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Oxamyl

Volume 3

B.9 (CA) Ecotoxicology data and assessment of risks for non-target species

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May 2016	First RAR of Italy	
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B.9 ECOTOXICOLOGY DATA A

Introduction

This document contains summaries of ecotoxicology tests completed for oxamyl (DPX-D1410) (the active substance of Oxamyl 10GR and Oxamyl 10SL) and metabolites IN-A2213, IN-D2708, IN-N0079, and IN-T2921. Test summaries cover the individual study types and a discussion of the primary ecotoxicological effects.

The test substance specification can be determined from the test substance code which is a research and development code number given to a specific batch of produced material (either technical or formulated). The test substances/materials used in ecotoxicology tests are listed in Table 1 below:

Table 1 Test item/material specifications

Test substance code	Type	Composition
Annex I inclusion EU approval review		
DPX-D1410-167	Oxamyl PAI (Pure Active Ingredient)	94.6%
DPX-D1410-196	Oxamyl PAI	96.9%, 97.1%, 97.2%
N.B 7577-46, 6689-176-5	Oxamyl PAI	97.1%
IN-A2213-11	IN-A2213 technical metabolite	100.0%
IN-D2708-6	IN-D2708 technical metabolite	99.9%, 99.87%
IN-N0079-8	IN-N0079 technical metabolite	99.6%, 99.57%
IN-T2921-2	IN-T2921 technical metabolite	98.7%
DPX-D1410-377	Oxamyl 10GR	100 g a.s./kg
DPX-D1410-381	Oxamyl 10SL	100 g a.s./L
DPX-D1410-421	Oxamyl 24SL	240 g a.s./L
2015 renewal submission		
DPX-D1410-196	Oxamyl technical	98%
DPX-D1410-490	Oxamyl technical	420 g a.s./L
DPX-D1410-532	Oxamyl technical	99.1%, 98%
IN-A2213-011	IN-A2213 technical metabolite	100%
IN-D2708-007	IN-D2708 technical metabolite	97.2%
IN-N0079-010	IN-N0079 technical metabolite	98.0%
IN-D1410-524	Oxamyl 24SL	240 g a.s./L

Unless specifically indicated, all reports in this section are submitted to address mandatory data requirements for the approval of active substance.

Unless specifically indicated, all tests submitted in this section that involve vertebrate animals address mandatory data requirements that could not be met with alternative methods. Studies were conducted according to prescribed guidelines.

Unless specifically indicated, this section does not contain reports of studies duplicating previous tests on vertebrate animals.

LITERATURE SEARCH:

A literature search was submitted by the Applicant (DuPont-40936_EU_MCA9) that ranged up to 10 years and within 6 months of the submission date. For ecotoxicology, out of the initial 352 records retrieved after all searches of peer-reviewed literature, 316 records excluded from the search results after rapid assessment for relevance. For the remaining potentially relevant 36 records, full-text documents have been assessed in detail, which resulted in total exclusion based on relevance. As consequence, the Applicant has not submitted any literature study.Effects on birds and other terrestrial vertebrates

B.9.1.1 Effects on birds

B.9.1.1.1 Acute oral toxicity to birds

Study submitted in the EU Dossier in 2001 and included in the first EU approval review.

B.9.1.1.1/01

Reference:	**Report:**	[redacted] (2000); Oxamyl technical: An acute oral toxicity study with the northern bobwhite **DuPont Report No.:** DuPont-2954 **Guidelines:** OPPTS 850.2100 (1996) **GLP**: yes
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1. Test material: Pure Oxamyl (PAI)
   Lot/Batch #: D1410-196
   Purity: 96.9%

Materials and methods:

Oxamyl was administered by single dose in water, which was orally intubated directly into the crop or proventriculus of fasted (Phase I: 19 hours & Phase II: 22 hours) northern bobwhite quail (*Colinus virginianus*). Birds were acclimated for 5 weeks for Phase I and 4 weeks for Phase II. Five quail/sex/dose received doses of 0, 0.8, 1.3, 2.2, 3.6, 6.0, or 10 mg a.s./kg bw at a dose volume of 4 ml/kg for Phase I of the study. After completion of Phase I, the highest rate, 10 mg a.s./kg bw, showed only 50% mortality. It was decided to add two additional rates. Phase II of the study consisted of a control and doses of Oxamyl at 16.7 and 27.8 mg a.s./kg bw. Average temperatures during the testing period were 23.0°C in Phase I and 19.5°C in Phase II. Birds were exposed to a 8h:16h photoperiod. Birds were observed for clinical signs of toxicity, body weight effects (on days 3, 7 & 14) and mortality for 14 days after dosing. Food consumption was measured between Days 0–3, 4–7 and 8 – 14. All birds were examined for gross pathological changes.

Findings:

The results are presented in Table 2 & Table 3 below. Treatment related mortalities occurred at 3.6, 6.0, 10, 16.7 and 27.8 mg a.s./kg bw Oxamyl. Clinical signs of toxicity most often observed at all doses above 0.8 mg/kg/bw, included loss of coordination, lower limb weakness, prostrate posture, loss of righting reflex, gaping, ruffled appearance, reduced reaction to stimuli (sound and motion) and lethargy. Other clinical signs of toxicity observed in male and/or females included lacrimation, convulsions, salivation, wing droop and lower limb rigidity. With the exception of 5 dead birds at the 10 mg a.s./kg bw Oxamyl, all surviving quail appeared normal by day 1 or earlier and throughout the remainder of the study. Compared to the Phase I control, there were no test substance-related body weight or food intake effects noted for the 0.8, 1.3, 2.2 or 3.6 mg a.s./kg dosage levels. Treatment related reduction in body weight and food consumption were noted at the 6.0 and 10.0 mg a.s./kg treatment group at the Day 0–3 interval (compared to Phase I control). In the 6.0 mg a.s./kg group there was approx. 8 and 9% reduction in bodyweight in males and females respectively and a decrease in food consumption in females of 76% and in males of 38%. In the 10.0 mg a.s./kg group there was approx. 7 and 9% reduction in bodyweight in males and females respectively and a decrease in food consumption in females of 76% and in males of 52%. When compared with the Phase II control, treatment group 27.8 mg a.s./kg showed treatment related reductions in food consumption (66% ↓ in males and 57% ↓ in females). Due to total mortality at treatment group 16.7 mg a.s./kg, weight and food consumption could not be determined.

Necropsy of birds that had died revealed that all of the birds found dead on dosing day (Day 0) in both Phase I and II had a clear coloured fluid in their crops, assumed to be the dosing solution. In addition, one bird in the 6.0 mg a.s./kg group and four birds in the 10.0 mg a.s./kg group had pale spleens. In the 16.7 mg a.s./kg group, three birds were noted with a clear fluid around the edges of their beaks, one bird was noted with an enlarged spleen,

one bird with a small pale spleen, one bird with brownish fluid in the crop and one bird with clear foamy liquid and feed in the crop. In the 27.8 mg a.s./kg group, one bird was noted with clear fluid around the beak. The necropsy results of all surviving birds were not remarkable. The time to effect following exposure occurred within 30 minutes of dosing in all treatments with the exception of the 0.8 mg a.s./kg treatment group.

Table 2 Summary of toxicological responses of northern bobwhite quail following a single oral dose of Oxamyl

Dose (mg as/kg body wt.)	Sex	Toxicological resultsa	Duration of clinical	Time of death
Control[b]	M	0/0/5	--	--
Control[b]	F	0/0/5	--	--
Phase I				
0.8[f]	M	0/0/5	--	--
0.8[f]	F	0/1*/5	2 days*	--
1.3[f]	M	0/0/5	--	--
1.3[f]	F	0/1/5	1 hour	--
2.2[f]	M	0/3[c]/5	1 day	--
2.2[f]	F	0/4[c]/5	1 day	--
3.6[f]	M	1/4/5	1 day[d]	Day 0
3.6[f]	F	0/5/5	1 day[d]	--
6.0[f]	M	1/4/5	1 day	Day 0
6.0[f]	F	3/2/5	1 day[e]	Day 0
10.0[f]	M	2/3/5	4 days	Day 0 and Day 1
10.0[f]	F	3/2/5	4 days	Day 0 and Day 1
Phase II				
16.7[g]	M	5/0/5	--	Day 0 and Day 1
16.7[g]	F	5/0/5	--	Day 0 and Day 1
27.8[g]	M	4/1/5	1 day	Day 0
27.8[g]	F	3/2/5	1 day	Day 0

[a] number of animals which died/number of animals with clinical signs/number of animals used
[b] numbers for the control are the average between Phase I control and Phase II Control.
[c] report did not differentiate between male and female affected at this particular rate
[d] one bird displayed clinical signs until Day 4, then returned to normal
[E] one hen displayed clinical signs through Day 3, then returned to normal
[f] this rate included in Phase I of the study
[g] this rate included in Phase II of the study
* this effect was not considered treatment-related

Table 3 Acute oral toxicity of Oxamyl to northern bobwhite quail

Test substance	Oxamyl
Test object	Northern bobwhite quail ♂and ♀
LD_{50}	9.5 mg a.s./kg bw
Lowest observed effect level (LOEL)	1.3 mg a.s./kg bw
Lethal Lethal dose (LLD)	3.6 mg a.s./kg bw

Highest tested dose without toxic effect (NOEL)	0.8 mg a.s./kg bw

Conclusion:

The acute oral LD_{50}value for northern bobwhite quail exposed to Oxamyl by single oral dose was calculated to be 9.5 mg a.s./kg bw. Based on signs of toxicity at the 1.3 mg a.s./kg bw dosage level, the NOEL was 0.8 mg a.s./kg bw the lowest dose tested.

RMS comments and conclusion

The acute oral toxicity to birds study DuPont-2954, originally submitted under EU Rev8 Point IIA 8.1.1 and conducted with test material pure oxamyl (PAI), was conducted under guideline OPPTS 850.2100 (1996). A review of this study indicates that it is in line with the current guideline (OPPTS 850.2100 [2012] or OECD 223 [2012]). A higher number of birds/dose was used, which increase the statistical power. Therefore this study is relied upon.

Study submitted in the EU Dossier in 2001 and included in the first EU approval review.

B.9.1.1.1/02

Reference:	**Report:**	
--		[redacted] (1981); Single-dose oral toxicity study in mallard ducks **DuPont Report No.:** HLO 89-81 **Guidelines:** Not given **GLP**: no.

1. Test material: Pure Oxamyl (PAI)
   Lot/Batch #: H-10,963-02
   Purity: 97.1%

Materials and methods:

Oxamyl was administered in 0.5% carboxymethylcellulose by single oral dose to fasted mallards (*Anas platyrhynchos*). Birds were acclimated to test conditions for 14 days. Five mallards/sex/dose received doses of 0, 1.0, 3.16, 10.0, 31.6, and 100 mg a.s./kg bw at a dose volume of 7.5 mL/kg. Average temperatures during the testing period was 7°C. Birds were exposed to a 12:12h photoperiod. Birds were observed for clinical signs of toxicity, body weight effects and mortality for 14 days after dosing (observation period may be longer than 14 days if effects continue to be observed). All birds were examined for gross pathological changes.

Findings:

The results are presented in Table 4 & Table 5. All control birds appeared normal throughout the study. Clinical observations in the treated birds consisted of loss of co- ordination at 1.00 and 3.16 mg a.s./kg, ruffled feathers at 1.00 mg a.s./kg, weakness and slow movement at 3.16 mg a.s./kg, discharge from the eyes and nose at 3.16 and 10.0 mg a.s./kg, and apparent leg paralysis at 3.16, 10.0. 31.6 and 100.0 mg a.s./kg. Within approximately 1 hour post dosing, 2 males and 3 females at the 3.16 mg a.s./kg and all males and females at 10.0, 31.6 and 100.0 mg a.s./kg were found dead. No gross pathological findings of note were observed in birds found dead during the study.

Table 4 Summary of toxicological responses of mallards following a single oral dose of Oxamyl

Dose (mg as/kg body wt.)	Sex	Toxicological results[a]	Duration of clinical signs	Time of death
0	M	0/0/5	--	--
0	F	0/0/5	--	--
1.0	M	0/1[b, c]/5	0-4 days	--
1.0	F	0/1[b]/5	0-4 days	--
3.16	M	2/3[b,c,d,e,f]/5	0-6 days	Day 1
3.16	F	3/2[b,d,e]/5	0-4 days	Day 1
10.0	M	5/4[d,e]/5	0-1 day	Day 1
10.0	F	5/5[d,e]/5	0-1 day	Day 1
31.6	M	5/0/5	--	0 hr
31.6	F	5/1[e]/5	0-1 day	0 hr
100	M	5/0/5	--	0 hr
100	F	5/2[e]/5	0-1 day	0 hr

[a] number of animals which died/number of animals with clinical signs/number of animals used
[b] loss of coordination
[c] feathers ruffled
[d] discharge from eyes and nose
[e] leg paralysis, bird cannot stand
[f] weak

Table 5 Acute oral toxicity of Oxamyl to mallards

Test substance	Oxamyl
Test object	Mallards ♂ and ♀
LD_{50}	3.16 mg a.s./kg bw
Lowest lethal dose (LLD)	3.16 mg a.s./kg bw
Highest tested dose without toxic effect (NOEL)	<1.0 mg a.s./kg bw

Conclusion:

The acute oral LD_{50}value for mallards exposed to Oxamyl by single oral dose was 3.16 mg a.s./kg bw. The no mortality dosage was 1.0 mg a.s./kg bw. The NOEL was ≤1.0 mg a.s./kg bw based on effects at 1.0 mg a.s./kg bw.

RMS comments and conclusion The acute oral toxicity to birds study HLO 89-81, originally submitted under EU Rev8 Point IIA 8.1.1 was conducted with test material pure oxamyl (PAI). Guidelines were according to U.S. EPA Code of Federal Regulations 40 CFR 163.71. A review of this study indicates that it is in line with the current guideline (OPPTS 850.2100 [2012] or OECD 223 [2012]) but no Certificate of Analysis for the test

material. The study was conducted as a non-GLP test. However, the study is considered scientifically valid and a reconduct is unlikely to yield a significantly different result because of a clear dose-response pattern. Therefore, this study is relied upon.

B.9.1.1.2 Short-term dietary toxicity to birds

Study submitted in the EU Dossier in 2001 and included in the first EU approval review.

B.9.1.1.2/01

Reference:	**Report:**	[redacted] (1988a); A dietary LC_{50} study with the bobwhite **DuPont Report No.:** HLO 47-88 **Guidelines:** U.S. EPA 71-2 (1988)
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1. Test material: Pure Oxamyl (PAI)
   Lot/Batch #: D1410-196
   Purity: 97.1%

Materials and methods:

Test substance was dissolved in acetone and corn oil, then blended into diet (final corn oil concentration in test and control diets: 2%). Birds (*Colinus virginianus*) (10 days old) were exposed to Oxamyl in feed over an exposure period of 5 days at nominal concentrations of 0, 39, 78, 156, 313 625, or 1250 mg a.s./kg diet. followed by a 3-day observation period. Ten birds (immature, undifferentiated by sex) were allocated to each dose level, with 4 control groups. The acclimation period was 10 days (from the day of hatching).

All animals were observed for mortality, signs of toxicity and abnormal behaviour once daily pre-treatment and twice daily during the treatment and observation periods. Group average bodyweights were recorded at initiation, then on Days 5 and 8. Average feed consumption was determined for each group Days 0-5 and 6-8. Ambient temperature was 38 ±2°C in the brooding compartment and 20 ±2°C in the test room, relative humidity was 37%. The photoperiod consisted of 16 h light per day during acclimation and throughout the test period.

Findings:

No mortalities or abnormal behaviour were observed in the control group throughout the study period. At the 39 mg a.s./kg concentration, there was no evidence of either clinical toxicity or treatment-related effects. At the 78 and 156 mg a.s./kg concentrations, transient behavioural effects were noted, with recovery occurring before study termination. At the 313 mg a.s./kg concentration, 50% mortality was recorded by the end of the study; the surviving birds showed signs of toxicity which included lethargy, loss of co-ordination, wing droop, depression, reduced reaction to external stimuli, lower limb weakness, salivation and regurgitation, between Day 0 and Day 7, but recovered prior to study termination. At the 625 mg a.s./kg concentration, 90% mortality was recorded by the end of the study; signs of toxicity in the sole surviving bird were apparent until Day 6. At the highest concentration (1250 mg a.s./kg), signs of toxicity were observed from Day 0, with 100% mortality occurring by Day 4. When compared to the controls, there was a concentration related reduction in body weight gain or bodyweight loss at all concentrations tested during the exposure period (Days 0 – 5). At all concentrations above 39 mg a.s./kg, there was a greater than 10% ↓ in average bodyweight compared to the control. Food consumption was reduced during the exposure period to between 39 and 70% of the control in all treatment groups. However, feed consumption increased post exposure to similar levels to the control. There was a corresponding reduction in feed consumption at all concentrations for the same period. The time to effect was observed within a few hours post dosing and continued until day 5 in the surviving birds of all treatment groups. The results are presented in Table 6 & Table 7.

