mean = 20°C |
| Photoperiod: | 24-hour photoperiod (400 to 758 lux) |

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
17-July-2002 to 05-August-2002

2. Test system

The acute toxicity of IN-KT413 to earthworms, *Eisenia fetida* (Savigny), was estimated in a 14-day soil exposure laboratory study. Four replicates of 10 clitellated adult earthworms each were exposed to nominal concentrations of 10, 32, 100, 316, and 1000 mg of IN-KT413/kg dry weight soil. The control was replicated four times, with ten earthworms in each replicate.

The toxic standard, 2-chloroacetamide, is tested once a year. The 14-day LC₅₀ of the most recent test was 30.4 mg 2-chloroacetamide/kg dry soil.

Earthworms were assessed for mortality and behavioural effects after 7 and 14 days of exposure and earthworm body weights were assessed at Days 0 and 14.

II. RESULTS AND DISCUSSION

A. FINDINGS

Table 179
Summary of results

Test species	<i>Eisenia fetida</i>					
Exposure	artificial soil					
NOEC	1000 mg/kg dry weight soil					
LOEC	higher than 1000 mg/kg dry weight soil					
LC₅₀	higher than 1000 mg/kg dry weight soil					
Test item	Control	IN-KT413				
IN-KT413 concentration [mg/kg dry soil wt]	Deionised water	10	32	100	316	1000
Mortality after 14 days	0	0	0	0	0	0
Significance	— ^a	—	—	—	—	—
Body weight change [%]	2.4	0.9	1.5	6.1	3.5	-5.0
Significance (Dunnett-test, $\alpha=0.05$)	—	n.s. ^b	n.s.	n.s.	n.s.	n.s.

^a Not applicable

^b Not significantly different compared to the control

III. CONCLUSION

The 14-day LC₅₀ was >1000 mg IN-KT413/kg dry weight soil. The NOEC = 1000 mg IN-KT413/kg dry weight soil.

(Luhrs, U., 2002e)

RMS comment

This study was submitted in the original DAR, AD3 2005. This study was conducted in compliance with the current guideline. The 14-day LC₅₀ was >1000 mg IN-KT413/kg dry weight soil. This study is still considered acceptable but only informative as acute toxicity studies are not required anymore.

Report: Lührs, U. (2014a); IN-KT413: Effects on reproduction and growth of the earthworm, *Eisenia fetida*, in artificial soil with 5% peat

DuPont Report No.: DuPont-36495

Guidelines: OECD 222 (2004), ISO 11268-2 (2012) **Deviations:** None

Testing Facility: IBACON, Rossdorf, Germany

Testing Facility Report No.: 75273022

GLP: Yes

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

Executive summary:

The sublethal toxicity of IN-KT413 to earthworms, *Eisenia fetida*, was 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 but with reduced organic matter) treated with IN-KT413 to obtain the nominal concentrations of 6.27, 12.54, 25.08, 50.15 and 100.3 mg/kg dry artificial soil (corresponding to 6.25, 12.5, 25.0, 50.0, and 100 mg/kg dry artificial soil, adjusted for purity) and to an untreated control (using the same amount of acetone and quartz sand as in the test item treated groups moistened with deionised water). 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 100 mg IN-KT413/kg dry artificial soil. The NOEC (No-Observable-Effect Concentration) for earthworms based on mortality, reproduction, growth and nominal concentrations was 100 mg IN-KT413/kg dry artificial soil, the highest concentration tested.

I. MATERIALS AND METHODS**A. MATERIALS**

- | | |
|--|---|
| 1. Test material: | IN-KT413 technical metabolite |
| Lot/Batch #: | KT413-003 |
| Purity: | 99.7% |
| CAS #: | Not assigned |
| Stability of test compound: | Not determined in the test system |
| 2. Control: | Untreated (using the same amount of acetone and sand as in the test item groups and moistened with deionised water) |
| Test vehicle: | Acetone |
| 3. Test organism | Earthworm |
| Species: | <i>Eisenia fetida</i> |
| Age at dosing: | Approximately 9 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 <i>ca.</i> 500 g artificial soil dry weight |
| Test medium: | Artificial soil prepared according to OECD 222 but with reduced content of peat (5%), maximum water holding capacity of the artificial soil, as measured: 41% |
| Diet: | Finely ground cattle manure |
| Water content of soil: | Initiation: 21.9 to 22.7% (equivalent to 53.4 to 55.3% of the maximum water holding capacity)
Termination: 22.5 to 24.6% (equivalent to 55.0 to 59.9% of the maximum water holding capacity) |
| Soil pH: | 5.9 at test start and 6.2 to 6.3 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
10-October-2013 to 09-December-2013

2. Experimental treatments

The sublethal toxicity of IN-KT413 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 but with reduced organic matter) treated with IN-KT413 to obtain the nominal concentrations of 6.27, 12.54, 25.08, 50.15 and 100.3 mg IN-KT413/kg dry artificial soil (corresponding to 6.25, 12.5, 25.0, 50.0, and 100 mg/kg dry artificial soil, adjusted for purity) and to an untreated control (using the same amount of acetone and quartz sand as in the test item treated groups moistened with deionised water). 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 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 and reproduction was performed using Williams t-test (multiple comparison, $\alpha = 0.05$, two-sided for weight changes, one-sided smaller for reproduction).

The LC₅₀ after 28 days was not determined by statistical analysis as no mortality was observed.

II. RESULTS AND DISCUSSION

A. FINDINGS

The EC₅₀ 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 LC₅₀ after 28 days was determined to be greater than 100 mg IN-KT413/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 exposure in any of the treatment groups. No statistically significant differences in weight change (28-day assessment) or reproduction (56-day assessment) of earthworms compared to the control were observed. Cumulative mortality and weight change (of adults) at 28 days, and reproduction at 56 days are reported in the summary table below.

Table 180
Sublethal toxicity of IN-KT413 to earthworms

Nominal IN-KT413 concentration (mg/kg dry soil)	28-day mortality (%) mean	28-day weight change (%) mean	56-day reproduction (# of juveniles) mean
Control (0.0)	0	21.0	279
6.25	0	21.6 ^{n.s.}	266 ^{n.s.}
12.5	0	25.9 ^{n.s.}	268 ^{n.s.}
25.0	0	21.3 ^{n.s.}	226 ^{n.s.}
50.0	0	23.7 ^{n.s.}	296 ^{n.s.}
100	0	23.0 ^{n.s.}	240 ^{n.s.}

^{n.s.} Not significantly different from the control

(Weight change: Williams t-test, two sided, $\alpha = 0.05$) (Reproduction: Williams t-test, one-sided smaller, $\alpha = 0.05$)

III. CONCLUSIONS

The No-Observable-Effect Concentration (NOEC) based on mortality, reproduction and growth and nominal concentrations was determined to be 100 mg IN-KT413/kg artificial soil dry weight, the highest concentration tested.

The LC₅₀ after 28 days was determined to be greater than 100 mg IN-KT413/kg soil dry weight.

The overall Lowest-Observable-Effect Concentration (LOEC) for IN-KT413 was determined to be >100 mg/kg artificial soil dry weight, the highest concentration tested. The overall No-Observable-Effect Concentration (NOEC) was determined to be 100 mg IN-KT413/kg artificial soil dry weight.

(Lührs, U., 2014a)

RMS comment

This study is valid.

The overall Lowest-Observable-Effect Concentration (LOEC) for IN-KT413 was determined to be >100 mg/kg artificial soil dry weight, the highest concentration tested. The overall No-Observable-Effect Concentration (NOEC) was determined to be 100 mg IN-KT413/kg artificial soil dry weight.

RMS calculated an EC10 value of 99.4 mg IN-KT413/kg dry artificial soil (95% confidence intervals: 22.74-432.55) based on the effects on reproduction. This EC10 is not considered reliable (large confidence interval) and is not used for the risk assessment. The NOEC of 100 mg IN-KT413/kg soil dry weight is used for the risk assessment.

Report: Lührs, U. (2002c); IN-MK638: Acute toxicity to the earthworm, *Eisenia fetida* in artificial soil

DuPont Report No.: DuPont-10070

Guidelines: OECD 207 (1984), ISO 11268-1 (1993) **Deviations:** None

Testing Facility: IBACON, Rossdorf, Germany

Testing Facility Report No.: 13391021

GLP: Yes

Certifying Authority: Hessisches Ministerium für Umwelt, Landwirtschaft und Forsten

I. MATERIALS AND METHODS

A. MATERIALS

- | | |
|--|---|
| 1. Test material: | IN-MK638 technical metabolite |
| Lot/Batch #: | MK638-002 |
| Purity: | 99.94%, by analysis |
| Description: | Solid white |
| CAS#: | Not available |
| Stability of test compound: | Test item is considered stable under test conditions |
| 2. Control: | Untreated (and moistened with deionised water) |
| Test vehicle: | Acetone and fine quartz sand |
| Toxic reference: | 2-Chloroacetamide |
| 3. Test organism: | Earthworm |
| Species: | <i>Eisenia fetida</i> |
| Age at dosing: | 8 to 9 months; life stage: adult with clitellum |
| Weight at dosing: | 371.1 to 463.1 mg |
| Source: | Laboratory, in-house culture (IBACON, Rossdorf, Germany) |
| Test chamber: | 1-L bottling jars, loosely covered by glass-lids, filled with approximately 500 g (dry weight equivalent) artificial soil. |
| Test medium: | Artificial soil prepared according to OECD 207 |
| Diet: | Unfed during test |
| Water content of soil: | Test initiation: 30.2% to 33.0% (equivalent to 47.2% to 51.5% of the maximum water holding capacity)
Test termination: 31.5% to 33.7% (equivalent to 49.2% to 52.6% of the maximum water holding capacity) |
| Soil pH: | Test initiation: 5.9–6.2; Test termination: 5.7–6.0 |
| 4. Environmental conditions (in-life period) | |
| Temperature: | 20 to 21°C; mean = 20.5°C |
| Photoperiod: | 24-hour photoperiod (400 to 665 lux) |

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
28-June-2002 to 15-July-2002
2. Test system
The acute toxicity of IN-MK638 to earthworms, *Eisenia fetida* (Savigny), was estimated in a 14-day soil exposure laboratory study. Four replicates of 10 clitellated adult earthworms each were exposed to nominal concentrations of 10, 32, 100, 316, and 1000 mg of IN-MK638/kg dry weight soil. The control was replicated four times, with 10 earthworms in each replicate.

The toxic standard, 2-chloroacetamide, is tested once a year. The 14-day LC₅₀ of the most recent test was 30.4 mg 2-chloroacetamide/kg dry soil.

Earthworms were assessed for mortality and behavioural effects after 7 and 14 days of exposure and earthworm body weights were assessed at Days 0 and 14.

II. RESULTS AND DISCUSSION

A. FINDINGS

The LC₅₀ was determined with the moving average interpolation. The NOEC was determined with Dunnett's test, $\alpha = 0.05$.

Table 181
Summary of results

Test species	<i>Eisenia fetida</i>					
Exposure	Artificial soil					
NOEC	316 mg/kg dry weight soil					
LOEC	1000 mg/kg dry weight soil					
LC₅₀ (14 days)	552.9 mg/kg soil dry weight (95% confidence limits of 510.4 and 598.9 mg/kg)					
Test item	Control	IN-MK638				
IN-MK638 concentration [mg/kg dry soil wt]	Deionised water	10	32	100	316	1000
Mortality after 14 days	0	0	0	0	2.5	100
Significance (Fisher exact test, $\alpha=0.05$)	— ^a	—	—	—	n.s.	^c
Body weight change [%]	-3.8	-2.3	-2.8	-2.0	-0.6	—
Significance (Dunnett-test, $\alpha=0.05$)	—	n.s. ^b	n.s.	n.s.	n.s.	—

^a Not applicable

^b Not significantly different compared to the control

^c Significantly different compared to the control

III. CONCLUSION

The 14-day LC₅₀ = 552.9 mg IN-MK638/kg dry soil (95% confidence limits of 510.4 and 598.9 mg/kg), the LOEC = 1000 mg IN-MK638/kg dry soil, and the NOEC = 316 mg IN-MK638/kg dry soil.

(Luhrs, U., 2002c)

RMS comment

This study was submitted in the original DAR, AD3 2005. This study was conducted in compliance with the current guideline. The 14-day LC₅₀ of the study report is of 552.9 mg IN-MK638/kg dry soil. However the LC₅₀ for the earthworm was recalculated in previous assessment of the substance (AD3, 2005) according to trimmed Spearman-Kärber method, based on Hamilton et al. (1977/1978), using data from the author, resulting in a 14 d nominal LC₅₀ of 546.2 mg/kg dry weight soil, 95% C.L. 516.0-578.0. This calculation was not revised by RMS as acute toxicity studies are not required anymore. This study is considered only informative.

Report: Lührs, U. (2013d); IN-MK638: Effects on reproduction and growth of the earthworm, *Eisenia Fetida*, in artificial soil with 5% peat

DuPont Report No.: DuPont-36498

Guidelines: OECD 222 (2004) **Deviations:** None

Testing Facility: IBACON, Rossdorf, Germany

Testing Facility Report No.: 75283022

GLP: Yes

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

Executive summary:

The sublethal toxicity of IN-MK638 to earthworms, *Eisenia fetida*, was 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 but with reduced organic matter) treated with the test item to obtain the nominal concentrations of 6.26, 12.51, 25.03, 50.05, and 100.1 mg IN-MK638/kg dry artificial soil (corresponding to 6.25, 12.5, 25.0, 50.0, and 100 mg IN-MK638/kg dry artificial soil, adjusted for purity) and to an untreated control (using the same amount of acetone and quartz sand as in the test item treated groups moistened with deionised water). 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 LC_{50} after 28 days was determined to be greater than 100 mg IN-MK638/kg dry artificial soil. The NOAEC (No-Observable-Adverse-Effect Concentration) for earthworms based on mortality, reproduction, growth and nominal concentrations was 100 mg IN-MK638/kg dry artificial soil, the highest concentration tested.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material	IN-MK638 technical metabolite
Lot/Batch #:	MK638-002
Purity:	99.9%
CAS #:	Not assigned
Stability of test compound:	Not determined in the test system
2. Control:	Untreated (using the same amount of acetone and sand as in the test item groups and moistened with deionised water)
Test vehicle:	Acetone
3. Test organism	Earthworm
Species:	<i>Eisenia fetida</i>
Age at dosing:	Approximately 8 months
Weight at dosing:	305 to 594 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 <i>ca.</i> 500 g artificial soil dry weight
Test medium:	Artificial soil prepared according to OECD 222 but with reduced content of peat (5%); maximum water holding capacity of the artificial soil, as measured: 40%
Diet:	Finely ground cattle manure
Water content of soil:	Initiation: 19.9 to 21.6% (equivalent to 49.8 to 54.1% of the maximum water holding capacity) Termination: 22.2 to 23.7% (equivalent to 55.6 to 59.3% of the maximum water holding capacity)
Soil pH:	5.8 to 5.9 at test start and 5.8 to 5.9 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

21-August-2013 to 17-October-2013

2. Experimental treatments

The sublethal toxicity of IN-MK638 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 but with reduced organic matter) treated with the test item to obtain the nominal concentrations of 6.26, 12.51, 25.03, 50.05, and 100.1 mg IN-MK638/kg dry artificial soil (corresponding to 6.25, 12.5, 25.0, 50.0, and 100 mg IN-MK638/kg dry artificial soil, adjusted for purity) and to an untreated control (using the same amount of acetone and quartz sand as in the test item treated groups moistened with deionised water). 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 2012 to October 2012.

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 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

Mortality data were analysed for significance by using Fisher's Exact Test (one-sided greater, $\alpha = 0.05$).

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 NOAEC for weight changes and reproduction was performed using Williams t-test (multiple comparison, $\alpha = 0.05$, two-sided for weight changes, one-sided smaller for reproduction).

The LC₅₀ after 28 days was not determined by statistical analysis as mortality was less than 50%.

II. RESULTS AND DISCUSSION

A. FINDINGS

The EC₅₀ for reproduction of the reference item in the most recent test was 1.7 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 except for one dead worm (2.5%) at the concentration of 6.25 mg IN-MK638/kg dry artificial soil, which was not statistically significantly different compared to the control. The LC₅₀ after 28 days was determined to be greater than 100 mg IN-MK638/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 exposure in any of the treatment groups. No statistically significant differences in weight change (28-day assessment) compared to the control were observed up to and including the concentration of 50.0 mg IN-MK638/kg dry artificial soil. At 100 mg IN-MK638/kg dry artificial soil there was a statistically significant body weight increase compared to the control, which was not considered to be an adverse effect.

No statistically significant differences in reproduction (56-day assessment) of earthworms compared to the control were observed up to and including the highest concentration of 100 mg IN-MK638/kg dry artificial soil. Cumulative mortality and weight change (of adults) at 28 days, and reproduction at 56 days are reported in the summary table below.

Table 182
Sublethal toxicity of IN-MK638 to earthworms

Nominal IN-MK638 concentration (mg/kg dry soil)	28-day mortality (%) mean	28-day weight change (%) mean	56-day reproduction (# of juveniles) mean
Control (0.0)	0	18.7	215
6.25	2.5 ^{n.s.}	18.9 ^{n.s.}	209 ^{n.s.}
12.5	0 ^{n.s.}	18.4 ^{n.s.}	245 ^{n.s.}
25.0	0 ^{n.s.}	18.3 ^{n.s.}	184 ^{n.s.}
50.0	0 ^{n.s.}	23.3 ^{n.s.}	211 ^{n.s.}
100	0 ^{n.s.}	36.5 [*]	178 ^{n.s.}

* significantly different from the control, but not considered to be adverse

n.s. not significantly different from the control

Mortality: Fisher's Exact Test, one-sided greater, $\alpha = 0.05$

Weight change: Williams t-test, two sided, $\alpha = 0.05$;

Reproduction: Williams t-test, one-sided smaller, $\alpha = 0.05$

III. CONCLUSIONS

IN-MK638 had no significant lethal effects or effects on reproduction or feeding activity of the earthworm *Eisenia fetida* when exposed to concentrations up to and including 100 mg/kg dry artificial soil. The statistically significant weight increase at 100 mg IN-MK638/kg dry artificial soil was not considered to be an adverse effect.

The overall NOAEC (No-Observable-Adverse-Effect Concentration) was determined to be 100 mg IN-MK638/kg dry artificial soil and the LOAEC (Lowest-Observable-Adverse-Effect Concentration) was estimated to be greater than 100 mg IN-MK638/kg dry artificial soil.

The LC₅₀ after 28 days was determined to be greater than 100 mg IN-MK638/kg dry artificial soil, the highest concentration tested.

(Lührs, U., 2013d)

RMS comment

This study is valid.

The NOEC based on reproduction (No-Observable-Effect Concentration) was determined to be 100 mg IN-MK638/kg dry artificial soil.

RMS notes that no significant effect was observed on the mean number of juveniles however, the values appear to be quite variable among test groups. No dose relationship can be found. RMS nevertheless calculated an EC10 value of 66.0 mg IN-MK638/kg dry artificial soil (95% confidence intervals: 2.02-1662.7) based on effects on reproduction. This EC10 is not considered reliable (large confidence interval) and is not used for the risk assessment. The NOEC of 50 mg IN-MK638/kg soil dry weight based on effects on weight change is used for the risk assessment.

Report: Lührs, U. (2002d); IN-MK643: Acute toxicity to the earthworm, *Eisenia fetida* in artificial soil

DuPont Report No.: DuPont-10072

Guidelines: OECD 207 (1984), ISO 11268-1 (1993) **Deviations:** None

Testing Facility: IBACON, Rossdorf, Germany

Testing Facility Report No.: 13381021

GLP: Yes

Certifying Authority: Hessisches Ministerium für Umwelt, Landwirtschaft und Forsten

I. MATERIALS AND METHODS

A. MATERIALS

- | | |
|--|--|
| 1. Test material: | IN-MK643 technical metabolite |
| Lot/Batch #: | MK643-002 |
| Purity: | 95.4%, by analysis |
| Description: | White solid |
| CAS#: | Not available |
| Stability of test compound: | Test item is considered stable under test conditions |
| 2. Control: | Untreated (and moistened with deionised water) |
| Test vehicle: | Fine quartz sand |
| Toxic reference: | 2-Chloroacetamide |
| 3. Test organism: | Earthworm |
| Species: | <i>Eisenia fetida</i> |
| Age at dosing: | 10 to 11 months; life stage: adult with clitellum |
| Weight at dosing: | 380 to 485 mg |
| Source: | Laboratory, in-house culture (Laboratory: IBACN, Rossdorf, Germany) |
| Test chamber: | 1-L bottling jars, loosely covered by glass-lids, filled with approximately 500 g (dry weight equivalent) artificial soil. |
| Test medium: | Artificial soil prepared according to OECD 207 |
| Diet: | Unfed during test |
| Water content of soil: | Test initiation: 31.8% to 34.3% (equivalent to 49.7% to 53.6% of the maximum water holding capacity)
Test termination: 32.4 to 35.9% (equivalent to 50.6% to 56.1% of the maximum water holding capacity) |
| Soil pH: | Test initiation: 5.6–5.7; Test termination: 5.9–6.2 |
| 4. Environmental conditions (in-life period) | |
| Temperature: | 20 to 21°C |
| Photoperiod: | 24 hour photoperiod (538 to 782 lux) |

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
27-June-2002 to 12-July-2002
2. Experimental treatments
The acute toxicity of IN-MK643 to earthworms, *Eisenia fetida* (Savigny), was estimated in a 14-day soil exposure laboratory study. Four replicates of 10 clitellated adult earthworms each were exposed to nominal concentrations of 10, 32, 100, 316, and 1000 mg of IN-MK643/kg dry weight soil. The control was replicated four times, with ten earthworms in each replicate.

The toxic standard, 2-chloroacetamide, is tested once a year. The 14-day LC₅₀ of the most recent test was 30.4 mg 2-chloroacetamide/kg dry soil.

Earthworms were assessed for mortality and behavioural effects after 7 and 14 days of exposure and earthworm body weights were assessed at Days 0 and 14. Morality was assessed with Fisher's exact test (2-sided) and body weight was assessed with Dunnett's test (2-sided), $\alpha = 0.05$.

II. RESULTS AND DISCUSSION

A. FINDINGS

Table 183
Summary of results

Test species	<i>Eisenia fetida</i>					
Exposure	Artificial soil					
NOEC	100 mg/kg dry weight soil					
LOEC	316 mg/kg dry weight soil					
LC₅₀ (14 days)	594.8 mg/kg soil dry weight (95% confidence limits of 524.4 and 674.7 mg/kg)					
Test item	Control	IN-MK643				
IN-MK643 concentration [mg/kg dry soil wt]	Deionised water	10	32	100	316	1000
Mortality after 14 days	0	0	0	0	0	97.5
Significance (Fisher exact test, $\alpha=0.05$)	— ^a	—	—	—	—	^c
Body weight change [%]	-0.1	-3.3	-4.2	-6.7	-19.0	-39.0
Significance (Dunnett-test, $\alpha=0.05$)	—	n.s. ^b	n.s.	n.s.	^c	^c

^a Not applicable

^b Not significantly different compared to the control

^c Significantly different compared to the control

III. CONCLUSION

The 14-day LC₅₀ = 594.8 mg IN-MK643/kg dry soil (95% confidence limits of 524.4 and 674.7 mg IN-MK643/kg dry soil by moving average procedure), the LOEC = 316 mg IN-MK643/kg dry soil (based on body weight), and the NOEC = 100 mg IN-MK643/kg dry soil (based on body weight).

(Lührs, U., 2002d)

RMS comment

This study was submitted in the original DAR, AD3 2005. This study was conducted in compliance with the current guideline. The 14-day LC₅₀ = 594.8 mg IN-MK643/kg dry soil. However the LC₅₀ for the earthworm was recalculated in previous assessment of the substance (AD3, 2005) according to trimmed Spearman-Kärber method, based on Hamilton et al. (1977/1978), using data from the author, resulting in a 14 d nominal LC₅₀ of 570.5 mg/kg dry weight soil, 95% C.L. 553.7-587.8. This study is still considered acceptable but only informative as acute toxicity studies are not required anymore.

Report: Lührs, U. (2013e); IN-MK643: Effects on reproduction and growth of the earthworm, *Eisenia fetida*, in artificial soil with 5% peat

DuPont Report No.: DuPont-36499

Guidelines: OECD 222 (2004), ISO 11268-2 (2012) **Deviations:** None

Testing Facility: IBACON, Rossdorf, Germany

Testing Facility Report No.: 75312022

GLP: Yes

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

Executive summary:

The sublethal toxicity of IN-MK643 to earthworms, *Eisenia fetida*, was 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 but with reduced organic matter) treated with the test item to obtain the nominal concentrations of 6.46, 12.93, 25.85, 51.71 and 103.4 mg IN-MK643/kg dry artificial soil (corresponding to 6.25, 12.5, 25.0, 50.0, and 100 mg IN-MK643/kg dry artificial soil, adjusted for purity) and to an untreated control (using the same amount of acetone and quartz sand as in the test item treated groups moistened with deionised water). Mortality and growth (body weight) of the earthworm were assessed after 28 days and the effect on reproduction (number of juveniles produced) was assessed after 56 days. The LC_{50} after 28 days was determined to be greater than 100 mg IN-MK643/kg dry artificial soil. The NOEC (No-Observable-Effect Concentration) for earthworms based on mortality, reproduction, growth and nominal concentrations was 25.0 mg IN-MK643/kg dry artificial soil. The LOEC (Lowest-Observable-Effect Concentration) was 50.0 mg IN-MK643/kg dry artificial soil.

I. MATERIALS AND METHODS

A. MATERIALS

- | | |
|--|---|
| 1. Test material | IN-MK643 technical metabolite |
| Lot/Batch #: | MK643-002 |
| Purity: | 96.7% |
| CAS #: | 877681-12-4 |
| Stability of test compound: | Not determined in the test system |
| 2. Control: | Untreated (using the same amount of acetone and quartz sand as in the test item treated groups moistened with deionised water) |
| Test vehicle: | Acetone |
| 3. Test organism | Earthworm |
| Species: | <i>Eisenia fetida</i> |
| Age at dosing: | Approximately 8 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 <i>ca.</i> 500 g artificial soil dry weight |
| Test medium: | Artificial soil prepared according to OECD 222 but with reduced content of peat (5%), maximum water holding capacity of the artificial soil, as measured: 40% |
| Diet: | Finely ground cattle manure |
| Water content of soil: | Initiation: 20.3 to 21.3% (equivalent to 50.8 to 53.2% of the maximum water holding capacity)
Termination: 23.0 to 25.4% (equivalent to 57.6 to 63.5% of the maximum water holding capacity) |
| Soil pH: | 5.7 at test start and 5.8 to 6.0 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
27-August-2013 to 23-October-2013
2. Experimental treatments
The sublethal toxicity of IN-MK643 to earthworms, *Eisenia fetida*, were 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 but with reduced organic matter) treated with the test item to obtain the nominal concentrations of 6.46, 12.93, 25.85, 51.71 and 103.4 mg IN-MK643/kg dry artificial soil (corresponding to 6.25, 12.5, 25.0, 50.0, and 100 mg IN-MK643/kg dry artificial soil, adjusted for purity) and to an untreated control (using the same amount of acetone and quartz sand as in the test item treated groups moistened with deionised water). 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 2012 to October 2012.
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, the soil (without the adult earthworms) 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 and reproduction was performed using Williams t-test (multiple comparison, $\alpha = 0.05$, two-sided for weight changes, one-sided smaller for reproduction).

The LC₅₀ after 28 days was not determined by statistical analysis as no mortality was observed.

II. RESULTS AND DISCUSSION

A. FINDINGS

The EC₅₀ for reproduction of the reference item in the most recent test was 1.7 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 LC₅₀ after 28 days was determined to be greater than 100 mg IN-MK643/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 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.

No significant effects on reproduction (56-day assessment) were observed up to and including the concentration of 25.0 mg IN-MK643/kg dry artificial soil. At the concentration of 50.0 mg IN-MK643/kg dry artificial soil and above reproduction was statistically significantly different compared to the control.

Cumulative mortality and weight change (of adults) at 28 days, and reproduction at 56 days are reported in the summary table below.

Table 184
Sublethal toxicity of IN-MK643 to earthworms

Nominal IN-MK643 concentration (mg test item/kg dry soil)	28-day mortality (%) mean	28-day weight change (%) mean	56-day reproduction (# of juveniles) mean
Control (0.0)	0	42.1	253
6.25	0	43.8 ^{n.s.}	271 ^{n.s.}
12.5	0	42.9 ^{n.s.}	269 ^{n.s.}
25.0	0	38.8 ^{n.s.}	219 ^{n.s.}
50.0	0	44.1 ^{n.s.}	191 [*]
100	0	43.9 ^{n.s.}	142 [*]

n.s. not significantly different from the control

* significantly different from the control

Weight change: Williams t-test, two sided, $\alpha = 0.05$; Reproduction: Williams t-test, one-sided smaller, $\alpha = 0.05$)

III. CONCLUSIONS

The overall NOEC (No-Observable-Effect Concentration) based on mortality, reproduction and growth and nominal concentrations was determined to be 25.0 mg IN-MK643/kg dry artificial soil and the LOEC (Lowest-Observable-Effect Concentration) was determined to be 50.0 mg IN-MK643/kg dry artificial soil.

The LC₅₀ after 28 days was determined to be greater than 100 mg IN-MK643/kg dry artificial soil, the highest concentration tested.

(Lühns, U., 2013e)

RMS comment

This study is valid.

RMS notes that the effects on reproduction were not significant at concentrations up to and including 25.0 mg IN-MK643/kg dry artificial soil but, as a clear dose-effect relationship was found on this parameter, an EC10 was calculated by RMS.

The EC₁₀ was determined to be 22.25 mg IN-MK643/kg dry artificial soil (95% confidence intervals: 5.93-56.16). The EC10 is used for the risk assessment.

B.9.4.2. Effects on non-target soil meso- and macrofauna (other than earthworms)

Report: Pavić, B. (2013c); Indoxacarb (DPX-KN128) technical: Effects on the collembola *Folsomia candida* in artificial soil with 5% peat

DuPont Report No.: DuPont-35311

Guidelines: OECD 232 (2009), ISO 11267 (1999) **Deviations:** None

Testing Facility: IBACON, Rossdorf, Germany

Testing Facility Report No.: 75242016

GLP: Yes

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

Executive summary:

The effects of indoxacarb (DPX-KN128) technical 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 12 days old Collembola were exposed for 28 days to artificial soil (prepared according to OECD 232) treated with five nominal concentrations of indoxacarb of 16.31, 32.62, 65.24, 130.5, and 261.0 mg a.s./kg soil dry weight (corresponding to 15.625, 31.25, 62.5, 125, and 250 mg a.s./kg dry artificial soil, adjusted for purity) and an untreated control (using the same amount of acetone and fine quartz sand per gram substrate as in the test item groups). Mortality and reproduction (number of juveniles produced) were assessed after 28 days. The overall 28-day NOEC (No-Observed-Effect Concentration) was determined to be 125 mg a.s./kg soil dry weight.

I. MATERIALS AND METHODS

A. MATERIALS

- | | | |
|----|------------------------------------|--|
| 1. | Test material: | Indoxacarb technical |
| | Lot/Batch #: | KN128-098 |
| | Purity: | 95.8% |
| | Description: | Solid |
| | CAS#: | 173584-44-6 |
| | Stability of test compound: | Not analyzed in the test system |
| 2. | Control: | Untreated (using the same amount of acetone and sand per g substrate as in the test item groups and moistened with deionised water) |
| | Test vehicle: | Acetone |
| | 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: 38% |
| | Diet: | Granulated dry yeast |
| | Water (deionised) content of soil: | Initiation: 20.3 to 20.7%, equivalent to 53.5 to 54.5% of the maximum water holding capacity
Termination: 16.9 to 24.9% equivalent to 44.4 to 65.5% of the maximum water holding capacity |
| | Soil pH: | 6.3 to 6.4 at test start; 6.4 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
28-September-2012 to 18-December-2012

2. Experimental treatments

A study was conducted to determine the effects of indoxacarb 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 of 16.31, 32.62, 65.24, 130.5 and 261.0 mg a.s./kg soil dry weight (corresponding to 15.625, 31.25, 62.5, 125, and 250 mg a.s./kg dry artificial soil, adjusted for purity) and an untreated control (using the same amount of acetone and fine quartz sand per gram substrate as in the test item groups). 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 from August 2012 to September 2012.

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 analyzed 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 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 59.9 mg/kg artificial soil dry weight.

No significant mortality relative to the control group was observed at any treatment level of indoxacarb (Fisher's Exact Test, one-sided greater, $\alpha = 0.05$). No abnormal behaviour was observed in the surviving adult or juvenile Collembola.

The mean number of juveniles produced in the highest test concentration was 232, which represented 67% of the control group reproduction. The effect on reproduction was statistically significant compared to the control only at the highest test concentration of 250 mg a.s./kg artificial soil. No significant reduced reproduction was observed at the lower test concentrations (Williams t-test, $\alpha = 0.05$, one-sided smaller).

Table 185
The effects on mortality and reproduction of Collembola, *Folsomia candida*, exposed to indoxacarb technical in artificial soil for 28 days

Indoxacarb concentration (mg a.s./kg soil dry weight adjusted for purity)	Mean% mortality	Reproduction	
		Mean juveniles per replicate	% of control
Untreated control (0.0)	16	348	-
15.625	25 ^{n.s.}	358 ^{n.s.}	103
31.25	18 ^{n.s.}	420 ^{n.s.}	120
62.5	15 ^{n.s.}	364 ^{n.s.}	104
125	35 ^{n.s.}	303 ^{n.s.}	87
250	35 ^{n.s.}	232 [*]	67

^{n.s.} Not statistically significant

^{*} Statistically significant

mortality: Fisher's Exact Test, one-sided greater, $\alpha = 0.05$

number of juveniles: Williams t-test, one-sided smaller, $\alpha = 0.05$)

III. CONCLUSIONS

Indoxacarb technical had no significant lethal effects on the Collembola *Folsomia candida* at concentrations up to and including 250 mg a.s./kg artificial soil dry weight. Indoxacarb technical had no significant reproductive effects when exposed to concentrations up to and including 125 mg a.s./kg artificial soil dry weight for 28 days.

The 28-day EC₅₀ for indoxacarb technical was estimated to be greater than 250 mg a.s./kg artificial soil dry weight. The overall Lowest-Observed-Effect Concentration (LOEC) for indoxacarb technical was determined to be 250 mg a.s./kg artificial soil dry weight. The overall No-Observed-Effect Concentration (NOEC) for indoxacarb technical was determined to be 125 mg a.s./kg artificial soil dry weight.

(Pavić, B., 2013c)

RMS comment

This study is valid.

RMS notes that the effects on reproduction were not significant at concentrations up to and including 125 mg a.s./kg artificial soil but, as a dose-effect relationship was found on this parameter, an EC10 was calculated by RMS.

The EC₁₀ was determined to be 106.8 mg as/kg dry artificial soil (95% confidence intervals: 22.43-224.51). The EC10 is used for the risk assessment.

Report: Pavić, B. (2013e); IN-JT333: Effects on the Collembola *Folsomia candida* in artificial soil with 5% peat

DuPont Report No.: DuPont-35312

Guidelines: ISO 11267 (1999), OECD 232 (2009) **Deviations:** None

Testing Facility: IBACON, Rossdorf, Germany

Testing Facility Report No.: 75252016

GLP: Yes

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

Executive summary:

The effects of IN-JT333 on the mortality and reproduction of Collembola (*Folsomia candida*) was determined in a 28-day soil exposure laboratory study according to OECD 232, 2009 and ISO 11267, 1999. Ten to 12 days old Collembola were exposed for 28 days to artificial soil (prepared according to OECD 232) treated with five nominal concentrations of IN-JT333 of 6.33, 12.65, 25.30, 50.61, and 101.2 mg/kg dry artificial soil (corresponding to 6.25, 12.5, 25.0, 50.0, and 100 mg/kg dry artificial soil, adjusted for purity) and an untreated control (using the same amount of acetone and fine quartz sand per g substrate as in the test item groups). Mortality and reproduction (number of juveniles produced) were assessed after 28 days. The overall 28-day NOEC (No-Observed-Effect Concentration) was determined to be 50.0 mg IN-JT333/kg soil dry weight.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: IN-JT333 technical metabolite
Lot/Batch #: JT333-017
Purity: 98.8%
Description: Solid
CAS#: Not available
Stability of test compound: Not analyzed in the test system
2. Control: Untreated (using the same amount of acetone and sand per g substrate as in the test item groups and moistened with deionised water)

Test vehicle: Acetone
Toxic reference: Boric acid
3. Test System: Collembola
Species: *Folsomia candida*, 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: 38%

Diet: Granulated dry yeast
Water (deionised) content of soil: Initiation: 21.1 to 22.1%, equivalent to 55.6 to 58.1% of the maximum water holding capacity
Termination: 17.8 to 20.5% equivalent to 46.8 to 53.9% of the maximum water holding capacity

Soil pH: 6.3 to 6.4 at test start; 6.1 to 6.2 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
24-October-2012 to 26-November-2012

2. Experimental treatments

A study was conducted to determine the effects of IN-JT333 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 IN-JT333 of 6.33, 12.65, 25.30, 50.61, and 101.2 mg/kg dry artificial soil (corresponding to 6.25, 12.5, 25.0, 50.0, and 100 mg/kg dry artificial soil, adjusted for purity) and an untreated control (using the same amount of acetone and fine quartz sand per gram substrate as in the test item groups). 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 from August 2012 to September 2012.

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 analyzed 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 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 59.9 mg/kg artificial soil dry weight.

No significant mortality relative to the control group was observed at any treatment level of IN-JT333 (Fisher's Exact Test, one-sided greater, $\alpha = 0.05$). No abnormal behaviour was observed in the surviving adult or juvenile Collembola.

The mean number of juveniles produced in the highest test concentration was 222, which represented 80% of the control group reproduction. This number of juveniles was statistically significant compared to the control where a mean number of 277 juveniles was produced (Williams t-test, $\alpha = 0.05$, one-sided smaller).

Table 186
The effects on mortality and reproduction of Collembola, *Folsomia candida*, exposed to IN-JT333 in artificial soil for 28 days

IN-JT333 concentration (mg/kg soil adjusted for purity)	Mean% mortality ^a	Reproduction	
		Mean juveniles per replicate	% of control
Untreated control (0.0)	11	277	-
6.25	28	271	98 ^{n.s.}
12.5	23	297	107 ^{n.s.}
25.0	18	289	104 ^{n.s.}
50.0	15	257	93 ^{n.s.}
100	23	222	80 [*]

^a There were no significant differences to the control (Fisher's Exact Test, $\alpha = 0.05$, one-sided greater)

n.s. Not statistically significant (Williams t-test, $\alpha = 0.05$, one-sided smaller)

* Statistically significant (Williams t-test, $\alpha = 0.05$, one-sided smaller)

III. CONCLUSIONS

IN-JT333 had no significant lethal effects on the Collembola *Folsomia candida* when exposed to concentrations up to and including 100 mg/kg artificial soil dry weight for 28 days and no reproductive effects when exposed to concentrations up to and including 50.0 mg/kg artificial soil dry weight for 28 days.

The 28-day EC₅₀ for IN-JT333 was estimated to be greater than 100 mg/kg artificial soil dry weight. The overall Lowest-Observed-Effect Concentration (LOEC) was determined to be 100 mg IN-JT333/kg artificial soil dry weight and the overall 28-day No-Observed-Effect Concentration (NOEC) was determined to be 50.0 mg IN-JT333/kg artificial soil dry weight.

(Pavić, B., 2013e)

RMS comment

This study is valid.

The overall Lowest-Observed-Effect Concentration (LOEC) was determined to be 100 mg IN-JT333/kg artificial soil dry weight and the overall 28-day No-Observed-Effect Concentration (NOEC) was determined to be 50.0 mg IN-JT333/kg artificial soil dry weight.

RMS calculated an EC₁₀ value of 64.17 mg IN-JT333/kg dry artificial soil (95% confidence intervals: 11.18-108.04) based on reproduction.

Report: Luhrs, U. (2005); IN-JU873: Effects on the collembola, *Folsomia candida* in artificial soil

DuPont Report No.: DuPont-16461

Guidelines: ISO 11267 (1999) **Deviations:** None

Testing Facility: IBACON, Rossdorf, Germany

Testing Facility Report No.: 13373016

GLP: Yes

Certifying Authority: Hessisches Ministerium für Umwelt, Landwirtschaft und Forsten

Executive summary:

A study was conducted to determine the effect of IN-JU873 on the mortality and reproduction of Collembola (*Folsomia candida*) according to ISO 11267, 1999. The Collembola were exposed for 28 days to artificial soil (prepared according to OECD 207) treated with nominal concentrations of 1, 10, and 100 mg IN-JU873/kg soil dry weight. The EC₅₀ and the Lowest-Observed-Effect Concentration (LOEC) for IN-JU873 were determined to be >100 mg/kg soil dry weight, the highest concentration tested. The overall NOEC (No-Observed-Effect Concentration) was determined to be 100 mg IN-JU873/kg soil dry weight.

I. MATERIALS AND METHODS

A. MATERIALS

- | | |
|---|---|
| 1. Test material: | IN-JU873 technical metabolite |
| Lot/Batch #: | JU873-005 |
| Purity: | 99.06% |
| Description: | Solid/white |
| CAS#: | Not assigned |
| Stability of test compound: | Considered to be stable under the conditions of the test |
| 2. Vehicle: | acetone |
| Toxic reference: | Phenmedipham (tested once per year) |
| 3. Test system: | Collembola |
| Species: | <i>Folsomia candida</i> Willem |
| Age at test start: | 10 to 12 days |
| Weight at test start: | Not specified |
| Source: | In-house laboratory culture |
| Acclimation period: | 12 days |
| Diet: | Granulated dry yeast |
| Test chamber: | Glass containers (volume: 100 mL; diameter: 5.0 cm),
closed, filled with 30 ± 1.5 g artificial soil fresh weight |
| Test medium: | Artificial soil prepared according to OECD 207 |
| Maximum water holding capacity of the
OECD 207 artificial soil, as measured: | 57% |
| Water content: | Initiation: 30% (equivalent to 52 to 53% of the maximum
water holding capacity)
Termination: 28 to 29% (equivalent to 49 to 51% of the
maximum water holding capacity) |
| 4. Environmental conditions | |
| Temperature: | 18 to 21°C |
| pH: | 5.5 at test start and at test termination |
| Photoperiod: | 16 hour light, 8 hour dark, photoperiod (470 to 590 lux) |

B. STUDY DESIGN AND METHODS

1. Experimental start/completion
27-April-2005 to 29-June-2005

2. Experimental treatments

A study was conducted to determine the effect of IN-JU873 on the mortality and reproduction of Collembola (*Folsomia candida*). Five replicates of ten Collembola each (total 50 individuals per treatment group) were each exposed to nominal concentrations of 1, 10, and 100 mg IN-JU873/kg artificial soil dry weight, an untreated (deionised water only) control and a solvent (acetone and deionised water) control. A toxic standard (Phenmedipham, tested at a concentration range of 3.6 to 140 mg/kg artificial soil dry weight) is tested once per year to ensure sensitivity of the test system. The most recent test to this study was conducted from April 2005 to May 2005.

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 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-exact test (two-sided, $\alpha = 0.05$).

Reproduction data were tested for normal distribution and homoscedascity using Kolmogoroff-Smirnov test and Cochran test ($\alpha = 0.05$). Control and solvent control were compared using Student-t test for homogeneous variances ($\alpha = 0.05$, one-sided smaller). Further statistical evaluation of the NOEC for reproduction was performed using Dunnett test (multiple comparison, $\alpha = 0.05$, one-sided smaller).

The LC_{50} and its 95% confidence limits were not determined by a statistical analysis as no mortality $\geq 50\%$ was observed.

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 6.64 mg phenmedipham/kg artificial soil dry weight, and the EC_{50} for reproduction was calculated to be 19.9 mg phenmedipham/kg artificial soil dry weight.

IN-JU873 caused no significant increase in mortality of Collembola, *Folsomia candida*, when exposed to concentrations up to and including 100 mg IN-JU873/kg artificial soil dry weight, the highest rate tested. Mean mortality was 9% in the pooled control and 10% at 100 mg IN-JU873/kg artificial soil dry weight.

IN-JU873 had no significant reproductive effects on the Collembola, *Folsomia candida*, when exposed to concentrations up to and including 100 mg IN-JU873/kg artificial soil dry weight, the highest concentration tested. The mean number of juveniles produced in the pooled control was 469 and in the highest test concentration it was 471. This represented an increase of 1% over the control group reproduction. IN-JU873 therefore had no significant reproductive or lethal effects on the Collembola, *Folsomia candida*, when exposed to concentrations up to and including 100 mg IN-JU873/kg artificial soil dry weight, the highest concentration tested.

Table 187
The effects on mortality and reproduction of collembola, *Folsomia candida*, exposed to IN-JU873 in artificial soil

Nominal IN-JU873 concentration (mg/kg soil)	Mean% mortality (\pm SD) ^a	Mean no. of juveniles (\pm SD) ^a	Reduction in reproduction (%) ^b
Untreated control (0.0)	12 (\pm 11)	472 (\pm 35)	-
Solvent control (0.0)	6 (\pm 9)	465 (\pm 63)	-
Pooled control	9 (\pm 10)	469 (\pm 48)	-
1	14 (\pm 11)	427 (\pm 49)	9
10	2 (\pm 4)	496 (\pm 63)	-6
100	10 (\pm 10)	471 (\pm 80)	-1

^a There were no significant differences from the control (mortality: Fisher Exact test, one-sided, $\alpha = 0.05$; number of juveniles: Dunnett Test, one-sided smaller, $\alpha = 0.05$)

^b Negative reduction represents an increase relative to the control group

III. CONCLUSIONS

The EC₅₀ and the Lowest-Observed-Effect Concentration (LOEC) for IN-JU873 were determined to be >100 mg/kg artificial soil dry weight, the highest concentration tested. The overall No-Observed-Effect Concentration (NOEC) was determined to be 100 mg IN-JU873/kg artificial soil dry weight.

(Lührs, U., 2005)

RMS comment

This study is valid according to the guideline ISO 11267 (1999). RMS notes that this guideline is not the one recommended for this kind of test in the Regulation (EU) No 283/2013 of 1 March 2013 setting out the data requirements for active substances, in accordance with Regulation (EC) No 1107/2009, however these guidelines are similar.

According to this Regulation, the test guideline OECD 232 is recommended. However, as the test clearly shows the absence of toxicity, the results above are considered acceptable.

The Lowest-Observed-Effect Concentration (LOEC) for IN-JU873 was determined to be >100 mg/kg artificial soil dry weight, the highest concentration tested. The overall No-Observed-Effect Concentration (NOEC) was determined to be 100 mg IN-JU873/kg artificial soil dry weight.

No dose related effect was found, therefore no EC10 was calculated.

Report: Lührs, U. (2011); IN-KB687: Effects on the collembola *Folsomia candida* in artificial soil with 5% peat

DuPont Report No.: DuPont-31721, Revision No. 1

Guidelines: OECD 232 (2009), ISO 11267 (1999) **Deviations:** None

Testing Facility: IBACON, Rossdorf, Germany

Testing Facility Report No.: 62862016

GLP: Yes

Certifying Authority: Hessisches Ministry for Environment, Energy, Family and Health (Wiesbaden, Germany)

Executive summary:

The effects of IN-KB687 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 12 day old Collembola were exposed for 28 days to artificial soil (prepared according to OECD 232) treated with five nominal concentrations of IN-KB687 of 6.26, 12.53, 25.05, 50.10, and 100.2 mg/kg dry artificial soil (corresponding to 6.25, 12.5, 25, 50, and 100 mg/kg dry artificial soil, adjusted for purity) and an untreated control (using the same amount of acetone and fine quartz sand per gram substrate as in the test item groups). Mortality and reproduction (number of juveniles produced) were assessed after 28 days. The overall 28-day NOEC (No-Observed-Effect Concentration) based on reproduction was determined to be 6.25 mg IN-KB687/kg soil dry weight.

I. MATERIALS AND METHODS

A. MATERIALS

- | | | |
|----|-----------------------------|--|
| 1. | Test material: | IN-KB687 technical metabolite |
| | Lot/Batch #: | KB687-002 |
| | Purity: | 99.8% |
| | CAS#: | 177905-10-1 |
| | Stability of test compound: | Not analysed in the test system |
| 2. | Control: | Untreated (using the same amount of acetone and sand per g substrate as in the test item groups and moistened with deionised water) |
| | Test vehicle: | Acetone |
| 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: | 10-12 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: 47% |
| | Diet: | Granulated dry yeast |
| | Water content of soil: | Initiation: 23.5 to 24.0%, equivalent to 50.1 to 51.1% of the maximum water holding capacity
Termination: 21.2 to 22.8% equivalent to 45.0 to 48.5% of the maximum water holding capacity |
| | Soil pH: | 6.4 to 6.5 at test start; 6.1 to 6.3 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
07-February-2011 to 09-March-2011

2. Experimental treatments

A study was conducted to determine the effects of IN-KB687 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 IN-KB687 of 6.26, 12.53, 25.05, 50.10, and 100.2 mg/kg dry artificial soil (corresponding to 6.25, 12.5, 25, 50, and 100 mg/kg dry artificial soil, adjusted for purity) and an untreated control (using the same amount of acetone and fine quartz sand per g substrate as in the test item groups). A reference item (boric acid, at a concentration range of 59.3 to 300 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 from September 2010 to October 2010.

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 Dunnett's t-test (multiple comparison, $\alpha = 0.05$, one-sided smaller). EC_{50} was determined by using the Probit Analysis.

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 70.7 mg/kg artificial soil dry weight.

No significant mortality relative to the control group was observed at the concentration of up to and including 12.5 mg IN-KB687/kg artificial soil dry weight (Fisher's Exact Test, one-sided greater, $\alpha = 0.05$). No abnormal behaviour was observed in the surviving adult or juvenile Collembola.

The mean number of juveniles produced in the test concentration of 6.25 mg IN-KB687/kg was 354, which represented 94% of the control group reproduction and was not statistically significant compared to the control. At the test concentrations of 12.5 and 25 mg IN-KB687/kg artificial soil dry weight the reproduction was statistically significantly reduced compared to the control (Dunnett's t-test, $\alpha = 0.05$).

Table 188
The effects on mortality and reproduction of Collembola, *Folsomia candida*, exposed to IN-KB687 in artificial soil for 28 days

Nominal IN-KB687 concentration adjusted for purity (mg/kg soil)	Mean% mortality ^a	Reproduction	
		Mean juveniles per replicate ^b	% of control
Untreated control (0.0)	10	376	-
6.25	13 ^{n.s.}	354 ^{n.s.}	94
12.5	18 ^{n.s.}	268 [*]	71
25	60 [*]	0 [*]	0
50	100 [*]	0	0
100	100 [*]	0	0

^a Fisher's Exact Test, one-sided greater, $\alpha = 0.05$

^b Dunnett's t-test, $\alpha = 0.05$, one-sided smaller

* Statistically significant

n.s. Not statistically significant

III. CONCLUSIONS

IN-KB687 had no significant effects on the Collembola *Folsomia candida* when exposed for 28 days to concentrations up to and including 12.5 mg/kg artificial soil dry weight (mortality) and 6.25 mg/kg artificial soil dry weight (reproduction), respectively. The 28-day EC_{50} for IN-KB687 was determined to be 13.9 mg/kg artificial soil dry weight. The overall 28-day the Lowest-Observed-Effect Concentration (LOEC) for IN-KB687 was determined to be 12.5 mg/kg artificial soil dry weight. The overall 28-day No-Observed-Effect Concentration (NOEC) based on reproduction was determined to be 6.25 mg/kg artificial soil dry weight.

(Lührs, U., 2011)

RMS comment

This study is valid.

IN-KB687 had no significant effects on the Collembola *Folsomia candida* when exposed for 28 days to concentrations up to and including 12.5 mg/kg artificial soil dry weight (mortality) and 6.25 mg/kg artificial soil dry weight (reproduction), respectively. The overall 28-day the Lowest-Observed-Effect Concentration (LOEC) for IN-KB687 was determined to be 12.5 mg/kg artificial soil dry weight. The overall 28-day No-Observed-Effect Concentration (NOEC) based on reproduction was determined to be 6.25 mg/kg artificial soil dry weight.

RMS calculated EC10 values for both mortality and reproduction:

EC10 = 14.7 mg IN-KB687/kg artificial soil dry weight (95% confidence intervals: 11.85-22.45) for mortality.

EC10 = 10.80 mg IN-KB687/kg artificial soil dry weight (95% confidence intervals: 9.95-12.13) for reproduction.

Report: Pavić, B. (2013h); IN-KG433: Effects on the Collembola *Folsomia candida* in artificial soil with 5% peat

DuPont Report No.: DuPont-35314

Guidelines: ISO 11267 (1999), OECD 232 (2009) **Deviations:** None

Testing Facility: IBACON, Rossdorf, Germany

Testing Facility Report No.: 75262016

GLP: Yes

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

Executive summary:

The effects of IN-KG433 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 12 day old Collembola were exposed for 28 days to artificial soil (prepared according to OECD 232) treated with five nominal concentrations of IN-KG433 of 6.26, 12.51, 25.03, 50.05 and 100.1 mg/kg dry artificial soil (corresponding to 6.25, 12.5, 25.0, 50.0, and 100 mg/kg dry artificial soil, adjusted for purity) and an untreated control (using the same amount of acetone and fine quartz sand per gram substrate as in the test item groups). Mortality and reproduction (number of juveniles produced) were assessed after 28 days. The 28-day NOEC (No-Observed-Effect Concentration) based on mortality was determined to be 100 mg IN-KG433/kg soil dry weight and based on reproduction to be 6.25 mg IN-KG433/kg soil dry weight.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: IN-KG433 technical metabolite
Lot/Batch #: KG433-004
Purity: 99.9%
CAS#: Not available
Stability of test compound: Not analyzed in the test system
2. Control: Untreated (using the same amount of acetone and sand per g substrate as in the test item groups and moistened with deionised water)

Test vehicle: Acetone
Toxic reference: Boric acid
3. Test System: Collembola
Species: *Folsomia candida*, 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: 38%

Diet: Granulated dry yeast
Water content of soil: Initiation: 20.5 to 21.3%, equivalent to 54.0 to 56.0% of the maximum water holding capacity
Termination: 18.9 to 21.4% equivalent to 49.8 to 56.3% of the maximum water holding capacity

Soil pH: 6.4 at test start; 6.4 to 6.5 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
27-August-2012 to 25-September-2012
2. Experimental treatments
A study was conducted to determine the effects of IN-KG433 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 IN-KG433 of 6.26, 12.51, 25.03, 50.05 and 100.1 mg/kg dry artificial soil (corresponding to 6.25, 12.5, 25.0, 50.0, and 100 mg/kg dry artificial soil, adjusted for purity) and an untreated control (using the same amount of acetone and fine quartz sand per g substrate as in the test item groups). 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 from August 2012 to September 2012.
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 analyzed 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 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 59.9 mg boric acid/kg artificial soil dry weight.

A significant increased mortality relative to the control group was observed at the concentrations of 50.0 mg IN-KG433/kg soil dry weight (Fisher's Exact Test, one-sided greater, $\alpha = 0.05$). However, this was not considered to be a treatment related effect since at the higher concentration of 100 mg IN-KG433/kg soil dry weight no statistically significant effect could be observed. No abnormal behaviour was observed in the surviving adult or juvenile Collembola.