Table 6 Summary of toxicological responses, body weight, and food intake of northern bobwhite chicks following dietary exposure to Oxamyl

Treatment (mg a.s./kg diet)	Toxicological resultsa	Duration of clinical signs	Time of death	Mean body mass (g/bird)			Mean intake (g/bird/day)	
				Day 0	Day 5	Day 8	Days 0-5	Days 6-8
0	0/0/40	--[b] shou	--	17	26	33	13	14
39	0/0/10	--	--	17	25	33	8	12
78	0/3/10	3-5 d	--	16	22	30	7	12
156	0/na[c]/10	1-7 d	--	17	20	28	6	11
313	5/na/10	0-7 d	1-5 d	17	16	24	4	14
625	9/na/10	0-6 d	0-3 d	17	14	20	5	28
1250	10/na/10	0-4 d	0-4 d	17	--	--	--	--

[a] number of animals which died/number of animals with clinical signs/number of animals used
[b] no data (either not applicable or no data due to 100% mortality)
[c] na = no data available in report

Table 7 Short-term dietary toxicity to northern bobwhite quail

Test substance	Oxamyl
Test object	Northern bobwhite quail chicks
LC_{50}	340 mg a.s./kg diet
Lowest lethal concentration (LLC)	313 mg a.s./kg diet
Lowest observed effect concentration (LOEC)	78 mg a.s./kg diet
Highest tested dose without toxic effect (NOEC)	39 mg a.s./kg diet

Conclusion:

The short-term dietary $LC_{50}$for northern bobwhite chicks exposed to Oxamyl in the diet for 5 days was 340 mg a.s./kg diet. The no mortality dosage was 156 mg a.s./kg diet. The short- term dietary NOEC was 39 mg a.s./kg diet based on food consumption and body weight effects in the 78 mg a.s./kg diet group.

RMS comments and conclusion

The short-term dietary toxicity to birds study HLO 47-88, originally submitted under EU Rev8 Point IIA 8.1.2 and conducted with test material pure oxamyl (PAI), was conducted under guideline U.S. EPA 71-2 (1988). A review of this study indicates that it fully meets the current guideline (OECD 205, 1984). Therefore this study is relied upon.

The LC50 has been recalculated to daily dose as 85 mg oxamyl/kg bw/day during in the previous Annex I review based on data in the monograph (see Addendum to Annex B, Ecotoxicology, May 2010). At this dose level and levels below the LC50, distinct food avoidance occurred in the test.

Study submitted in the EU Dossier in 2001 and included in the first EU approval review.

B.9.1.1.2/02

Reference:	Report:	[redacted] (1988b); A dietary LC_{50} study with the mallard **DuPont Report No.:** HLO 48-88 **Guidelines:** U.S. EPA 71-2 (1988)
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1. Test material: Pure Oxamyl (PAI)
   Lot/Batch #: D1410-196
   Purity: 97.1%

Materials and methods:

Test substance was dissolved in acetone and corn oil, then blended into diet (final corn oil concentration in test and control diets: 2%). Mallard ducklings (*Anas platyrhynchos*; 10 birds/dose) (10 days old) were exposed to Oxamyl in feed over an exposure period of 5 days at nominal concentrations of 0, 78, 156, 313, 625, 1250, or 2500 mg a.s./kg diet. followed by a 3-day observation period. Ten birds (immature, undifferentiated by sex) were allocated to each dose level, with 4 control groups. The acclimation period was 9 days (from the day of hatching).

All animals were observed for mortality, signs of toxicity and abnormal behaviour once daily pre-treatment and twice daily during the treatment and observation periods. Group average bodyweights were recorded at initiation, then on Days 5 and 8. Average feed consumption was determined for each group Days 0-5 and 6-8. Ambient temperature was 33 ±3°C in the brooding compartment and 20 ±2°C in the test room, relative humidity was 37%. The photoperiod consisted of 16 h light per day during acclimation and throughout the test period.

Findings:

No mortalities or abnormal behaviour were observed in the control group throughout the study period. At the 78 and 156 mg a.s./kg concentrations, there was no mortality and any signs of toxicity had disappeared by Day 6. At the 313 mg a.s./kg concentration, 10% mortality was recorded by the end of the study; the surviving birds showed signs of toxicity between Days 0 and 7, but recovered prior to study termination. At the 625 and 1250 mg a.s./kg concentrations, respective mortality of 40% and 70% were recorded by the end of the study; signs of toxicity in the surviving birds were apparent until Day 7 and Day 6, respectively. At the highest concentration (2500 mg a.s./kg), signs of toxicity were observed from Day 0, with 100% mortality occurring by Day 6. When compared to the controls, there was a concentration related reduction in body weight gain or bodyweight loss at all concentrations tested during the exposure period (Days 0 – 5). At all concentrations above 78 mg a.s./kg, there was a greater than 20% ↓ in average bodyweight during the exposure period compared to the control, however, all groups displayed an increase in bodyweight post the exposure period. Food consumption was reduced during the exposure period between 34 and 80% compared to the control in all treatment groups. However, feed consumption increased post exposure to levels similar to the control. There was a corresponding reduction in feed consumption at all concentrations for the same period. The time to effect was observed within a few hours post dosing and continued until day 5 in the surviving birds of all treatment groups. The results are presented in Table 8 & Table 9.

Table 8 Summary of toxicological responses, body weight, and food intake of mallard ducklings following dietary exposure to Oxamyl

Treatment (mg a.s./kg diet)	Toxicologic al resultsa	Duration of clinical signs	Time of death	Mean body mass (g/bird)			Mean intake (g/bird/day)	
				Day 0	Day 5	Day 8	Days 0-5	Days 6-8
0	0/0/40	None	--[b]	159	294	399	66	86
78	0/0/10	1-5 d	--	163	238	351	43	83
156	0/na[c]/10	0-5 d	--	174	188	315	30	85
313	1/na/10	0-6 d	5 d	155	135	251	19	77
625	4/na/10	0-7 d	4-5 d	156	111	209	14	71
1250	7/na/10	0-6 d	0-5 d	153	105	217	15	103
2500	10/na/10	0-6 d	0-6 d	152	91	--	7	0[b]

a number of animals which died/number of animals with clinical signs/number of animals used
b no data due to 100% mortality in this treatment group
c na = Not available (not reported in document)

Table 9 Short-term dietary toxicity of Oxamyl to mallard ducklings

Test substance	Oxamyl
Test object	Mallard ducklings
LC_{50}	766 mg a.s./kg bw
Lowest lethal concentration (LLC)	313 mg a.s./kg bw
Lowest observed effect concentration (LOEC)	78 mg a.s./kg bw
Highest tested dose without toxic effect (NOEC)	<78 mg a.s./kg bw

The short-term dietary $LC_{50}$for mallard ducklings exposed to Oxamyl in the diet for 5 days was 766 mg a.s./kg diet. The no mortality dosage was 156 mg a.s./kg diet. The short-term dietary NOEC was <78 mg a.s./kg diet based on effects on bodyweight (19%□)in the 78 mg a.s./kg diet group.

RMS comments and conclusion

The short-term dietary toxicity to birds study HLO 48-88, originally submitted under EU Rev8 Point IIA 8.1.2 and conducted with test material pure oxamyl (PAI), was conducted under guideline U.S. EPA 71-2 (1988). A review of this study indicates that it fully meets the current guideline (OECD 205, 1984). Therefore this study is relied upon.

The LC50 has been recalculated to daily dose as 96.6 mg oxamyl/kg bw/day. during in the previous Annex I review (see Addendum to Annex B, Ecotoxicology, May 2010).

B.9.1.1.3 Sub-chronic toxicity and reproductive to birds

Study submitted in the EU Dossier in 2001 and included in the first EU approval review.

B.9.1.1.3/01

Reference:	**Report:**	[redacted] (1982b); The effects of dietary inclusion of oxamyl on reproduction in the bobwhite quail **DuPont Report No.:** HLO 453-82 **Guidelines:** U.S. EPA 163.71-4 (1978)
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1. Test material: Pure Oxamyl (PAI)
   Lot/Batch #: N.B 7577-46, 6689-176-5
   Purity: 97.1%

Materials and methods:

Groups of young adult bobwhite quail (*Colinus virginianus*), were offered diet containing technical Oxamyl (purity 97.1%; corn-oil at a final concentration of 0.1% was used to aid dispersion of test material) at concentrations of 0, 10 or 50 mg a.s./kg diet for 28 weeks (sixteen weeks prior to the start of egg production and twelve weeks during egg production). Analysis of the diet revealed that concentration of test material was >90% of the nominal at weeks 1,3,5 and >85% of nominal at weeks 9,14 17, 21 amd 25. Each treatment group comprised 14 replicates of one male and two females. Starting on Week 17, and weekly thereafter, eggs were incubated to hatching and chicks observed over a 14-day period. The test incubation conditions were: 7 hours light, 17 hours dark until Day 46; 8 hours light, 16 hours darkness until Day 53; 9 hours light, 15 hours darkness until Day 67; 12 hours light, 12 hours darkness until Day 74; 13 hours light, 11 hours darkness until Day 81 and then 14 hours light, 10 hours darkness until study termination; a mean maximum temperature of 19°C (range 11-25°C), and a mean minimum temperature of 14°C (range 8-22°C); mean relative humidity 67% (range 48-85%).

Eggs were collected daily and the number recorded during the 12-week egg production period. Eggs were stored at room temperature during a 7-day period, candled and any broken or cracked eggs recorded and discarded. The remaining eggs (except those used for shell thickness measurement) were incubated at 37.7°C, 63% relative humidity. Eggs were turned automatically every 45 minutes. Eggs were candled on Day 11 and Day 18 of the incubation period and all infertile eggs and eggs showing embryonic death were recorded and discarded. After 20 days incubation, the eggs were transferred to a hatcher at 37.5°C.

All hatched chicks were individually weighed, tagged and transferred to wooden floor pens within 24 hours of hatching. Food and water were provided ad libitum.

Individual adult body weights were recorded on Days 0, 14, 28, 42, 56, 70, 84 and 196; food consumption in each replicate was measured weekly throughout the pre-treatment and treatment periods of the adult phase. Individual chick body weights were recorded within 24 hours of hatching and on Day 14 after hatching; egg shell thickness was measured on the first two days of Weeks 1, 3, 5, 7, 9 and 11 of the egg production period. Adult birds and chicks were observed daily for mortality and clinical signs; all adult birds were subjected to post mortem examination.

Parental Findings:

Four birds died or were sacrificed (two in the control and two in the 50 mg a.s./kg diet treatment group) during the pre-egg production period; all were replaced with spare birds. There was no treatment-related effect on adult mortality; clinical observations and post mortem changes were consistent with long-term housing of birds in cages, and were not treatment-related. Adult body weight changes over the treatment period were similar in all groups and there was no evidence of any statistically significant treatment-related effect. Adult food consumption was significantly lower in the two Oxamyl treated groups compared to the control during both pre-

egg production and egg production periods ($p<0.05$; Williams Test). When the two treatment groups were compared to the control over the full study period, food consumption was statistically significantly different also ($p<0.01$; Williams Test).

Table 10 Bodyweight and food consumption of bobwhite quail exposed to oxamyl.

Treatment (ppm)	Day of Study								
Bodyweight (g)									
	0	**14**	**28**	**42**	**56**	**70**	**84**	**196**	**Mean ± SD**
Control	200	202	211	208	209	202	205	208	206 ± 4
Oxamyl 10	200	204	211	209	211	204	207	213	207 ± 4
Oxamyl 50	201	202	210	207	210	203	206	212	206 ± 4
Food Consumption (g/bird/day)									
Control	18	19	18	17	17	14	21	22	18 ± 3
Oxamyl 10	17	18	17	15	17	13	19	22	17 ± 3
Oxamyl 50	16	18	18	16	17	14	20	22	18 ± 3

Table 11 Parental Findings

Test Substance	TG a.i.
Test Object	Bobwhite Quail
Exposure	28 weeks
Lowest tested concentration with effect (LOEC) mg a.i./kg feed	10
Highest concentration without toxic effect (NOEC) mg a.i./kg feed	<10

Reproductive Findings:

Initial chick body weights were similar overall in all treatment groups; chicks that died after hatching during the 14-day observation period were examined and no abnormalities were detected. The results for the main reproduction parameters are presented in Table 12.

Table 12 Effects of dietary administration of Oxamyl on a range of reproductive parameters in Bobwhite Quails.

Endpoints	Treatment Groups (mg Oxamyl/kg)		
	Control	**10 mg a.i./kg diet**	**50 mg a.i./kg diet**
Eggs laid	1094	1121	1233
Eggs damaged	299	162	269
% damaged eggs laid	28	14.6	22.4
Eggs set	631	769	769
Viable embryos	533	648	657
Hatchlings	414	550	541
14-day old survivors	334	437	425
Eggs laid/female	42	44	47
Egg shell thickness (mm)	0.19	0.20	0.20

Of the reproductive parameters measured, differences were detected between the control and Oxamyl treatments. The proportion of damaged eggs was approx. 45.8% lower in the 10 mg a.s./kg diet treated group compared to the control, however this was not the case in the highest treatment group where there was a reduction of only 10% compared to the control. There was an increase in the number of eggs set by 21% in both of the treatment groups when compared to the control. The proportion of chicks surviving to 14 days was significantly higher

(30%) in the 10 mg a.s./kg diet treatment and 27% higher in the 50 mg a.s./kg diet compared to the control (Williams test, P<0.05). It is not clear from the results obtained whether oxamyl has hormetic effects on reproductive parameters measured in the bobwhite quail. In some case, such as the number of eggs laid and the number of eggs set, these parameters were significantly higher in the oxamyl treatment groups compared to the control group.

Conclusion:

The reproductive NOEC for bobwhite quail exposed to Oxamyl in the diet for 28 weeks was >50 mg a.s./kg diet, based on absence of reproductive effects observed at this dose level.

RMS comments and conclusion

The sub-chronic toxicity and reproductive to birds study HLO 453-82, originally submitted under EU Rev8 Point IIA 8.1.3 and conducted with test material pure oxamyl (PAI), was conducted under guideline U.S. EPA 163.71-4 (1978). A review of this study indicates that it is in line with the current guideline (OECD 206, 1984).

This study is relied upon.

The reproductive 28weeks NOEC is ≥ 50 mg a.s./kg diet.

The 28weeks NOEC has been recalculated to daily dose as 4.36 mg a.s./kg bw/day during the previous Annex I review (see Addendum to Annex B, Ecotoxicology, May 2010). Due to the test design the EC10/20 cannot be calculated.

Study submitted in the EU Dossier in 2001 and included in the first EU approval review.

B.9.1.1.3/02

Reference:	**Report:**	[redacted] (1982a); The effects of dietary inclusion of oxamyl on reproduction in the mallard duck **DuPont Report No.:** HLO 337-82 **Guidelines:** U.S. EPA 163.71-4 (1978)
--		

1. Test material: Pure Oxamyl (PAI)
   Lot/Batch #: N.B 7577-46, 6689-176-5
   Purity: 97.1%

Materials and methods:

Groups of young adult mallard ducks (*Anas platyrhynchos*), were offered diet containing technical Oxamyl (purity 97.1%; corn-oil at a final concentration of 0.1% was used to aid dispersion of test material) at concentrations of 0, 10 or 50 mg a.s./kg diet for 22 weeks (ten weeks prior to the start of egg production and during twelve weeks of egg production). Analysis of the diet revealed that concentration of test material was >90% of the nominal at weeks 1,3,5 and >85% of nominal at weeks 9,14 17, and 21. Each treatment group comprised six replicates of two male and five females. Starting from the beginning of Week 11, and weekly thereafter, eggs were incubated to hatching and chicks observed over a 14-day period. The test incubation conditions were: 7 hours light, 17 hours darkness until Day 44; 8 hours light, 16 hours darkness until Day 51; 9 hours light, 15 hours darkness until Day 65; 12 hours light, 12 hours darkness until Day 72; 13 hours light, 11 hours darkness until Day 79 and then 14 hours light, 10 hours darkness until study termination; a mean maximum temperature of 18°C (range 12-24°C), and a mean minimum temperature of 14°C (range 8-20°C); mean relative humidity 67% (range 49-85%).