The range of mean numbers of juveniles produced in the IN-KG433 treated groups were from 431 to 626 juvenile Collembola, which was statistically significantly different at the concentration of 12.5 mg/kg soil dry weight and above compared to the control, where a mean of 626 juvenile Collembola was counted (Williams t-test, $\alpha = 0.05$, one-sided smaller).

Table 189

The effects on mortality and reproduction of Collembola, *Folsomia candida*, exposed to IN-KG433 in artificial soil for 28 days

IN-KG433 concentration (mg/kg soil)	Mean% mortality ^a	Reproduction	
		Mean juveniles per replicate ^b	% of control
Untreated control (0.0)	9	626	-
6.25	18 ^{n.s.}	626 ^{n.s.}	100
12.5	5 ^{n.s.}	492*	78
25.0	20 ^{n.s.}	482*	77
50.0	28 *	500*	80
100	20 ^{n.s.}	431*	69

^a Fisher's Exact Test, one-sided greater, $\alpha = 0.05$

^b William's t-test, $\alpha = 0.05$, one-sided smaller

^{n.s.} Not significant different compared to the control

*

III. CONCLUSIONS

IN-KG433 had no significant lethal treatment related effects on the Collembola *Folsomia candida* when exposed to concentrations up to and including 100 mg/kg artificial soil dry weight for 28 days and no reproductive effects on the Collembola *Folsomia candida* when exposed to concentrations up to and including 6.25 mg/kg artificial soil dry weight for 28 days.

The 28-day EC₅₀ for IN-KG433 was estimated to be greater than 100 mg/kg artificial soil dry weight.

The 28-day Lowest-Observed-Effect Concentration (LOEC) for mortality was determined to be greater than 100 mg IN-KG433/kg artificial soil dry weight and for reproduction 12.5 mg IN-KG433/kg artificial soil dry weight. The 28-day No-Observed-Effect Concentration (NOEC) based on mortality was determined to be 100 mg IN-KG433/kg artificial soil dry weight and based on reproduction to be 6.25 mg IN-KG433/kg artificial soil dry weight.

(Pavić, B., 2013h)

RMS comment

This study is valid.

The 28-day Lowest-Observed-Effect Concentration (LOEC) for mortality was determined to be greater than 100 mg IN-KG433/kg artificial soil dry weight and for reproduction 12.5 mg IN-KG433/kg artificial soil dry weight. The 28-day No-Observed-Effect Concentration (NOEC) based on mortality was determined to be 100 mg IN-KG433/kg artificial soil dry weight and based on reproduction to be 6.25 mg IN-KG433/kg artificial soil dry weight.

RMS calculated EC10 values for effects on reproduction:

EC10 = 5.97 mg IN-KG433/kg artificial soil dry weight (95% confidence intervals: 0.06-54.14) for reproduction. The confidence interval seems rather large and the EC10 is considered poorly reliable by RMS. The NOEC value of 6.25 mg IN-KG433/kg artificial soil dry weight (which is slightly higher than the EC10) can be used for the risk assessment.

Report: Pavić, B. (2013g); IN-KT413: Effects on the collembola *Folsomia candida* in artificial soil with 5% peat

DuPont Report No.: DuPont-35313

Guidelines: OECD 232 (2009), ISO 11267 (1999) **Deviations:** None

Testing Facility: IBACON, Rossdorf, Germany

Testing Facility Report No.: 75272016

GLP: Yes

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

Executive summary:

The effects of IN-KT413 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 12 day old Collembola were exposed for 28 days to artificial soil (prepared according to OECD 232) treated with five nominal concentrations of IN-KT413 of 6.27, 12.54, 25.08, 51.15 and 100.3 mg/kg dry artificial soil (corresponding to 6.25, 12.5, 25.0, 50.0, and 100 mg/kg dry artificial soil, adjusted for purity) and an untreated control (using the same amount of acetone and fine quartz sand per g substrate as in the test item groups). 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 100 mg IN-KT413/kg soil dry weight.

I. MATERIALS AND METHODS

A. MATERIALS

- | | | |
|----|------------------------------------|--|
| 1. | Test material: | IN-KT413 technical metabolite |
| | Lot/Batch #: | KT413-003 |
| | Purity: | 99.7% |
| | Description: | Solid |
| | CAS#: | Not available |
| | Stability of test compound: | Not analyzed in the test system |
| 2. | Control: | Untreated (using the same amount of acetone and sand per g substrate as in the test item groups and moistened with deionised water) |
| | Test vehicle: | Acetone |
| | 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: 38% |
| | Diet: | Granulated dry yeast |
| | Water (deionised) content of soil: | Initiation: 20.5 to 21.1%, equivalent to 53.9 to 55.5% of the maximum water holding capacity
Termination: 19.7 to 20.8% equivalent to 51.8 to 54.9% of the maximum water holding capacity |
| | Soil pH: | 6.1 to 6.4 at test start; 6.0 to 6.1 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
04-September-2012 to 09-October-2012

2. Experimental treatments

A study was conducted to determine the effects of IN-KT413 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 IN-KT413 of 6.27, 12.54, 25.08, 51.15 and 100.3 mg/kg dry artificial soil (corresponding to 6.25, 12.5, 25.0, 50.0, and 100 mg/kg dry artificial soil, adjusted for purity) and an untreated control (using the same amount of acetone and fine quartz sand per g substrate as in the test item groups). 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 from August 2011 to October 2011.

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 analyzed 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 Bonferroni-Welch t-test (multiple comparison, $\alpha = 0.05$, one-sided smaller). EC_{50} was not determined by statistical analysis as 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 75.7 mg/kg artificial soil dry weight.

No significant mortality relative to the control group was observed at any treatment level of IN-KT413 (Fisher's Exact Test, one-sided greater, $\alpha = 0.05$). No abnormal behaviour was observed in the surviving adult or juvenile Collembola.

The mean number of juveniles produced in the highest test concentration was 318, which represented 86% of the control group reproduction. The effect on reproduction was not statistically significant compared to the control (Bonferroni-Welch t-test, $\alpha = 0.05$).

Table 190
The effects on mortality and reproduction of Collembola, *Folsomia candida*, exposed to IN-KT413 in artificial soil for 28 days

IN-KT413 concentration (mg/kg soil)	Mean% mortality ^a	Reproduction	
		Mean juveniles per replicate ^a	% of control
Untreated control (0.0)	13	370	-
6.25	25	364	98
12.5	15	411	111
25.0	20	401	108
50.0	20	384	104
100	20	318	86

^a There were no significant differences from the control
(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

IN-KT413 had no significant lethal or reproductive effects on the Collembola *Folsomia candida* when exposed to concentrations up to and including 100 mg/kg artificial soil dry weight for 28 days.

The 28-day EC₅₀ and the Lowest-Observed-Effect Concentration (LOEC) for IN-KT413 were determined to be greater than 100 mg/kg artificial soil dry weight. The overall 28-day No-Observed-Effect Concentration (NOEC) was determined to be 100 mg IN-KT413/kg artificial soil dry weight.

(Pavić, B., 2013g)

RMS comment

This study is valid.

The Lowest-Observed-Effect Concentration (LOEC) for IN-KT413 was determined to be greater than 100 mg/kg artificial soil dry weight. The overall 28-day No-Observed-Effect Concentration (NOEC) was determined to be 100 mg IN-KT413/kg artificial soil dry weight.

RMS calculated EC10 values for effects on reproduction:

EC10 = 89.62 mg IN-KG413/kg artificial soil dry weight (95% confidence intervals: 6.03-588.21) for reproduction. This EC10 is not considered reliable by RMS (confidence interval too large). The NOEC of 100 mg IN-KT413/kg artificial soil dry weight is used for the risk assessment.

Report: Pavić, B. (2013j); IN-MK638: Effects on the collembola *Folsomia candida* in artificial soil with 5% peat

DuPont Report No.: DuPont-35315

Guidelines: OECD 232 (2009), ISO 11267 (1999) **Deviations:** None

Testing Facility: IBACON, Rossdorf, Germany

Testing Facility Report No.: 75282016

GLP: Yes

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

Executive summary:

The effects of IN-MK638 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 12 day old Collembola were exposed for 28 days to artificial soil (prepared according to OECD 232) treated with five nominal concentrations of IN-MK638 of 3.128, 6.26, 12.51, 25.03 and 50.05 mg/kg dry artificial soil (corresponding to 3.125, 6.25, 12.5, 25.0 and 50.0 mg/kg dry artificial soil, adjusted for purity) and an untreated control (using the same amount of acetone and fine quartz sand per g substrate as in the test item groups). 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 25.0 mg IN-MK638/kg soil dry weight.

I. MATERIALS AND METHODS

A. MATERIALS

- | | | |
|----|------------------------------------|--|
| 1. | Test material: | IN-MK638 technical metabolite |
| | Lot/Batch #: | MK638-002 |
| | Purity: | 99.9% |
| | Description: | Solid, Crystalline |
| | CAS#: | 82971-90-2 |
| | Stability of test compound: | Not analyzed in the test system |
| 2. | Control: | Untreated (using the same amount of acetone and sand per g substrate as in the test item groups and moistened with deionised water) |
| | Test vehicle: | Acetone |
| | 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: 38% |
| | Diet: | Granulated dry yeast |
| | Water (deionised) content of soil: | Initiation: 20.0 to 21.0%, equivalent to 52.7 to 55.3% of the maximum water holding capacity
Termination: 17.6 to 20.1% equivalent to 46.3 to 52.9% of the maximum water holding capacity |
| | Soil pH: | 7.1 to 7.3 at test start; 6.3 to 6.5 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
22-October-2012 to 15-January-2013
2. Experimental treatments
A study was conducted to determine the effects of IN-MK638 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 IN-MK638 of 3.128, 6.26, 12.51, 25.03 and 50.05 mg/kg dry artificial soil (corresponding to 3.125, 6.25, 12.5, 25.0 and 50.0 mg/kg dry artificial soil, adjusted for purity) and an untreated control (using the same amount of acetone and fine quartz sand per gram substrate as in the test item groups). A reference item (boric acid) 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 2012 to September 2012.
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 analyzed 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 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 59.9 mg boric acid/kg artificial soil dry weight.

No significant mortality relative to the control group was observed up to and including the concentration of 25.0 mg IN-MK638/kg soil dry weight. At 50.0 mg IN-MK638/kg soil dry weight, the highest concentration tested, mortality was statistically significantly increased compared to the control (Fisher's Exact Test, $\alpha = 0.05$, one-sided greater).

No abnormal behaviour was observed in the surviving adult or juvenile Collembola.

The mean number of juveniles produced at the concentration of 25.0 mg IN-MK638/kg soil dry weight was 197, which represented 90% of the control group reproduction. The effect on reproduction was not statistically significant compared to the control. At the concentration of 50.0 mg IN-MK638/kg artificial soil the number of juveniles was statistically significantly reduced compared to the control (Williams t-test, $\alpha = 0.05$, one-sided smaller).

Table 191
The effects on mortality and reproduction of Collembola, *Folsomia candida*, exposed to IN-MK638 in artificial soil for 28 days

IN-MK638 concentration (mg/kg soil dry weight)	Mean% mortality	Reproduction	
		Mean juveniles per replicate	% of control
Untreated control (0.0)	19	220	-
3.125	28 n.s.	231 n.s.	105
6.25	30 n.s.	207 n.s.	94
12.5	38 n.s.	172 n.s.	78
25.0	38 n.s.	197 n.s.	90
50.0	50*	159*	72

n.s. Not statistically significant

* Statistically significant

mortality: Fisher's Exact Test, one-sided greater, $\alpha = 0.05$; number of juveniles: Williams t-test, one-sided smaller, $\alpha = 0.05$

III. CONCLUSIONS

IN-MK638 had no significant lethal or reproductive effects on the Collembola *Folsomia candida* when exposed to concentrations up to and including 25.0 mg/kg artificial soil dry weight for 28 days.

The 28-day EC₅₀ was estimated to be greater than 50.0 mg IN-MK638/kg artificial soil dry weight.

The overall Lowest-Observed-Effect Concentration (LOEC) for IN-MK638 was determined to be 50.0 mg/kg artificial soil dry weight. The overall No-Observed-Effect Concentration (NOEC) was determined to be 25.0 mg/kg artificial soil dry weight.

(Pavić, B., 2013j)

RMS comment

This study is valid.

RMS notes that the effects on reproduction were not significant at concentrations up to and including 25 mg IN-MK638/kg artificial soil but, as a dose-effect relationship was found on this parameter, an EC10 was calculated by RMS.

The EC₁₀ was determined to be 10.15 mg IN-MK638/kg dry artificial soil (95% confidence intervals: 0.032-47.53). The confidence interval seems rather large, the reliability of the EC10 value is therefore low.

The overall Lowest-Observed-Effect Concentration (LOEC) for IN-MK638 was determined to be 50.0 mg/kg artificial soil dry weight. The overall No-Observed-Effect Concentration (NOEC) was determined to be 25.0 mg/kg artificial soil dry weight.

The EC10 is used for the risk assessment.

Report: Lührs, U. (2006); IN-MK643: Effects on the collembola, *Folsomia candida* in artificial soil

DuPont Report No.: DuPont-16462

Guidelines: ISO 11267 (1999) **Deviations:** None

Testing Facility: IBACON, Rossdorf, Germany

Testing Facility Report No.: 13383016

GLP: Yes

Certifying Authority: Hessisches Ministerium für Umwelt, Landwirtschaft und Forsten

Executive summary:

A study was conducted to determine the effect of IN-MK643 on the mortality and reproduction of Collembola (*Folsomia candida*) according to ISO 11267, 1999. The Collembola were exposed for 28 days to artificial soil (prepared according to OECD 207) treated with nominal concentrations of 1, 10, and 100 mg IN-MK643/kg soil dry weight and to an untreated and a solvent control. The EC₅₀ for IN-MK643 was determined to be >100 mg/kg soil dry weight, the highest concentration tested. The overall Lowest-Observed-Effect Concentration (LOEC) was determined to be 100 mg IN-MK643/kg soil dry weight, the overall NOEC (No-Observed-Effect Concentration) was determined to be 10 mg IN-MK643/kg soil dry weight. A previous test with IN-MK643 that was repeated because a solvent control was not included showed no statistically significant effect on mortality or reproduction at concentrations up to and including 100 mg/kg soil dry weight. Taken together, the tests suggest that the overall NOEC is most likely 100 mg IN-MK643/kg artificial soil dry weight.

I. MATERIALS AND METHODS

A. MATERIALS

- | | |
|---|---|
| 1. Test material: | IN-MK643 technical metabolite |
| Lot/Batch #: | MK643-002 |
| Purity: | 96.6% |
| Description: | Solid/white |
| CAS#: | Not assigned |
| Stability of test compound: | Considered to be stable under the conditions of the test |
| 2. Vehicle: | Acetone |
| Toxic reference: | Phenmedipham (tested once per year) |
| 3. Test System: | Collembola |
| Species: | <i>Folsomia candida</i> Willem |
| Age at test start: | 10 to 12 days |
| Weight at test start: | Not specified |
| Source: | In-house laboratory culture |
| Acclimation period: | 12 days |
| Diet: | Granulated dry yeast |
| Test chamber: | Glass containers (volume: 100 mL; diameter: 5.0 cm),
closed, filled with 30 ± 1.5 g artificial soil fresh weight |
| Test medium: | Artificial soil prepared according to OECD 207 |
| Maximum water holding capacity of the
OECD 207 artificial soil, as measured: | 57% |
| Water content: | Initiation: 31 to 32% (equivalent to 55 to 56% of the
maximum water holding capacity)
Termination: 29 to 30% (equivalent to 51 to 53% of the
maximum water holding capacity) |
| 4. Environmental conditions | |
| Temperature: | 18 to 21°C |
| pH: | 5.5 at test start and at test termination |
| Photoperiod: | 16 hour light, 8 hour dark, photoperiod (480 to 590 lux) |

B. STUDY DESIGN AND METHODS

1. Experimental start/completion
11-April-2005 to 08-July-2005

2. Experimental treatments

A study was conducted to determine the effect of IN-MK643 on the mortality and reproduction of Collembola (*Folsomia candida*). Five replicates of ten Collembola each (total 50 individuals per treatment group) were each exposed to nominal concentrations of 1, 10, and 100 mg IN-MK643/kg artificial soil dry weight and an untreated (deionised water only) control and a solvent control (same amount of acetone treated sand as in the test item groups). A toxic standard (Phenmedipham, tested at a concentration range of 3.6 to 140 mg a.s./kg artificial soil dry weight) is tested once per year to ensure sensitivity of the test system. The most recent test to this study was conducted from April 2005 to May 2005.

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 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-exact test (two-sided, $\alpha = 0.05$).

Reproduction data were tested for normal distribution and homoscedascity using Kolmogoroff-Smirnov test and Cochran test ($\alpha = 0.05$). Control and solvent control were compared with Student-t test (pairwise comparison, $\alpha = 0.05$, two sided). Further statistical evaluation of the NOEC for reproduction was performed using Dunnett test (multiple comparison, $\alpha = 0.05$, one-sided smaller).

The LC_{50} and its 95% confidence limits were not determined by a statistical analysis as no mortality $\geq 50\%$ was observed.

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 6.64 mg phenmediphan/kg artificial soil dry weight and the EC_{50} for reproduction was calculated to be 19.9 mg phenmedipham/kg artificial soil dry weight.

IN-MK643 caused no significant increase in mortality of Collembola, *Folsomia candida*, when exposed to concentrations up to and including 100 mg/kg artificial soil dry weight, the highest rate tested. Mean mortality was 8% in the controls and 10% at 100 mg IN-MK643/kg artificial soil dry weight.

IN-MK643 had no significant reproductive effects on the Collembola, *Folsomia candida*, when exposed to concentrations up to and including 10 mg/kg artificial soil dry weight. At the concentration of 100 mg IN-MK643/kg artificial soil dry weight a slight (13%) but statistically significant reduction of reproduction was observed. The mean number of juveniles produced in the pooled control was 665, in the test concentration of 1 mg IN-MK643/kg was 659, in the test concentration of 10 mg IN-MK643/kg was 658, and in the test concentration of 100 mg IN-MK643/kg was 577. This represented reductions of 1, 1 and 13%, respectively, compared to the pooled control group. IN-MK643 therefore had no significant lethal effects on the Collembola, *Folsomia candida*, when exposed to concentrations up to and including 100 mg/kg artificial soil dry weight, the highest concentration tested. A previous test with IN-MK643 that was repeated because a solvent control was not included showed no statistically significant effect on mortality or reproduction at concentrations up to and including 100 mg IN-MK643/kg soil dry weight. Taken together, the tests suggest that the overall NOEC is most likely 100 mg/kg artificial soil dry weight.

Table 192
The effects on mortality and reproduction of Collembola, *Folsomia candida*, exposed to IN-MK643 in artificial soil

Nominal IN-MK643 concentration (mg/kg soil)	Mean% mortality (\pm SD) ^a	Mean no. of juveniles (\pm SD)	Reduction in reproduction (%)
Untreated control (0.0)	8 (\pm 8)	668 (\pm 45)	-
Solvent control (0.0)	8 (\pm 8)	662(\pm 67)	
pooled control	8 (\pm 8)	665 (\pm 54)	
1	0 (\pm 0)	659 (\pm 35)	1
10	6 (\pm 9)	658 (\pm 53)	1
100	10 (\pm 7)	577 (\pm 56) ^b	13

^a There were no significant differences from the control (Fisher Exact test, two-sided, $\alpha = 0.05$)

^b Statistically different compared to pooled controls (Dunnett Test, one-sided smaller, $\alpha = 0.05$)

III. CONCLUSIONS

The EC₅₀ and the Lowest-Observed-Effect Concentration (LOEC) for IN-MK643 were determined to be >100 mg/kg artificial soil dry weight, the highest concentration tested. The overall No-Observed-Effect Concentration (NOEC) was determined to be 10 mg IN-MK643/kg artificial soil dry weight based on a slight, but statistically significant reduction in reproduction. The overall NOEC is proposed to be 100 mg IN-MK643/kg artificial soil dry weight.

(Lührs, U., 2006)

RMS comment

It is noted in the study report that a previous test with IN-MK643 (test was repeated because a solvent control was not included) showed no statistically significant effect on mortality or reproduction at concentrations up to and including 100 mg IN-MK643/kg soil dry weight. Taken together, the tests suggest that the overall NOEC is most likely 100 mg/kg artificial soil dry weight. It is agreed by RMS that effects at this concentration are expected to be low (reproduction equivalent to 91% of control in the first experiment, not significant), however RMS cannot ascertain the absence of effect at this concentration as significant effects were observed in the second experiment. Therefore as a conservative assumption, the NOEC of 10 mg IN-MK643/kg artificial soil dry weight (based on a slight, but statistically significant reduction in reproduction), should be considered relevant.

This study is valid according to the guideline ISO 11267 (1999). RMS notes that this guideline is not the one recommended for this kind of test in the Regulation (EU) No 283/2013 of 1 March 2013 setting out the data requirements for active substances, in accordance with Regulation (EC) No 1107/2009. According to this Regulation, the test guideline OECD 232 is recommended, however these guidelines are similar.

As the test clearly shows the absence of toxicity at the tested concentration of 10 mg IN-MK643/kg artificial soil dry weight, the results above are considered acceptable.

RMS calculated an EC10 value of 80.28 mg IN-MK643/kg artificial soil dry weight (95% confidence intervals: 24.98-137.02).

Report: Pavić, B. (2013d); Indoxacarb (DPX-KN128) technical: Effects on reproduction of the predatory mite *Hypoaspis aculeifer* in artificial soil with 5% peat

DuPont Report No.: DuPont-35304

Guidelines: OECD 226 (2008) **Deviations:** None

Testing Facility: IBACON, Rossdorf, Germany

Testing Facility Report No.: 75241089

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 (DPX-KN128) technical 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 indoxacarb to obtain the nominal concentrations of 65.24, 130.5, 261.0, 521.9, and 1043.8 mg indoxacarb/kg soil dry weight (corresponding to 62.5, 125, 250, 500, and 1000 mg a.s./kg dry artificial soil, adjusted for purity) and to an untreated control (using the same amount of acetone and fine quartz sand per gram substrate as in the test item groups). Indoxacarb had no significant lethal or reproductive effects on the predatory

mite *Hypoaspis aculeifer* when exposed to concentrations up to and including 1000 mg a.s./kg artificial soil dry weight for 14 days, the highest dose tested.

The 14-day EC₅₀ and the Lowest-Observed-Effect Concentration (LOEC) for indoxacarb were determined to be greater than 1000 mg a.s./kg artificial soil dry weight. The overall 14-day No-Observed-Effect Concentration (NOEC) based on mortality and reproduction was determined to be 1000 mg a.s./kg artificial soil dry weight.

I. MATERIALS AND METHODS

A. MATERIALS

- | | | |
|----|------------------------------------|--|
| 1. | Test material: | Indoxacarb technical |
| | Lot/Batch #: | DPX-KN128-098 |
| | Purity: | 95.8% |
| | Description: | Solid |
| | CAS#: | 173584-44-6 |
| | Stability of test compound: | Not analyzed in the test system |
| 2. | Control: | Untreated (using the same amount of acetone and sand per g substrate as in the test item groups and moistened with deionised water) |
| | Test vehicle: | Acetone |
| | Toxic reference: | Dimethoate |
| 3. | Test System: | Predatory soil mites (adult females) |
| | Species: | <i>Hypoaspis aculeifer</i> |
| | Age at dosing: | Adults, approximately 7 days after reaching the adult stage (28 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: | 28 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: 38% |
| | Diet: | Cheese mite (<i>Tyrophagus putrescentiae</i>) |
| | Water (deionised) content of soil: | Initiation: 20.5 to 20.9%, equivalent to 54.1 to 55.0% of the maximum water holding capacity
Termination: 18.9 to 20.2% equivalent to 49.7 to 53.1% of the maximum water holding capacity |
| | Soil pH: | 6.2 to 6.4 at test start; 6.0 to 6.1 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
28-September-2012 to 16-October-2012
2. Experimental treatments
A study was conducted to determine the effect of indoxacarb 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 65.24, 130.5, 261.0, 521.9, and 1043.8 mg a.s./kg soil dry weight (corresponding to 62.5, 125, 250, 500, and 1000 mg a.s./kg dry artificial soil, adjusted for purity) and to an untreated control (using the same amount of acetone and fine quartz sand per gram substrate as in the test item groups). 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 from May 2012 to June 2012.

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 analyzed for significance by using Fisher's Exact Test (one-sided greater, $\alpha = 0.05$). Reproduction data were tested for normal distribution and homoscedascity using Kolmogorov-Smirnov 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).

The EC₅₀ was not determined by a statistical analysis as reduction of reproduction was less than 50%.

II. RESULTS AND DISCUSSION

A. FINDINGS

All validity criteria were met. The reference item caused statistically significant effects on reproduction at a concentration of 1.7 mg dimethoate/kg soil dry weight and above and the EC₅₀ for reproduction was 4.0 mg dimethoate/kg artificial soil dry weight.

A summary of the results is provided in the table below:

Table 193
The effects on mortality and reproduction of the soil mite, *Hypoaspis aculeifer*, exposed to indoxacarb technical in artificial soil for 14 days

Indoxacarb concentration (mg a.s./kg soil dry weight adjusted for purity)	Mean% mortality ^a	Reproduction	
		Mean juveniles per replicate ^a	% of control
Untreated control (0.0)	11	222	-
62.5	10	205	92
125	3	227	102
250	5	191	86
500	8	212	96
1000	5	215	97

^a There were no significant differences from the control
(mortality: Fisher's Exact Test, one-sided greater, $\alpha = 0.05$; number of juveniles: Williams t-test, one-sided smaller, $\alpha = 0.05$)

III. CONCLUSIONS

Indoxacarb technical had no significant lethal or reproductive effects on the soil mite *Hypoaspis aculeifer* when exposed to concentrations up to and including 1000 mg a.s./kg artificial soil dry weight.

The 14-day EC₅₀ and the Lowest-Observed-Effect Concentration (LOEC) for indoxacarb technical, based on reproduction were determined to be greater than 1000 mg a.s./kg artificial soil dry weight. The overall 14-day No-Observed-Effect Concentration (NOEC) for indoxacarb technical was determined to be 1000 mg a.s./kg artificial soil dry weight.

(Pavić, B., 2013d)

RMS comment

This study is valid.

The Lowest-Observed-Effect Concentration (LOEC) for indoxacarb technical, based on reproduction was determined to be greater than 1000 mg a.s./kg artificial soil dry weight. The overall 14-day No-Observed-Effect Concentration (NOEC) for indoxacarb technical was determined to be 1000 mg a.s./kg artificial soil dry weight. No EC10 was necessary as no dose related effect was observed.

Report: Pavić, B. (2013f); IN-JT333: Effects on reproduction of the predatory mite *Hypoaspis aculeifer* in artificial soil with 5% peat

DuPont Report No.: DuPont-35305

Guidelines: OECD 226 (2008) **Deviations:** None

Testing Facility: IBACON, Rossdorf, Germany

Testing Facility Report No.: 75251089

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 IN-JT333 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 IN-JT333 to obtain the nominal concentrations of 6.33, 12.65, 25.30, 50.61, 101.2 mg/kg dry artificial soil (corresponding to 6.25, 12.5, 25.0, 50.0, and 100 mg/kg artificial soil dry weight, adjusted for purity) and to an untreated control (using the same amount of acetone and fine quartz sand per gram substrate as in the test item groups). IN-JT333 had no significant lethal or reproductive effects on the predatory mite *Hypoaspis aculeifer* when exposed to concentrations up to and including 100 mg/kg artificial soil dry weight for 14 days, the highest dose tested.

The 14-day EC₅₀ and the Lowest-Observed-Effect Concentration (LOEC) for IN-JT333 were determined to be greater than 100 mg/kg artificial soil dry weight. The overall 14-day No-Observed-Effect Concentration (NOEC) based on mortality and reproduction was determined to be 100 mg IN-JT333/kg artificial soil dry weight.

I. MATERIALS AND METHODS

A. MATERIALS

- | | | |
|----|------------------------------------|--|
| 1. | Test material: | IN-JT333 technical metabolite |
| | Lot/Batch #: | JT333-017 |
| | Purity: | 98.8% |
| | Description: | Solid |
| | CAS#: | Not available |
| | Stability of test compound: | Not analyzed in the test system |
| 2. | Control: | Untreated (using the same amount of acetone and sand per g substrate as in the test item groups and moistened with deionised water) |
| | Test vehicle: | Acetone |
| | Toxic reference: | Dimethoate |
| 3. | Test System: | Predatory soil mites (adult females) |
| | Species: | <i>Hypoaspis aculeifer</i> |
| | Age at dosing: | Adults, approximately 12 days after reaching the adult stage (33 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: | 33 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: 38% |
| | Diet: | Cheese mite (<i>Tyrophagus putrescentiae</i>) |
| | Water (deionised) content of soil: | Initiation: 21.1 to 22.1%, equivalent to 55.6 to 58.1% of the maximum water holding capacity
Termination: 19.4 to 21.7% equivalent to 51.1 to 57.1% of the maximum water holding capacity |
| | Soil pH: | 6.3 to 6.4 at test start; 5.8 to 6.1 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
24-October-2012 to 13-November-2012

2. Experimental treatments

A study was conducted to determine the effect of IN-JT333 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 6.33, 12.65, 25.30, 50.61, and 101.2 mg IN-JT333/kg dry artificial soil (corresponding to 6.25, 12.5, 25.0, 50.0, and 100 mg IN-JT333/kg artificial soil dry weight, adjusted for purity) and to an untreated control (using the same amount of acetone and fine quartz sand per gram substrate as in the test item groups). 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 from May 2012 to June 2012.

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 analyzed 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). The EC_{50} was not determined by a statistical analysis as reduction of reproduction was less than 50%.

II. RESULTS AND DISCUSSION

A. FINDINGS

All validity criteria were met. The reference item caused statistically significant effects on reproduction at a concentration of 1.7 mg dimethoate/kg soil dry weight and above and the EC_{50} for reproduction was 4.0 mg dimethoate/kg artificial soil dry weight.

A summary of the results is provided in the table below.

Table 194
The effects on mortality and reproduction of the soil mite, *Hypoaspis aculeifer*, exposed to IN-JT333 in artificial soil for 14 days

IN-JT333 concentration (mg/kg soil dry weight adjusted for purity)	Mean% mortality ^a	Reproduction	
		Mean juveniles per replicate ^a	% of control
Untreated control (0.0)	15	172	-
6.25	18	192	112
12.5	28	157	92
25.0	15	187	109
50.0	13	208	121
100	8	201	117

^a There were no significant differences to the control
(mortality: Fisher's Exact Test, $\alpha = 0.05$, one-sided greater; number of juveniles: Williams t-test, $\alpha = 0.05$, one-sided smaller)

III. CONCLUSIONS

IN-JT333 had no significant lethal or reproductive effects on the soil mite *Hypoaspis aculeifer* when exposed to concentrations up to and including 100 mg/kg artificial soil dry weight.

The 14-day EC_{50} and the Lowest-Observed-Effect Concentration (LOEC) for IN-JT333, based on reproduction were determined to be greater than 100 mg/kg artificial soil dry weight. The overall 14-day No-Observed-Effect Concentration (NOEC) was determined to be 100 mg IN-JT333/kg artificial soil dry weight.

(Pavić, B., 2013f)

RMS comment

This study is valid.

The Lowest-Observed-Effect Concentration (LOEC) for IN-JT333, based on reproduction was determined to be greater than 100 mg/kg artificial soil dry weight. The overall 14-day No-Observed-Effect Concentration (NOEC) was determined to be 100 mg IN-JT333/kg artificial soil dry weight. No EC10 was necessary as no dose related effect was observed.

Report: Pavić, B. (2013a); IN-JU873: Effects on the reproduction of the predatory mite *Hypoaspis aculeifer* in artificial soil with 5% peat

DuPont Report No.: DuPont-35308

Guidelines: OECD 226 (2008) **Deviations:** None

Testing Facility: IBACON, Rossdorf, Germany

Testing Facility Report No.: 75291089

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 IN-JU873 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 IN-JU873 to obtain the nominal concentrations of 6.31, 12.61, 25.23, 50.45 and 100.9 mg/kg dry artificial soil (corresponding to 6.25, 12.5, 25, 50, and 100 mg/kg artificial soil dry weight, adjusted for purity) and to an untreated control (using the same amount of acetone and fine quartz sand per gram substrate as in the test item groups). IN-JU873 had no significant lethal or reproductive effects on the predatory mite *Hypoaspis aculeifer* when exposed to concentrations up to and including 100 mg/kg artificial soil dry weight for 14 days, the highest dose tested.

The 14-day EC₅₀ and the Lowest-Observed-Effect Concentration (LOEC) for IN-JU873 were determined to be greater than 100 mg/kg artificial soil dry weight. The overall 14-day No-Observed-Effect Concentration (NOEC) based on mortality and reproduction was determined to be 100 mg IN-JU873/kg artificial soil dry weight.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: IN-JU873 technical metabolite
 Lot/Batch #: JU873-005
 Purity: 99.1%
 Description: Solid, crystalline
 CAS#: 144172-25-8
 Stability of test compound: Not analysed in the test system
2. Control: Untreated (using the same amount of acetone and sand per g substrate as in the test item groups and moistened with deionised water)
 Test vehicle: Acetone
 Toxic reference: Dimethoate
3. Test System: Predatory soil mites (adult females)
 Species: *Hypoaspis aculeifer*
 Age at dosing: Adults, approximately 12 days after reaching the adult stage (33 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: 33 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: 44%
 Diet: Cheese mite (*Tyrophagus putrescentiae*)
 Water (deionised) content of soil: Initiation: 23.8 to 24.1%, equivalent to 54.0 to 54.8% of the maximum water holding capacity
 Termination: 23.0 to 24.0% equivalent to 52.3 to 54.6% of the maximum water holding capacity
 Soil pH: 6.5 at test start; 5.3 to 5.8 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
 22-August-2012 to 11-September-2012

2. Experimental treatments

A study was conducted to determine the effect of IN-JU873 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 6.31, 12.61, 25.23, 50.45 and 100.9 mg IN-JU873/kg dry artificial soil (corresponding to 6.25, 12.5, 25, 50, and 100 mg IN-JU873/kg artificial soil dry weight, adjusted for purity) and to an untreated control (using the same amount of acetone and fine quartz sand per gram substrate as in the test item groups). 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 from May 2012 to June 2012.

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 additionally Cochran'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).

The EC₅₀ was not determined by a statistical analysis as reduction of reproduction was less than 50%.

II. RESULTS AND DISCUSSION

A. FINDINGS

All validity criteria were met. The reference item caused statistically significant effects on reproduction at a concentration of 1.7 mg dimethoate/kg soil dry weight and above and the EC₅₀ for reproduction was 4.0 mg dimethoate/kg artificial soil dry weight.

A summary of the results is provided in Table 195.

Table 195
The effects on mortality and reproduction of the soil mite, *Hypoaspis aculeifer*, exposed to IN-JU873 in artificial soil for 14 days

IN-JU873 (mg/kg soil dry weight)	Mean% mortality ^a	Reproduction	
		Mean juveniles per replicate ^a	% of control
Untreated control (0.0)	9	262	-
6.25	8	246	94
12.5	10	215	82
25	5	200	76
50	10	312	119
100	13	261	100

^a There were no significant differences from the control
(mortality: Fisher's Exact Test, one-sided greater, $\alpha = 0.05$; number of juveniles: Williams t-test, one-sided smaller, $\alpha = 0.05$)

III. CONCLUSIONS

The 14-day EC₅₀ and the Lowest-Observed-Effect Concentration (LOEC) for IN-JU873, based on reproduction were determined to be greater than 100 mg/kg artificial soil dry weight, the highest concentration tested. The overall 14-day No-Observed-Effect Concentration (NOEC) was determined to be 100 mg IN-JU873/kg artificial soil dry weight.

(Pavić, B., 2013a)

RMS comment

This study is valid.

The Lowest-Observed-Effect Concentration (LOEC) for IN-JU873, based on reproduction was determined to be greater than 100 mg/kg artificial soil dry weight, the highest concentration tested. The overall 14-day No-Observed-Effect Concentration (NOEC) was determined to be 100 mg IN-JU873/kg artificial soil dry weight. No EC10 was necessary as no dose related effect was observed.

Report: Pavić, B. (2013m); IN-KB687: Effects on reproduction of the predatory mite *Hypoaspis aculeifer* in artificial soil with 5% peat

DuPont Report No.: DuPont-35364

Guidelines: OECD 226 (2008) **Deviations:** None

Testing Facility: IBACON, Rossdorf, Germany

Testing Facility Report No.: 75301089

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 IN-KB687 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 IN-KB687 to obtain the nominal concentrations of 3.13, 6.26, 12.53, 25.05, and 51.10 mg/kg dry artificial soil (corresponding to 3.125, 6.25, 12.5, 25.0, and 51.0 mg/kg artificial soil dry weight, adjusted for purity) and to an untreated control (using the same amount of acetone and fine quartz sand per gram substrate as in the test item groups). IN-KB687 had no significant lethal effects on the predatory mite *Hypoaspis aculeifer* when exposed to concentrations up to and including 51.0 mg/kg artificial soil dry weight and no significant reproductive effects when exposed to a concentration of 3.125 mg/kg artificial soil dry weight for 14 days.

The 14-day EC₅₀ for IN-KB687 was determined to be 48.43 mg/kg artificial soil dry weight (95% confidence limits of 32.15 to 120.74 mg/kg artificial soil dry weight). The Lowest-Observed-Effect Concentration (LOEC) based on reproduction was determined to be 6.25 mg IN-KB687/kg artificial soil dry weight. The overall 14-day No-Observed-Effect Concentration (NOEC) was determined to be 3.125 mg IN-KB687/kg artificial soil dry weight.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: IN-KB687 technical metabolite
 Lot/Batch #: KB687-002
 Purity: 99.8%
 Description: Solid
 CAS#: Not assigned
 Stability of test compound: Not analyzed in the test system
2. Control: Untreated (using the same amount of acetone and sand per g substrate as in the test item groups and moistened with deionised water)
 Test vehicle: Acetone
 Toxic reference: Dimethoate
3. Test System: Predatory soil mites (adult females)
 Species: *Hypoaspis aculeifer*
 Age at dosing: Adults, approximately 14 days after reaching the adult stage (35 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: 35 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: 38%
 Diet: Cheese mite (*Tyrophagus putrescentiae*)
 Water (deionised) content of soil: Initiation: 20.6 to 21.0%, equivalent to 54.2 to 55.4% of the maximum water holding capacity
 Termination: 19.7 to 20.4% equivalent to 51.9 to 53.6% of the maximum water holding capacity
 Soil pH: 6.4 to 6.5 at test start; 6.2 to 6.4 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
 15-October-2012 to 31-October-2012

2. Experimental treatments

A study was conducted to determine the effect of IN-KB687 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 3.13, 6.26, 12.53, 25.05, and 51.10 mg IN-KB687/kg dry artificial soil (corresponding to 3.125, 6.25, 12.5, 25.0, and 51.0 mg IN-KB687/kg artificial soil dry weight, adjusted for purity) and to an untreated control (using the same amount of acetone and fine quartz sand per gram substrate as in the test item groups). 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 from May 2012 to June 2012.

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 analyzed 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).

The EC_{50} of reproduction and its 95% confidence limits were determined by applying Probit-Analysis (Finney, 1971).

II. RESULTS AND DISCUSSION

A. FINDINGS

All validity criteria were met. The reference item caused statistically significant effects on reproduction at a concentration of 1.7 mg dimethoate/kg soil dry weight and above; and the EC_{50} for reproduction was 4.0 mg dimethoate/kg artificial soil dry weight.

A summary of the results is provided in Table 196.

Table 196
The effects on mortality and reproduction of the soil mite, *Hypoaspis aculeifer*, exposed to IN-KB687 in artificial soil for 14 days

IN-KB687 concentration (mg/kg soil dry weight adjusted for purity)	Mean% mortality	Reproduction	
		Mean juveniles per replicate	% of control
Untreated control (0.0)	5	197	-
3.125	8 ^b	209	106 ^b
6.25	8 ^b	175	89 ^a
12.5	5 ^b	146	74 ^a
25.0	5 ^b	136	69 ^a
51.0	8 ^b	95	48 ^a

^a Statistically significant (number of juveniles: Williams t-test, $\alpha = 0.05$, one-sided smaller)

^b Not 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)

III. CONCLUSIONS

IN-KB687 had no significant lethal effects on the soil mite *Hypoaspis aculeifer* when exposed to concentrations up to and including 51.0 mg/kg artificial soil dry weight and no significant reproductive effects when exposed to concentrations up to and including 3.125 mg/kg artificial soil dry weight.

The 14-day EC₅₀ for IN-KB687 based on reproduction was determined to be 48.43 mg/kg artificial soil dry weight (95% confidence limits of 32.15 to 120.74 mg/kg artificial soil dry weight). The Lowest-Observed-Effect Concentration (LOEC) based on reproduction was determined to be 6.25 mg IN-KB687/kg artificial soil dry weight. The overall 14-day No-Observed-Effect Concentration (NOEC) was determined to be 3.125 mg IN-KB687/kg artificial soil dry weight.

(Pavić, B., 2013m)

RMS comment

This study is valid.

The Lowest-Observed-Effect Concentration (LOEC) based on reproduction was determined to be 6.25 mg IN-KB687/kg artificial soil dry weight. The overall 14-day No-Observed-Effect Concentration (NOEC) was determined to be 3.125 mg IN-KB687/kg artificial soil dry weight. RMS calculated an EC10 value of 5.44 mg IN-KB687/kg artificial soil dry weight (95% confidence intervals: 2.44-9.94) based on the effects on reproduction.

Report: Pavić, B. (2013i); IN-KG433: Effects on reproduction of the predatory mite *Hypoaspis aculeifer* in artificial soil with 5% peat

DuPont Report No.: DuPont-35307

Guidelines: OECD 226 (2008) **Deviations:** None

Testing Facility: IBACON, Rossdorf, Germany

Testing Facility Report No.: 75260189

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 IN-KG433 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 IN-KG433 to obtain the nominal concentrations of 6.26, 12.51, 25.03, 50.05 and 100.1 mg/kg soil dry weight (corresponding to 6.25, 12.5, 25.0, 50.0, and 100 mg/kg artificial soil dry weight, adjusted for purity) and to an untreated control (using the same amount of acetone and fine quartz sand per gram substrate as in the test item groups). IN-KG433 had no significant lethal or reproductive effects on the predatory mite *Hypoaspis aculeifer* when exposed to concentrations up to and including 100 mg/kg artificial soil dry weight for 14 days, the highest dose tested.

The 14-day EC₅₀ and the Lowest-Observed-Effect Concentration (LOEC) for IN-KG433 were determined to be greater than 100 mg/kg artificial soil dry weight. The overall 14-day No-Observed-Effect Concentration (NOEC) based on mortality and reproduction was determined to be 100 mg IN-KG433/kg artificial soil dry weight.

I. MATERIALS AND METHODS

A. MATERIALS

- | | | |
|----|------------------------------------|--|
| 1. | Test material: | IN-KG433 technical metabolite |
| | Lot/Batch #: | KG433-004 |
| | Purity: | 99.9% |
| | Description: | Solid |
| | CAS#: | Not assigned |
| | Stability of test compound: | Not analyzed in the test system |
| 2. | Control: | Untreated (using the same amount of acetone and sand per g substrate as in the test item groups and moistened with deionised water) |
| | Test vehicle: | Acetone |
| | Toxic reference: | Dimethoate |
| 3. | Test System: | Predatory soil mites (adult females) |
| | Species: | <i>Hypoaspis aculeifer</i> |
| | Age at dosing: | Adults, approximately 10 days after reaching the adult stage (31 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: | 31 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: 38% |
| | Diet: | Cheese mite (<i>Tyrophagus putrescentiae</i>) |
| | Water (deionised) content of soil: | Initiation: 20.5 to 21.3%, equivalent to 54.0 to 56.0% of the maximum water holding capacity
Termination: 20.1 to 21.5% equivalent to 53.0 to 56.6% of the maximum water holding capacity |
| | Soil pH: | 6.4 at test start; 5.8 to 6.1 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
27-August-2012 to 11-September-2012

2. Experimental treatments

A study was conducted to determine the effect of IN-KG433 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 6.26, 12.51, 25.03, 50.05 and 100.1 mg IN-KG433/kg soil dry weight (corresponding to 6.25, 12.5, 25.0, 50.0, and 100 mg IN-KG433/kg artificial soil dry weight, adjusted for purity) and to an untreated control (using the same amount of acetone and fine quartz sand per gram substrate as in the test item groups). 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 from May 2012 to June 2012.

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 analyzed 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). The EC_{50} was not determined by a statistical analysis as reduction of reproduction was less than 50%.

II. RESULTS AND DISCUSSION

A. FINDINGS

All validity criteria were met. The reference item caused statistically significant effects on reproduction at a concentration of 1.7 mg dimethoate/kg soil dry weight and above, and the EC_{50} for reproduction was 4.0 mg dimethoate/kg artificial soil dry weight.

A summary of the results is provided in Table 197.

Table 197
The effects on mortality and reproduction of the soil mite, *Hypoaspis aculeifer*, exposed to IN-KG433 in artificial soil for 14 days

IN-KG433 concentration (mg/kg soil dry weight adjusted for purity)	Mean% mortality ^a	Reproduction	
		Mean juveniles per replicate ^a	% of control
Untreated control (0.0)	4	169	-
6.25	8	162	96
12.5	10	161	95
25.0	15	184	109
50.0	18	184	109
100	15	182	108

^a There were no significant differences from the control
(mortality: Fisher's Exact Test, one-sided greater, $\alpha = 0.05$; number of juveniles: Williams t-test, one-sided smaller, $\alpha = 0.05$)

III. CONCLUSIONS

IN-KG433 had no significant lethal or reproductive effects on the soil mite *Hypoaspis aculeifer* when exposed to concentrations up to and including 100 mg/kg artificial soil dry weight.

The 14-day EC_{50} and the Lowest-Observed-Effect Concentration (LOEC) for IN-KG433, based on reproduction were determined to be greater than 100 mg/kg artificial soil dry weight. The overall 14-day No-Observed-Effect Concentration (NOEC) was determined to be 100 mg IN-KG433/kg artificial soil dry weight.

(Pavić, B., 2013i)

RMS comment

This study is valid.

The Lowest-Observed-Effect Concentration (LOEC) for IN-KG433, based on reproduction was determined to be greater than 100 mg/kg artificial soil dry weight. The overall 14-day No-Observed-Effect Concentration (NOEC) was determined to be 100 mg IN-KG433/kg artificial soil dry weight. No EC10 was necessary as no dose related effect was observed.

Report: Pavić, B. (2013b); IN-KT413: Effects on reproduction of the predatory mite *Hypoaspis aculeifer* in artificial soil with 5% peat

DuPont Report No.: DuPont-35306

Guidelines: OECD 226 (2008) **Deviations:** None

Testing Facility: IBACON, Rossdorf, Germany

Testing Facility Report No.: 75271089

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 IN-KT413 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 IN-KT413 to obtain the nominal concentrations of 6.27, 12.54, 25.08, 50.15 and 100.3 mg/kg dry artificial soil (corresponding to 6.25, 12.5, 25, 50, and 100 mg/kg artificial soil dry weight, adjusted for purity) and to an untreated control (using the same amount of acetone and fine quartz sand per gram substrate as in the test item groups). IN-KT413 had no significant lethal or reproductive effects on the predatory mite *Hypoaspis aculeifer* when exposed to concentrations up to and including 100 mg/kg artificial soil dry weight for 14 days, the highest dose tested.

The 14-day EC₅₀ and the Lowest-Observed-Effect Concentration (LOEC) for IN-KT413 were determined to be greater than 100 mg/kg artificial soil dry weight. The overall 14-day No-Observed-Effect Concentration (NOEC) based on mortality and reproduction was determined to be 100 mg IN-KT413/kg artificial soil dry weight.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: IN-KT413 technical metabolite
 Lot/Batch #: KT413-003
 Purity: 99.7%
 Description: Solid
 CAS#: Not available
 Stability of test compound: Not analysed in the test system
2. Control: Untreated (using the same amount of acetone and sand per g substrate as in the test item groups and moistened with deionised water)
 Test vehicle: Acetone
 Toxic reference: Dimethoate
3. Test System: Predatory soil mites (adult females)
 Species: *Hypoaspis aculeifer*
 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: 38%
 Diet: Cheese mite (*Tyrophagus putrescentiae*)
 Water (deionised) content of soil: Initiation: 20.5 to 21.1%, equivalent to 53.9 to 55.5% of the maximum water holding capacity
 Termination: 19.6 to 20.9% equivalent to 51.5 to 55.0% of the maximum water holding capacity
 Soil pH: 6.1 to 6.4 at test start; 6.4 to 6.5 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
 04-September-2012 to 25-September-2012

2. Experimental treatments

A study was conducted to determine the effect of IN-KT413 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 6.27, 12.54, 25.08, 50.15 and 100.3 mg IN-KT413/kg dry artificial soil (corresponding to 6.25, 12.5, 25, 50, and 100 mg IN-KT413/kg artificial soil dry weight, adjusted for purity) and to an untreated control (using the same amount of acetone and fine quartz sand per gram substrate as in the test item groups). 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 from May 2012 to June 2012.

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 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).

The EC₅₀ was not determined by a statistical analysis as reduction of reproduction was less than 50%.

II. RESULTS AND DISCUSSION

A. FINDINGS

All validity criteria were met. The reference item caused statistically significant effects on reproduction at a concentration of 1.7 mg dimethoate/kg soil dry weight and above, and the EC₅₀ for reproduction was 4.0 mg dimethoate/kg artificial soil dry weight.

A summary of the results is provided in Table 198.

Table 198
The effects on mortality and reproduction of the soil mite, *Hypoaspis aculeifer*, exposed to IN-KT413 in artificial soil for 14 days

IN-KT413 (mg/kg soil dry weight)	Mean% mortality ^a	Reproduction	
		Mean juveniles per replicate ^a	% of control
Untreated control (0.0)	9	206	-
6.25	8	222	108
12.5	18	217	106
25	10	211	103
50	15	182	88
100	18	183	89

^a There were no significant differences from the control
(mortality: Fisher's Exact Test, one-sided greater, $\alpha = 0.05$; number of juveniles: Williams t-test, one-sided smaller, $\alpha = 0.05$)
- not applicable

III. CONCLUSIONS

The 14-day EC₅₀ and the Lowest-Observed-Effect Concentration (LOEC) for IN-KT413, based on reproduction were determined to be greater than 100 mg/kg artificial soil dry weight, the highest concentration tested. The overall 14-day No-Observed-Effect Concentration (NOEC) was determined to be 100 mg IN-KT413/kg artificial soil dry weight.

(Pavić, B., 2013b)

RMS comment

This study is valid.

The Lowest-Observed-Effect Concentration (LOEC) for IN-KT413, based on reproduction was determined to be greater than 100 mg/kg artificial soil dry weight, the highest concentration tested. The overall 14-day No-Observed-Effect Concentration (NOEC) was determined to be 100 mg IN-KT413/kg artificial soil dry weight. RMS calculated an EC10 value of 62.7 mg IN-KT413/kg artificial soil dry weight (95% confidence intervals: 7.91-146.2).

Report: Pavić, B. (2013k); IN-MK638: Effects on reproduction of the predatory mite *Hypoaspis aculeifer* in artificial soil with 5% peat

DuPont Report No.: DuPont-35309

Guidelines: OECD 226 (2008) **Deviations:** None

Testing Facility: IBACON, Rossdorf, Germany

Testing Facility Report No.: 75281089

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 IN-MK638 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 IN-MK638 to obtain the nominal concentrations of 6.26, 12.51, 25.03, 50.05 and 100.1 mg/kg dry artificial soil (corresponding to 6.25, 12.5, 25.0, 50.0, and 100 mg/kg artificial soil dry weight, adjusted for purity) and to an untreated control (using the same amount of acetone and fine quartz sand per gram substrate as in the test item groups). IN-MK638 had no significant lethal or reproductive effects on the predatory mite *Hypoaspis aculeifer* when exposed to concentrations up to and including 100 mg/kg artificial soil dry weight for 14 days, the highest dose tested.

The 14-day EC₅₀ and the Lowest-Observed-Effect Concentration (LOEC) for IN-MK638 were determined to be greater than 100 mg/kg artificial soil dry weight. The overall 14-day No-Observed-Effect Concentration (NOEC) based on mortality and reproduction was determined to be 100 mg IN-MK638/kg artificial soil dry weight.

I. MATERIALS AND METHODS

A. MATERIALS

- | | | |
|----|------------------------------------|--|
| 1. | Test material: | IN-MK638 technical metabolite |
| | Lot/Batch #: | MK638-002 |
| | Purity: | 99.9% |
| | Description: | Solid, Crystalline |
| | CAS#: | 82971-90-2 |
| | Stability of test compound: | Not analyzed in the test system |
| 2. | Control: | Untreated (using the same amount of acetone and sand per g substrate as in the test item groups and moistened with deionised water) |
| | Test vehicle: | Acetone |
| | Toxic reference: | Dimethoate |
| 3. | Test System: | Predatory soil mites (adult females) |
| | Species: | <i>Hypoaspis aculeifer</i> |
| | Age at dosing: | Adults, approximately 7 days after reaching the adult stage (28 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: | 28 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: 38% |
| | Diet: | Cheese mite (<i>Tyrophagus putrescentiae</i>) |
| | Water (deionised) content of soil: | Initiation: 21.1 to 21.7%, equivalent to 55.4 to 57.1% of the maximum water holding capacity
Termination: 18.9 to 20.0% equivalent to 49.7 to 52.6% of the maximum water holding capacity |
| | Soil pH: | 6.2 to 6.3 at test start; 6.1 to 6.4 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
22-October-2012 to 04-December-2012

2. Experimental treatments

A study was conducted to determine the effect of IN-MK638 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 6.26, 12.51, 25.03, 50.05 and 100.1 mg IN-MK638/kg dry artificial soil (corresponding to 6.25, 12.5, 25.0, 50.0, and 100 mg IN-MK638/kg artificial soil dry weight, adjusted for purity) and to an untreated control (using the same amount of acetone and fine quartz sand per g substrate as in the test item groups). 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 from May 2012 to June 2012.

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 analyzed for significance by using Fisher's Exact Test ($\alpha = 0.05$, one-sided greater). 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).

The EC_{50} was not determined by a statistical analysis as reduction of reproduction was less than 50%.

II. RESULTS AND DISCUSSION

A. FINDINGS

All validity criteria were met. The reference item caused statistically significant effects on reproduction at a concentration of 1.7 mg dimethoate/kg soil dry weight and above; and the EC_{50} for reproduction was 4.0 mg dimethoate/kg artificial soil dry weight.

A summary of the results is provided in Table 199.

Table 199
The effects on mortality and reproduction of the soil mite, *Hypoaspis aculeifer*, exposed to IN-MK638 in artificial soil for 14 days

IN-MK638 concentration (mg/kg soil dry weight adjusted for purity)	Mean% mortality ^a	Reproduction	
		Mean juveniles per replicate ^a	% of control
Untreated control (0.0)	5	282	-
6.25	10	264	94
12.5	8	275	98
25.0	5	277	98
50.0	5	272	97
100	5	255	91

^a There were no significant differences from the control
(mortality: Fisher's Exact Test, $\alpha = 0.05$, one-sided greater; number of juveniles: Williams t-test, $\alpha = 0.05$, one-sided smaller)

III. CONCLUSIONS

IN-MK638 had no significant lethal or reproductive effects on the soil mite *Hypoaspis aculeifer* when exposed to concentrations up to and including 100 mg/kg artificial soil dry weight.