Eggs were collected daily and the number recorded during the 12-week egg production period. Eggs were stored at room temperature during a 7-day period, candled and any broken or cracked eggs recorded and discarded. The remaining eggs (except those used for shell thickness measurement) were incubated at 37.7°C, 64% relative humidity. The eggs were turned automatically every 45 minutes. Eggs were candled on Days 14 and 21 of the incubation period and all infertile eggs and eggs showing embryonic death were recorded and discarded. On Day 23 of the incubation period, the eggs were transferred to a hatcher at 37.5°C.

All hatched ducklings were individually weighed, tagged and transferred to wooden floor pens within 24 hours of hatching. Food and water were provided ad libitum.

Individual adult body weights were recorded on Days 0, 14, 28, 42, 56 and 154; food consumption in each replicate was measured weekly throughout the pre-treatment and treatment periods of the adult phase. Individual chick body weights were recorded within 24 hours of hatching and on Day 14 after hatching; egg shell thickness was measured on the first two days of Weeks 1, 3, 5, 7, 9 and 11 of the egg production period. Adult birds and chicks were observed daily for mortality and clinical signs; all adult birds were subjected to post mortem examination.

Parental Findings:

Seven birds died or were sacrificed during the pre-egg production period mainly as a result of bullying (two in the 10 mg a.s./kg diet treatment and five in the 50 mg a.s./kg diet treatment), all of which were replaced with spare birds. During the egg production period, six birds died in the control, two birds died in the 10 mg a.s./kg diet treatment and nine birds died in the 50 mg a.s./kg diet treatment, of which one was sacrificed. Six birds died in the Control group (14%) however, it was reported by the notifier that one bird in this group displayed signs of being bullied. Also, in examination of the eggshell thickness of control birds, it is apparent that the eggshell thickness was lower, than that quoted in the USEPA Guidelines state which states that the eggshell thickness of eggs produced by control birds should be 0.34mm. However, there is only a 3% difference in thickness which could be attributed to error in measurements taken. When individual measurements were evaluated the Standard Error was in the region of 0.03mm. There were no treatment-related effects on adult mortality, food consumption or body weight Table 13.

Table 13 Bodyweight and food consumption of the mallard duck exposed to oxamyl

Treatment (ppm)	Day of Study						
Bodyweight (g/bird)							
	0	**14**	**28**	**42**	**56**	**154**	**Mean ±SD**
Control	1000	1037	1071	1089	1079	1166	1074 ± 56
Oxamyl 10	1008	1063	1088	1102	1075	1121	1076 ± 39
Oxamyl 50	1017	1000	1047	1082	1065	1064	1046 ± 31
Food Consumption (g/bird/day)							
Control	144	156	165	150	159	240	169 ± 36
Oxamyl 10	140	164	160	147	140	213	161 ± 28
Oxamyl 50	79	141	153	137	135	248	149 ± 55

Table 14 Parental Findings

Test Substance	TG a.i.
Test Object	Mallard Duck
Exposure	22 weeks
Lowest tested concentration with effect (LOEC) mg a.i./kg feed	10
Highest concentration without toxic effect (NOEC) mg a.i./kg feed	<10

Initial duckling body weights were similar in all treatment groups and statistical analysis of the results showed that the only significant difference was in Week 4, when body weights in the 50 mg a.s./kg diet treatment group were higher than the control (Williams test, P<0.05). Ducklings that died during the 14-day observation period after hatching were examined and no abnormalities were detected. The results for the main reproduction parameters are presented in Table 15 – Table Table 19.

Table 15 Reproduction toxicity of Oxamyl to mallard ducks: Number of eggs laid

Dose	Week of egg production												Total
(mg a.s./kg diet)	1	2	3	4	5	6	7	8	9	10	11	12	
Number of eggs laid													
0	78	110	159	182	169	170	173	162	151	125	86	92	1657
10	92	141	159	154	158	159	147	130	125	137	102	87	1591
50	76	100	126	140	125	134	143	100	95	81	45	54	1219
Number of eggs laid per female													
0	2.6	3.7	5.3	6.1	5.6	5.7	5.8	5.4	5.0	4.2	3.4	3.8	56.6
10	3.1	4.7	5.3	5.1	5.3	5.3	4.9	4.3	4.2	4.7	3.6	3.1	53.6
50	2.6	3.4	4.4	5.0	4.5	4.9	5.3	3.7	3.5	3.0	1.8	2.2	44.3

Table 16 Reproduction toxicity of Oxamyl to mallard ducks: Group mean egg shell thickness (mm)

Dose	Week of production					
(mg a.s./kg diet)	1	3	5	7	9	11
0	0.33	0.34	0.34	0.33	0.33	0.32
10	0.33	0.33	0.34	0.33	0.33	0.33
50	0.33	0.32	0.33	0.32	0.32	0.32

Table 17 Reproduction toxicity of Oxamyl to mallard ducks: Damaged eggs

Dose	Week of egg production												Total
(mg a.s./kg	1	2	3	4	5	6	7	8	9	10	11	12	
Number of eggs cracked or broken													
0	3	3	12	10	11	17	7	4	8	3	2	6	86
10	3	9	12	13	12	15	6	13	4	9	3	2	101
50	2	7	5	10	8	0	8	7	5	2	1	1	56
% damaged of eggs laid													
0	3.8	2.7	7.5	5.5	6.5	10.0	4.0	2.5	5.3	2.4	2.3	6.5	5.2
10	3.3	6.4	7.5	8.4	7.6	9.4	4.1	10.0	3.2	6.6	2.9	2.3	6.3
50	2.6	7.0	4.0	7.1	6.4	0	5.6	7.0	5.3	2.5	2.2	1.9	4.6

Table 18 Reproduction toxicity of Oxamyl to mallard ducks: Percentages of infertile eggs, and early and late embryonic deaths

Parameter	Control	10 mg a.s./kg diet	50 mg a.s./kg diet
No. of eggs set	1342	1283	1013
% of infertile eggs	7.0	9.7	18.3
% early embryonic deaths of total fertile eggs	4.5	5.5	4.3
% late embryonic deaths of total eggs set after Day 14	4.9	5.9	5.7

Table 19 Reproduction toxicity of Oxamyl to mallard duck: Duckling hatching, mean body weights and survival results

Parameter	Control	10 mg a.s./kg diet	50 mg a.s./kg diet
Total no. of fertile eggs	1248	1158	828
No. Hatched	684	593	417
% Hatched	54.8	51.2	50.4
Mean initial body weights (g)	35	37	35
No of ducklings surviving at 14 days	624	561	385
% Survival at 14 days	91.2	94.6	92.3
Mean 14-day body weights (g)	191	191	185

There were no statistically significant effects of treatment on the proportion of eggs cracked, mean egg weight, embryonic deaths, hatchability of fertile eggs, or survival of ducklings to 14 days (Williams test, $p>0.05$). However, in the 50 mg a.s./kg diet treatment group, statistically significant reductions compared to the control were recorded on the numbers of eggs laid, total egg mass and numbers of infertile eggs produced (Williams test, $p<0.05$).

Conclusion:

The NOEC for mallard ducks exposed to Oxamyl in the diet for 22 weeks was 10 mg a.s./kg diet, based on egg production and egg viability effects observed at the highest dose level tested (50 mg a.s./kg diet).

RMS comments and conclusion

The sub-chronic toxicity and reproductive to birds study HLO 337-82, originally submitted under EU Rev8 Point IIA 8.1.3 and conducted with test material pure oxamyl (PAI), was conducted under guideline U.S. EPA 163.71-4 (1978). A review of this study indicates that it is in line with the current guideline (OECD 206, 1984). The study is relied upon.

B.9.1.1.4 The **22weeks NOEC has been recalculated to daily dose as 1.5 mg a.s./kg bw/day during the previous Annex I review (see Addendum to Annex B, Ecotoxicology, May 2010). Due to the test design the EC10/20 cannot be calculated.Summary and conclusion**

A summary of the avian toxicity testing values obtained with oxamyl is presented in Table 20.

Table 20 Summary of avian toxicity endpoints for oxamyl

Test	Species	Measurement endpoint	Endpoint value	Reference[a]
Single oral dose	Bobwhite quail	LD_{50}	9.5 mg/kg bw	DuPont-2954
Single oral dose	Mallard duck	LD_{50}	3.16 mg/kg bw	HLO 89-81
Short-term dietary (5 day)	Bobwhite quail	LC_{50}	85 mg/kg bw (340 ppm in feed)	HLO 47-88
Short-term dietary (5 day)	Mallard duck	LC_{50}	96.6 mg/kg bw (766 ppm in feed)	HLO 48-88
Reproduction (21 week)	Bobwhite quail	NOEC	4.36 mg a.s./kg bw/day (50 ppm in feed)	HLO 453-82
Reproduction (21 week)	Mallard duck	NOEC	1.5 mg a.s./kg bw/day (10 ppm in feed)	HLO 337-82

[a] All studies cited or summarised in this document.

B.9.1.2 Effects on terrestrial vertebrates other than birds

B.9.1.2.1 Acute oral toxicity to mammals

A study summary for the acute oral toxicity study to rat may be found in chapter B.6 and the Oxamyl EU Renewal Dossier, Document M-CA, Section 5, DuPont-40932 EU, Point CA 5.2.1.

B.9.1.2.2 Long-term and reproductive toxicity to mammals

A study summary for the rat 2-generation reproduction study may be found in chapter B.6 and the Oxamyl EU Renewal Dossier, Document M-CA, Section 5, DuPont-40932 EU, Point CA 5.6.1.

B.9.1.2.3 Summary and conclusion

A summary of the avian toxicity testing values obtained with oxamyl is presented in Table 21.

Table 21 Summary of mammal toxicity endpoints for oxamyl

Test	Measurement endpoint	Norwegian rat	Reference[a]
Single oral dose	LD_{50}	2.5 mg/kg bw	DuPont-26931
Reproduction (2 generation)	NOEC	1.43 mg a.s./kg bw/day (25 mg a.s./kg feed)	HLR 423-90

[a] All studies cited or summarised in the Oxamyl EU Renewal Dossier, Document M-CA, Section 5, DuPont-40932 EU.

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

The log K_{ow} of oxamyl is -0.44, indicating no potential for bioconcentration in prey of birds and mammals. A study of bioconcentration in earthworms was conducted (DuPont-38477) and is summarised in Point **Errore. L'origine riferimento non è stata trovata.** in this document. The worst case, measured BCF was 0.03, which documented a lack of bioconcentration of oxamyl active substance in earthworms.

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

Oxamyl is a carbamate insecticide with a reversible cholinesterase inhibition mode of action. Acute single-dose exposures in oral toxicity tests to birds, mammals, and other terrestrial vertebrate wildlife elicit rapid onset of

toxic responses with steep dose-response slopes and low LD_{50} values. Short-term dietary exposures to birds and mammals elicit moderate toxic responses with low dose-response slopes and moderate LC_{50} values. Long-term dietary exposures to birds and mammals elicit reproductive NOECs at doses that are toxic to parents. Birds and mammals are able to metabolize oxamyl after dietary exposures, thus reducing any toxic effects. Similar responses are anticipated for reptiles and amphibians.

B.9.1.5 Potential for endocrine disruption

Oxamyl does not have any endocrine effects on birds, mammals, and amphibians, as documented in Points B.9.1.2 and B.9.2.3 of this document (DuPont-40935 EU) and in the Oxamyl EU Renewal Dossier, Document M-CA, Section 5, DuPont-40932 EU, Point CA 5.6. Oxamyl is not anticipated to have endocrine effects in reptiles.

Annex II, 3.6.5 of Regulation (EC) 1107/2009 provides the framework criteria for approval of an active substance related to endocrine disruption and highlights that the following interim criteria shall be used until the final criteria are in place:

- Active substances that are or have to be classified as category 2 for carcinogenicity ('C-2') and reproductive toxicity ('R-2') shall be considered to have endocrine disrupting properties, and

- Active substances that are or have to be classified as category 2 for reproductive toxicity and which have toxic effects on the endocrine organs may be considered to have endocrine disrupting properties.

Oxamyl is not classified nor is expected to be classified as R2 or C2 for mammals, thus it does not qualify to be suspected to have endocrine properties.

However, a study with vertebrate wildlife (frogs) was conducted with oxamyl active ingredient at the request of the United States Environmental Protection Agency to assist in development of the U.S. EPA Endocrine Disruption Screening and Testing Program and is summarized in Point B.9.2.3 of this document (DuPont-40935 EU). No endocrine effects were documented on the amphibian thyroid in this study.

B.9.2 Effects on aquatic organisms

B.9.2.1 Acute toxicity to fish

Active substance

Study submitted in the EU Dossier in 2001 and included in the first EU approval review.

B.9.2.1/01

Reference:	**Report:**	[redacted] (2000a); Oxamyl technical: Static, acute, 96-hour, (LC_{50}) test to rainbow trout, *Oncorhynchus mykiss* **DuPont Report No.:** DuPont-2907 **Guidelines:** EEC Method C.1. (1992), U.S. EPA 72-1 (1988), OECD 203 (1992)
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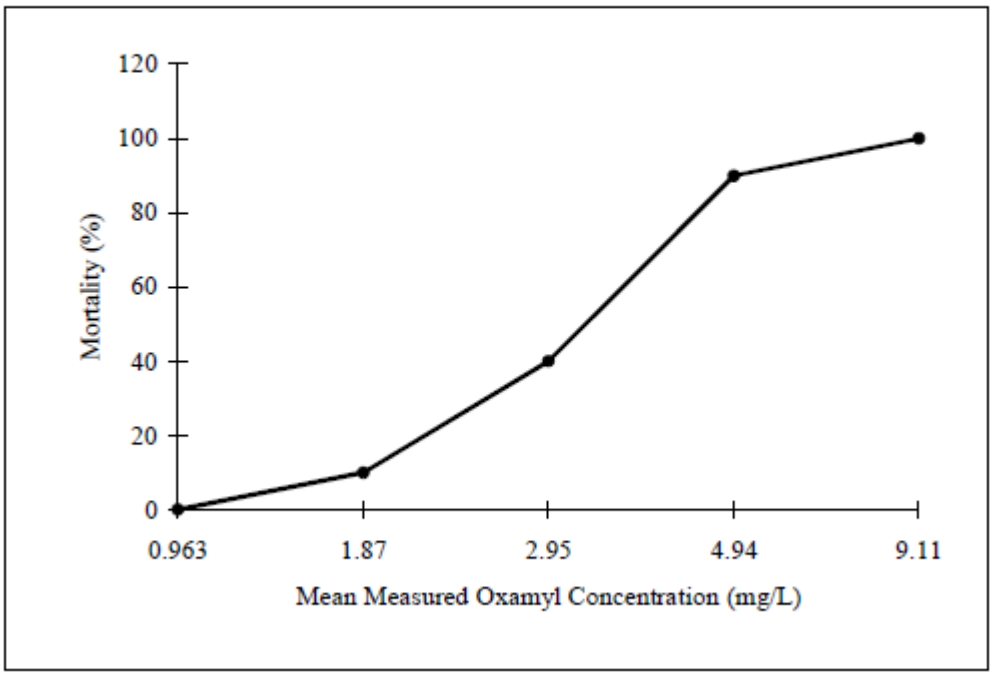
1. Test material: Pure Oxamyl (PAI)
   Lot/Batch #: D1410-196
   Purity: 96.9%

Materials and methods:

The acute toxicity of Oxamyl to unfed fingerling rainbow trout, *Oncorhynchus mykiss*, was determined in an unaerated, static, 96-hour test. Treatments consisted of a dilution water control and nominal concentrations of 1.3, 2.2, 2.6, 3.3, 6.0, and 10 mg a.s./l (mean measured concentrations were 0.0, 0.963, 1.87, 2.95, 4.94 and 9.11). Two replicates containing 5 fish were exposed to each treatment concentration and control. Test chambers were glass aquaria which held 15 L of test solution. Test solutions were maintained between 11.5 and 12.7°C maintained with recirculating water bath. In addition, a continuously-recording thermometer was used to check for temperature variation in one control replicate. Fish in the Control ranged from 3.27 – 3.77cm in total length (mean=3.46cm) and 0.31 – 0.47g in wet weight, blotted dry (mean=0.38g) at test termination. Loading in the water control was 0.13g/l at test termination. Water temperature, pH, dissolved oxygen and conductivity were measured in all replicates of the control and the test concentrations. Measurements were taken at test initiation and every 24 hours thereafter. Total alkalinity and EDTA hardness of the water control was measured at the beginning of the test in the control. A photoperiod of 16 hours light and 8 hours dark was employed during the test. Mortality and behavioural observations were made every 24 hours. Dead fish were removed from test chambers when observed. Analysis of test solutions were made by HPLC equipped with UV. The Binomial method(8) was used to calculate 24 and 48 hour LC50s and 95% confidence limits, and the probit method was used to calculate the 72 and 96 hour LC50s and 95% confidence limits.