The 14-day EC_{50} and the Lowest-Observed-Effect Concentration (LOEC) for IN-MK638, based on reproduction were determined to be greater than 100 mg/kg artificial soil dry weight. The overall 14-day No-Observed-Effect Concentration (NOEC) was determined to be 100 mg IN-MK638/kg artificial soil dry weight.

(Pavić, B., 2013k)

RMS comment

This study is valid.

The Lowest-Observed-Effect Concentration (LOEC) for IN-MK638, based on reproduction was determined to be greater than 100 mg/kg artificial soil dry weight. The overall 14-day No-Observed-Effect Concentration (NOEC) was determined to be 100 mg IN-MK638/kg artificial soil dry weight. No EC10 was necessary as no dose related effect was observed.

Report: Pavić, B. (2013); IN-MK643: Effects on reproduction of the predatory mite *Hypoaspis aculeifer* in artificial soil with 5% peat

DuPont Report No.: DuPont-35310

Guidelines: OECD 226 (2008) **Deviations:** None

Testing Facility: IBACON, Rossdorf, Germany

Testing Facility Report No.: 75311089

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 IN-MK643 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 IN-MK643 to obtain the nominal concentrations of 6.46, 12.93, 25.85, 51.71, and 103.4 mg/kg dry artificial soil (corresponding to 6.25, 12.5, 25.0, 50.0, and 100 mg/kg artificial soil dry weight, adjusted for purity) and to an untreated control (using the same amount of acetone and fine quartz sand per gram substrate as in the test item groups). IN-MK643 had no significant lethal or reproductive effects on the predatory mite *Hypoaspis aculeifer* when exposed to concentrations up to and including 100 mg/kg artificial soil dry weight for 14 days, the highest dose tested.

The 14-day EC₅₀ and the Lowest-Observed-Effect Concentration (LOEC) for IN-MK643 were determined to be greater than 100 mg/kg artificial soil dry weight. The overall 14-day No-Observed-Effect Concentration (NOEC) based on mortality and reproduction was determined to be 100 mg IN-MK643/kg artificial soil dry weight.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: IN-MK643 technical metabolite
 Lot/Batch #: MK643-002
 Purity: 96.7%
 Description: Solid
 CAS#: Not assigned
 Stability of test compound: Not analyzed in the test system
2. Control: Untreated (using the same amount of acetone and sand per g substrate as in the test item groups and moistened with deionised water)
 Test vehicle: Acetone
 Toxic reference: Dimethoate
3. Test System: Predatory soil mites (adult females)
 Species: *Hypoaspis aculeifer*
 Age at dosing: Adults, approximately 12 days after reaching the adult stage (33 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: 33 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: 38%
 Diet: Cheese mite (*Tyrophagus putrescentiae*)
 Water content of soil: Initiation: 21.3 to 21.7%, equivalent to 56.2 to 57.2%* of the maximum water holding capacity
 Termination: 18.7 to 20.2% equivalent to 49.3 to 53.1% of the maximum water holding capacity
 * The water content in the test item concentration of 12.5 mg/kg soil dry weight at experimental start was 62.5% and considered to be due to a weighing error since reduction of water content at end of experiment is within range. Therefore, this value was not considered for the water content min-max range.
 Soil pH: 6.3 to 6.5 at test start; 5.8 to 6.1 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
 24-October-2012 to 13-November-2012

2. Experimental treatments

A study was conducted to determine the effect of IN-MK643 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 6.46, 12.93, 25.85, 51.71, and 103.4 mg IN-MK643/kg dry artificial soil (corresponding to 6.25, 12.5, 25.0, 50.0, and 100 mg IN-MK643/kg artificial soil dry weight, adjusted for purity) and to an untreated control (using the same amount of acetone and fine quartz sand per gram substrate as in the test item groups). 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 from May 2012 to June 2012.

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 analyzed 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). The EC_{50} was not determined by a statistical analysis as reduction of reproduction was less than 50%.

II. RESULTS AND DISCUSSION

A. FINDINGS

All validity criteria were met. The reference item caused statistically significant effects on reproduction at a concentration of 1.7 mg dimethoate/kg soil dry weight and above and the EC_{50} for reproduction was 4.0 mg dimethoate/kg artificial soil dry weight.

A summary of the results is provided in the table below.

Table 200
The effects on mortality and reproduction of the soil mite, *Hypoaspis aculeifer*, exposed to IN-MK643 in artificial soil for 14 days

IN-MK643 concentration (mg/kg soil dry weight adjusted for purity)	Mean% mortality ^a	Reproduction	
		Mean juveniles per replicate ^a	% of control
Untreated control (0.0)	15	172	-
6.25	20	187	109
12.5	10	216	126
25.0	10	218	127
50.0	25	192	112
100	23	225	131

^a There were no significant differences to the control
(mortality: Fisher's Exact Test, $\alpha = 0.05$, one-sided greater; number of juveniles: Williams t-test, $\alpha = 0.05$, one-sided smaller)

III. CONCLUSIONS

IN-MK643 had no significant lethal or reproductive effects on the soil mite *Hypoaspis aculeifer* when exposed to concentrations up to and including 100 mg/kg artificial soil dry weight.

The 14-day EC_{50} and the Lowest-Observed-Effect Concentration (LOEC) for IN-MK643, based on reproduction were determined to be greater than 100 mg/kg artificial soil dry weight. The overall 14-day No-Observed-Effect Concentration (NOEC) was determined to be 100 mg IN-MK643/kg artificial soil dry weight.

(Pavić, B., 20131)

RMS comment

This study is valid.

The Lowest-Observed-Effect Concentration (LOEC) for IN-MK643, based on reproduction was determined to be greater than 100 mg/kg artificial soil dry weight. The overall 14-day No-Observed-Effect Concentration (NOEC) was determined to be 100 mg IN-MK643/kg artificial soil dry weight. No EC10 was necessary as no dose related effect was observed.

B.9.5. EFFECTS ON SOIL NITROGEN TRANSFORMATION

Report: Carter, J.N. (1997); DPX-MP062 (a racemic mixture of 75% DPX-KN128 and 25% IN-KN127): Effects on soil non-target micro-organisms

DuPont Report No.: AMR 4134-96

Guidelines: SETAC - EUROPE (1995) **Deviations:** None

Testing Facility: Huntingdon Life Sciences Ltd., Huntingdon, Cambridgeshire, England, UK

Testing Facility Report No.: DPT 376/963428

GLP: Yes

Certifying Authority: Department of Health (U.K.)

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: DPX-MP062 technical
 Lot/Batch#: MP062-51A
 Purity: 94.54%
 Description: White powder
 CAS#: DPX-MP062: 144171-61-9
 DPX-KN128: 173584-44-6
 Stability of test compound: Not determined in the test system
2. Control: Soil treated with distilled water
 Test vehicle: Distilled water
 Toxic standard: None
3. Test organism: Natural assemblage of soil microflora
 Source: Common sandy agricultural soil collected from a fallow grassland near Clifton, Nottinghamshire, UK
 Test chamber: Nitrogen transformation test: 500 mL glass jars with perforated plastic lid containing approximately 100 g soil
 Respiration test: 1000 mL respirometer flasks, with a gas scrubbing system, containing approximately 100 g soil
 Substrates: Lucerne meal (nitrogen determination),
 Lucerne meal (short-term respiration study)
 Soil: Natural soil
 Soil type: Sandy loam
 Soil pH: 7.1
 % Total organic carbon: 1.18
 CEC (mEq/100 g): 16.2
 Water holding capacity (%): 34.98
 Microbial biomass 1.00
 (% of total soil organic carbon):
4. Environmental conditions
 Temperature: 18 to 22°C
 Photoperiod: Continuous dark

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
 29-October-1996 to 02-December-1996
2. Experimental treatments
 The effects of DPX-MP062 on soil microbial respiration (carbon mineralisation) and nitrogen transformations (ammonification and nitrification) were examined in a typical agricultural soil during a 28-day exposure in a laboratory study. The soil was a sandy loam obtained from Clifton, Nottinghamshire, UK, and had 66% sand, 1.2% organic carbon, pH 7.1, and 1% microbial biomass as a percent of soil organic carbon. The soil was divided into three groups: unamended control soil, amended control soil, and amended soil treated with DPX-MP062 at a rate equivalent to 250 g DPX-MP062/ha. Each treatment group was divided into four replicates. Amended soils contained 0.5% Lucerne meal added as a source of nitrogen and carbon. Production of carbon dioxide by soil microbes and concentrations of ammonium, nitrate, and nitrite were determined periodically.

II. RESULTS AND DISCUSSION

A. FINDINGS

Effects of DPX-MP062 on soil ammonium, nitrate, and carbon mineralisation are summarised in Table 201, Table 202, and Table 203.

Table 201
Summary of effects of DPX-MP062 on soil ammonium levels as µg N/g soil

Day	Soil only A ^a	Soil + Lucerne B ^a	Soil + Lucerne + DPX-MP062 C ^a	% Effect ^b
0	0.88	9.56	10.52	+10.04
14	1.00	0.96	0.94	-2.08
21	0.39	0.30	0.87	+190
28	1.39	0.52	0.54	+3.85

^a Each value is a mean of four replicate analyses

^b % Effect = [(C/B)-1] × 100

Table 202
Summary of effects of DPX-MP062 on soil nitrate levels as µg N/g soil

Day	Soil only A ^a	Soil + Lucerne B ^a	Soil + Lucerne + DPX-MP062 C ^a	% Effect ^b
0	9.32	11.45	11.65	+1.75
14	10.85	14.02	15	+6.99
21	11.55	18.77	17.8	-5.17
28	15.82	28.99	35.58	+22.73

^a Each value is a mean of four replicate analyses

^b % Effect = [(C/B)-1] × 100

Table 203
Summary of effects of DPX-MP062 on carbon mineralisation
(cumulative mean µg C as CO₂/g soil)

Day	Soil only ^a	Soil + Lucerne ^a	Soil + Lucerne + DPX-MP062 ^a	% Effect ^b
0	0.96	8.14	14.33	+76.04
2	18.48	227.45	270.26	+18.82
7	48.25	567.10	602.77	+6.29
14	81.37	766.73	789.66	+2.99
21	106.92	853.71	875.43	+2.54
28	125.38	908.75	928.02	+2.12

^a Each value is a mean of three replicate analyses

^b % Effect = [(C/B)-1] × 100

III. CONCLUSION

DPX-MP062 had no significant effect on carbon mineralisation or nitrogen transformation in Lucerne-amended soil (<25% deviation from controls) when applied at up to 250 g a.s./ha (equivalent to a concentration of 0.333 mg DPX-MP062/kg soil dry weight). Under anticipated conditions of field use, DPX-MP062 is categorised as having low risk to soil microflora.

(Carter, J. N., 1997)

RMS comment

This study was submitted in the original DAR (2000). This study was not conducted according to current guideline. The applicant indicates that it partially meets the current guideline (OECD 216) but includes one test concentration instead of two. However, reconducting the study is unlikely to yield a significantly different result because the test concentration tested was 250 g a.s./ha, which is between the recommended 5-10x of the single application rate that is recommended for the higher concentration rate in this test. No effects were seen at this rate. Repeating the test with 1 time the maximum single application rate concentration would not add any additional knowledge to the study, and therefore it is relied upon.

According to OECD 216 and 217, the variation between replicate control samples should be less than $\pm 15\%$. This validity criterion could not be checked as results are not available for each replicate. This test however, was not conducted according to these guidelines.

DPX-MP062 had no significant effect on carbon mineralisation or nitrogen transformation in Lucerne-amended soil (<25% deviation from controls) when applied at up to 250 g a.s./ha (equivalent to a concentration of 0.333 mg DPX-MP062/kg soil dry weight). This study is still considered acceptable.

Report: Carter, J.N. (1996); IN-JT333 effects on soil non-target micro-organisms

DuPont Report No.: AMR 3910-96

Guidelines: SETAC - EUROPE (1995) **Deviations:** None

Testing Facility: Huntingdon Life Sciences Ltd., Huntingdon, Cambridgeshire, England, UK

Testing Facility Report No.: DPT 355/961366

GLP: Yes

Certifying Authority: Department of Health (U.K.)

I. MATERIALS AND METHODS

A. MATERIALS

- | | |
|-----------------------------------|--|
| 1. Test material: | IN-JT333 technical metabolite |
| Lot/Batch#: | IN-JT333-20 |
| Purity: | 98.7% |
| Description: | Off-white powder |
| CAS#: | DPX-MP062: 144171-61-9
DPX-KN128: 173584-44-6 |
| Stability of test compound: | Not determined in the test system |
| 2. Control: | Soil treated with distilled water |
| Test vehicle: | Distilled water and 1 mL acetone |
| Toxic standard: | Dinoseb acetate |
| 3. Test organism: | Natural assemblage of soil microflora |
| Source: | Common agricultural soil collected from a fallow grassland near Nottinghamshire, UK |
| Test chamber | Nitrogen transformation test: glass jars containing approximately 100 g soil
Respiration test: glass jars containing approximately 100 g soil |
| Substrates: | Lucerne meal (3.0 % nitrogen)
Glucose (short-term respiration study) |
| Soil: | Natural soil |
| Soil type: | Loamy sand |
| Soil pH: | 6.6 |
| % Total organic carbon: | 1.3 |
| CEC (mEq/100 g): | 15.3 |
| Water holding capacity (%): | 37.96 |
| Microbial biomass | 1.28 |
| (% of total soil organic carbon): | |
| 4. Environmental conditions | |
| Temperature: | 20 ± 2°C |
| Photoperiod: | Continuous dark |

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
17-April-1996 to 04-June-1996
2. Experimental treatments
The effects of IN-JT333 on soil nitrogen transformation and microbial respiration were examined in a loamy sand soil in a laboratory study for a total of 42 (nitrogen) and 28 days (respiration) according to the SETAC-Europe test guideline. The soil was treated with water (control) or IN-JT333 at a single rate of 0.08 mg IN-JT333/kg soil dry weight. For the assessment of nitrogen turnover the soil was amended with Lucerne meal before application of the test material and nitrate, ammonium, and nitrite levels of the soil were determined periodically up to 42 days after applying the test material. The cumulative rate of respiration in Lucerne meal-amended soil was measured periodically up to 28 days after application of the test material.

II. RESULTS AND DISCUSSION

A. FINDINGS

The test was considered valid because the toxic standard, dinoseb acetate resulted in >25% deviation in nitrogen transformation and respiration compared to controls. Summaries of the findings for nitrate and soil respiration are

presented in Table 204 and Table 205. There was no deviation in the level of ammonium between IN-JT333-treated and control soil at the end of the study. Nitrite levels were negligible at all sampling points. Levels of nitrate and ammonium and the rate of respiration were not statistically different from controls at the end of the study and deviations in these parameters were <25% compared to control soil at the end of the study.

The cumulative rate of nitrate formation was not determined in the original study, but is included here for comparison. The deviation in the rate of nitrate formation at the end of the study did not exceed the 25% effect threshold.

Table 204
Summary of effects of IN-JT333 on the nitrate and ammonium levels and rate of nitrate formation in a loamy sand soil amended with Lucerne meal

Time (d)	Treatment group		
	0.08 mg IN-JT333/kg soil dry weight		
	Nitrate level (% deviation from control) ^a	Ammonium level (% deviation from control) ^a	Rate of nitrate formation (% deviation from control) ^{ab}
0	-1.7	-11.5	-
14	29.6	-53.8	59.3
21	-11.5	-100	37.2
28	-7.4	-35.3	-10.4
42	14.0	0.0	19.9

^a % Effect is expressed as deviation compared to control:
[(measured parameter in treated soil/measured parameter in control soil)-1] x100

^b % Deviation is calculated as the difference in the rate of nitrate formation in the control at 0-14, 0-21 and 0-28 and 0-42 days from the rates in IN-JT333-treated soil.

Table 205
Effect of IN-JT333 on respiration in a loamy sand soil amended with Lucerne meal

Time (d)	Treatment group (0.08 mg IN-JT333/kg soil dry weight)
	Respiration rate (% deviation from control) ^a
0	6.3
2	0.1
7	0.9
14	0.8
21	-0.2
28	-0.8

^a % Effect is expressed as deviation compared to control:
[(measured parameter in treated soil/measured parameter in control soil)-1] x100

III. CONCLUSION

IN-JT333 had no significant effect on soil respiration and nitrogen transformation at up to 0.08 mg IN-JT333/kg soil dry weight (<25% deviation from controls). IN-JT333, therefore, can be categorised as having low risk to soil microflora.

(Carter, J.N., 1996)

RMS comment

This study was submitted in the original DAR (2000). This study was not conducted according to current guideline. The applicant indicates that it partially meets the current guideline (OECD 216) but includes one test concentration used instead of two. However, reconducting the study is unlikely to yield a significantly different result because the test concentration tested was 60 g a.s./ha, which is above the recommended single application rate in this test. No effects were seen at this rate. Repeating the test with a higher concentration would not add any additional knowledge to the study, and therefore it is relied upon.

According to OECD 216 and 217, the variation between replicate control samples should be less than $\pm 15\%$. This validity criterion could not be checked as results are not available for each replicate. This test however, was not conducted according to these guidelines.

IN-JT333 had no significant effect on soil respiration and nitrogen transformation at up to 0.08 mg IN-JT333/kg soil dry weight (<25% deviation from controls). This study is still considered acceptable.

Report: Kolzer, U. (2002a); IN-JU873: Assessment of the effects on soil microflora

DuPont Report No.: DuPont-10069

Guidelines: OECD 216 (2000), OECD 217 (2000), BBA Part VI 1990 1-1 **Deviations:** None

Testing Facility: GAB Biotechnologie, GmbH, Neifern-Oschelbronn, Germany

Testing Facility Report No.: 20021243/01-ABMF

GLP: Yes

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

I. MATERIALS AND METHODS

A. MATERIALS

- | | |
|--|--|
| 1. Test material: | IN-JU873 technical metabolite |
| Lot/Batch#: | JU873-005 |
| Purity: | 99.06% |
| Description: | White crystalline solid |
| CAS#: | Not available |
| Stability of test compound: | Not determined in the test system |
| 2. Control: | Soil treated with deionised water |
| Test vehicle: | Acetone, quartz sand |
| Toxic standard: | Dinoterb |
| 3. Test organism: | Natural assemblage of soil microflora |
| Source: | Common agricultural soil collected from a fallow grassland near Offenbach, Germany |
| Test chamber | Nitrogen transformation test: Appropriate glass bottles closed loosely with screw caps containing approximately 3300 g soil
Respiration test: Appropriate glass bottles closed loosely with screw caps containing approximately 6000 g soil |
| Substrates: | Lucerne meal (nitrogen determination),
Glucose (short-term respiration study) |
| Soil: | Natural soil |
| Soil type: | Loamy sand |
| Soil pH: | 6.00 |
| % Total organic carbon: | 1.1 |
| CEC (mval/100 g): | 9.1 |
| Water holding capacity (g H ₂ O/100 g soil dry weight): | 34.1 |
| Microbial biomass (mg C/100 g dry weight): | 11.3 |
| 4. Environmental conditions | |
| Temperature: | 20 ± 2°C |
| Photoperiod: | Continuous dark |

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
22-August-2002 to 20-September-2002

2. Experimental treatments

The effects of IN-JU873 on soil nitrogen transformation and microbial respiration were examined in a loamy sand soil in a laboratory study for a total of 28 days. The soil was treated with water (control) or IN-JU873 at rates of 0.087 and 0.87 mg IN-JU873/kg soil dry weight (corresponding to 1× and 10× maximum predicted environmental concentration in soil [PEC_{soil}]). The PEC_{soil} was estimated as the maximum potential concentration of the degradation product in the top 5 cm soil based on a maximum application rate of the parent molecule, corrected for molecular mass, and assuming 100% conversion to a single degradation product and no subsequent degradation. Each treatment group contained three replicates. For the assessment of nitrogen turnover the soil was amended with Lucerne meal before application of the test material and nitrate, ammonium, and nitrite levels of the soil were determined periodically up to 28 days after applying the test material. The cumulative rate of nitrate formation was calculated by comparing the rates of nitrate formation at three intervals: 0–7, 0–14, and 0–28 days between the controls and treatments. The rate of short-term, glucose-induced respiration was measured in soil samples taken periodically up to 28 days after application of the test material.

II. RESULTS AND DISCUSSION

A. FINDINGS

The test was considered valid because variation between replicate control samples was <15%. A summary of the effects of IN-JU873 on nitrate levels, rate of nitrate formation, and soil respiration is presented in Table 206 and Table 207. Deviations in the levels of ammonium and nitrite at the end of the study were not determined because measured levels were below the limit of quantitation at both 1× and 10× the maximum PEC_{soil} at the end of the study. Deviations in all measured parameters were <25% compared to control soil at the end of the study.

Table 206

Summary of effects of IN-JU873 on the nitrate levels and rate of nitrate formation in a loamy sand soil amended with Lucerne meal

Time	0.087 mg IN-JU873/kg soil dry weight (1× maximum PEC_{soil})		0.87 mg IN-JU873/kg soil dry weight (10× maximum PEC_{soil})	
	Nitrate level (% deviation from control) ^a	Rate of nitrate formation (% deviation from control) ^{a,b}	Nitrate level (% deviation from control) ^a	Rate of nitrate formation (% deviation from control) ^{a,b}
0 d	5.5	—	5.5	—
7 d	0	33	4.8	9.1
14 d	4.9	3	15.4	44.8
28 d	4.2	3	-4.5	-14.4

^a % Effect is expressed as deviation compared to control:

$[(\text{measured parameter in treated soil}/\text{measured parameter in control soil}) - 1] \times 100$

^b % Deviation is calculated as the difference in the rate of nitrate formation in the control at 0–7, 0–14, and 0–28 days from the rates in IN-JU873-treated soil.

Table 207

Summary of effects of IN-JU873 on rate of glucose-induced respiration in a loamy sand soil

Time	Treatment group	
	0.087 mg IN-JU873/kg soil dry weight (1× maximum PEC_{soil})	0.87 mg IN-JU873/kg soil dry weight (10× maximum PEC_{soil})
	Respiration (%) (deviation from control) ^a	Respiration (%) (deviation from control) ^a
0 d	3.25	-6.00
7 d	2.06	-10.3
14 d	4.90	-5.45
28 d	5.81	2.33

^a % Effect is expressed as deviation compared to control:

$[(\text{measured parameter in treated soil}/\text{measured parameter in control soil}) - 1] \times 100$

III. CONCLUSION

IN-JU873 had no significant effect on soil respiration and nitrogen transformation at up to 0.87 mg IN-JU873/kg soil dry weight (equivalent to 10x its maximum PEC_{soil}) (<25% deviation from controls). IN-JU873, therefore, can be categorised as having low risk to soil microflora.

(Kolzer, U., 2002a)

RMS comment

This study was submitted in the original DAR (AD3, 2005). This study was conducted in compliance with the current guideline. The study is valid according to validity criteria.

IN-JU873 had no significant effect on soil respiration and nitrogen transformation at up to 0.87 mg IN-JU873/kg soil dry weight (<25% deviation from controls). IN-JU873. This study is still considered acceptable.

Report: Feil, N. (2011); IN-KB687: Assessment of the effects on soil microflora

DuPont Report No.: DuPont-31719

Guidelines: OECD 216 (2000), OECD 217 (2000) **Deviations:** None

Testing Facility: IBACON, Rossdorf, Germany

Testing Facility Report No.: 62861080

GLP: Yes

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

Executive summary:

A laboratory soil microflora study was conducted in a mid loamy sand soil to determine the effects of IN-KB687 on nitrogen transformation and soil respiration. This study was conducted according to OECD 216 and OECD 217. IN-KB687 was applied with acetone treated quartz sand to the soil at nominal test concentrations of 0.13, 0.67, and 1.33 mg/kg soil dry weight. These concentrations were equivalent to 1-, 5- and 10-times the maximum field rate for IN-KB687. The control consisted of soil mixed with acetone treated quartz sand. At the end of 28 days for soil respiration and 28 days for nitrogen transformation, deviations in soil containing up to 1.33 mg of soil dry weight of IN-KB687 when compared to the control were <25%, the effect threshold specified by the OECD test guidelines.

I. MATERIALS AND METHODS

A. MATERIALS

- | | |
|---------------------------------|---|
| 1. Test material: | IN-KB687 technical metabolite |
| Lot/Batch#: | KB687-002 |
| Purity: | 99.8% |
| Description: | solid |
| CAS#: | Not assigned |
| Stability of test compound: | The test item is considered to be stable under test conditions. |
| 2. Control: | Untreated soil/deionised water |
| Test vehicle: | Acetone treated quartz sand |
| Reference item: | Sodium chloride (tested once per year) |
| 3. Test organism: | Soil microflora in a natural soil |
| Source: | Fallow land near Rossdorf, Germany |
| Test chambers: | Nitrogen transformation test: 500 mL plastic boxes with perforated plastic lids containing approximately 400 g soil dry weight
Respiration test: 1000 mL plastic boxes, with perforated plastic lids containing approximately 800 grams of soil dry weight |
| Substrates: | Lucerne meal: 5 g/kg soil dry weight (nitrogen determination),
Glucose: 2 g/kg soil wet weight (short-term respiration study) |
| Acclimation period: | 29 days |
| 4. Environmental conditions | |
| Temperature: | 20 to 22°C |
| Photoperiod: | Continuous darkness |
| Soil: | Natural soil |
| Soil type: | Silty loamy sand |
| Soil pH: | 7.0 |
| % total organic carbon: | 1.11 |
| CEC (meg)/100 g: | 61 |
| Water holding capacity (%): | 41.4 |
| Soil moisture range during test | |
| % of water holding capacity: | 45-49% |

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
18-May-2011 to 15-June-2011

2. Experimental treatments

A laboratory study was conducted in a silty loamy sand soil to determine the effects of IN-KB687 on nitrogen transformation and soil respiration. IN-KB687 was dissolved in acetone and applied drop-wise to quartz sand. After evaporation of the solvent, the IN-KB687-treated sand was mixed with the soil at nominal test concentrations of 0.13, 0.67, and 1.33 mg/kg soil dry weight. Assuming the test material is uniformly distributed in the top 5 cm of soil and a soil bulk density of 1.5 g dry weight, these rates were equivalent to 1-, 5- and 10-times the maximum field rate for IN-KB687. The control consisted of soil treated with acetone treated quartz sand. The reference item (positive control), 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 for nitrogen transformation and for 28 days for carbon transformation.

3. Observations

Samples were collected for determination of nitrogen transformation and soil respiration at Days 0, 7, 14, and 28 following application of the test item.

4. Statistics

R/S-Test and Cochran's-Test ($\alpha = 0.05$): Normality and homogeneity of variance

Student t-test + Welch t-test, two sided, $\alpha = 0.05$: Test for significant differences between the treatment groups and the control group.

Calculations:

% deviation from the control = $[(C - T)/C] \times 100$;

Nitrate formation rate cumulative (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;

Nitrate formation rate interval (mg/day) = The difference between the $\text{NO}_3\text{-N}$ (mg/kg soil dry weight) content between the sampling days in intervals and Day 0, divided by the difference of the number of sampling days.

II. RESULTS AND DISCUSSION

A. FINDINGS

IN-KB687, at 0.13, 0.67, and 1.33 mg/kg soil dry weight (equivalent to 1-, 5- and 10-times maximum field rate for IN-KB687), had no effects on all concentrations in the nitrate content in soil. At the end of the 28-day study, the deviations in nitrate content compared to the control soil (-4.24, 0.35, and 2.71%, respectively) were below the 25% trigger value in accordance with OECD guideline 216.

The two rates of nitrate formation were below the 25% trigger value according to the OECD guideline 216. On Day 28, the cumulative nitrate formation rate deviated -3.17, 1.59, and 7.94%, respectively, and for the incremental formation rate -6.49, 0.00, and -0.65%, respectively, when compared to the control for the test concentrations of 0.13, 0.67, and 1.33 mg/kg soil dry weight, respectively.