Findings:

Mortality of Rainbow Trout, *Oncorhynchus mykiss*, at the Conclusion of an Unaerated Static, Acute, 96-Hour, (LC_{50}) Test with Oxamyl Technical



A summary of cumulative mortality and sublethal effects is presented in Table 22. No mortality occurred in the control. The highest concentration causing no mortality was 0.963 mg/L and the lowest concentration causing 100% mortality was 9.11 mg/L. After 72 hours exposure to 0.963 mg oxamyl/l, one fish exhibited one or more of the following effects – lethargy; loss of equilibrium; change in condition or misshapen body. However, after a further 24 hours, all effects had disappeared. Total mortality occurred at 9.11 mg/L by 24 hours.

Table 22 Summary of mortality and sublethal effects of Oxamyl on Rainbow Trout, Oncorhynchus mykiss, exposed for 96 hours in an unaerated, static, acute test

Test substance	TG a.i.
Test object	Rainbow trout
Exposure	96 h, static
LC_{50} mg a.i./l	3.13 (95% CI: 2.49 – 3.98)
Lowest observed effect concentration (LOEC) mg a.i./l	1.87
Highest tested conc. without toxic effect (NOEC) mg a.i./l	0.963

Observations:

Mean measured concentrations were 74% to 91% of the nominal values after correction for test substance purity of 96.9%. The results reported are based on mean measured concentrations. During the test, water temperature was in the range 11.5 – 12.7°C (mean=12.3°C), dissolved oxygen ranged from 8.0 to 9.3 mg/l (mean=8.7mg/l) and pH was between 7.4 and 8.0. The value for total hardness and conductivity of the dilution water at the start of the test were 44 mg/l as CaCO3 and 150 – 160 µmhos/cm respectively. The $LC_{50}$and NOEC values for rainbow trout are detailed in Table 22.

Conclusion:

The 96-hour $LC_{50}$for Oxamyl to the rainbow trout was 3.13 mg a.s./L, based on mean measured concentrations. Therefore, oxamyl is toxic to the rainbow trout.

RMS comments and conclusion The acute toxicity to fish study DuPont-2907, originally submitted under EU Rev8 Point IIA 8.2.1 and conducted with test material pure oxamyl (PAI), was conducted under guidelines EEC Method C.1. (1992), U.S. EPA 72-1 (1988), and OECD 203 (1992). A review of this study against the current OECD 203 (1992) indicates that the validity criteria regarding control mortality, constant conditions, DO concentration were fulfilled. Since the concentrations of the test substance in the test solutions were not satisfactorily maintained, the results are based on mean measured concentrations.

The following deviations from OECD 203 (1992) are noted:

Temperature range was 11.5 – 12.7°C , i.e. slightly lower then the recommended 13-17°C;
The fish length was 3.27 – 3.77cm, i.e. shorter than the recommended 5.0 ±1.0 cm.
Fish were starved for 2 d prior the test instead of 1d.

Conclusion: the noted deviations are not considered severe, hence also taking into account issues of animal welfare, the study is judged acceptable.

Study submitted in the EU Dossier in 2001 and included in the first EU approval review.

B.9.2.1/02

Reference: --	**Report:**	[redacted] (2000b); Oxamyl technical: Static-renewal, acute, 96-hour, (LC_{50}) test to bluegill sunfish, *Lepomis macrochirus* **DuPont Report No.:** DuPont-2908 **Guidelines:** EEC Method C.1. (1992), OECD 203 (1992), U.S. EPA 72-1 (1988)

1. Test material: Pure Oxamyl (PAI)
   Lot/Batch #: D1410-196
   Purity: 96.9%

Materials and methods:

The acute toxicity of Oxamyl to unfed fingerling bluegill sunfish, *Lepomis macrochirus*, was determined in an unaerated, static-renewal, 96-hour test. Fish were purchased and acclimated for 89 d at 22±2°C Treatments consisted of a dilution water control, and nominal concentrations of 1.3, 2.3, 3.7, 6.2, and 10.3 mg a.s./L. Two replicates containing 5 fish were exposed to each treatment concentration and control. Test chambers were glass aquaria which held 10 L of test solution. Test solutions were maintained between 21.1 and 22.3 °C maintained with recirculating water bath. In addition, a continuously-recording thermometer was used to check for temperature variation in one control replicate. At the end of the study, weight of fish ranged between 0.25 to 0.47g wet weight, total length of fish was 2.90 to 3.5cm (mean=3.24) and fish loading was 0.19g/l at test conclusion. Water temperature, pH, dissolved oxygen and conductivity were measured in all replicates of the control and the test concentrations. Measurements were taken at test initiation and every 24 hours thereafter. Total alkalinity and EDTA hardness of the water control was measured at the beginning of the test in the control. A photoperiod of 16 hours light and 8 hours dark was employed during the test. Mortality and behavioural observations were made every 24 hours. Dead fish were removed from test chambers when observed. Analysis of test solutions were made by HPLC.The probit method was used to calculate the LC50s and 95% confidence limits.

Findings:

Mortality of Bluegill Sunfish, *Lepomis macrochirus*, in an Unaerated Static-Renewal, Acute, 96-Hour, (LC_{50}) Test with Oxamyl Technical

Mean, Measured Oxamyl Concentration (mg/L)	Cumulative Mortality (%)				
	0 Hours	24 Hours	48 Hours	72 Hours	96 Hours
Water Control A†	0	0	0	0	0
Water Control B†	0	0	0	0	0
1.24 A†	0	0	0	0	0
1.24 B†	0	0	0	0	0
2.12 A†	0	0	0	0	0
2.12 B†	0	0	0	0	0
3.52 A†	0	0	0	0	0
3.52 B†	0	0	0	0	0
5.80 A†	0	0	0	40	60
5.80 B†	0	20	40	40	40
9.27 A†	0	60	80	80	80
9.27 B†	0	60	80	80	100

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

A summary of cumulative mortality and sublethal effects is presented in Table 23. Mean, measured concentrations of Oxamyl were 1.24, 2.12, 3.52, 5.80, and 9.27 mg a.s./L and ranged from 93 to 98% of nominal concentrations. There were no mortality or sublethal effects below 5.80 mg/L and in the control. There was 0% mortality at 96 hours in test vessels containing 1.24, 2.12, and 3.52 mg a.s./L, 50% mortality in test vessels containing 5.80 mg a.s./L and 90% mortality in test vessels containing 9.27 mg a.s./L. Several fish exposed to 5.80 and 9.27 mg a.s./L exhibited signs of lethargy at 24, 72 and 96 hours. No other sublethal effects were

observed at any tested concentration during the definitive toxicity test. Time to first observed mortality was 24 hours.

Table 23 Summary of mortality and sublethal effects of Oxamyl on bluegill sunfish, *Lepomis macrochirus*, exposed for 96 hours in an unaerated, static-renewal, acute test

Test substance	TG a.i.
Test object	Bluegill Sunfish
Exposure	96 h, static
LC_{50} mg a.i./l	6.12 (95% CI: 4.97 – 7.52)
Lowest observed effect concentration (LOEC) mg a.i./l	5.8
Highest tested conc. without toxic effect (NOEC) mg a.i./l	3.52

Observations:

The results reported are based on mean measured concentrations. During the test, water temperature was in the range 21.1 – 22.3°C (mean=21.9°C), dissolved oxygen ranged from 7.8 to 8.9 mg/l (mean=8.5mg/l) and pH was between 7.3 and 7.8. The value for EDTAhardness and conductivity of the dilution water at the start of the test were 44 mg/l as CaCO3 and 130 – 160 µmhos/cm respectively.

Conclusion:

The 96-hour $LC_{50}$for Oxamyl to bluegill sunfish was 6.12 mg a.s./L (based on mean measured concentrations).

RMS comments and conclusion

The acute toxicity to fish study DuPont-2907, originally submitted under EU Rev8 Point IIA 8.2.1 and conducted with test material pure oxamyl (PAI), was conducted under guidelines EEC Method C.1. (1992), U.S. EPA 72-1 (1988), and OECD 203 (1992). A review of this study against the current OECD 203 (1992) indicates that the validity criteria regarding control mortality, constant conditions, DO concentration were fulfilled.

The following deviations from OECD 203 (1992) are noted:

The fish length was 2.90 to 3.5cm, i.e. slightly longer than the recommended 2.0 ±1.0 cm.
Fish were starved for 2 d prior the test instead of 1d.

Conclusion: the noted deviations are not considered severe, hence, also taking into account issues of animal welfare, the study is judged acceptable.

Metabolites

Study submitted in the EU Dossier in 2001 and included in the first EU approval review.

B.9.2.1/03

Reference: --	**Report:**	[redacted] (1999a); IN-A2213: Static, acute, 96-hour limit test to rainbow trout, *Oncorhynchus mykiss* **DuPont Report No.:** DuPont-2500 **Guidelines:** OECD 203 (1992), EEC Method C.1. (1992), U.S. EPA 72-1 (1988)

1. Test material: IN-A2213 technical metabolite
   Lot/Batch #: A2213-11
   Purity: 100%

Materials and methods:

The acute toxicity of IN-A2213 to unfed fingerling rainbow trout, *Oncorhynchus mykiss*, was determined in an unaerated, static, 96-hour test. Fish were acclimated for 17d after purchase. Treatments consisted of a dilution water control and a single nominal concentration of 130 mg/L. Three replicates, each containing 10 fish, were exposed to the treatment concentration and control. Test chambers were glass aquaria which held 15 L of test solution. Test solutions were maintained between 11.9 and 12.6°C. The mean temperature during the study was 12.2°C. Fish in the water control ranged from 3.10 – 3.78 cm in total length (mean=3.48cm) and 0.23– 0.50g in wet weight, blotted dry (mean = 0.35g) at test termination. Water temperature, pH, dissolved oxygen and conductivity were measured in all replicates of the control and the test concentrations. Measurements were taken at test initiation and every 24 hours thereafter. Total alkalinity and EDTA hardness of the water control was measured at the beginning of the test in the control. A photoperiod of 16 hours light and 8 hours dark was employed during the test. Mortality and behavioural observations were made every 24 hours. Dead fish were removed from test chambers when observed. Samples were analyzed using a Hewlett Packard Series 1100 HPLC equipped with a UV detector.

Findings:

A summary of cumulative mortality and sublethal effects is presented in Table B.9.2.1.2-1. The mean, measured concentration of IN-A2213 was 132 mg/L. This mean measured value of IN-A2213 was 102% of the targeted nominal concentration. There was no mortality at any concentration including the control during the definitive study and no sublethal effects were observed.

Table B.9.2.1/03-1: Summary of mortality and sublethal effects of IN-A2213 on rainbow trout, *Oncorhynchus mykiss*, exposed for 96 hours in an unaerated, static, acute test

Test substance	Metabolite IN-A2213
Test object	Rainbow Trout
Exposure	96 h, static
LC_{50} mg a.i./l	>132
Lowest observed effect concentration (LOEC) mg a.i./l	132
Highest tested conc. without toxic effect (NOEC) mg a.i./l	132

Observations:

The results reported are based on mean mesaured concentrations. During the test, water temperature was in the range 11.9 – 12.6°C (mean=12.3°C), dissolved oxygen ranged from 8.9 to 9.7 mg/l (mean=9.2mg/l) and pH was between 7.2 and 7.8. The value for EDTA hardness and conductivity of the dilution water at the start of the test were 48 mg/l as CaCO3 and 160 – 200 µmhos/cm respectively. Loading in the water control was 0.23 g/L at test conclusion.

Conclusion:

The 96-hour LC50 for IN-A2213 to the rainbow trout was >132 mg/L, based on mean, measured concentrations.

RMS comments and conclusion

The acute toxicity to fish study DuPont-2907, originally submitted under EU Rev8 Point IIA 8.2.1 and conducted with test material pure oxamyl (PAI), was conducted under guidelines EEC Method C.1. (1992), U.S. EPA 72-1 (1988), and OECD 203 (1992). A review of this study against the current OECD 203 (1992) indicates that the validity criteria regarding control mortality, constant conditions, DO concentration were fulfilled.

The following deviations from OECD 203 (1992) are noted:

Temperature range was 11.9 – 12.6°C °C , i.e. slightly lower then the recommended 13-17°C;
The fish length was 3.10 – 3.78 cm in total length (mean=3.48cm), i.e. shorter than the recommended 5.0 ±1.0 cm.
Fish were starved for 2 d prior the test instead of 1d.

Conclusion: the noted deviations are not considered severe, hence, also taking into account issues of animal welfare, the study is judged acceptable.

Study submitted in the EU Dossier in 2001 and included in the first EU approval review.

B.9.2.1/04

Reference: --	**Report:**	[redacted] (1999); IN-D2708: Static, acute, 96-hour, (LC_{50}) test to rainbow trout, *Oncorhynchus mykiss* **DuPont Report No.:** DuPont-2507 **Guidelines:** EEC Method C.1. (1992), OECD 203 (1992), U.S. EPA 72-1 (1988)

1. Test material: IN-D2708 technical metabolite
   Lot/Batch #: D2708-6
   Purity: 99.9%

Materials and methods:

The acute toxicity of IN-D2708 to unfed fingerling rainbow trout, *Oncorhynchus mykiss*, was determined in an unaerated, static, 96-hour test. Fish were acclimated for 14 d at 12 ± 1°C. Treatments consisted of a dilution water control, and nominal concentrations of 17, 28, 47, 78 and 130 mg/L (Mean measured concentrations were 0.0, 17.1, 28.7, 49.1, 79.3 and 135 mg/l). Two replicates containing 5 fish were exposed to each treatment concentration and control. Test chambers were glass aquaria which held 7 L of test solution Test solutions were maintained between 11.4 and 12.3°C. The mean temperature during the study was 11.9°C. In addition, a continuously-recording thermometer was used to check for temperature variation in one replicate of the dilution water control. Fish in the water control ranged from 3.35 – 4.0 cm in total length (mean=3.69cm) and 0.32 – 0.54g in wet weight, blotted dry (mean = 0.43g) at test termination. Loading in the water control was 0.31 g/l at test termination. Water temperature, pH, dissolved oxygen and conductivity were measured in all replicates of the control and the test concentrations. Measurements were taken at test initiation and every 24 hours thereafter. Total alkalinity and EDTA hardness of the water control was measured at the beginning of the test in the control. A photoperiod of 16 hours light and 8 hours dark was employed during the test. Mortality and behavioural observations were made every 24 hours. Dead fish were removed from test chambers when observed. Samples were analyzed using a Hewlett Packard Series 1100 HPLC equipped with a UV detector. The binomial method was used to calculate LC50s and 95% confidence limits.

Findings:

Mortality of Rainbow Trout, *Oncorhynchus mykiss*, in an Unaerated Static, Acute, 96-Hour, (LC_{50}) Test with IN-D2708

Mean, Measured IN-D2708 Concentration (mg/L)	Cumulative Mortality (%)				
	0 Hours	24 Hours	48 Hours	72 Hours	96 Hours
Water Control A†	0	0	0	0	0
Water Control B†	0	0	0	0	0
17.1 A†	0	0	0	0	0
17.1 B†	0	0	0	0	0
28.7 A†	0	0	0	0	0
28.7 B†	0	0	0	0	0
49.1 A†	0	0	0	0	0
49.1 B†	0	0	0	0	0
79.3 A†	0	0	0	0	0
79.3 B†	0	0	0	0	40
135 A†	0	100	100	100	100
135 B†	0	100	100	100	100

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

pH of IN-D2708 Test Solutions

Mean, Measured IN-D2708 Concentration (mg/L)	0 Hours	24 Hours	48 Hours	72 Hours	96 Hours
Water Control A†	7.2	7.5	7.6	7.5	7.8
Water Control B†	7.3	7.5	7.6	7.5	7.8
17.1 A†	7.2	7.5	7.6	7.5	7.8
17.1 B†	7.2	7.5	7.6	7.5	7.8
28.7 A†	7.1	7.2	7.5	7.5	7.8
28.7 B†	7.0	7.2	7.5	7.5	7.8
49.1 A†	6.3	6.9	7.3	7.3	7.6
49.1 B†	6.3	6.9	7.2	7.3	7.6
79.3 A†	4.2	4.3	4.5	4.5	4.9
79.3 B†	4.1	4.2	4.3	4.3	4.6
135 A†	3.3	3.3	--	--	--
135 B†	3.3	3.3	--	--	--

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

A summary of cumulative mortality and sublethal effects is presented in Table 24. Mean, measured concentrations of IN-D2708 were 17.1, 28.7, 49.1, 79.3, and 135 mg/L and ranged from 101 to 104% of nominal concentrations. There were no mortality or sublethal effects below 79.3 mg/L. The highest concentration causing no mortality was 49.1 mg/L and the lowest concentration causing 100% mortality was 135 mg/L. Several fish exposed to 79.3 mg/L exhibited a loss of equilibrium, erratic swimming, change in coloration and lethargy at 48, 72 and 96 hours. No other sublethal effects were observed at any tested concentration during the definitive toxicity test. Time to first observed mortality was 24 hours. No mortality or sublethal effects were recorded in the control.

Table 24 Summary of mortality and sublethal effects of IN-D2708 on rainbow trout, *Oncorhynchus mykiss*, exposed for 96 hours in an unaerated, static, acute test

Test substance	Metabolite IN-D2708
Test object	Rainbow Trout
Exposure	96 h, static
LC_{50} mg a.i./l	93.8
Lowest observed effect concentration (LOEC) mg a.i./l	79.3
Highest tested conc. without toxic effect (NOEC) mg a.i./l	49.1

Observations:

The results reported are based on mean measured concentrations. During the test, water temperature was in the range 11.4 – 12.3°C (mean=11.9°C), dissolved oxygen ranged from 9.3 to 10.4 mg/l (mean=9.7mg/l) and pH was between 3.3 and 7.8. The value for EDTA hardness and conductivity of the dilution water at the start of the test were 48 mg/l as CaCO3 and 150 – 340 µmhos/cm respectively. Loading in the water control was 0.31 g/L at test conclusion.

Conclusion:

The 96-hour $LC_{50}$for IN-D2708 to the rainbow trout was 93.8 mg/L, based on mean, measured concentrations (no confidence interval available).

RMS comments and conclusion

The acute toxicity to fish study DuPont-2907, originally submitted under EU Rev8 Point IIA 8.2.1 and conducted with test material pure oxamyl (PAI), was conducted under guidelines EEC Method C.1. (1992), U.S. EPA 72-1 (1988), and OECD 203 (1992). A review of this study against the current OECD 203 (1992) indicates that the validity criteria regarding control mortality, constant conditions, DO concentration were fulfilled.

The following deviations from OECD 203 (1992) are noted:

Temperature range was 11.4 – 12.3°C (mean=11.9°C),, i.e. slightly lower then the recommended 13-17°C;
The fish length was 3.35 – 4.0 cm in total length (mean=3.69cm), i.e. shorter than the recommended 5.0 ±1.0 cm.
Fish were starved for 2 d prior the test instead of 1d.

In addition, the pH varied considerably among the tested concentrations, showing a marked decrease at increasing concentrations (from pH= 7.2-7.8 of the control and the two lowest concentrations down to 3.3 of the highest concentration). This was observed already at the start of test (day 0).

Conclusion: the noted deviations are not considered severe such to invalidate the test. Anyhow, the low pH at the two highest concentations might account for the mortality observed. The results of the study are not fully reliable, but are informative of the low toxicity of the metabolite to fish (0% mortality at 49.1 mg/L). Supportive information.Study submitted in the EU Dossier in 2001 and included in the first EU approval review.

B.9.2.1/05

Reference: --	**Report:**	[redacted] (1999b); IN-N0079: Static, acute, 96-hour, (LC_{50}) test to rainbow trout, *Oncorhynchus mykiss* **DuPont Report No.:** DuPont-2512 **Guidelines:** OECD 203 (1992), EEC Method C.1. (1992), U.S. EPA 72-1

		(1982)

1. Test material: IN-N0079 technical metabolite
   Lot/Batch #: N0079-8
   Purity: 99.6%

Materials and methods:

The acute toxicity of IN-N0079 to unfed fingerling rainbow trout, *Oncorhynchus mykiss*, was determined in an unaerated, static, 96-hour test. Fish were acclimated for 12 d at 12 ± 2°C. Treatments consisted of a dilution water control and nominal concentrations of 10, 17, 28, 49, 78, and 130 mg/L. Two replicates containing 5 fish were exposed to each treatment concentration and control. Test chambers were glass aquaria which held 15 L of test solution Test solutions were maintained between 11.8 and 12.5°C using a water bath. The mean temperature during the study was 11.9°C. In addition, a continuously-recording thermometer was used to check for temperature variation in one replicate of the dilution water control. Fish in the water control ranged from 3.02 – 3.85 cm in total length (mean=3.39cm) and 0.20 – 0.53g in wet weight, blotted dry (mean = 0.32g) at test termination. Loading in the water control was 0.11 g/l at test termination. Water temperature, pH, dissolved oxygen and conductivity were measured in all replicates of the control and the test concentrations. Measurements were taken at test initiation and every 24 hours thereafter. Total alkalinity and EDTA hardness of the water control was measured at the beginning of the test in the control. A photoperiod of 16 hours light and 8 hours dark was employed during the test. Mortality and behavioural observations were made every 24 hours. Dead fish were removed from test chambers when observed. Samples were analyzed using a Hewlett Packard Series 1100 HPLC equipped with a UV detector. The binomial method was used to calculate LC50s and 95% confidence limits.

Findings:

Mortality of Rainbow Trout, *Oncorhynchus mykiss*, in an Unaerated Static, Acute, 96-Hour, (LC_{50}) Test with IN-N0079

Mean, Measured IN-N0079 Concentration (mg/L)	Cumulative Mortality (%)				
	0 Hours	24 Hours	48 Hours	72 Hours	96 Hours
Water Control A†	0	0	0	0	0
Water Control B†	0	0	0	0	0
9.85 A†	0	0	0	0	0
9.85 B†	0	0	0	0	0
17.2 A†	0	0	0	0	0
17.2 B†	0	0	0	0	0
29.3 A†	0	0	0	100	100
29.3 B†	0	0	0	100	100
49.4 A†	0	0	100	100	100
49.4 B†	0	0	100	100	100
79.7 A†	0	100	100	100	100
79.7 B†	0	100	100	100	100
135 A†	0	100	100	100	100
135 B†	0	100	100	100	100

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

A summary of cumulative mortality and sublethal effects is presented in Table 25. Mean, measured concentrations of IN-N0079 were 9.85, 17.2, 29.3, 49.4, 79.7, and 135 mg/L and ranged from 99 to 105% of nominal concentrations. There were no mortality or sublethal effects below 29.3 mg/L. The highest concentration causing no mortality was 17.2 mg/L and the lowest concentration causing 100% mortality was

29.3 mg/L. No sublethal effects were observed at any tested concentration during the definitive toxicity test. Time to first observed mortality was 24 hours. No mortality or sublethal effects were recorded in the control.

Table 25 Summary of mortality and sublethal effects of IN-N0079 on rainbow trout, *Oncorhynchus mykiss*, exposed for 96 hours in an unaerated, static, acute test

Test substance	Metabolite IN-N0079
Test object	Rainbow Trout
Exposure	96 h, static
LC_{50} mg a.i./l	22.4
Lowest observed effect concentration (LOEC) mg a.i./l	29.3
Highest tested conc. without toxic effect (NOEC) mg a.i./l	17.2

Observations:

The results reported are based on mean measured concentrations. During the test, water temperature was in the range 11.8 – 12.5°C (mean=12.2°C), dissolved oxygen ranged from 9.2 to 9.7 mg/l (mean=9.5mg/l) and pH was between 7.2 and 8.0. The value for EDTA hardness and conductivity of the dilution water at the start of the test were 44 mg/l as CaCO3 and 150 – 160 µmhos/cm respectively.

Conclusion:

The 96-hour $LC_{50}$for IN-N0079 to the rainbow trout was 22.4 mg/L, based on mean, measured concentrations.

The acute toxicity to fish study DuPont-2512, originally submitted under EU Rev8 Point IIA 8.2.1 and conducted with test material IN-N0079 technical metabolite, was conducted under guidelines OECD 203 (1992), EEC Method C.1. (1992), and U.S. EPA 72-1 (1982). A review of this study indicates that it fully meets the current guideline, OECD 203 (1992). Therefore this study is relied upon.

RMS comments and conclusion

The acute toxicity to fish study DuPont-2907, originally submitted under EU Rev8 Point IIA 8.2.1 and conducted with test material pure oxamyl (PAI), was conducted under guidelines EEC Method C.1. (1992), U.S. EPA 72-1 (1988), and OECD 203 (1992). A review of this study against the current OECD 203 (1992) indicates that the validity criteria regarding control mortality, constant conditions, DO concentration were fulfilled.

The following deviations from OECD 203 (1992) are noted:

Temperature range was 11.8 – 12.5°C (mean=12.2°C), i.e. slightly lower then the recommended 13-17°C;
The fish length was 3.02 – 3.85 cm in total length (mean=3.39cm), i.e. shorter than the recommended 5.0 ±1.0 cm.
Fish were starved for 2 d prior the test instead of 1d.

Conclusion: the noted deviations are not considered severe, hence, also taking into account issues of animal welfare, the study is judged acceptable.Study submitted in the EU Dossier in 2001 and included in the first EU approval review.

B.9.2.1/06

Reference: --	**Report:**	[redacted] (2000); IN-T2921: Acute, 96-hour $LC_{50}$to rainbow trout, *Oncorhynchus mykiss* **DuPont Report No.:** DuPont-4439

		Guidelines: OECD 203 (1992), U.S. EPA 72-1 (1982), EEC Method C.1. (1992)

1. Test material: IN-T2921 technical metabolite
   Lot/Batch #: T2921-2
   Purity: 98.7%

Materials and methods:

The acute toxicity of IN-T2921 to unfed fingerling rainbow trout, *Oncorhynchus mykiss*, was determined in an unaerated, static, 96-hour limit test. Fish were acclimated for 17 d at 12 ± 1°C. Treatments consisted of a dilution water control and a single nominal concentration of 120 mg IN-T2921/L. Three replicates containing 10 fish were exposed to the single treatment concentration and control. Test chambers were glass aquaria which held 15 L of test solution Test solutions were maintained between 11.7 and 12.4°C. The mean temperature during the study was 12.0°C. Fish in the water control ranged from 2.88 – 4.07 cm in total length (mean=3.56cm) and 0.16 – 0.58g in wet weight, blotted dry (mean = 0.35g) at test termination. Loading in the water control was 0.23 g/l at test termination. Water temperature, pH, dissolved oxygen and conductivity were measured in all replicates of the control and the test concentrations. Measurements were taken at test initiation and every 24 hours thereafter. Total alkalinity and EDTA hardness of the water control was measured at the beginning of the test in the control. A photoperiod of 16 hours light and 8 hours dark was employed during the test. Mortality and behavioural observations were made every 24 hours. Dead fish were removed from test chambers when observed. Samples were analyzed using a Hewlett Packard Series 1100 HPLC equipped with a UV detector.

Findings:

Summaries of cumulative mortality and sublethal effects are presented in Table 26. The mean, measured concentration of IN-T2921 was 127 mg/L. There were no mortality or sublethal effects at the single tested concentration. Mortality was not seen in any water control fish and none of the surviving control fish were affected at the end of the study.

Table 26 Summary of mortality and sublethal effects of IN-T2921 on rainbow trout, *Oncorhynchus mykiss,* exposed for 96 hours in an unaerated, static, acute test

Test substance	Metabolite IN-T2921
Test object	Rainbow Trout
Exposure	96 h, static
LC_{50}mg a.i./l	>127
Lowest observed effect concentration (LOEC) mg a.i./l	127
Highest tested conc. without toxic effect (NOEC) mg a.i./l	127

Observations:

The results reported are based on mean measured concentrations. During the test, water temperature was in the range 11.7 – 12.4°C (mean=12.0°C), dissolved oxygen ranged from 9.0 to 9.8 mg/l (mean=9.4mg/l) and pH was between 7.3 and 7.8.The value for EDTA hardness and conductivity of the dilution water at the start of the test were 48 mg/l as CaCO3 and 130 – 140 µmhos/cm respectively.

Conclusion:

The 96-hour $LC_{50}$for IN-T2921 to rainbow trout was greater than 127 mg/L, based on mean measured concentrations.

RMS comments and conclusion

The acute toxicity to fish study DuPont-4439, originally submitted under EU Rev8 Point IIA 8.2.1 and conducted with test material IN-T2921 technical metabolite, was conducted under guidelines OECD 203 (1992), U.S. EPA 72-1 (1982), and EEC Method C.1. (1992).

A review of this study against the current OECD 203 (1992) indicates that the validity criteria regarding control mortality, constant conditions, DO concentration were fulfilled.

The following deviations from OECD 203 (1992) are noted:

Temperature range was 11.7 – 12.4°C (mean=12.0°C),, i.e. slightly lower then the recommended 13-17°C;
The fish length was 2.88 – 4.07 cm in total length (mean=3.56cm), , i.e. shorter than the recommended 5.0 ±1.0 cm.

Fish were starved for 2 d prior the test instead of 1d.

Conclusion: the noted deviations are not considered severe, hence, also taking into account issues of animal welfare and the nature of a limit test, the study is judged acceptable.

B.9.2.2 Long-term and chronic toxicity to fish

B.9.2.2.1 Fish early life stage toxicity test

Study submitted in the EU Dossier in 2001 and included in the first EU approval review.

B.9.2.2.1/01

Reference:	**Report:**	[redacted] (1988); Early life stage toxicity of IN D1410-196 (oxamyl) to rainbow trout **DuPont Report No.:** HLR 468-88 **Guidelines:** U.S. EPA 72-4 (1988)
--		

1. Test material: Pure Oxamyl (PAI)
   Lot/Batch #: D1410-196
   Purity: 97.2%

Materials and methods:

Rainbow trout (*Salmo gairdnerii*, equivalent to *Oncorhynchus mykiss*) embryos (approx. 24 hours old) and larvae were continuously exposed to Oxamyl (purity: 97.2%) in a flow- through test system at measured concentrations of 0.06, 0.10, 0.23, 0.40, 0.77 and 1.5 mg a.s./L for 61 days. Observations were made on embryo hatching and larval survival and growth.

The test vessels (aquaria made of stainless steel) were filled with 7 litres of filtered well water (water depth: 17 cm), maintained at the test temperature (11.8 – 13.9°C), with a dissolved oxygen concentration of 9.4 to 10.0 mg/L. Each aquarium was partitioned in the middle to give two replicates (four replicates per test concentration). For the embryo phase, two 200 mL screen bottomed glass cups were suspended in each aquarium replicate (water depth: 2 to 5 cm), and twenty embryos were added to each cup (80 embryos per test concentration). The

first larvae hatched on Day 20 and hatching was considered complete by Day 29. As the larvae hatched, they were transferred from the embryo cup to the appropriate aquarium, with up to 15 larvae per replicate; excess larvae were counted and then discarded. Survival of the remaining larvae was assessed for 32 days, and at the end of the study all surviving larvae were weighed and measured.

Findings:

The mean measured concentrations of Oxamyl in the test vessels ranged from 75% to 100% of the nominal values.

The results are presented in Table 27. No statistically significant effects on embryo hatching, larval survival, first day of larval swim-up, or larval growth were observed at measured test concentrations of 0.06 to 0.77 mg a.s./L. However, the % embryo hatch was <66% as stated in the guidelines. At 1.5 mg a.s./L (the highest concentration tested), statistically significant effects on embryo hatching and a delay in the first day of larval swim- up were observed compared to the control.

Dissolved oxygen ranged from 8.6 – 10.4 mg/l during the study (mean = 9.55 ±0.3). pH ranged from 7.5 – 8.8 during the study. Mean Total hardness was 81 ±4 mg CaCO3/l. Conductivity was 159 ±9 µmhos/cm.

Table 27 Embryo viability, larval survival, total length and wet weight of rainbow trout during early life-stage exposure to Oxamyl

Mean measured concentration	Percent embryo hatch	Percent larval survival	Mean 1st day of larval	Larval length (cm) Mean ± SD	Wet weight (mg) Mean ± SD
Water control	49	96	33	2.9±0.4	401±109
0.06	65	96	33	2.7±0.2	425±84
0.10	40	93	33	2.8±0.2	469±83
0.23	60	96	34	2.6±0.2	393±88
0.40	52	96	33	2.9±0.2	409±99
0.77	35	92	34	2.9±0.2	441±94
1.5	2*	100	38*	2.9±0.1	384±63

* Treatment group mean significantly different (P <0.05) from control group by Dunnett Test.

Conclusion: The NOEC for rainbow trout exposed to Oxamyl was 0.77 mg a.s./L, based on statistically significant effects on embryo hatching and larval swim-up at 1.5 mg a.s./L (mean measured concentrations).