The short-term respiration rate in soil treated with IN-KB687 was not statistically significantly different from the control at the end of the study (Day 28). At the end of the study, deviations in respiration rates at concentrations up to and including 1.33 mg/kg soil (dry weight equivalent) compared to the control were <25%, the effect threshold specified by the OECD guidelines.

Table 208
Summary of effects of IN-KB687 on nitrate formation and short-term respiration in soil

IN-KB687 concentration ^a (mg/kg/kg sdw)	NO ₃ -N levels (Day 28)		Nitrate formation rate (Day 0 to 28)		Nitrate formation rate (Day 14 to 28)		Respiration rate (Day 28)	
	mg/kg dry soil	% Dev. from control ^{b,c}	mg/kg dry soil/day	% Dev. from control ^{b,c}	mg/kg dry soil/day	% Dev. from control ^{b,c}	mg CO ₂ /hr/ kg dry soil	% Dev. from control ^{b,c}
Control	33.542	---	0.63	---	1.54	---	14.809	---
0.13	32.119	-4.24	0.61	-3.17	1.44	-6.49	14.961	1.03
0.67	33.661	0.35	0.64	1.59	1.54	0.00	15.219	2.77
1.33	34.451	2.71	0.68	7.94	1.53	-0.65	14.617	-1.30

^a Test item concentrations correspond to 1-, 5- and 10-times the maximum field rate for IN-KB687

^b Negative value =% inhibition, positive value =% stimulation

^c Statistical evaluation (Student t-test + Welch t-test, two sided, $\alpha = 0.05$)

* significant differences from the control

sdw: soil dry weight

III. CONCLUSION

It can be concluded that IN-KB687 has no long term effect on soil microbial nitrification or respiration at concentrations up to and including 1.33 mg/kg soil dry weight (deviations between treatments and controls for both nitrogen transformation and respiration were <25% at the end of the study, the effect threshold specified by the OECD guidelines).

(Feil, N., 2011)

RMS comment

This study is valid.

Deviations between treatments and controls for both nitrogen transformation and respiration were <25% at the end of the study. IN-KB687 has no long term effect on soil microbial nitrification or respiration at concentrations up to and including 1.33 mg/kg soil dry weight.

Report: Carter, J.N. (1997); IN-KG433 technical: Effects on soil non-target micro-organisms

DuPont Report No.: AMR 4436-97

Guidelines: SETAC - EUROPE (1995) **Deviations:** None

Testing Facility: Huntingdon Life Sciences Ltd., Huntingdon, Cambridgeshire, England, UK

Testing Facility Report No.: DPT 416/971736

GLP: Yes

Certifying Authority: Department of Health (U.K.)

Executive summary:

The effects of IN-KG433, a soil metabolite of DPX-MP062, on nitrogen transformation and soil respiration were investigated in a sandy loam soil in a laboratory study. The study was conducted according to the SETAC - Europe (1995) guideline “*Procedure for Assessing the Environmental Fate and Ecotoxicity of Pesticides.*”

IN-KG433 was applied in aqueous solution to the soil at a nominal test concentration of 0.076 mg/kg of soil (dry weight equivalent). The application targeted 50 g IN-KG433/ha, 1 times the maximum predicted environmental concentration based on assumptions of maximum application rate of parent DPX-MP062 (stated in the report as 250 g a.s./ha/season), a conversion of 20% of the applied to the metabolite, and uniform distribution in the top 5 cm of soil. The control consisted of soil treated with distilled water.

At the end of 28 days, deviations in respiration rates at a concentration of 0.076 mg IN-KG433/kg of soil (dry weight equivalent) compared to the control were <25%, the effect threshold specified by the guidelines. IN-KG433 also had no significant effect on soil nitrogen transformation (<25% deviation from controls), based on the levels of both nitrate and ammonium. The cumulative nitrate formation rate suggests that nitrification may be affected at the tested concentration. However, the very small deviation in nitrate levels between control and IN-KG433 treatments after 28 days suggests that the effect on nitrification is negligible. IN-KG433, therefore, can be categorised as having low risk to soil microflora.

I. MATERIALS AND METHODS**A. MATERIALS**

- | | |
|--|---|
| 1. Test material: | IN-KG433 technical metabolite |
| Lot/Batch #: | KG433-3 |
| Purity: | 98.0% |
| Description: | Off-white solid |
| CAS#: | 526224-31-7 |
| Stability of test compound: | Not determined in the test system |
| 2. Control: | Soil treated with distilled water |
| Test vehicle: | Distilled water |
| Toxic standard: | Dinoseb acetate |
| 3. Test organism: | Natural assemblage of soil microflora |
| Source: | Common agricultural soil collected from Clifton, Nottinghamshire, UK |
| Test chamber | Nitrogen transformation test: Glass jars containing approximately 100 g soil
Respiration test: Respirometer flasks containing approximately 100 g soil connected to a CO ₂ scrubbing system (air flow through 1 M NaOH traps) |
| Substrate: | Lucerne meal |
| Soil: | Natural soil |
| Soil type: | Sandy loam |
| Soil pH: | 6.3 |
| % Total organic carbon: | 0.8 |
| CEC (meq/100 g): | 7.6 |
| Water holding capacity (%): | 34.9 |
| Microbial biomass
(% of total soil organic carbon): | 3.05 |
| 4. Environmental conditions | |
| Temperature: | 20 ± 2°C |
| Photoperiod: | Continuous dark |

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed

16-April-1997 to 15-May-1997

2. Experimental treatments

The effects of IN-KG433 on nitrogen transformation and soil respiration were investigated in a sandy loam soil in a laboratory study. IN-KG433 was applied in aqueous solution to the soil at a nominal test concentration of 0.076 mg/kg of soil (dry weight equivalent). The control consisted of soil treated with distilled water. Each treatment group contained four replicates. The toxic standard, dinoseb acetate, tested once a year was applied to the soil at 40 mg/kg soil dry weight (Nov-Dec 1996). Samples for nitrogen determination were incubated for 28 days and samples for respiration determination were incubated for 28 days.

3. Observations

Samples were collected for determination of nitrate content at Days 0, 14, 21 and 28 following application of the test item.

Samples were collected for soil respiration determination at Days 0, 2, 7, 14, 21 and 28 following application of the test item.

4. Statistics

Differences between the control and treated soils were evaluated statistically using one-way analyses of variance ($p < 0.05$). No significant differences were found in levels of ammonium, nitrate and carbon between the control and treated soils.

Calculations:

Nitrate formation rate (mg/day/kg soil dry weight) = 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

In the most recent test with the toxic reference, the deviation between the control and the toxic reference was >25% in both the nitrogen transformation test and soil respiration test. All validation criteria were met indicating the validity of this study.

Summaries of the findings for nitrate and soil respiration are presented in Table 209. Deviation in the levels of ammonium at the end of the study was 15.8% compared to the control. Nitrite levels were negligible at all sampling points. Levels of nitrate and ammonium and the rate of mineralisation were not statistically different from controls at the end of the study and deviations in these parameters were <25% compared to the control soil at the end of the study.

Table 209
Summary of effects of IN-KG433 on nitrate formation and short-term respiration in soil

Nominal IN-KG433 conc. (mg/kg dry soil)	Time (Days)	Nitrate level (% deviation from control)	Ammonium level (% deviation from control)	Carbon mineralisation (% deviation from control)
0.076	0	3.9	-3.8	101.8
	7		---	12.2
	14	12.3	48.7	11.9
	21	-4.5	40.8	12.3
	28	-14.9	15.8	12.4

--- Levels not tested on Day 7.

III. CONCLUSION

IN-KG433 had no significant effect on soil respiration at 0.076 mg IN-KG433/kg soil dry weight. IN-KG433 had no significant effect on soil nitrogen transformation (<25% deviation from controls) based on the levels of both nitrate and ammonium. The cumulative rate of nitrate formation suggests that nitrification may be affected. However, the very small deviation in nitrate levels between control and IN-KG433 treatments after 28 days suggests that the effect on nitrification is negligible. IN-KG433, therefore, can be categorised as having low risk to soil microflora.

(Carter, J.N., 1997)

RMS comment

This study was not conducted according to the current guideline. The applicant indicates that it partially meets the current guideline (OECD 216) but includes one test concentration used instead of two. However, reconducting the study is unlikely to yield a significantly different result because the test concentration tested was 0.076 mg IN-KG433/kg soil dry weight, which is above the expected exposure concentration for the uses intended.

According to OECD 216 and 217, the variation between replicate control samples should be less than $\pm 15\%$. This validity criterion could not be checked as results are not available for each replicate. This test however, was not conducted according to these guidelines.

IN-KG433 had no effect $\geq 25\%$ from controls on soil respiration at 0.076 mg IN-KG433/kg soil dry weight at the end of the study. IN-KG433 had no effect $\geq 25\%$ from controls on soil nitrogen transformation based on the levels of both nitrate and ammonium.

This study is considered acceptable.

Report: Kolzer, U. (2002d); IN-KT413: Assessment of the effects on soil microflora

DuPont Report No.: DuPont-11051

Guidelines: OECD 216 (2000), OECD 217 (2000), BBA Part VI 1990 1-1 **Deviations:** None

Testing Facility: GAB Biotechnologie, GmbH, Neifern-Oschelbronn, Germany

Testing Facility Report No.: 20021300/01-ABMF

GLP: Yes

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

I. MATERIALS AND METHODS

A. MATERIALS

- | | |
|--|---|
| 1. Test material: | IN-KT413 technical metabolite |
| Lot/Batch#: | KT413-003 |
| Purity: | 97% |
| Description: | White powder |
| CAS#: | Not available |
| Stability of test compound: | Not determined in the test system |
| 2. Control: | Deionised water |
| Test vehicle: | Acetone, quartz sand |
| Toxic standard: | Dinoterb acetate |
| 3. Test organism: | Natural assemblage of soil microflora |
| Source: | Common agricultural soil collected from a fallow grassland near Offenbach, Germany |
| Test chamber | Nitrogen transformation test: 500 mL glass bottle with screw cap containing approximately 400 g soil
Respiration test: 1000 mL glass bottle with screw cap containing approximately 800 g soil |
| Substrates: | Lucerne meal (nitrogen determination),
Glucose (short-term respiration study) |
| Soil: | Natural soil |
| Soil type: | Loamy sand |
| Soil pH: | 6.06 |
| % Total organic carbon: | 1.1 |
| CEC (mval/100 g): | 9.1 |
| Water holding capacity (g H ₂ O/100 g soil dry weight): | 34.1 |
| Microbial biomass (mg C/100 g dry weight): | 11.3 |
| 4. Environmental conditions | |
| Temperature: | 18 to 22°C |
| Photoperiod: | Continuous dark |

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
22-August-2002 to 20-September-2002

2. Experimental treatments

The effects of IN-KT413 on soil nitrogen transformation and microbial respiration were examined in a loamy sand soil in a laboratory study for a total of 28 days. The soil was treated with water (control) or IN-KT413 at rates of 0.102 and 1.02 mg IN-KT413/kg soil dry weight (corresponding to 1× and 10× maximum predicted environmental concentration in soil [PEC_{soil}]). The PEC_{soil} was estimated as the maximum potential concentration of the degradation product in the top 5 cm soil based on a maximum application rate of the parent molecule, corrected for molecular mass, and assuming 100% conversion to a single degradation product and no subsequent degradation. Each treatment group contained three replicates. For the assessment of nitrogen turnover the soil was amended with Lucerne meal before application of the test material and nitrate, ammonium and nitrite levels of the soil were determined periodically up to 28 days after applying the test material. The cumulative rate of nitrate formation was calculated by comparing the rates of nitrate formation at three intervals: 0–7, 0–14, and 0–28 days between the controls and treatments. The rate of short-term, glucose-induced respiration was measured in soil samples taken periodically up to 28 days after application of the test material.

II. RESULTS AND DISCUSSION

A. FINDINGS

The test was considered valid because variation between replicate control samples was <15%. A summary of the effects of IN-KT413 on nitrate levels and soil respiration is presented in Table 210 and Table 211. Deviations in the levels of ammonium and nitrite at the end of the study were not determined, because measured levels were below the limit of quantitation at both 1× and 10× the maximum PEC_{soil} at the end of the study. Deviations in all measured parameters were <25% compared to control soil at the end of the study.

Table 210
Summary of effects (%) of IN-KT413 on the nitrate levels in a loamy sand soil amended with Lucerne meal

Time	Treatment group			
	0.102 mg IN-KT413/kg soil dry weight (1× maximum PEC _{soil})		1.02 mg IN-KT413/kg soil dry weight (10× maximum PEC _{soil})	
	Nitrate level (% deviation from control) ^a	Rate of nitrate formation (% deviation from control) ^{a,b}	Nitrate level (% deviation from control) ^a	Rate of nitrate formation (% deviation from control) ^{a,b}
0 d	-3.92	—	0.49	—
7 d	-10.6	29.4	19.4	-94.1
14 d	-9.77	-29	8.27	33.9
28 d	12	28.7	-2.01	-4.6

^a % Effect is expressed as deviation compared to control:

$[(\text{measured parameter in treated soil}/\text{measured parameter in control soil}) - 1] \times 100$

^b % Deviation is calculated as the difference in the rate of nitrate formation in the control at 0–7, 0–14, and 0–28 days from the rates in IN-KT413-treated soil

Table 211
Summary of effects (%) of IN-KT413 on glucose-induced respiration in a loamy sand soil

Time	Treatment group	
	0.102 mg IN-KT413/kg soil dry weight (1× maximum PEC _{soil})	1.02 mg IN-KT413/kg soil dry weight (10× maximum PEC _{soil})
	Respiration (%) (deviation from control) ^a	Respiration (%) (deviation from control) ^a
0 d	-8.75	-4.5
7 d	-11.1	-12.3
14 d	-0.272	-0.272
28 d	-3.49	-9.01

^a % Effect is expressed as deviation compared to control:

$[(\text{measured parameter in treated soil}/\text{measured parameter in control soil}) - 1] \times 100$

III. CONCLUSION

IN-KT413 had no significant effect on soil respiration and nitrogen transformation at up to 1.02 mg IN-KT413/kg soil dry weight (equivalent to 10× its maximum PEC_{soil}) (<25% deviation from controls). IN-KT413, therefore, can be categorised as having low risk to soil microflora.

(Kölzer, U., 2002d)

RMS comment

This study was submitted in the original DAR (AD3, 2005). This study was conducted in compliance with the current guideline. The study is valid according to validity criteria.

IN-KT413 had no significant effect on soil respiration and nitrogen transformation at up to 1.02 mg IN-KT413/kg soil dry weight (<25% deviation from controls). This study is still considered acceptable.

Report: Kolzer, U. (2002b); IN-MK638: Assessment of the effects on soil microflora

DuPont Report No.: DuPont-10071

Guidelines: OECD 216 (2000), OECD 217 (2000), BBA Part VI 1990 1-1 **Deviations:** None

Testing Facility: GAB Biotechnologie, GmbH, Neifern-Oschelbronn, Germany

Testing Facility Report No.: 20021245/01-ABMF

GLP: Yes

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

I. MATERIALS AND METHODS

A. MATERIALS

- | | |
|--|--|
| 1. Test material: | IN-MK638 technical metabolite |
| Lot/Batch#: | MK638-002 |
| Purity: | 99.94% |
| Description: | White crystalline solid |
| CAS#: | Not available |
| Stability of test compound: | Not determined in the test system |
| 2. Control: | Soil treated with deionised water |
| Test vehicle: | Acetone, quartz sand |
| Toxic standard: | Dinoterb |
| 3. Test organism: | Natural assemblage of soil microflora |
| Source: | Common agricultural soil collected from a fallow grassland near Offenbach, Germany |
| Test chamber | Nitrogen transformation test: Appropriate glass bottles closed loosely with screw caps containing approximately 3300 g soil
Respiration test: Appropriate glass bottles closed loosely with screw caps containing approximately 6000 g soil |
| Substrates: | Lucerne meal (nitrogen determination),
Glucose (short-term respiration study) |
| Soil: | Natural soil |
| Soil type: | Loamy sand |
| Soil pH: | 6.31 |
| % Total organic carbon: | 1.1 |
| CEC (mval/100 g): | 9.1 |
| Water holding capacity (g H ₂ O/100 g soil dry weight): | 34.1 |
| Microbial biomass (mg C/100 g dry weight): | 11.3 |
| 4. Environmental conditions | |
| Temperature: | 20 ± 2°C |
| Photoperiod: | Continuous dark |

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
22-August-2002 to 20-September-2002

2. Experimental treatments

The effects of IN-MK638 on soil nitrogen transformation and microbial respiration were examined in a loamy sand soil in a laboratory study for a total of 28 days. The soil was treated with water (control) or IN-MK638 at rates of 0.042 and 0.42 mg IN-MK638/kg soil dry weight (corresponding to 1× and 10× maximum predicted environmental concentration in soil [PEC_{soil}]). The PEC_{soil} was determined to be the maximum potential concentration of the degradation product in the top 5 cm soil based on a maximum application rate of the parent molecule, corrected for molecular mass, and assuming 100% conversion to a single degradation product and no subsequent degradation. Each treatment group contained three replicates. For the assessment of nitrogen turnover, the soil was amended with Lucerne meal before application of the test material and nitrate, ammonium, and nitrite levels of the soil were determined periodically up to 28 days after applying the test material. The rate of short-term, glucose-induced respiration was measured in soil samples taken periodically up to 28 days after application of the test material.

II. RESULTS AND DISCUSSION

A. FINDINGS

The test was considered valid because variation between replicate control samples was <15%. Summaries of the effects of IN-MK638 on nitrate levels and soil respiration is presented in Table 212 and Table 213. Deviations in the levels of ammonium and nitrite at the end of the study were not determined because measured levels were below the limit of quantitation at both 0.042 and 0.42 mg IN-MK638/kg soil dry weight at the end of the study. Deviations in all measured parameters were <25% compared to control soil at the end of the study.

Table 212

Summary of effects of IN-MK638 on the nitrate levels and rate of nitrate formation in a loamy sand soil amended with Lucerne meal

Time	0.042 mg IN-MK638/kg soil dry weight (1× maximum PEC _{soil})		0.42 mg IN-MK638/kg soil dry weight (10× maximum PEC _{soil})	
	Nitrate level (% deviation from control) ^a	Rate of nitrate formation (% deviation from control) ^{a,b}	Nitrate level (% deviation from control) ^a	Rate of nitrate formation (% deviation from control) ^{a,b}
0 d	-3.9	—	2.0	—
7 d	13.4	75.4	22.0	80.7
14 d	13.9	218.0	13.2	50.0
28 d	5.8	15.7	9.0	15.7

^a % Effect is expressed as deviation compared to control:

$[(\text{measured parameter in treated soil}/\text{measured parameter in control soil})-1] \times 100$

^b % Deviation is calculated as the difference in the rate of nitrate formation in the control at 0–7, 0–14, and 0–28 days from the rates in IN-MK638-treated soil.

Table 213

Effect of IN-MK638 on glucose-induced respiration in a loamy sand soil

Time	Treatment group	
	0.042 mg IN-MK638/kg soil dry weight (1× maximum PEC _{soil})	0.42 mg IN-MK638/kg soil dry weight (10× maximum PEC _{soil})
	Respiration (%) (deviation from control) ^a	Respiration (%) (deviation from control) ^a
0 d	-8.75	-5.25
7 d	1.29	2.06
14 d	2.45	-4.90
28 d	-6.69	-5.81

^a % Effect is expressed as deviation compared to control:

$[(\text{measured parameter in treated soil}/\text{measured parameter in control soil})-1] \times 100$

III. CONCLUSION

IN-MK638 had no significant effect on soil respiration and nitrogen transformation at up to 0.42 mg IN-MK638/kg soil dry weight (equivalent to 10× its maximum PEC_{soil}) (<25% deviation from controls). IN-MK638, therefore, can be categorised as having low risk to soil microflora.

(Kolzer, U., 2002b)

RMS comment

This study was submitted in the original DAR (AD3, 2005). This study was conducted in compliance with the current guideline. The study is valid according to validity criteria.

IN-MK638 had no significant effect on soil respiration and nitrogen transformation at up to 0.42 mg IN-MK638/kg soil dry weight (<25% deviation from controls). This study is still considered acceptable.

Report: Kolzer, U. (2002c); IN-MK643: Assessment of the effects on soil microflora

DuPont Report No.: DuPont-10073

Guidelines: OECD 216 (2000), OECD 217 (2000), BBA Part VI 1990 1-1 **Deviations:** None

Testing Facility: GAB Biotechnologie, GmbH, Neifern-Oschelbronn, Germany

Testing Facility Report No.: 20021244/01-ABMF

GLP: Yes

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: IN-MK643 technical metabolite
Lot/Batch#: MK643-002
Purity: 95.4%
Description: White powder
CAS#: Not available
Stability of test compound: Not determined in the test system
2. Control: Soil treated with deionised water
Test vehicle: Acetone, quartz sand
Toxic standard: Dinoterb
3. Test organism: Natural assemblage of soil microflora
Source: Common agricultural soil collected from a fallow field near Offenbach, Germany

Test chamber: Nitrogen transformation test: Appropriate glass bottles closed loosely with screw caps containing approximately 3300 g soil
Respiration test: Appropriate glass bottles closed loosely with screw caps containing approximately 6000 g soil

Substrates: Lucerne meal (nitrogen determination),
Glucose (short-term respiration study)

Soil: Natural soil
Soil type: Loamy sand
Soil pH: 6.31
% Total organic carbon: 1.1
CEC (mval/100 g): 9.1
Water holding capacity (g H₂O/100 g soil dry weight): 34.1
Microbial biomass (mg C/100 g dry weight): 11.3
4. Environmental conditions
Temperature: 20 ± 2°C
Photoperiod: Continuous dark

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
22-August-2002 to 20-September-2002

2. Experimental treatments

The effects of IN-MK643 on soil nitrogen transformation and microbial respiration were examined in a loamy sand soil in a laboratory study for a total of 28 days. The soil was treated with water (control) or IN-MK643 at rates of 0.041 and 0.41 mg IN-MK643/kg soil dry weight (corresponding to 1× and 10× maximum predicted environmental concentration in soil [PEC_{soil}]). The PEC_{soil} was determined to be the maximum potential concentration of the degradation product in the top 5 cm soil based on a maximum application rate of the parent molecule, corrected for molecular mass, and assuming 100% conversion to a single degradation product and no subsequent degradation. Each treatment group contained three replicates. For the assessment of nitrogen turnover, the soil was amended with Lucerne meal before application of the test material and nitrate, ammonium, and nitrite levels of the soil were determined periodically up to 28 days after applying the test material. The rate of short-term, glucose-induced respiration was measured in soil samples taken periodically up to 28 days after application of the test material.

II. RESULTS AND DISCUSSION

A. FINDINGS

The test was considered valid because variation between replicate control samples was <15%. A summary of the effects of IN-MK643 on nitrate levels and soil respiration is presented in Table 214 and Table 215. Deviations in the levels of ammonium and nitrite at the end of the study were not determined because measured levels were below the limit of quantitation at both 1× and 10× the maximum PEC_{soil} at the end of the study. Deviations in all measured parameters were <25% compared to control soil at the end of the study.

Table 214

Summary of effects of IN-MK643 on the nitrate levels and rate of nitrate formation in a loamy sand soil amended with Lucerne meal

Time	0.041 mg IN-MK643/kg soil dry weight (1× maximum PEC _{soil})		0.41 mg IN-MK643/kg soil dry weight (10× maximum PEC _{soil})	
	Nitrate level (% deviation from control) ^a	Rate of nitrate formation (% deviation from control) ^{a,b}	Nitrate level (% deviation from control) ^a	Rate of nitrate formation (% deviation from control) ^{a,b}
0 d	5.0	—	-4.0	—
7 d	1.8	-21.7	25.6	>100
14 d	22.1	72.9	9.0	47.9
28 d	-11.9	-29.2	-0.8	2.8

^a % Effect is expressed as deviation compared to control:

$[(\text{measured parameter in treated soil}/\text{measured parameter in control soil})-1]\times 100$

^b % Deviation is calculated as the difference in the rate of nitrate formation in the control at 0–7, 0–14, and 0–28 days from the rates in IN-MK643-treated soil.

Table 215

Effect of IN-MK643 on glucose-induced respiration in a loamy sand soil

Time (d)	Treatment group	
	0.041 mg IN-MK643/kg soil dry weight (1× maximum PEC _{soil})	0.41 mg IN-MK643/kg soil dry weight (10× maximum PEC _{soil})
	Respiration rate (%) (deviation from control) ^a	Respiration rate (%) (deviation from control) ^a
0	4.50	-33.3
7	0.771	4.11
14	-5.72	-10.1
28	-1.45	-10.2

^a % Effect is expressed as deviation compared to control:

$[(\text{measured parameter in treated soil}/\text{measured parameter in control soil})-1] \times 100$

III. CONCLUSION

IN-MK643 had no significant effect on soil respiration and nitrogen transformation at up to 0.41 mg IN-MK643/kg soil dry weight (equivalent to 10× its maximum PEC_{soil}) (<25% deviation from controls). IN-MK643, therefore, can be categorised as having low risk to soil microflora.

(Kolzer, U., 2002c)

RMS comment

This study was submitted in the original DAR (AD3, 2005). This study was conducted in compliance with the current guideline. The study is valid according to validity criteria.

IN-MK643 had no significant effect on soil respiration and nitrogen transformation at up to 0.41 mg IN-MK643/kg soil dry weight (<25% deviation from controls). This study is still considered acceptable.

B.9.6. EFFECTS ON TERRESTRIAL NON-TARGET HIGHER PLANTS

B.9.6.1. Summary of screening data

According to the applicant, non-target terrestrial plant response to indoxacarb (DPX-KN128) was evaluated on common plant species in the form of DPX-JW062 as a screening step to determine herbicidal activity if any. DPX-JW062 (50% DPX-KN128, 50% IN-KN127) was tested at either 1 or 0.5 kg/ha equivalent rate for post emergence and pre emergence herbicidal activity on *Ipomoea hederacea* (morning glory), *Xanthium persylvanicum* (Cocklebur), *Abutilon theophrasti* (velvet leaf), *Digitaria sanguinalis* (large crabgrass), *Setaria faberici* (Giant foxtail), *Echinochloa crusgalli* (barnyard grass), *Bromus tectorum* (cheatgrass), *Avena fatua* (wild oats), *Sorghum halpense* (Sorghum).

RMS comment

No report was provided for screening data. The absence of herbicidal activity could not be checked by RMS. The risk assessment is based of two vegetative vigor studies conducted with the representative formulation (see Volume 3 CP).

B.9.6.2. Testing on non-target plants

Non target plant data were submitted with the formulated product, Indoxacarb 150 g/L EC to fulfil the requirements of this data point. Please refer to RAR Volume_3_CP for the study summaries.

B.9.7. EFFECTS ON OTHER TERRESTRIAL ORGANISMS (FLORA AND FAUNA)

No further testing is necessary.