RMS comments and conclusion

The fish early life stage toxicity test study HLR 468-88, originally submitted under EU Rev8 Point IIA 8.2.2.2 and conducted with test material pure oxamyl (PAI), was conducted under guideline U.S. EPA 72-4 (1988). A review of this study indicates that it partially meets the current guideline, OECD 210 (2013); deviations include lack of observations for abnormal behavior.

Validity criteria:

-The DO should be >60% of air saturation throughout the test: fulfilled.
-The water temperature should not differ by more than ± 1.5°C between test chambers or between successive days and should be within 10± 1.5°C for rainbow trout: the temperature was continuously monitored and also checked daily, it is reported as ranging from 11.8 to 13.9. with a mean of 12.7°C. It cannot be ascertained if

temperature was checked in all the test vessels. The range is outside the recommended one for rainbow trout. Not fulfilled.
-The analytical measure of the concentrations is compulsory. Fulfilled.
-Hatching success in the control should be at least 75%. It was 49 %. Not fulfilled.
-Post-hatch success should be at least 75%: it was 96%. Fulfilled.

Conclusion: the study is not valid.

Study submitted in the EU Dossier in 2001 and included in the first EU approval review.

B.9.2.2.1/02

Reference: --	**Report:**	[redacted] (1982); Early life stage toxicity of oxamyl to fathead minnow **DuPont Report No.:** HLR 877-81 **Guidelines:** Draft No. 1 ASTM E-47 (February 1981) **GLP**: no

1. Test material: Pure Oxamyl (PAI)
   Lot/Batch #: D1410-196
   Purity: 97.1%

Materials and methods:

Duplicate groups of 50 fathead minnow (*Pimephales promelas*) eggs (fertilised embryos, less than twenty four hours old at study initiation) were exposed to Oxamyl (purity 97.1%) at nominal concentrations of 0 (control), 0.25, 0.5, 1.0, 2.0 and 4.0 mg a.s./L in a flow through system. Egg viability was assessed daily until hatching was complete; any dead embryos or embryos covered in fungi were removed.

To initiate the 28-day larval exposure study, 20 newly hatched fathead minnows were randomly selected from each embryo replicate and transferred to the respective test aquarium, with two replicates per concentration; the excess larvae were counted and then discarded. The test larvae were fed at least once daily on live brine shrimp nauplii (*Artemia salina*). The test conditions were 16 hours light, 8 hours dark; a temperature of 25°C; pH 7.5 to 7.7; and dissolved oxygen 7.7 – 7.8 mg/L. Daily observations were made on larval mortality, behaviour and appearance. The number of live fry in each aquarium was recorded weekly. At test termination, surviving larvae were measured (mean standard length) and individually weighed.

Findings:

Analytical determinations of Oxamyl concentrations were within 25% of nominal levels. However, analytical difficulties at the lowest Oxamyl concentration prevented precise determination, therefore all results are based on nominal concentrations.

The results for the biological parameters assessed are presented in Table 28.

Table 28 Embryo viability, 28-day larval survival, mean standard length and wet weight of fathead minnow (*Pimephales promelas*) during early life-stage exposure to Oxamyl

Nominal concentration (mg a.s./L)	Embryo viability (%)	Larvae (28 days post-hatch)			
		Number of	Percent larval	Mean standard length (mm)	Mean wet weight (g)
Control	94	20	100	15.2	39.7

0.25	77	20	100	15.9	47.0
0.5	93	18	90	15.4	39.0
1.0	95	15	75[b]	14.2	37.3
2.0	85	13	65[b]	13.7	34.8
4.0	71	4	20[ab]	13.0[b]	27.5

[a] Diluter malfunction in one replicate only, therefore results were excluded from statistical analysis.
[b] Treatment group mean significantly different (P <0.05) from control group by Dunnett Test.

Percent embryo hatch was reduced in one or both replicates at nominal test concentrations of 0.25, 2.0, and 4.0 mg a.s./L; however these reductions, which were not statistically significant, were considered to be a result of fungal growth rather than a treatment related effect.

Significant reductions in larval survival were detected at nominal test concentrations of 1.0 mg a.s./L and above. However significant effects on larval growth (mean standard length) occurred only at the highest concentration tested (4.0 mg a.s./L). No treatment-related effects on egg viability, larval survival or growth were detected at nominal concentrations of 0.5 mg a.s./L or below.

Conclusion:

The NOEC of Oxamyl to the early life-stages of fathead minnow was 0.5 mg a.s./L, based on the reduced survival of fathead minnow larvae exposed for 28 days. The LOEC was 1.0 mg a.s./L.

RMS comments and conclusion

The fish early life stage toxicity test study HLR 877-81, originally submitted under EU Rev8 Point IIA 8.2.2.2 was conducted according to an old draft guidance. The study is not GLP. A review of this study indicates that it partially meets the current guideline, OECD 210 (2013); deviations include lack of observations of abnormal behaviour and the use of two replicates instead of a minimum of 4. At the end of the study, the mean standard length of control fish was 15.2 mm, while the OECD 210 (2013) indicates a minimum length of 18 cm.

Validity criteria:

-The DO should be >60% of air saturation throughout the test: fulfilled.
-The water temperature should not differ by more than ± 1.5°C between test chambers or between successive days and should be within 25± 1.5°C for fathead minnow: the temperature was measured at 4h intervals on working days in the controls, resulting in 25 °C (SD 0.3). Aquaria were immersed in a water bath thermostatically controlled. Assumed to be fulfilled.
-The analytical measure of the concentrations is compulsory. Fulfilled, but at the four highest concentrations, mean measured concentrations were outside the 20% of the nominals, hence the results should be expressed as mean measured concentrations.

-Hatching success in the control should be at least 75%.. Fulfilled.

-Post-hatch success should be at least 75%. Fulfilled.

Conclusion: the study can be used as supportive information. Results should be recalculated based on mean measured concentration.

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

B.9.2.2.1/03

Reference: **CA 8.2.2.1/01**	**Report:**	[redacted] (2012); Oxamyl (DPX-D1410) Technical (98% w/w): Early life-stage toxicity test with the sheepshead minnow, *Cyprinodon variegatus*, under flow-through conditions **DuPont Report No.:** DuPont-34270 **Guidelines:** OPPTS 850.1400 (1996), OECD 210 (1992) **Deviations:** None **Testing Facility:** [redacted] **Testing Facility Report No.:** 68029 **GLP:** Yes **Certifying Authority:** Laboratories in the USA are not certified by any government agency, but are subject to regular inspections.

Executive summary:

The early life-stage toxicity of oxamyl to fed Sheepshead Minnow (*Cyprinodon variegatus*) was determined in a 29-day post-hatch flow-through test. The test was conducted in accordance with the U.S. EPA, Office of Prevention, Pesticides and Toxic Substance (OPPTS), Ecological Effects Test Guideline 850.1400 and the Organization for Economic Cooperation and Development (OECD), Guideline 210. Treatments consisted of a dilution water control and six nominal concentrations of 0.025, 0.050, 0.10, 0.20, 0.40, and 0.80 mg a.s./L. Based on mean measured concentrations of oxamyl, the NOEC value for egg hatchability, post-hatch survival, standard length, and blotted wet weight was 0.356 mg a.s./L. The most sensitive endpoints were egg hatchability, fry survival and fry growth, with an MATC value of 0.506 mg a.s./L.

I. MATERIALS AND METHODS

A. MATERIALS

1.	Test material:	Pure Oxamyl (PAI)
	Lot/Batch #:	D1410-196
	Purity:	98.0% by analysis
	Description:	White crystalline solid
	CAS#:	23135-22-0
	Stability of test compound:	Stable at normal temperatures and storage conditions
2.	Control:	Dilution (laboratory blended water) water
	Solvent control:	None
	Test vehicle:	Dilution water (laboratory salt water)
	Toxic reference:	None
3.	Test organism:	Sheepshead Minnow
	Species:	*Cyprinodon variegatus*
	Age at dosing:	<24 hours
	Initial population:	20 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 15 cm wide by 22 cm long by 24 cm high with a test solution depth of 14 cm
4.	Environmental conditions (in-life period)	
	Temperature:	24.1 to 24.9°C for fry
	Photoperiod:	16 hr photoperiod (643 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

   28-March-2012 to 05-May-2012

2. Experimental treatments

   The early life-stage toxicity of oxamyl to fed *Cyprinodon variegatus* was determined in an unaerated, flow-through, 29-day post-hatch test. Treatments consisted of a dilution water control, and six nominal concentrations of 0 (control), 0.025, 0.050, 0.10, 0.20, 0.40, and 0.80 mg a.s./L. Twenty embryos were used per replicate with four replicates per test concentration 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 behavioral or physical changes, including abnormalities, were recorded. At the end of the 29-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 oxamyl 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, 31, 35, and 38 of the definitive test. The analysis of the samples for oxamyl 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

The NOEC for egg hatchability and fish survival (29-day post-hatch) data were determined by using a Fisher's exact test. A Hochberg adjustment was used to control the experiment-wise error rate for the Fisher's test at the same alpha level. In addition, the NOEC for these parameters was estimated using a one-way analysis of variance (ANOVA) procedure and a one-tailed Dunnett's test with the alternate hypothesis being the mean for the parameter was reduced in comparison to the control mean. The time to start of hatch and time to complete hatch was evaluated using an ANOVA procedure and a one-tailed Dunnett's test with the alternate hypothesis being the mean for the parameter was greater in comparison to the control mean. The NOEC values for standard length and blotted wet weight, were determined using a nested ANOVA procedure, where the treatment means are weighted for number of fish in each chamber, and a one-tailed Dunnett's test with the alternate hypothesis being the mean for the parameter was reduced in comparison to the control mean. 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. When the Shapiro-Wilk's and Levene's tests indicated normality and homogeneity of variance the non-transformed data were used in the parametric ANOVA and Dunnett's test. The transformed (*i.e.*, ranks) data were used when the Shapiro-Wilk's and Levene's tests indicated non–normality and/or heterogeneity of variance. 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 sensitive endpoints.

II. RESULTS AND DISCUSSION

A. FINDINGS

The mean measured concentrations of oxamyl in the control and test substance treatments during the study were <LOD (control), 0.0198, 0.0424, 0.0826, 0.169, 0.356, and 0.718 mg a.s./L, which represented 78 to 90% of the nominal concentrations. No residues of oxamyl were detected in the control above the LOD of 0.00000537 mg a.s./L. All test acceptability criteria were met.

Egg hatch began in the control and the 0.0198, 0.0424, 0.0826 mg a.s./L treatments on study Day 5. Hatch in the 0.169, 0.356, and 0.718 mg a.s./L treatments began on study days 6, 6, and 7, respectively. Day 0 post-hatch (*i.e.*, ≥95% hatch) in the control treatment was determined to be Day 9. Hatch was completed in all treatment replicates between study Days 9 and 13, with the exception of one replicate in each of the 0.0826, 0.169, and 0.356 mg a.s./L treatments where complete hatch was achieved on study Days 15, 14, and 16, respectively. Overall hatching success in the control treatment was 86% which met the acceptability criterion for this endpoint. Hatching success in the test substance treatments ranged from 68% in the 0.718 mg a.s./L treatment to 81% in the 0.0198 and 0.0424 mg a.s./L treatments. There was no statistically significant delay in time to start or completion of hatch observed in the test substance treatments as compared to the control. There was a statistically significant reduction in hatch success observed in the 0.718 mg a.s./L test substance treatment as compared to the control.

Mean standard length in the control and test substance treatments was 14.6, 14.7, 15.5, 15.2, 14.4, 14.0, and 8.2 mm in the control, 0.0198, 0.0424, 0.0826, 0.169, 0.356, and 0.718 mg a.s./L treatments, respectively. Mean blotted wet weight in the control and test substance treatments was 0.1099, 0.1100, 0.1228, 0.1278, 0.1074, 0.0997, and 0.0267 g in the control, 0.0198, 0.0424, 0.0826, 0.169, 0.356, and 0.718 mg a.s./L treatments, respectively. There was a statistically significant reduction of mean standard length and mean blotted wet weight at the 0.718 mg a.s./L treatment as compared to the control.

A summary of hatching and survival is presented in the following table.

Table 29 Summary of observed mortality of *Cyprinodon variegatus* exposed to oxamyl in a flow-through test

Mean measured oxamyl 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	19/20	17/20	17/20	16/20	17/19	17/17	17/17	13/16
0.0198	15/20	14/20	17/20	19/20	14/15	10/14	14/17	18/19
0.0424	18/20	17/20	16/20	14/20	15/18	17/17	15/16	14/14
0.0826	13/20	16/20	15/20	14/20	10/13	14/16	10/15	14/14
0.169	16/20	13/20	16/20	16/20	14/16	11/13	14/16	14/16
0.356	19/20	16/20	15/20	14/20	16/19	16/16	15/15	11/14
0.718	13/20	13/20	13/20	15/20	7/13	9/13	8/13	7/15

The results are summarized as follows:

Biological Parameter	No-Observed-Effect Concentration (NOEC) [a]	Lowest-Observed-Effect Concentration (LOEC) [a]	MATC[a]
Day Hatch Start	0.718	>0.718	NA
Day Hatch Completed	0.718	>0.718	NA
Egg Hatchability	0.356	0.718	0.506
Fry Survival [b]	0.356	0.718	0.506
Standard Length	0.356	0.718	0.506
Blotted Wet Weight	0.356	0.718	0.506

[a] Expressed as mean measured concentration of oxamyl (mg a.s./L).
[b] Fry survival based on number of hatched fry surviving on day 29 post-hatch.
NA Not applicable

III. CONCLUSION

Based on mean measured concentrations of oxamyl, the NOEC value was 0.356 mg a.s./L based on egg hatchability, post-hatch survival, standard length, and blotted wet weight.

([redacted] 2012)

RMS comments and conclusion

The mean standard length of control fish was 14.6 mm and ranged from 8.2 to 15.5 mm , while the OECD 210 (2013) indicates a minimum length of 17 cm.

Validity criteria:

-The DO should be >60% of air saturation throughout the test: fulfilled.
-The water temperature should not differ by more than ± 1.5°C between test chambers or between successive days and should be within 10± 1.5°C for rainbow trout: Temperature was 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. It was in the range 24.1 to 24.9°C. A continuous recording of temperature was made using a data

logger and thermistor probe placed in a centrally-located test chamber, that demonstrated that the temperature of the test solutions remained within the 25 ± 2 °C. This criterion is not verifiable. Assumed to be fulfilled.
-The analytical measure of the concentrations is compulsory. Fulfilled.
-Hatching success in the control should be at least 75%: fulfilled.
-Post-hatch success should be at least 80%: fulfilled.

Conclusion: the study is acceptable although one of the validity criteria is only assumed to be fulfilled.

B.9.2.2.2 Fish full life cycle test

A fish life cycle study was not performed based on the following criteria:

The bioconcentration factor is expected to be low, since the log P_{ow} is -0.44 for a pH-value of 7.

Oxamyl is also not expected to persist in water or sediments. The aquatic/sediment persistence endpoints for the total system at two sites are:

- Red Oak Stream DT_{50}=0.82, DT_{90}=8.31 days
- Town Park Pond DT_{50}=0.69, DT_{90}=2.28 days.

In the field, these rates are expected to be faster due to additional dissipation mechanisms such as photolysis (Oxamyl EU Renewal Dossier, Document M-CA, Section 7, DuPont-40934 EU).

B.9.2.2.3 Bioconcentration in fish

Oxamyl

The triggers for this study are log P_{ow} ≥3 and potential exposure that may lead to bioconcentration where the DT_{90} is ≤24 hours *via* hydrolysis. The log P_{ow} for oxamyl is -0.44.

Based on the log P_{ow}, the short residual in aquatic systems, and the unlikelihood of continuous and repeated exposure, no studies on bioconcentration potential of the active substance in fish are required.

B.9.2.3 Endocrine disrupting properties

Oxamyl does not have any endocrine effects on aquatic organisms, as documented in Points B.9.2.2 and CA 8.3.4 of this document (DuPont-40935 EU).

Annex II, 3.6.5 of Regulation (EC) 1107/2009 provides the framework criteria for approval of an active substance related to endocrine disruption and highlights that the following interim criteria shall be used until the final criteria are in place:

- Active substances that are or have to be classified as category 2 for carcinogenicity (‘C-2’) and reproductive toxicity (‘R-2’) shall be considered to have endocrine disrupting properties, and
- Active substances that are or have to be classified as category 2 for reproductive toxicity and which have toxic effects on the endocrine organs may be considered to have endocrine disrupting properties.

Oxamyl is not classified R2 or C2 for mammals, thus does not qualify as a suspected endocrine disrupter.

However, two studies with vertebrates (amphibians and fish) were conducted with oxamyl active substance at the request of the United States Environmental Protection Agency to assist in development of the U.S. EPA Endocrine Disruption Screening and Testing Program and are summarized below. From the screening Amphibian Metamorphosis Assay (AMA) with the frog *Xenopus laevis* there are no clear evidence the oxamyl

may interfere with the normal function of the hypothalamic-pituitary-thyroid (HPT) axis. Apical endpoints are not affected, but the incidence and severity of the recorded follicular cell hypertrophy found in tyroid hystopatology might represent an indicator of hormonal activity as a possible treatment related effect cannot be excluded with certainty. Therefore the possible interference with the normal function of the hypothalamic-pituitary-thyroid (HPT) axis should be further addressed. In this respect, the RMS highlights that, looking at the Human health section of this DAR, the complete data package support the conclusion that oxamyl is not an endocrine disrupter. According to OECD guidance 150 (Table C.3.6), a longer-term amphibian test would be the next step which could be taken in case the indicators of hormonal activity are positive with "Strong evidence for in vivo thyroid activity in amphibians". In the available AMA study, the relevance of the recorded follicular cell hypertrophy is debatable.

The 21d fish short term reproduction assay (OPPTS 890.1350 (2009) resulted in no effect for apical endpoints or indicators related to endocrine disruption. This test (according to OECD 229) is proposed as Level 3 test in the OECD conceptual framework (Guidance document on standardised test guideline for evaluating chemicals for endocrine disruption, N° 150, 2012). Study submitted to the EU for the first time in this submission.

B.9.2.3/01

Reference: CA 8.2.3/01	**Report:**	[redacted] (2015); Oxamyl (DPX-D1410) technical: 21-D amphibian metamorphosis assay (AMA) with south African clawed frog, *Xenopus laevis* **DuPont Report No.:** DuPont-31032, Revision No. 1 **Guidelines:** OPPTS 890.1100 (2009) **Deviations:** None **Testing Facility:** [redacted] **Testing Facility Report No.:** DUPO01-00270 **GLP:** Yes **Certifying Authority:** Laboratories in the USA are not certified by any government agency, but are subject to regular inspections.

Executive summary:

The effects of oxamyl on the amphibian metamorphosis in the African clawed frog (*Xenopus laevis*) were determined under unaerated, continuous-flow conditions for 21 days. A dilution water control and nominal test item concentrations of 13, 40, and 120 µg a.s./L were used during the study. The mean measured concentrations were 10.5, 39.9, and 130.1 µg a.s./L. The NOEC for *X. laevis* exposed to oxamyl was 130.1 µg a.s./L based on time-weighted mean measured concentrations and AMA endpoints.

I. MATERIALS AND METHODS

A. MATERIALS

1.	Test material:	Pure Oxamyl (PAI)
	Lot/Batch #:	D1410-196
	Purity:	98.0%
	Description:	Colorless clear liquid, stable in water (25°C), K_{ow} = 0.36, volatility = 3.8×10^{-7} mm Hg (25°C)
	CAS#:	23135-22-0
	Stability of test compound:	Shown to be stable under the conditions of the test
2.	Vehicle and/or positive control:	Dilution (laboratory well water) water control
3.	Test animals:	African clawed frog
	Species:	*Xenopus laevis*
	Age at dosing:	NF stage 51
	Source:	In-house culture originating from [redacted]
	Acclimation period:	14 days
	Diet:	Sera Micron®
	Water:	Dechlorinated tap water
	Housing:	50 L tanks
4.	Environmental conditions	
	Temperature:	22 ± 1°C
	Relative humidity:	Not relevant to this study
	Photoperiod:	12 h light:12 h dark photoperiod (600 to 2000 lux)

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed

7-July-2011 to 1-July-2011

2. Experimental treatments

The effects of oxamyl on metamorphosis of African clawed frog (*Xenopus laevis*) larvae were determined under unaerated continuous-flow conditions for 21 days. A dilution water control and nominal test item concentrations of 13, 40, and 120 µg a.s./L were used during the study. A total of 80 (20/replicate) Nieuwkoop and Faber (NF) stage 51 larvae per treatment were exposed per concentration. On exposure day 7, 5 larvae were subsampled to a total of 20 larvae per test item concentration for developmental stage, growth (wet body weight and snout-vent length [SVL]), and hind limb length endpoints. Test solutions were maintained between 21 and 23°C. Analytical verification of oxamyl concentrations was made on test solutions sampled during pre-exposure (diluter equilibration), on Day 0, 7, 14, and at test end (Day 21).

3. Observations

Daily observations were made to assess survival and estimated developmental stage for each treatment replicate. Individual developmental stage and growth (wet weight and SVL) were measured in a subset of 5 larvae per replicate (20 per treatment) on exposure day 7. These larvae were fixed in modified Davidson's solution and preserved in 10% (v/v) neutral buffered formalin. At test conclusion (exposure Day 21), larval survival, developmental stage, wet weight, SVL length, and hind limb length were measured and the larvae fixed in modified Davidson's solution and preserved in 10% (v/v) neutral buffered formalin. Five specimens stage matched across all treatments to the median developmental stage at test termination were selected for histopathological examination. [redacted], under the direction of pathologist [redacted], performed the tissue preparation, histology, and histopathological interpretation in accordance with appropriate facility guidance documents (SOPs) and the relevant guidance documents on histopathology for the AMA. Histological evaluation of the thyroid included, but was not limited to: thyroid gland hypertrophy/atrophy, follicular cell hypertrophy, follicular cell hyperplasia, and as additional qualitative criteria: follicular lumen area, colloid quality and follicular cell height/shape. Severity grading (4 grades) was reported in accordance with OECD Guidance Document on Amphibian Thyroid Histopathology (2007) and Grim et al (2009).

4. Statistics

Following enumeration of survival and morbidity data from the rangefinding study, the 96-hour LC_{50} and EC_{50} (morbidity) were determined using the trimmed Spearman-Karber method.

For all continuous quantitative endpoints (HLL, SVL, wet weight) that followed a monotonic concentration-response, the Jonckheere-Terpstra test was applied in step-down manner to establish significant treatment effects. For continuous endpoints that were not consistent with a monotonic concentration-response, the data was evaluated for normality (Shapiro-Wilk's test) and homogeneity (Levene's test). If a data set was found to have a non-normal distribution or a heterogeneous distribution of variance, a normalizing, variance stabilizing transformation was used. If data sets were normally distributed with homogeneous variance following transformation, the data sets were evaluated using Dunnett's test. If the data sets were normally distributed with heterogeneous variance following data transformation, the Tamhane-Dunnett, (T3 test) or the Mann-Whitney-Wilcoxon U test were used to evaluate the data. Where no normalizing transformation could be found, the Mann-Whitney-Wilcoxon U test using a Bonferroni-Holm adjustment to the p-values was used to evaluate the data sets.

A Cochran-Armitage test was applied if the data set had a consistent concentration-response. Alternatively, non-monotonic mortality data sets were evaluated using Fisher's Exact test with a Bonferroni-Holm adjustment. A significant treatment effect for developmental stage was determined on the replicate median values using the Jonckheere-Terpstra or Mann-Whitney U test. In the event median values could not be determined, replicate mean stage values were used and evaluated using Dunnett's test. Concentration-response monotonicity was assessed visually from the replicate and treatment medians or means. The statistical significance of all tests indicated was assessed at $p = 0.05$.

The No Observable Effect Concentration (NOEC) was defined as the highest measured concentration at or below which no statistically significant effect was observed. The Lowest Observed Effect Concentration (LOEC) was the lowest measured concentration at which a statistically significant effect occurred relative to the dilution water control.

II. RESULTS AND DISCUSSION

A. FINDINGS

Nominal oxamyl concentrations selected for the rangefinding study were 0.1, 0.5, 1.0, 5.0, 10.0, and 25.0 mg/L oxamyl. A dilution water control was used in this study. The corresponding time-weighted mean measured concentrations of oxamyl were 0.12, 0.54, 1.07, 5.46, 10.1, and 26.6 mg a.s./L. The dilution water control solutions showed no detectable levels (<0.03 mg a.s./L) of oxamyl. Rangefinding testing indicated that the 4-d LC_{50} value of oxamyl in NF stage 51 larvae was 2.42 mg a.s./L, and the EC_{50} (morbidity) was 0.25 mg a.s./L.

In the main study, time-weighted mean measured concentrations of oxamyl were 10.6, 39.9, and 130.1 µg/L. All test solutions were clear and colorless with no precipitate throughout the study. All chemical and physical parameters for the 21-day study were within acceptable ranges. The test system was maintained at 22.4-22.9°C and a pH of 6.8-

7.7.

Larval Mortality in Xenopus laevis

Treatment (µg a.i./L) [measured][1]	Larval Mortality					
	Day 7[2]			Day 21		
	n	Mortality #	Mortality %	n	Mortality #	Mortality %
0.0 [<LOD]	80	0	0	60	1	1.7
13 [10.6]	80	0	0	60	2	3.3
40 [39.9]	80	0	0	60	0	0
120 [130.1]	80	0	0	60	2	3.3

Oxamyl had no significant effect on the developmental stage obtained at test conclusion, hind limb length, occurrence of asynchronous development, treatment related histopathological effects on the thyroid, SVL, or whole body weight. Therefore, the NOEC for oxamyl was 130.1 µg/L based on mean measured time weighted average measured concentrations and AMA endpoints.

A summary of developmental and thyroid histopathology-related endpoints is presented in Table 30.

Table 30 Developmental and thyroid gross pathology/histopathology endpoints in the AMA with *Xenopus laevis*

Nominal concentration[a] (µg a.s./L) [measured]	NF developmental stage				Hind limb length[b]				Asynchronous development				Thyroid gross and histo-pathology
	Day 7		Day 21		Day 7		Day 21		Day 7		Day 21		Day 21
	Median	p	Median	p	Mean (mm)	p	Mean (mm)	p	% Diff.	p	% Diff.	p	Treatment-related effects? (Yes/No)[c,d]
0.0 (<LOD)[e]	54	NAf	64	NA	2.8	NA	20.4	NA	0	NA	0	NA	NA
13 (10.6)	54	>0.05	64	>0.05	3.0	>0.05	22.0	>0.05	0	NA	0	NA	No
40 (39.9)	54	>0.05	64	>0.05	2.8	>0.05	21.4	>0.05	0	NA	0	NA	No
120 (130.1)	54	>0.05	63.5	>0.05	2.9	>0.05	20.8	>0.05	0	NA	0	NA	No

[a] Time-weighted mean measured concentrations

[b] Hind-limb length is normalized to snout-vent length (SVL)

[c] Effects are reported based on comparison to the dilution water control. Conclusions regarding histopathology are based on the expert opinion of a board-certified pathologist.

[d] Effects are considered statistically significant at ($p<0.05$) as compared to dilution water control.

[e] LOD = 8.5 µg a.s./L

[f] Not applicable

A common diagnosis in this study was follicular cell hypertrophy (mild to moderatre). Two of the 20 stage-matched control specimens were found to have mild follicular cell hypertrophy. Follicular cell hypertrophy was recorded were, in >20% of the tissue: 1) the typically spherical follicular cell nuclei become oval or elongated, and/or 2) the heights of follicular cells were at least three times the diameter of their nuclei (approximately). Based on the stage control development reached at test conclusion (median stage 64), the response observed was not unexpected or atypical. The prevalence and severity of this finding were slightly increase in frogs of the 120

dose group as compared to controls, but the magnitude of the increased prevalence and severity was insufficient to conclude that this necessarily represented a treatment effect.

Table 2. Prevalence and Severity of Selected Histopathologic Findings in the Thyroid				
Oxamyl Dose Group (µg/L)	Control (0)	13	40	120
n	20	20	20	20
Follicular Cell Hypertrophy	2	2	3	5
mild	2	2	3	4
moderate	-	-	-	1

The only additional finding in this study was follicular hyperplasia (mild), which was diagnosed in a single frog in eazch of the 13 and 120 ug/L dose groups. Because this finding was only present in individual animals per group, and because a low degree of follicular cella hyperplasia has been documented to occur in control tadpoles used in AMA studies (Grim, 2007), this finding was not considered to be related to oxamyl exposure.

A summary of growth-related effects is presented in Table 31.

Table 31 Growth endpoints in the AMA with *Xenopus laevis*

Nominal concentration (µg a.s./L) [measured][a]	Snout-vent length				Body weight			
	Day 7		Day 21		Day 7		Day 21	
	Mean (mm)	p[b]	Mean (mm)	p[b]	Mean (g)	p[c]	Mean (g)	p[b]
0.0 (<LOD)	13.0	NA[d]	18.6	NA	0.246	0.0 (<LOD)	0.860	NA
13 (10.6)	12.6	>0.05	18.7	>0.05	0.235	>0.05	0.840	>0.05
40 (39.9)	12.7	>0.05	18.5	>0.05	0.239	>0.05	0.760	>0.05
120 (130.1)	12.6	>0.05	18.8	>0.05	0.236	>0.05	0.777	>0.05

[a] Time-weighted mean measured concentrations
[b] Ranked ANOVA; p = 0.05
[c] ANOVA; p = 0.05
[d] Not applicable

III. CONCLUSION

The NOEC for *X. laevis* exposed to oxamyl in a 21 day amphibian metamorphosis assay (AMA) was 130.1 µg a.s./L based on time-weighted mean measured concentrations and AMA endpoints. The 4-day LC_{50} was 2.42 mg a.s./L.

([redacted] 2015)

RMS comment:

The study was carried out according to OPPTS 890.1100 (2009). The design, procedure and endpoints match well the reccomendations in OECD TG 231: Amphibian Metamorphosis Assay (AMA).
The validity criteria relative to mortality in controls, Minimum median developmental stage of controls at end of test (57), Spread of development stage in control group, Dissolved Oxygen, pH, Water temperature, test concentrations without overt toxicity, replicate performance, are fulfilled. The concentrations are calculated as time-weighted mean measured concentrations.
The reported 4-day LC_{50} = 2.42 mg a.s./L refers to the range finding test. In the definitive test, mortality was up to 3.3%.

The conclusion of the histopatology report from [redacted], states that:
"*There were no histomorphologic effects that were substantially more prevalent in oxamyl treated frogs as compared to controls. There was a slight increase in the prevalence and severity of follicular cell hypertrophy*

in frogs in the 120 ug/L dose group compared to controls, but the magnitude of the increased prevalence and severity was insufficient to conclude that this necessarily represented a treatment effect."

The RMS concludes that based on the incidence and severity of the recorded follicular cell hypertrophy, a possible treatment related effect cannot be excluded.

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

B.9.2.3/02

Reference: **CA 8.2.3/02**	**Report:**	[redacted] (2012); Oxamyl technical (DPX-D1410): Short term reproduction assay with the fathead minnow, *Pimephales promelas*, determined under flow-through conditions **DuPont Report No.:** DuPont-31031 **Guidelines:** OPPTS 890.1350 (2009) **Deviations:** None **Testing Facility:** [redacted] **Testing Facility Report No.:** 66763 **GLP:** Yes **Certifying Authority:** Laboratories in the USA are not certified by any government agency, but are subject to regular inspections.

Executive summary:

The short-term fish reproduction assay employed in this study is a Tier 1 assay in the U.S. EPA's Endocrine Disruptor Screening Program's (EDSP) 2-tiered approach to implement the statutory testing requirements of FFDCA section 408(p) (21 U.S.C. 346a). This assay is intended to be used in conjunction with other assays in the OPPTS 890 test guideline series that comprise the full Tier 1 screening battery under the EDSP.

A flow-through test with the fathead minnow (*Pimephales promelas*) exposed to a control and three sublethal concentrations (*i.e.*, nominal 0.090, 0.30, and 1.0 mg a.s./L) of pure oxamyl (PAI; oxamyl) was conducted to assess the potential effects of the test substance on fish reproduction, secondary sexual characteristics, and plasma vitellogenin (VTG) concentrations. Gonadal histopathology was performed and the findings from these evaluations were used to assess the biological significance of the quantifiable endpoints (*i.e.*, reproduction, secondary sexual characteristic as tubercle score, and VTG concentrations).

The study consisted of two phases; a pre-exposure phase and a chemical-exposure phase. During the pre-exposure phase (*i.e.*, without test substance), a total of 30 spawning groups of 5.5-month old adult fathead minnow were assessed for a 14-day period. Each spawning group was comprised of four females and two males at the start of the pre-exposure phase. The wet weight of each individual fish at the start of the pre-exposure phase was ± 20% of the arithmetic mean wet weight of the same gender fish in all treatment groups. Fecundity data was collected daily and at the end of the pre-exposure phase the spawning groups that demonstrated a regular spawning pattern (*i.e.*, spawning occurring every 3 to 4 days) and exceeded 15 eggs/female/day were randomly assigned to a control or chemical-exposure chamber at the conclusion of the pre-exposure phase.