B.9.8. EFFECTS ON BIOLOGICAL METHODS FOR SEWAGE TREATMENT

Report: Lebertz, H. (1999); Indoxacarb: Activated sludge, respiration inhibition test

DuPont Report No.: DuPont-2533

Guidelines: OECD 209 (1984) **Deviations:** None

Testing Facility: Institut Fresenius Chemische und Biologische/GmbH, Taunusstein, Germany

Testing Facility Report No.: IF-99/16975-00

GLP: Yes

Certifying Authority: Hessisches Ministry for Environment, Energy, Family and Health (Wiesbaden, Germany)

I. MATERIALS AND METHODS

A. MATERIALS

- | | |
|-----------------------------|---|
| 1. Test material: | DPX-MP062 technical |
| Lot/Batch #: | MP062-216A |
| Purity: | 95.8% |
| Description: | White, granular |
| CAS#: | DPX-MP062: 114171-61-9 |
| | DPX-KN128 (the active isomer): 173584-44-6 |
| Stability of test compound: | Not determined in the test system |
| 2. Control: | Tap water |
| Test vehicle: | Tap water |
| Toxic reference: | 3,5-dichlorophenol 97% |
| 3. Test organisms: | Municipal sewage treatment plant activated sludge |
| Source: | Activated sludge from the municipal wastewater treatment plant of Taunusstein-Bleidenstadt, Germany |
| Test chambers: | 250 mL glass bottles |
| 4. Environmental conditions | |
| Temperature: | 20.0 to 21.0°C |
| pH of sludge: | Test initiation: 7.0 |
| | Test termination: 8.13 to 8.36 |

B. STUDY DESIGN AND METHODS

1. In-life initiated/completion
15-June-1999 to 23-June-1999
2. Experimental treatments
Toxicity of DPX-MP062 to sewage sludge was determined. Sewage sludge was obtained from a municipal treatment plant, washed, adjusted to a dry mass of 4 g/L and pH 7, and fed synthetic diet. Three test concentrations were prepared: 10, 100, and 1000 mg DPX-MP062/L drinking water, with water and 3,5-dichlorophenol controls. A volume of 250 mL test solution was prepared and incubated at 20-21°C. Respiration was determined by measuring oxygen concentration in test solutions after 3 hours.

II. RESULTS AND DISCUSSION

A. FINDINGS

Respiration rate of activated sewage sludge decreased on average 13% in the treatment units compared to the control units. Comment on effect of positive controls and validity of test (post test and deviation in controls). The criteria of validity of results given by the OECD guideline 209 were kept within this study:

Table 216
Toxicity to activated sewage sludge

Mean respiration rate--controls	1.76 (0.12)
Mean respiration rate – 1000 mg DPX-MP062/L	1.53 (0.23)
% Reduction in respiration rate in 3 h	13%
EC ₂₀	>1000 mg/L
EC ₅₀	>1000 mg/L

III. CONCLUSIONS

DPX-MP062 technical at 1000 mg/L drinking water is classified as harmless to activated sewage sludge in this worst-case laboratory study. Neither the EC₂₀ nor EC₅₀ could be determined.

(Lebertz, H., 1999)

RMS comment

Study summarised in Indoxacarb DAR, Volume 3, B9, AD3, 2005. This study was conducted in compliance with the current guideline. DPX-MP062 technical at 1000 mg/L drinking water is harmless to activated sewage sludge in this worst-case laboratory study. This study is still acceptable.

B.9.9. MONITORING DATA

No monitoring data were provided by the applicant. Such data are not considered required for indoxacarb.

B.9.10. BIOLOGICAL ACTIVITY OF METABOLITES POTENTIALLY OCCURRING IN GROUNDWATER

No testing necessary.

B.9.11. REFERENCES RELIED ON

Literature review for indoxacarb

Relevance criteria

Peer reviewed open literature relevant to the dossier may satisfy or partially satisfy data requirements as set out in Regulation (EC) No 1107/2009. The relevance criteria chosen for the selection of peer reviewed scientific open literature is consistent with the OECD guidance and does not restrict the selection of literature (Table 217). The relevance criteria guide the selection of literature dealing with the side effects on health, environment and non-target species for indoxacarb, and its relevant metabolites when used according to the legally registered label. Non-GLP studies in open literature may be considered relevant if the design and execution of the study is consistent with generally accepted scientific practice and guidelines. Clearly non-relevant studies are excluded.

Only literature search concerning the section ecotoxicology is reported below.

Table 217 lists the selection criteria applied to the results of the search for peer reviewed open literature relevant to indoxacarb and relevant metabolites. Relevant metabolites of a particular active substance as defined by Regulation (EC) No 1107/2009 can only be definitively identified at the end of a risk assessment. Scientific literature search will focus on

metabolites, degradation products, or transformation products of an active substance formed either in the organism or in the environment.

Table 217
Relevance criteria

Data requirement(s) (indicated by the correspondent data point number(s) as identified in Commission Regulation (EU) 283/2013)	Criteria for relevance
All Data Points	The dose levels or application rates reflect the proposed GAP.
	The test system, target crop, or species are prescribed by Regulation (EC) No 1107/2009 or the relevance is explained if not standard.
	Well identified test material, including its purity and impurity profile, is described.
	Study design and/or execution are consistent with relevant study guidelines.
	The endpoint is relevant to an EU data point as prescribed by Regulations (EU) No 283/2013 and 284/2013
Ecotoxicological studies	A relevant route of exposure is presented.

Search criteria

Reasonable effort was taken to locate all sources of relevant peer reviewed open literature concentrated on comprehensive databases containing worldwide coverage of biology, chemistry, biomedical, agricultural and environmental fields. The search ranged up to 10 years and within 6 months of the submission date. The initial search is a single concept search capturing all data points using search terms and synonyms for the active substance. If a large number of search results are returned from the single concept search making assessment for relevance impractical, a separate, focussed search is conducted for grouped data points. A separate single concept search is also conducted for each relevant metabolite.

Table 218 lists the literature search details for the active substance: indoxacarb

Table 218
Details of literature search for indoxacarb

Indoxacarb/DPX-KN128 173584-44-6 (initial search)	
	Databases Searched in STN: AGRICOLA, BIOSIS, CABA, CAplus, Ecotox
Justification for using these sources:	AGRICOLA – A bibliographic database containing selective worldwide coverage of agriculture and related fields. (4.2+ million records) BIOSIS - Contains information on life sciences, including biological and biomedical areas. (18.7+ million records) CABA – Covers worldwide literature from all areas of agriculture and related applied and life sciences. (5.3+ million records) CAplus – Covers worldwide literature from all areas of chemistry, biochemistry, chemical engineering, and related sciences. (28.6+ million records)
Date of the search:	March 1, 2013
Date range of the search:	2005-2013
Search strategies used:	1. 83120 s 173584-44-6 2. 39751 s L1 and 2005-2013/py 3. 38957 s L2 not p/dt 4. 2063 s L3 and (pesticide? Or herbicide? Or fungicide? Or insecticide?) 5. 1432 dup rem L4 (631 duplicates removed) 6. 388 s L5 and (AVIAN, TROUT, ONCORHYNCHUS MYKISS, CARP, CYPRINUS CARPIO, FATHEAD MINNOW, PIMEPHALES PROMELAS, ZEBRAFISH, DANIO RERIO, MEDAKA, ORYZIAS LATIPES, BLUEGILL, LEPOMIS MACROCHIRUS, DAPHNIA, ALGAE, AQUATIC, PSEUDOKIRCHNERIELLA SUBCAPITATA, ANABAENA FLO-AQUAE, SELENASTRUM CAPRICORNUTUM, LEMNA, MYRIOPHYLLUM, CERATOPHYLLUM, VALLISNERIA, CABOMBA, ELODEA, AQUATIC, NON-TARGET ARTHROPOD, HONEY BEE, APIS MELLIFERA, TYPHLODROMUS, APHIDIUS, POECILUS, CHRYSOPERLA, COCCINELLA, ALLEOCHARA, WASP, MITE, LACEWING, EARTHWORM, EISENIA FETIDA, COLLEMBOLA, FORSOMIA CANDIDA, MICROBIAL, MICRO-ORGANISM, NON-TARGET PLANTS, RYEGRASS, CORN, OAT?, WHEAT, SORGHUM, ONION, CUCUMBER, PEA, TOMATO?, OILSEED RAPE, SOYBEAN, SUGAR BEET)
Total number of original records retrieved:	388

Indoxacarb/DPX-KN128 173584-44-6 (Final search)

Databases Searched	Databases Searched in STN: AGRICOLA, BIOSIS, CABA, CAplus, Ecotox
Date of the final search:	18 November 2014
Date range for final search	2012-2014

Search strategies used:	<p>FILE 'CAPLUS, CABA, BIOSIS, AGRICOLA' ENTERED AT 15:43:50 ON 18 NOV 2014</p> <p>L1 3481 S INDOXACARB OR KN128 OR 173584-44-6</p> <p>L2 793 S L1 AND 2013-2014/PY</p> <p>L3 427 S L2 NOT P/DT</p> <p>L4 401 S L3 AND (PESTICID? OR HERBICID? OR INSECTICID? OR FUNGICID?)</p> <p>L5 280 DUP REM L4 (121 DUPLICATES REMOVED)</p> <p>L6. 79 s L5 and (AVIAN OR TROUT OR ONCORHYNCHUS MYKISS OR CARP OR CYPRINUS CARPIO OR FATHEAD MINNOW OR PIMEPHALES PROMELAS OR ZEBRAFISH OR DANIO RERIO OR MEDAKA OR ORYZIAS LATIPES OR BLUEGILL OR LEPOMIS MACROCHIRUS OR DAPHNIA OR ALGAE OR PSEUDOKIRCHNERIELLA SUBCAPITATA OR ANABAENA FLOS-AQUAE OR SELENASTRUM CAPRICORNUTUM OR LEMNA OR MYRIOPHYLLUM OR CERATOPHYLLUM OR VALLISNERIA OR CABOMBA OR ELODEA OR AQUATIC OR NON-TARGET ARTHROPOD? OR HONEYBEE OR APIS MELLIFERA OR TYPHLODROMUS OR APHIDIUS OR POECILUS OR CHRYSOPERLA OR COCCINELLA OR ALLEOCHARA OR WASP OR MITE OR LACEWING OR EARTHWORM OR EISENIA FETIDA OR COLLEMBOLA OR FOLSOMIA CANDIDA OR MICROBIAL OR MICRO-ORGANISM OR NON-TARGET PLANT? OR RYEGRASS OR CORN OR OAT? OR WHEAT OR SORGHUM OR ONION OR CUCUMBER OR PEA OR TOMATO? OR OILSEED RAPE OR SOYBEAN OR SUGAR BEET OR ECOTOX? OR ECOSYSTEM?)</p>
Total number of original records retrieved:	<p>79</p>

DPX-JW062 144171-61-9 (Initial search)	
Data requirement(s) captured in the search	Databases Searched in STN: AGRICOLA, BIOSIS, CABA, CAPlus, Ecotox
Justification for using these sources:	AGRICOLA – A bibliographic database containing selective worldwide coverage of agriculture and related fields. (4.2+ million records) BIOSIS - Contains information on life sciences, including biological and biomedical areas. (18.7+ million records) CABA – Covers worldwide literature from all areas of agriculture and related applied and life sciences. (5.3+ million records) CAPlus – Covers worldwide literature from all areas of chemistry, biochemistry, chemical engineering, and related sciences. (28.6+ million records)
Date of the search:	March 1, 2013
Date range of the search:	2005-2013
Search strategies used:	<ol style="list-style-type: none"> 1. 803 S 144171-61-9, DPX-JW062, Tornado 2. 2433 S L1 and 2005-2013/py 3. 1947 s L2 not p/dt 4. 1334 Dup rem l3 (613 duplicates removed) 5. 1016 s L4 and (pesticide? Or herbicide? Or fungicide? Or insecticide?) 6. 261 s L5 and (AVIAN, TROUT, ONCORHYNCHUS MYKISS, CARP, CYPRINUS CARPIO, FATHEAD MINNOW, PIMEPHALES PROMELAS, ZEBRAFISH, DANIO RERIO, MEDAKA, ORYZIAS LATIPES, BLUEGILL, LEPOMIS MACROCHIRUS, DAPHNIA, ALGAE, PSEUDOKIRCHNERIELLA SUBCAPITATA, ANABAENA FLO-AQUAE, SELENASTRUM CAPRICORNUTUM, LEMNA, MYRIOPHYLLUM, CERATOPHYLLUM, VALLISNERIA, CABOMBA, ELODEA, AQUATIC, NON-TARGET ARTHROPOD, HONEY BEE, APIS MELLIFERA, TYPHLODROMUS, APHIDIUS, POECILUS, CHRYSOPERLA, COCCINELLA, ALLEOCHARA, WASP, MITE, LACEWING, EARTHWORM, EISENIA FETIDA, COLLEMBOLA, FORSOMIA CANDIDA, MICROBIAL, MICRO-ORGANISM, NON-TARGET PLANTS, RYEGRASS, CORN, OAT?, WHEAT, SORGHUM, ONION, CUCUMBER, PEA, TOMATO?, OILSEED RAPE, SOYBEAN, SUGAR BEET)
Total number of original records retrieved:	261

IN-KN127 185608-75-7 (First search)

Databases Searched	Databases Searched in STN: AGRICOLA, BIOSIS, CABA, CAplus, Ecotox
Justification for using this source:	<p>AGRICOLA – A bibliographic database containing selective worldwide coverage of agriculture and related fields. (4.2+ million records)</p> <p>BIOSIS - Contains information on life sciences, including biological and biomedical areas. (18.7+ million records)</p> <p>CABA – Covers worldwide literature from all areas of agriculture and related applied and life sciences. (5.3+ million records)</p> <p>CAplus – Covers worldwide literature from all areas of chemistry, biochemistry, chemical engineering, and related sciences. (28.6+ million records)</p>
Date of the search:	March 1, 2013
Date range of the search:	2005-2013/py
Search strategies used:	<p>1. 17 s 185608-75-7, DPX-KN127</p> <p>2. 8 s L1 and 2005-2013/py</p> <p>3. 8 Dup Rem L2 (0 duplicates removed)</p>
Total number of records retrieved:	8

DPX-JW062 144171-61-9 and IN-KN127 185608-75-7 (Final search)

Databases Searched	Databases Searched in STN: AGRICOLA, BIOSIS, CABA, CAplus, Ecotox
Date of the final search:	18 November 2014
Date range for final search	2012-2014
Search strategies used:	<p>FILE 'CAPLUS, CABA, BIOSIS, AGRICOLA' ENTERED 15:59:38 ON 18 NOV 2014</p> <p>L1 94 S 144171-61-9 OR JW062</p> <p>L2 21 S 185608-75-7 OR KN127</p> <p>L3 107 S L1 OR L2</p> <p>L4 8 S L3 AND 2013-2014/PY</p> <p>L5 4 S L4 NOT P/DT</p> <p>L6 4 DUP REM L5 (0 DUPLICATES REMOVED))</p>
Total number of records retrieved:	4

The published literature searches for DPX-KN128 and DPX-JW062 created a lot of duplicate responses which were removed giving the following grand totals:

Grand Total (both searches, DPX-KN128, IN-JW062 and IN-KN1127	740 after removing duplicates 314
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Relevant study selection-results of the selection process

Obviously non-relevant studies in open literature search were excluded by applying the relevance criteria previously presented in **Table 217** of this document.

Table 219 summarises the results of the selection process including the number of summary records and full text documents assessed.

Table 219
Literature search results: Ecotoxicology

Data requirement(s) captured in the search	Number
Total number of summary records retrieved after all searches of peer-reviewed literature (excluding duplicates)	314
Number of summary records excluded from the search results after rapid assessment for relevance	267
Total number of full-text documents assessed in detail	47
Number of studies excluded from further consideration after detailed assessment for relevance	46
Number of studies not excluded for relevance after detailed assessment (<i>i.e.</i> , relevant studies and studies of unclear relevance)	1

RMS comment:

A single study was considered relevant by the applicant for effects on non-target species.

Table 220
Literature to be included after detailed assessment: Ecotoxicology.

Data Requirement No., Reference No.	Author(s)	Year	Title	Source
CP, 10.3.1.6/19	van der Steen, J.J.M., Dinter, A.	2006	A monitoring study to assess the acute mortality effects of indoxacarb on honey bees (<i>Apis mellifera</i> L.) in flowering apple orchards PPO Bijen, DuPont de Nemours (Deutschland) GmbH	Pest Manag Sci 63 1095-1099 (2007)

A detailed assessment of this publication was made by RMS in Volume 3 CP.

Besides for some of the retrieved papers, RMS considered that the non-relevance for environmental assessment purpose was not so clear. For the following studies at least, detailed reasons for exclusions were requested.

Author(s)	Year	Title	Source	Reason (s) for non-inclusion
Heylen, Kevin; Gobin, Bruno; Arckens, Lutgarde; Huybrechts, Roger; Billen, Johan	2011	The effects of four crop protection products on the morphology and ultrastructure of the hypopharyngeal gland of the European honeybee, <i>Apis mellifera</i>	Apidologie	Applicant: Not an accepted study design RMS: Agreed. There is no validated testing method available for effects on hypopharyngeal gland.
Ding, Yuping; Weston, Donald P.; You, Jing; Rother, Amanda K.; Lydy, Michael J.	2011	Toxicity of Sediment-Associated Pesticides to <i>Chironomus dilutus</i> and <i>Hyalella azteca</i>	Archives of Environmental Contamination and Toxicology	Applicant: Not done according to OECD study design RMS: Agreed.
Yu, Ruixian; Zhao, Xueping; Wu, Changxing; Wu, Shenggan; Cang, Tao; Chen, Liping; Wang, Qiang	2009	Evaluation of indoxacarb to environmental organisms	Nongyao	Applicant: Unclear test methods RMS : Agreed. Publication written in chinese.
Belien, T.; Kellers, J.; Heylen, K.; Billen, J.; Arckens, L.; Huybrechts, R.; Gobin, B. Editor(s): Jansen, J. P.	2010	Identification and evaluation of sublethal effects of crop protection products on honey bees (<i>Apis mellifera</i>).	IOBC/WPRS Bulletin (2010) Volume 55, pp. 55-59 Published by: International Organization for Biological and Integrated Control of Noxious Animals and Plants (IOBC/OILB), West Palaearctic Regional Section (WPRS/SROP), Dijon Conference: Working Group "Pest	Applicant: dose rates do not reflect proposed GAP RMS: Agreed.
Liang, Hongwu; Qiu, Jing; Wang, Chengju; Li, Xuefeng; Qiu, Lihong; Zhou, Zhiqiang	2014	Enantioselectivity bioaccumulation and toxic effects of indoxacarb in zebrafish (<i>Danio rerio</i>)	Abstracts of Papers, 248th ACS National Meeting & Exposition, San Francisco, CA, United States, August 10-14, 2014 (2014), AGRO-649. American Chemical Society: Washington, D. C. CODEN: 69SZG4	Applicant: This is an oral presentation abstract, unclear what was said in the presentation RMS: No details available.
Pei, Hui; Ou, Xiao-ming; Yu, Wei-li; Yi, Zheng-hua; Bai, Jian-jun; Gao, De-liang	2013	Acute toxicity of four insecticides to honeybee <i>Apis mellifera</i>	Shijie Nongyao	Applicant: Unclear study methods RMS : Agreed. Publication written in chinese.

On RMS's request, a new search was required for ecotoxicology to address potential effects on bumble bees, reptiles and amphibians.

Literature Search: 11/7/2015

Databases: CAPlus, CABA, BIOSIS, Agricola






Strategy: indoxacarb or 173584-44-6 or DPX-KN128 or DPX-MP062 or DPX-JW062 and PY 2005-2015 and Bumble bees or reptiles or amphibians

Data requirement(s) captured in the search	Number
Total number of summary records retrieved after all searches of peer-reviewed literature (excluding duplicates)	12
Number of summary records excluded from the search results after rapid assessment for relevance	11
Total number of full-text documents assessed in detail	1
Number of studies excluded from further consideration after detailed assessment for relevance	1
Number of studies not excluded for relevance after detailed assessment (<i>i.e.</i> , relevant studies and studies of unclear relevance)	1






An other study was considered relevant by the applicant for effects on non-target species.

Author(s)	Year	Title	Source	Reason(s) for non-inclusion
Steen, J. J. M. van der; Dinter, A	20009	A monitoring study confirming the safe use of DuPont Steward insecticide (a.s. indoxacarb) for natural bumblebee populations in flowering apple orchards and recommendations for the use of commercial bumble bee hives in flowering apple and pear orchards treated with Steward	Julius-Kuehn-Archiv (2009), Number 423, 67 p. ISSN: 1868-9892 Published by: Julius Kuehn Institut, Bundesforschungsinstitut fuer Kulturpflanzen, Quedlinburg Conference: Hazards of pesticides to bees. 10th International Symposium of the ICP-Bee Protection Group. Bucharest, Romania, 8-10 October, 2008. URL (Availability): http://pub.jki.bund.de/index.php/JKA/issue/archive	Applicant: Presentation RMS: Only an abstract was provided. However the formulation Steward is not the representative formulation and the results of this monitoring are not taken into consideration.

REFERENCES LIST

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
CA, 8.1.1.1		1996	IN-JT333-20: An acute oral toxicity study with the northern bobwhite  AMR 3890-96 Study submitted in the EU Dossier in 1997 and included in the first EU approval review. Published: No	Y	✗ N	<u>Comment from RMS:</u> Study from the original submission	DuPont	<i>In DAR (2000)</i>
CA, 8.1.1.1		1997	DPX-MP062 technical (approximately 75% DPX- KN128, 25% DPX-KN127): An acute oral toxicity study with the northern bobwhite  AMR 3940-96, Revision No. 2 Study submitted in the EU Dossier in 1997 and included in the first EU approval review. Published: No	Y	✗ N	<u>Comment from RMS:</u> Study from the original submission	DuPont	<i>In DAR (2000)</i>
CA, 8.1.1.2		1997a	DPX-MP062 technical	Y	✗ N	<u>Comment from RMS:</u>	DuPont	<i>In DAR (2000)</i>


			(approximately 75% DPX-KN128, 25% DPX-KN127): A dietary LC ₅₀ study with the northern bobwhite [REDACTED] AMR 4094-96 Study submitted in the EU Dossier in 1997 and included in the first EU approval review. Published: No			Study from the original submission		
CA, 8.1.1.2		1997b	DPX-MP062 technical (approximately 75% DPX-KN128, 25% DPX-KN127): A dietary LC ₅₀ study with the mallard [REDACTED] AMR 4093-96 Study submitted in the EU Dossier in 1997 and included in the first EU approval review. Published: No	Y	✗ N	<u>Comment from RMS:</u> Study from the original submission	DuPont	<i>In</i> <i>DAR</i> (2000)
CA, 8.1.1.3		1997a	DPX-MP062 technical (approximately 75% DPX-KN128, 25% DPX-KN127): A reproduction study with the mallard (<i>Anas platyrhynchos</i>) [REDACTED] AMR 4095-96 Study submitted in	Y	✗ N	<u>Comment from RMS:</u> Study from the original submission	DuPont	<i>In</i> <i>DAR</i> (2000)

			the EU Dossier in 1997 and included in the first EU approval review. Published: No					
CA, 8.1.1.3		1997b	DPX-MP062 technical (approximately 75% DPX-KN128, 25% DPX-KN127): A reproduction study with the northern bobwhite (<i>Colinus virginianus</i>)  AMR 4096-96 Study submitted in the EU Dossier in 1997 and included in the first EU approval review. Published: No	Y	✗ N	<u>Comment from RMS:</u> Study from the original submission	DuPont	In (2000) <i>DAR</i>
CA, 8.2.1		1997a	DPX-MP062 (approximately 75% DPX-KN128, 25% IN-KN127): Flow-through, acute, 96-hour LC ₅₀ to bluegill sunfish, <i>Lepomis macrochirus</i>  HLR 912-96, Revision No. 2 Study submitted in the EU Dossier in 1997 and included in the first EU approval review. Published: No	Y	✗ N	<u>Comment from RMS:</u> Study from the original submission	DuPont	In (2000) <i>DAR</i>
CA, 8.2.1		1997b	IN-JT333-20: Flow-through,	Y	✗ N	<u>Comment from RMS:</u>	DuPont	In (2000) <i>DAR</i>







			acute, 96-hour LC ₅₀ to rainbow trout, <i>Oncorhynchus mykiss</i> [REDACTED] HL-1997- 00180 Study submitted in the EU Dossier in 1997 and included in the first EU approval review. Published: No			Study from the original submission		
CA, 8.2.1	[REDACTED]	1999	IN-KT413, a metabolite of DPX-MP062: Acute, static, 96-hour toxicity test to rainbow trout, <i>Oncorhynchus mykiss</i> [REDACTED] DuPont-1311 Study submitted in the EU Dossier and included in the first EU approval review. Published: No	Y	✗ N	<u>Comment from RMS:</u> Study from the original submission	DuPont	<i>In addendum to DAR (2001)</i>
CA, 8.2.1	[REDACTED]	2003a	IN-MP819: Flow-through, acute, 96-hour LC ₅₀ to rainbow trout, <i>Oncorhynchus mykiss</i> [REDACTED] DuPont-11492 Study submitted in the EU Dossier in 2005 and included in the first EU approval review.	Y	✗ N	<u>Comment from RMS:</u> Study from the original submission	DuPont	<i>In addendum to DAR (2005)</i>

CA, 8.2.1		2003b	Published: No IN-MS775: Static, acute, 96-hour limit test to rainbow trout, <i>Oncorhynchus mykiss</i> [REDACTED] DuPont-12091 Study submitted in the EU Dossier in 2005 and included in the first EU approval review. Published: No	Y	✗ N	<u>Comment from RMS:</u> Study from the original submission	DuPont	<i>In addendum to DAR (2005)</i>
CA, 8.2.1/01		2013	IN-MK643: Acute toxicity to the rainbow trout, <i>Oncorhynchus mykiss</i> , determined under static test conditions [REDACTED] DuPont-36162 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>
CA, 8.2.1/03		2013	IN-KB687: Acute toxicity to the rainbow trout, <i>Oncorhynchus mykiss</i> , determined under static test conditions [REDACTED] DuPont-35828 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>
CA, 8.2.1/04		2010	Indoxacarb (DPX-KN128)	Y	Y	The study is necessary for	DuPont	<i>Submitted for the purpose</i>

	F		technical: A 96-hour flow-through acute toxicity test with the rainbow trout (<i>Oncorhynchus mykiss</i>) [REDACTED] DuPont-29541 GLP: Yes Published: No			the regulatory decision, conducted according to GLP and has not previously been protected or if previously protected the period of data protection has not expired at the time of submission of this dossier.		<i>of renewal</i>
CA, 8.2.1/05	[REDACTED]	1997	IN-KG433 technical: Flow-through, acute, 96-hour limit test to rainbow trout, <i>Oncorhynchus mykiss</i> [REDACTED] HL-1997-00412 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>
CA, 8.2.1/06	[REDACTED]	2013	IN-MK638: Acute toxicity to the rainbow trout, <i>Oncorhynchus mykiss</i> , determined under static test conditions [REDACTED] DuPont-35827 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>
CA, 8.2.1/08	[REDACTED]	2014	IN-JU873: Acute toxicity to the rainbow trout, <i>Oncorhynchus mykiss</i> , determined	Y	Y	The study is necessary for the regulatory decision, conducted according to GLP and has	DuPont	<i>Submitted for the purpose of renewal</i>

			under static-renewal test conditions  DuPont-35826 GLP: Yes Published: No			not previously been protected or if previously protected the period of data protection has not expired at the time of submission of this dossier.		
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CA, 8.2.1/09		2015	IN-U8E24: Acute toxicity to the rainbow trout, <i>Oncorhynchus mykiss</i> , determined under static test conditions DuPont-43485 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>
CA, 8.2.1/10		2015a	IN-KN124: Acute toxicity to the rainbow trout, <i>Oncorhynchus mykiss</i> , determined under static- renewal test conditions DuPont-43113 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>
CA, 8.2.1/11		2015b	IN UYG24: Acute toxicity to the rainbow trout, <i>Oncorhynchus mykiss</i> , determined under static test conditions DuPont-43422 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>
CA, 8.2.1/12		2015	IN-KN125: Acute toxicity to the rainbow trout, <i>Oncorhynchus</i>	Y	Y	The study is necessary for the regulatory decision, conducted	DuPont	<i>Submitted for the purpose of renewal</i>

			<i>mykiss</i> , determined under static- renewal test conditions  DuPont-43104 GLP: Yes Published: No			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.		
CA, 8.2.2.1		1997	DPX-MP062 (approximately 75% DPX- KN128, 25% IN-KN127): Early life-stage toxicity to rainbow trout, <i>Oncorhynchus mykiss</i>  HLR 598-96, Revision No. 1 Study submitted in the EU Dossier in 1997 and included in the first EU approval review. Published: No	Y	✕ N	<u>Comment from RMS:</u> Study from the original submission	DuPont	<i>In DAR (2000)</i>
CA, 8.2.2.1/0 2		2014	IN-JT333: Early life-stage toxicity test with the fathead minnow, <i>Pimephales promelas</i> , under flow- through conditions  DuPont-41669 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>
CA, 8.2.2.1/0 3		2014	Indoxacarb (DPX-KN128): Early life-stage toxicity test	Y	Y	The study is necessary for the regulatory decision,	DuPont	<i>Submitted for the purpose of renewal</i>

			with the fathead minnow, <i>Pimephales promelas</i> , under flow-through conditions [REDACTED] DuPont-41426 GLP: Yes Published: No			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.		
CA, 8.2.2.3	[REDACTED]	2002	Bioconcentration and metabolism of [indanone-1- ¹⁴ C]DPX-JW062 and [trifluoromethoxyphenyl(U)- ¹⁴ C]DPX-JW062 in fish (a racemic mixture of DPX-KN128 and IN-KN127) [REDACTED] AMR 3663-95, Revision No. 1 Study submitted in the EU Dossier in 2005 and included in the first EU approval review. Published: No	Y	✗ N	<u>Comment from RMS:</u> Study from the original submission	DuPont	<i>In addendum to DAR (2005)</i>
CA, 8.2.4.1	Boeri, R.L., Magazu, J.P., Ward, T.J.	1999	IN-KT413, a metabolite of DPX-MP062: Acute, static, 48-hour toxicity (EC ₅₀) test to <i>Daphnia magna</i> T.R. Wilbury Laboratories, Inc. DuPont-1309 Study	N	✗ N	<u>Comment from RMS:</u> Study from the original submission	DuPont	<i>In addendum to DAR (2001)</i>

			submitted in the EU Dossier in 2001 and included in the first EU approval review. Published: No					
CA, 8.2.4.1	Hoke, R.A.	1997	IN-JT333-20: Static-renewal, acute, 48-hour EC ₅₀ to <i>Daphnia magna</i> DuPont Haskell Laboratory HL-1997-00006 Study submitted in the EU Dossier in 1997 and included in the first EU approval review. Published: No	N	¥ N	<u>Comment from RMS:</u> Study from the original submission	DuPont	<i>In DAR (2000)</i>
CA, 8.2.4.1	Samel, A.	2003a	IN-MP819: Flow-through, acute, 48-hour EC ₅₀ to <i>Daphnia magna</i> DuPont Haskell Laboratory DuPont-11491 Study submitted in the EU Dossier in 2005 and included in the first EU approval review. Published: No	N	¥ N	<u>Comment from RMS:</u> Study from the original submission	DuPont	<i>In addendum to DAR (2005)</i>
CA, 8.2.4.1	Samel, A.	2003b	IN-MS775: Static, acute, 48-hour EC ₅₀ to <i>Daphnia magna</i> DuPont Haskell Laboratory DuPont-12090 Study submitted in the EU Dossier	N	¥ N	<u>Comment from RMS:</u> Study from the original submission	DuPont	<i>In addendum to DAR (2005)</i>

			in 2005 and included in the first EU approval review. Published: No					
CA, 8.2.4.1/01	Bergfield, A.	2013	IN-MK643: 48-Hour static, acute toxicity test with the Cladoceran, <i>Daphnia magna</i> ABC Laboratories, Inc. (Missouri) DuPont-36163 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>
CA, 8.2.4.1/02	Gaertner, K.	2013	IN-KB687: 48-Hour static, acute toxicity test with the Cladoceran, <i>Daphnia magna</i> ABC Laboratories, Inc. (Missouri) DuPont-35831 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>
CA, 8.2.4.1/03	Gallagher, S.P., Kendall, T.Z., Krueger, H.O.	2010	Indoxacarb (DPX-KN128) technical: A 48-hour flow-through acute toxicity test with the Cladoceran (<i>Daphnia magna</i>) Wildlife International, Ltd. DuPont-29542 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	DuPont	<i>Submitted for the purpose of renewal</i>

CA, 8.2.4.1/0 4	Hoke, R.A.	1997	IN-KG433 technical: Static-renewal, acute, 48-hour limit test to <i>Daphnia magna</i> DuPont Haskell Laboratory HLO-1997- 00363 GLP: Yes Published: No	N	Y	this dossier. 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>
CA, 8.2.4.1/0 5	Holou, M.	2013	IN-MK638: 48-Hour static, acute toxicity test with the Cladoceran, <i>Daphnia magna</i> ABC Laboratories, Inc. (Missouri) DuPont-35830 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>
CA, 8.2.4.1/0 7	Rebstock, M.	2014	IN-JU873: 48- Hour static- renewal, acute toxicity test with the Cladoceran, <i>Daphnia magna</i> ABC Laboratories, Inc. (Missouri) DuPont-35829 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>

CA, 8.2.4.1/0 8	Bradbury, N.	2015a	IN-U8E24: 48- hour static, acute toxicity test with the cladoceran, <i>Daphnia magna</i> ABC Laboratories, Inc. (Missouri) DuPont-43486 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.	DuPon t	<i>Submitted for the purpose of renewal</i>
CA, 8.2.4.1/0 9	Bradbury, N.	2015b	IN-UYG24: 48-Hour static, acute toxicity test with the cladoceran, <i>Daphnia magna</i> ABC Laboratories, Inc. (Missouri) DuPont-43423 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.	DuPon t	<i>Submitted for the purpose of renewal</i>
CA, 8.2.4.1/1 0	Goudie, O.J.	2015	IN-KN124: 48- hour static- renewal, acute toxicity test with the cladoceran, <i>Daphnia magna</i> ABC Laboratories, Inc. (Missouri) DuPont-43106 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.	DuPon t	<i>Submitted for the purpose of renewal</i>
CA, 8.2.4.1/1 1	Mays, C.	2015	IN-KN125: 48- Hour static- renewal, acute toxicity test with the	N	Y	The study is necessary for the regulatory decision, conducted	DuPon t	<i>Submitted for the purpose of renewal</i>

			cladoceran, <i>Daphnia magna</i> ABC Laboratories, Inc. (Missouri) DuPont-43105 GLP: Yes Published: No			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.		
CA, 8.2.4.2/0 1	Brougher, D.S., Martin, K.H., Gallagher, S.P., Bodle, E.S.	2014	IN-KT413: A 96-hour static acute toxicity test with the saltwater mysid (<i>Americamysis bahia</i>) Wildlife International Ltd. (USA) DuPont-38347 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>
CA, 8.2.4.2/0 2	Dinehart, S.	2013a	IN-MK643: Acute toxicity with the mysid shrimp, <i>Americamysis bahia</i> , determined under static test conditions ABC Laboratories, Inc. (Missouri) DuPont-36476 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>
CA, 8.2.4.2/0 3	Dinehart, S.	2013b	IN-MK638: Acute toxicity with the mysid shrimp, <i>Americamysis bahia</i> , determined under static test conditions ABC	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>

			Laboratories, Inc. (Missouri) DuPont-36475 GLP: Yes Published: No			previously protected the period of data protection has not expired at the time of submission of this dossier.		
CA, 8.2.4.2/0 4	Dinehart, S.	2013c	IN-KB687: Acute toxicity with the mysid shrimp, <i>Americamysis bahia</i> , determined under static- renewal test conditions ABC Laboratories, Inc. (Missouri) DuPont-36477 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>
CA, 8.2.4.2/0 5	Dinehart, S.	2013d	IN-JT333: Acute toxicity with the mysid shrimp, <i>Americamysis bahia</i> , determined under static- renewal conditions ABC Laboratories, Inc. (Missouri) DuPont-36489 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>
CA, 8.2.4.2/0 6	Dinehart, S.	2013e	IN-JU873: Acute toxicity with the mysid shrimp, <i>Americamysis bahia</i> , determined under static- renewal conditions ABC Laboratories, Inc. (Missouri) DuPont-36474 GLP: Yes	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	DuPont	<i>Submitted for the purpose of renewal</i>

			Published: No			the time of submission of this dossier.		
CA, 8.2.4.2/07	Dinehart, S.	2014a	Indoxacarb (DPX-KN128): Acute toxicity with the mysid shrimp, <i>Americamysis bahia</i> , determined under flow-through test conditions ABC Laboratories, Inc. (Missouri) DuPont-38440 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>
CA, 8.2.5.1/01	Boeri, R.L., Wyskiel, D.C., Ward, T.J.	2003	IN-KT413: Chronic, static-renewal toxicity to the daphnid, <i>Daphnia magna</i> T.R. Wilbury Laboratories, Inc. DuPont-12041 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>
CA, 8.2.5.1/02	Lamichhane, K.	2014	Indoxacarb (DPX-KN128): Static renewal, chronic toxicity test with the cladoceran, <i>Daphnia magna</i> ABC Laboratories, Inc. (Missouri) DuPont-41661 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>
CA, 8.2.5.2/0	Boeri, R.L.,	1997	Chronic toxicity of	N	Y	The study is necessary for	DuPont	<i>Submitted for the purpose</i>

1	Magazu, J.P., Ward, T.J.		DPX-MP062 to the mysid, <i>Mysidopsis bahia</i> T.R. Wilbury Laboratories, Inc. HLO-1997-00206, Revision No. 1 GLP: Yes Published: No			the regulatory decision, conducted according to GLP and has not previously been protected or if previously protected the period of data protection has not expired at the time of submission of this dossier.		<i>of renewal</i>
CA, 8.2.5.4	Aufderheide, J.	2004a	IN-MP819: Chronic toxicity test with midge larvae (<i>Chironomus riparius</i>) using spiked sediment ABC Laboratories, Inc. DuPont-13231 Study submitted in the EU Dossier in 2005 and included in the first EU approval review. Published: No	N	Y N	<u>Comment from RMS:</u> Study from the original submission	DuPont	<i>In addendum to DAR (2005)</i>
CA, 8.2.5.4	Aufderheide, J.	2004b	IN-MS775: Chronic toxicity test with midge larvae (<i>Chironomus riparius</i>) using spiked sediment ABC Laboratories, Inc. DuPont-13232 Study submitted in the EU Dossier in 2005 and included in the first EU approval review.	N	Y N	<u>Comment from RMS:</u> Study from the original submission	DuPont	<i>In addendum to DAR (2005)</i>

CA, 8.2.5.4	Radford, K.	2001	Published: No IN-JT333: To assess the toxicity to the sediment dwelling phase of the midge <i>Chironomus riparius</i> Huntingdon Life Sciences Ltd. DuPont-4054 Study submitted in the EU Dossier in 2005 and included in the first EU approval review. Published: No	N	✖ N	<u>Comment from RMS:</u> Study from the original submission	DuPont	<i>In addendum to DAR (2005)</i>
CA, 8.2.5.3/01	Thomas, S.T., Kendall, T.Z., Martin, K.H., Gallagher, S.P., Krueger, H.O.	2014	IN-KT413: A prolonged sediment toxicity test with <i>Chironomus riparius</i> using spiked water Wildlife International Ltd. (USA) DuPont-36116 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>
CA, 8.2.5.3/02	Thomas, S.T., Martin, K.H., Gallagher, S.P., Bodle, E.S.	2014	¹⁴ C DPX-KN128: A prolonged sediment toxicity test with <i>Chironomus riparius</i> using spiked water Wildlife International Ltd. (USA) DuPont-35832, 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>
CA,	Thomas,	2015	¹⁴ C-Indoxacarb	N	Y	The study is	DuPont	<i>Submitted for</i>

8.2.5.3/03	S.T., Siddiqui, A.I., Gallagher, S.P., Krueger, H.O.		(DPX-KN128) technical: A prolonged sediment toxicity test with the midge (<i>Chironomus riparius</i>) using spiked water Wildlife International Ltd. (USA) DuPont-41246 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>
CA, 8.2.5.4/01	Thomas, S.T., Kendall, T.Z., Martin, K.H., Gallagher, S.P., Krueger, H.O.	2013	IN-KT413: A prolonged sediment toxicity test with <i>Chironomus riparius</i> using spiked sediment Wildlife International Ltd. (USA) DuPont-35824 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>
CA, 8.2.5.4/02	Thomas, S.T., Martin, K.H., Gallagher, S.P., Krueger, H.O.	2014	IN-KG433: A prolonged sediment toxicity test with <i>Chironomus riparius</i> using spiked sediment Wildlife International Ltd. (USA) DuPont-35825 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>
CA, 8.2.6.1	Boeri, R.L., Ward, T.J.	2000	IN-KT413: Influence on growth and growth rate of the alga, <i>Selenastrum</i>	N	¥ N	<u>Comment from RMS:</u> Study from the original submission	DuPont	<i>In addendum to DAR (2005)</i>

			<i>capricornutum</i> T.R. Wilbury Laboratories, Inc. DuPont-3935 Study submitted in the EU Dossier in 2005 and included in the first EU approval review. Published: No					
CA, 8.2.6.1	Sloman, T.L., Leva, S.E.	1997b	IN-JT333 (metabolite of DPX-MP062): Influence on growth and growth rate of the green alga <i>Pseudokirchneriella</i> <i>subcapitata</i> (formerly called <i>Selenastrum</i> <i>capricornutum</i>) DuPont Stine- Haskell Research Center AMR 4259-96 Study submitted in the EU Dossier in 1997 and included in the first EU approval review. Published: No	N	✗ N	<u>Comment from RMS:</u> Study from the original submission	DuPont	<i>In</i> <i>DAR</i> (2000)
CA, 8.2.6.1	Sloman, T.L.	2003a	IN-MP819: Influence on growth and growth rate of the green alga <i>Selenastrum</i> <i>capricornutum</i> DuPont Haskell Laboratory DuPont-11493 Study submitted in the EU Dossier in 2005 and included in the first EU	N	✗ N	<u>Comment from RMS:</u> Study from the original submission	DuPont	<i>In addendum</i> <i>to</i> <i>DAR</i> (2005)

			approval review. Published: No					
CA, 8.2.6.1	Sloman, T.L.	2003b	IN-MS775: Influence on growth and growth rate of the green alga <i>Selenastrum capricornutum</i> DuPont Haskell Laboratory DuPont-12092 Study submitted in the EU Dossier in 2005 and included in the first EU approval review. Published: No	N	✗ N	<u>Comment from RMS:</u> Study from the original submission	DuPont	<i>In addendum to DAR (2005)</i>
CA, 8.2.6.1/01	Aufderheide, J.	2014	Indoxacarb (DPX-KN128): Growth inhibition test with the unicellular green alga, <i>Pseudokirchneriella subcapitata</i> ABC Laboratories, Inc. (Missouri) DuPont-38349 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>
CA, 8.2.6.1/02	Bergfield, A.	2013	IN-MK643: Growth inhibition test with the unicellular green alga, <i>Pseudokirchneriella subcapitata</i> ABC Laboratories, Inc. (Missouri) DuPont-36161 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>

CA, 8.2.6.1/0 3	Gaertner, K.	2013	IN-KB687: Growth inhibition test with the unicellular green alga, <i>Pseudokirchne riella subcapitata</i> ABC Laboratories, Inc. (Missouri) DuPont-35822 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.	DuPon t	<i>Submitted for the purpose of renewal</i>
CA, 8.2.6.1/0 4	Holou, M.	2013	IN-MK638: Growth inhibition test with the unicellular green alga, <i>Pseudokirchne riella subcapitata</i> ABC Laboratories, Inc. (Missouri) DuPont-35821 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.	DuPon t	<i>Submitted for the purpose of renewal</i>
CA, 8.2.6.1/0 5	Rebstock, M.	2014	IN-JU873: Growth inhibition test with the unicellular green alga, <i>Pseudokirchne riella subcapita</i> ABC Laboratories, Inc. (Missouri) DuPont-35820 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.	DuPon t	<i>Submitted for the purpose of renewal</i>

CA, 8.2.6.1/0 7	Amoroso, T.	2015	IN-KN125: Growth inhibition test with the unicellular green alga, <i>Pseudokirchneriella subcapitata</i> ABC Laboratories, Inc. (Missouri) DuPont-43103, 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>
CA, 8.2.6.1/0 8	Goudie, O.J.	2015a	IN-UYG24: Growth inhibition test with the unicellular green alga, <i>Pseudokirchneriella subcapitata</i> ABC Laboratories, Inc. (Missouri) DuPont-43421 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>
CA, 8.2.6.1/0 9	Goudie, O.J.	2015b	IN-U8E24: Growth inhibition test with the unicellular green alga, <i>Pseudokirchneriella subcapitata</i> ABC Laboratories, Inc. (Missouri) DuPont-43484 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>
CA, 8.2.6.1/1 0	Mays, C.	2015	IN-KN124: Growth inhibition test with the unicellular	N	Y	The study is necessary for the regulatory decision, conducted	DuPont	<i>Submitted for the purpose of renewal</i>

			green alga, <i>Pseudokirschneriella subcapitata</i> ABC Laboratories, Inc. (Missouri) DuPont-43112 GLP: Yes Published: No			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.		
CA, 8.3.1.1.1/01	Haupt, S.	2014	Indoxacarb (DPX-KN128) technical: Acute oral and contact toxicity to the bumblebee, <i>Bombus terrestris</i> L. (Hymenoptera) IBACON DuPont-38350 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
CA, 8.3.1.1.1/02	Kling, A.	2014	Indoxacarb (DPX-KN128) technical: Acute oral and contact toxicity to the honeybee, <i>Apis mellifera</i> L. under laboratory conditions Eurofins Agrosience Services EcoChem GmbH DuPont-36500 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
CA, 8.3.1.2/01	Kling, A.	2014	Indoxacarb (DPX-KN128) technical: Assessment of chronic effects to the honeybee, <i>Apis mellifera</i> L., in a 10 days	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been	DuPont	Submitted for the purpose of renewal

			continuous laboratory feeding test Eurofins Agrosience Services EcoChem GmbH DuPont-36490 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.		
CA, 8.3.1.3/01	Kleinhenz, M.	2014	Indoxacarb (DPX-KN128) technical: A feeding study to evaluate effects on the brood of honey bees (<i>Apis mellifera</i> ; Hymenoptera, Apidae) in Germany 2013 Eurofins Agrosience Services EcoChem GmbH DuPont-36493 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>
CA, 8.3.1.3/02	Berg, C.	2015	Indoxacarb (DPX-KN128) technical: A feeding study to evaluate effects on the brood of honey bees (<i>Apis Mellifera</i> , Hymenoptera, Apidae) in Germany 2015 Eurofins Agrosience Services EcoChem GmbH DuPont-43111 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>
CA, 8.4.1/01	Lührs, U.	2013a	IN-KG433: Effects on reproduction and growth of the earthworm, <i>Eisenia fetida</i> , in artificial soil with 5% peat	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>

			IBACON DuPont-36496 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.		
CA, 8.4.1/02	Lührs, U.	2013b	IN-JU873: Effects on reproduction and growth of the earthworm, <i>Eisenia fetida</i> , in artificial soil with 5% peat IBACON DuPont-36497 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>
CA, 8.4.1/03	Lührs, U.	2013c	IN-JT333: Effects on reproduction and growth of the earthworm, <i>Eisenia fetida</i> , in artificial soil with 5% peat IBACON DuPont-36494 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>
CA, 8.4.1/04	Lührs, U.	2013d	IN-MK638: Effects on reproduction and growth of the earthworm, <i>Eisenia fetida</i> , in artificial soil with 5% peat IBACON DuPont-36498 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	DuPont	<i>Submitted for the purpose of renewal</i>

						protection has not expired at the time of submission of this dossier.		
CA, 8.4.1/05	Lühns, U.	2013e	IN-MK643: Effects on reproduction and growth of the earthworm, <i>Eisenia fetida</i> , in artificial soil with 5% peat IBACON DuPont-36499 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>
CA, 8.4.1/06	Lühns, U.	2014a	IN-KT413: Effects on reproduction and growth of the earthworm, <i>Eisenia fetida</i> , in artificial soil with 5% peat IBACON DuPont-36495 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>
CA, 8.4.1/07	Lühns, U.	2014b	IN-JT333: Effects on reproduction and growth of the earthworm, <i>Eisenia fetida</i> , in artificial soil, 2014 IBACON DuPont-39714 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>

CA, 8.4.1/08	Pavic, B.	2013	Indoxacarb (DPX-KN128) technical: Effects on reproduction and growth of the earthworm, <i>Eisenia fetida</i> , in artificial soil with 5% peat IBACON DuPont-36101 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>
CA, 8.4.1/09	Shanmugasundaram, R.	2011	IN-KB687: Effects on reproduction and growth of the earthworm, <i>Eisenia fetida</i> , in artificial soil with 5% peat International Institute of Biotechnology and Toxicology (IIBAT) DuPont-31720 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>
CA, 8.4.2.1/01	Luhrs, U.	2005	IN-JU873: Effects on the collembola, <i>Folsomia candida</i> in artificial soil IBACON DuPont-16461 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>
CA, 8.4.2.1/02	Luhrs, U.	2006	IN-MK643: Effects on the collembola, <i>Folsomia candida</i> in	N	Y	The study is necessary for the regulatory decision, conducted	DuPont	<i>Submitted for the purpose of renewal</i>

			artificial soil IBACON DuPont-16462 GLP: Yes Published: No			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.		
CA, 8.4.2.1/0 3	Luhrs, U.	2011	IN-KB687: Effects on the collembola <i>Folsomia candida</i> in artificial soil with 5% peat IBACON DuPont-31721, 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>
CA, 8.4.2.1/0 4	Pavić, B.	2013a	IN-JU873: Effects on the reproduction of the predatory mite <i>Hypoaspis aculeifer</i> in artificial soil with 5% peat IBACON DuPont-35308 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>
CA, 8.4.2.1/0 5	Pavić, B.	2013b	IN-KT413: Effects on reproduction of the predatory mite <i>Hypoaspis aculeifer</i> in artificial soil with 5% peat IBACON DuPont-35306	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>

			GLP: Yes Published: No			previously protected the period of data protection has not expired at the time of submission of this dossier.		
CA, 8.4.2.1/06	Pavić, B.	2013c	Indoxacarb (DPX-KN128) technical: Effects on the collembola <i>Folsomia candida</i> in artificial soil with 5% peat IBACON DuPont-35311 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>
CA, 8.4.2.1/07	Pavić, B.	2013d	Indoxacarb (DPX-KN128) technical: Effects on reproduction of the predatory mite <i>Hypoaspis aculeifer</i> in artificial soil with 5% peat IBACON DuPont-35304 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>
CA, 8.4.2.1/08	Pavić, B.	2013e	IN-JT333: Effects on the Collembola <i>Folsomia candida</i> in artificial soil with 5% peat IBACON DuPont-35312 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	DuPont	<i>Submitted for the purpose of renewal</i>

						the time of submission of this dossier.		
CA, 8.4.2.1/09	Pavić, B.	2013f	IN-JT333: Effects on reproduction of the predatory mite <i>Hypoaspis aculeifer</i> in artificial soil with 5% peat IBACON DuPont-35305 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>
CA, 8.4.2.1/10	Pavić, B.	2013g	IN-KT413: Effects on the collembola <i>Folsomia candida</i> in artificial soil with 5% peat IBACON DuPont-35313 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>
CA, 8.4.2.1/11	Pavić, B.	2013h	IN-KG433: Effects on the Collembola <i>Folsomia candida</i> in artificial soil with 5% peat IBACON DuPont-35314 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>
CA, 8.4.2.1/1	Pavić, B.	2013i	IN-KG433: Effects on	N	Y	The study is necessary for	DuPont	<i>Submitted for the purpose</i>

2			reproduction of the predatory mite <i>Hypoaspis aculeifer</i> in artificial soil with 5% peat IBACON DuPont-35307 GLP: Yes Published: No			the regulatory decision, conducted according to GLP and has not previously been protected or if previously protected the period of data protection has not expired at the time of submission of this dossier.		<i>of renewal</i>
CA, 8.4.2.1/13	Pavić, B.	2013j	IN-MK638: Effects on the collembola <i>Folsomia candida</i> in artificial soil with 5% peat IBACON DuPont-35315 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>
CA, 8.4.2.1/14	Pavić, B.	2013k	IN-MK638: Effects on reproduction of the predatory mite <i>Hypoaspis aculeifer</i> in artificial soil with 5% peat IBACON DuPont-35309 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>
CA, 8.4.2.1/15	Pavić, B.	2013l	IN-MK643: Effects on reproduction of the predatory mite <i>Hypoaspis aculeifer</i> in artificial soil	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has	DuPont	<i>Submitted for the purpose of renewal</i>

			with 5% peat IBACON DuPont-35310 GLP: Yes Published: No			not previously been protected or if previously protected the period of data protection has not expired at the time of submission of this dossier.		
CA, 8.4.2.1/1 6	Pavić, B.	2013m	IN-KB687: Effects on reproduction of the predatory mite <i>Hypoaspis aculeifer</i> in artificial soil with 5% peat IBACON DuPont-35364 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>
CA, 8.5	Carter, J.N.	1997	DPX-MP062 (a racemic mixture of 75% DPX-KN128 and 25% IN- KN127): Effects on soil non-target micro-organisms Huntingdon Life Sciences Ltd. AMR 4134-96 Study submitted in the EU Dossier in 1997 and included in the first EU approval review. Published: No	N	Y N	<u>Comment from RMS:</u> Study from the original submission	DuPont	<i>In DAR (2000)</i>
CA, 8.5	Carter, J.N.	1996	IN-JT333 effects on soil non-target micro- organisms Huntingdon Life Sciences	N	Y N	<u>Comment from RMS:</u> Study from the original submission	DuPont	<i>In DAR (2000)</i>

			Ltd. AMR 3910-96 Study submitted in the EU Dossier in 1997 and included in the first EU approval review. Published: No					
CA, 8.5	Kolzer, U.	2002a	IN-JU873: Assessment of the effects on soil microflora GAB Biotechnologie , GmbH DuPont-10069 Study submitted in the EU Dossier in 2005 and included in the first EU approval review. Published: No	N	¥ N	<u>Comment from RMS:</u> Study from the original submission	DuPont	<i>In addendum to DAR (2005)</i>
CA, 8.5	Kolzer, U.	2002b	IN-MK638: Assessment of the effects on soil microflora GAB Biotechnologie , GmbH DuPont-10071 Study submitted in the EU Dossier in 2005 and included in the first EU approval review. Published: No	N	¥ N	<u>Comment from RMS:</u> Study from the original submission	DuPont	<i>In addendum to DAR (2005)</i>
CA, 8.5	Kolzer, U.	2002c	IN-MK643: Assessment of the effects on soil microflora GAB Biotechnologie , GmbH DuPont-10073 Study submitted in the EU Dossier in 2005 and included in the first EU	N	¥ N	<u>Comment from RMS:</u> Study from the original submission	DuPont	<i>In addendum to DAR (2005)</i>

			approval review. Published: No					
CA, 8.5	Kolzer, U.	2002d	IN-KT413: Assessment of the effects on soil microflora GAB Biotechnologie, GmbH DuPont-11051 Study submitted in the EU Dossier in 2005 and included in the first EU approval review. Published: No	N	✗ N	<u>Comment from RMS:</u> Study from the original submission	DuPont	<i>In addendum to DAR (2005)</i>
CA, 8.5/01	Carter, J.N.	1997	IN-KG433 technical: Effects on soil non-target micro-organisms Huntingdon Life Sciences Ltd. AMR 4436-97 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>
CA, 8.5/02	Feil, N.	2011	IN-KB687: Assessment of the effects on soil microflora IBACON DuPont-31719 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>
CA, 8.8	Lebertz, H.	1999	Indoxacarb: Activated sludge,	N	✗ N	<u>Comment from RMS:</u> Study from	DuPont	<i>In addendum to DAR (2005)</i>

			respiration inhibition test Institut Fresenius Chemische und Biologische/G mbH DuPont-2533 Study submitted in the EU Dossier in 2005 and included in the first EU approval review. Published: No			the original submission		
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