The randomly assigned spawning groups were exposed to oxamyl for three weeks to assess impacts on survival and reproductive performance (number of spawns, number of eggs produced, number of eggs per spawn and percent fertilization). At the end of the chemical-exposure phase, the exposed fish were weighed (*i.e.*, blotted wet weight) and then sampled for plasma vitellogenin (VTG), gonad size determinations expressed as gonado-somatic index (GSI) and histological endpoints (gonad histopathology) to evaluate effects potentially associated with endocrine disrupting chemicals. The control (dilution water without chemical) and each of the three test substance treatments were replicated four times (*i.e.*, replicates A-D) and were evaluated concurrently.

Test solutions were supplied to the test chambers through an intermittent-flow diluter system. Test solutions were delivered to replicate test chambers at a rate approximately equal to 100 L/replicate/day (10 volume

additions per day). The mean measured concentrations of oxamyl during the 21-day chemical exposure period were 0.0866, 0.305, and 0.989 mg a.s./L and ranged from 96 to 102% of the nominal concentrations.

Adult survival was not statistically different from control in any of the test substance concentrations following 21 days of exposure to oxamyl. Mean percent survival values were 96, 92, 96, and 88% in the control, 0.0866, 0.305, and 0.989 mg a.s./L treatments, respectively.

A statistically significant decrease in mean wet weight of females was observed at 0.989 mg a.s./L. The mean wet weight of the males at termination was not statistically different from control at any test substance concentration.

The mean cumulative fecundity, mean number of spawns, mean number of eggs per female per day, and percent fertility were not statistically different from control at any test substance concentration. The tubercle scores and size of the gonads, expressed as GSI, for both males and females were not statistically different from control at any test substance concentration. The mean measured plasma VTG concentrations in both males and females at termination were not statistically different from control at any test substance concentration.

There were no histological findings in either the evaluated testes or ovaries that were related to oxamyl administration. Findings were generally present in comparable numbers of control and oxamyl-treated fish and/or were anticipated background-type changes (*e.g.*, mineralization of the testis or collecting ducts in males). The study NOEC based on the endocrine-relevant endpoints (*i.e.*, reproductive parameters, VTG, GSI, gonad histopathology) was 0.989 mg a.s./L. The overall study NOEC based on the most sensitive endpoint, female wet weight, was 0.305 mg a.s./L

The overall study NOEC based on the most sensitive endpoint, female wet weight, was 0.305 mg a.s./L.

MATERIALS AND METHODS

A. MATERIALS

1.	Test material:	Pure Oxamyl (PAI)
	Lot/Batch #:	D1410-196
	Purity:	98.0% by analysis
	Description:	White crystalline solid
	CAS#:	23135-22-0
	Stability of test compound:	Stable in the test system
2.	Control:	Dilution (laboratory blended water) water
	Solvent control:	None
	Test vehicle:	Dilution (laboratory blended water) water
	Toxic reference:	None
3.	Test organism:	Fathead minnow
	Species:	*Pimephales promelas*
	Age at dosing:	5.5 to 6 months
	Initial population:	2 males and 4 females per replicate (8 males and 16 females per treatment)
	Source:	In-house culture
	Diet:	Frozen adult brine shrimp (*Artemia* sp.) and live brine shrimp (*Artemia* sp.) nauplii *ad libitum* three times daily. Food was withheld at least 12 hours prior to termination of the chemical exposure phase.
	Test chamber:	Glass aquaria measuring approximately 22 cm wide × 38 cm long × 22 cm high with a test solution depth of 12 cm
4.	Environmental conditions (in-life period)	
	Temperature:	24.3 to 25.4°C for pre-exposure 24.6 to 25.1°C for oxamyl exposure
	Dissolved Oxygen (mg/L)	4.84 to 8.38 for oxamyl exposure
	Photoperiod:	16 hr photoperiod (520.7 to 797.8 lux) and 8 hr darkness that included 30 min transitional light preceding and following the 16-hr light interval

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed (exposure test)

31-May-2011 to 21-June-2011

2. Experimental treatments

A flow-through test with the fathead minnow (*Pimephales promelas*) exposed to a control and three sublethal concentrations (*i.e.*, nominal 0.090, 0.30, and 1.0 mg a.s./L) of oxamyl was conducted to assess the potential effects of the test substance on fish reproduction, secondary sexual characteristics, and plasma vitellogenin (VTG) concentrations. Test solutions were delivered to 4 replicate test chambers per treatment at a rate approximately equal to 100 L/replicate/day (10 volume additions per day). Gonadal histopathology was performed and the findings from these evaluations were used to assess the biological significance of the quantifiable endpoints (*i.e.*, reproduction, secondary sexual characteristic as tubercle score, and VTG concentrations).

3. Observations

Mortality, physical appearance, and behavioural observations were made daily throughout the exposure.

4. Statistics

Descriptive statistics, including the mean, standard deviation, minimum, maximum, and quartiles, were determined for each endpoint measured in the tests. Experimental units, on which observations or measurements were made, were the replicated test chambers. Measurements of growth (*i.e.*, weight) and VTG were made on the organisms; however, the organisms were not treated as the experimental unit and rather treated as subsamples measured within the experimental unit. Growth and VTG analyses were gender specific. All statistical analyses were performed using SAS software. Inferences of statistical significance were based upon a $p = 0.05$ unless otherwise noted. Chemical dosing regimens were considered classifications of fixed effects (*i.e.*, control and three test substance treatments).

The no-observed-effect-concentration (NOEC) for survival was determined by using a one-tailed Dunnett's test and a Fisher's exact test with the alternate hypothesis being the mean for the parameter was reduced in comparison to the control mean. A Hochberg adjustment was used to control the experiment-wise error rate for the Fisher's test at the same alpha level. The NOEC for blotted wet weight, GSI, tubercle score, and VTG, as well as the reproductive endpoints was determined using an ANOVA procedure and either a one-tailed or two-tailed Dunnett's test. A one-tailed Dunnett's test was performed when all test substance treatments were either greater or less than the control with the alternate hypothesis being the mean for the parameter was increased or reduced in comparison to the control mean. A two-tailed Dunnett's test was performed when there were test substance treatments greater and less than the control with the alternate hypothesis being the mean for the parameter was not equal to the control mean. 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. When the results from the Shapiro-Wilk's and Levene's tests indicated normality ($p > 0.01$) and homogeneity of variance ($p > 0.01$; equal treatment variances), a parametric analysis was performed using the non-transformed data. When the results from the Shapiro-Wilk's and Levene's tests indicated non-normality ($p < 0.01$) and/or unequal treatment variances ($p < 0.01$), a non-parametric analysis was performed on the ranks of the data.

II RESULTS AND DISCUSSION

A. FINDINGS

Measured Oxamyl Concentration as mg a.s./L (Percent of Nominal Concentration)

Study Day	Control	Level 1 (0.090) [a]	Level 2 (0.30) [a]	Level 3 (1.0) [a]	Diluter Stock (200) [a]
-N	<LOD	0.0849 (94)	0.304 (101)	0.914 (91)	198 (99)
0	<LOD	0.0812 (90)	0.282 (94)	0.886 (89)	237 (119)
7	<LOD	0.0876 (97)	0.310 (103)	1.09 (109)	211 (106)
14	<LOD	0.0865 (96)	0.312 (104)	0.970 (97)	203 (102)
21	<LOD	0.0910 (101)	0.315 (105)	1.01 (101)	211 (106)
Mean	<LOD	0.0866 (96)	0.305 (102)	0.989 (99)	216 (108)

LOD: Limit of Detection = 0.0085 mg a.s./L.
[a] Target nominal concentration of oxamyl in mg a.s./L.

Adult survival was not adversely affected in any of the test treatments following 21 days of exposure to oxamyl. Mean percent survival values were 96, 92, 96, and 88% in the control, 0.0866, 0.305, and 0.989 mg a.s./L treatments, respectively.

A statistically significant decrease in mean wet weight of females was observed at 0.989 mg a.s./L. The mean wet weight of the males at termination was not statistically different from control at any test substance concentration.

The mean cumulative fecundity, mean number of spawns, mean number of eggs per female per day, and percent fertility were not statistically different from control at any test substance concentration. The tubercle scores and size of the gonads, expressed as GSI, for both males and females were not statistically different from control at any test substance concentration. The mean measured plasma VTG concentrations in both males and females at termination were not statistically different from control at any test substance concentration.

There were no histological findings in either the evaluated testes or ovaries that were related to oxamyl administration. Findings were generally present in comparable numbers of control and oxamyl-treated fish and/or were anticipated background-type changes (*e.g.*, mineralization of the testis or collecting ducts in males).

Prevalence and severity of histopathologic findings in male fathead minnows

Oxamyl Mean Measured Concentration (mg a.s./L)	0	0.0866	0.305	0.989
Number Examined	7	7	8	7
Testicular Stage Scores				
Stage 1.5	1	2	2	2
Stage 2.0	3	3	1	2
Stage 2.5	2	2	2	2
Stage 3.0	-	-	2	1
Stage 3.5	1	-	1	-
Mean Score	2.3	2.0	2.4	2.1

Prevalence and severity of histopathologic findings in female fathead minnows

Oxamyl Mean Measured Concentration (mg a.s./L)	0	0.0866	0.305	0.989
Number Examined	16	15	15	14
Ovarian Stage Scores				
Stage 1.0	1	-	-	-
Stage 2.0	1	1	2	1
Stage 2.5	-	1	1	-
Stage 3.0	4	5	6	7
Stage 3.5	9	6	4	4
Stage 4.0	1	2	2	2
Mean Score	3.2	3.2	3.1	3.2

The study NOEC based on the endocrine-relevant endpoints (*i.e.*, reproductive parameters, VTG, GSI, gonad histopathology) was 0.989 mg a.s./L. The overall study NOEC based on the most sensitive endpoint, female wet weight, was 0.305 mg a.s./L.

The results of the analyses of all biological endpoints evaluated during the study are presented in the following table for comparative purposes.

Table 32 Results of the analyses of all biological endpoints evaluated during the study

Endpoint	Control mean	0.0866 mg a.s./L treatment mean	0.305 mg a.s./L treatment mean	0.989 mg a.s./L treatment mean
Adult survival	96%	92%	96%	88%
Female weight	1.828 g	1.673 g	1.710 g	1.585 g *
Male weight	3.943 g	3.861 g	4.098 g	4.571 g
Female tubercle score	0	0	0	0
Male tubercle score	32	33	37	33
Cumulative number of eggs	4322	4020	4813	3819
Number of spawns	17	19	18	18
Number of eggs/female/day	51	48	61	47
Percent fertility	91%	90%	87%	92%
Female GSI	14	13	14	15
Male GSI	1.03	1.09	1.17	1.34
Female VTG	5546 µg/mL	5543 µg/mL	5535 µg/mL	5183 µg/mL
Male VTG	0.70 µg/mL	1.52 µg/mL	5.32 µg/mL	1.00 µg/mL

[a] Statistically determined to be significantly less than the control (Dunnett's Test; $p = 0.05$).

III. CONCLUSIONS

Adult survival was not adversely affected following 21 days of exposure to oxamyl at any treatment level. Mean percent survival values were 96, 92, 96, and 88% in the control, 0.0866, 0.305, and 0.989 mg a.s./L treatments, respectively.

A statistically significant decrease in mean wet weight of females was observed at 0.989 mg a.s./L. The mean wet weight of the males at termination was not adversely affected in any test substance concentration.

The mean cumulative fecundity, mean number of spawns, mean number of eggs per female per day, and percent fertility were not statistically different from control at any test substance concentration. The tubercle scores and size of the gonads, expressed as GSI, for both males and females were not statistically different from control at any test substance concentration. The mean measured plasma VTG concentrations in both males and females at termination were not statistically different from control at any test substance concentration.

There were no histological findings in either the evaluated testes or ovaries that were related to oxamyl administration. Findings were generally present in comparable numbers of control and oxamyl-treated fish and/or were anticipated background-type changes (*e.g.*, mineralization of the testis or collecting ducts in males).

The study NOEC based on the endocrine-relevant endpoints (*i.e.*, reproductive parameters, VTG, GSI, gonad histopathology) was 0.989 mg a.s./L. The overall study NOEC based on the most sensitive endpoint, female wet weight, was 0.305 mg a.s./L.

([redacted] 2012)

RMS comments and conclusion

The study conducted according to OPPTS 890.1350 (2009) includes also gonadal histology. The study design, conduct and endpoints are in line with the 21d fish short term reproduction assay (OECD 229).

The test is valid and acceptable. The results are expressed as mean measured concentrations.

B.9.2.4 Acute toxicity to aquatic invertebrates

B.9.2.4.1 Acute toxicity to *Daphnia magna*

Chemical active

Study submitted in the EU Dossier in 2001 and included in the first EU approval review.

B.9.2.4.1/01

Reference: --	**Report:**	Boeri, R.L., Magazu, J.P., Ward, T.J. (1999b); Oxamyl technical: Acute, 48-hour EC_{50} to *Daphnia magna* **DuPont Report No.:** DuPont-2553 **Guidelines:** EEC Method C.2. (1992), OECD 202 (1984), U.S. EPA 72-2 (1988)

1. Test material: Pure Oxamyl (PAI)
   Lot/Batch #: D1410-196
   Purity: 96.9% by analysis

Materials and methods:

The acute toxicity of Oxamyl to unfed *Daphnia magna* neonates (<24-hour old) was determined in an unaerated, static-renewal, 48-hour test. Treatments consisted of a dilution water control, and nominal concentrations of 0.26, 0.43, 0.72, 1.2, and 2.0 mg a.s./L. The dilution water control met OECD and ASTM dilution water criteria and specifications and contained no solvent. Ten daphnids were used per replicate with two replicates per test concentration and control. *Daphnia magna* (first instar, <24 hours old, collected 19 days after the first appearance of neonates in the parent culture) were exposed to ZA1963 in a static system for 48 hours at 20 ±1°C. Glass beakers (300-mL) containing 200 mL of test solution were used as test chambers (two replicate chambers per concentration). A photoperiod of 16 hours light (approximately 490 LUX) and 8 hours darkness was employed which included approximately 15 minutes of transitional light preceding and following the 16-hour light interval.

Actual concentrations of oxamyl to which the Daphnia were exposed were determined by chemical analysis at 0 and 48 hours using a Hewlett Packard Series 1100 HPLC equipped with a UV detector. Daphnia were assessed for effects in terms of signs of toxicity and immobility at 0, 24 and 48 hours. Water temperature, pH and dissolved oxygen were monitored as required during the test.

The binomial method was used to calculate 24 hour EC50 and 95% confidence limits, and the probit method was used to calculate the 48 hour EC50 and 95% confidence limits.

Findings:

During the test, water temperature was in the range 19.2 – 19.9°C, dissolved oxygen ranged between 8.5 and 8.7 mg/l and pH was between 7.4 and 7.6. Conductivity ranged from 520 – 600 µmhos/cm during the study. Total alkalinity and EDTA hardness of the dilution water control were 63 and 168 mg/l as CaCO3 mg/l.

Mean, measured concentrations of Oxamyl were 0.227, 0.379, 0.634, 0.991, and 1.70 mg a.s./L and ranged from 83 to 88% of nominal concentrations. During the 48h test, the test solution concentrations were maintained within 20% of nominals. Immobility was observed at all concentrations and the lowest concentration causing 100% immobility was 0.634 mg a.s./L. Immobile *Daphnia magna* were observed in test vessels containing 0.227, 0.379, 0.634, 0.991, and 1.70 mg a.s./L Oxamyl at 24 and 48 hours.

Immobility and Sublethal Effects in *Daphnia magna* at 24 and 48 Hours in an Unaerated Static Renewal, Acute Toxicity (EC_{50}) Test with Oxamyl

Mean, Measured Oxamyl Concentration (mg/L)	Immobility (%)[a,b]		
	0 Hours	24 Hours	48 Hours
Water Control A[†]	0	0	0
Water Control B[†]	0	0	0
0.227 A[†]	0	0	10
0.227 B[†]	0	0	0
0.379 A[†]	0	0	70
0.379 B[†]	0	0	90
0.634 A[†]	0	70	100
0.634 B[†]	0	100	100
0.991 A[†]	0	100	100
0.991 B[†]	0	100	100
1.70 A[†]	0	100	100
1.70 B[†]	0	100	100

[†] A and B represent replicates; each replicate contained 10 daphnids (total 20 daphnids per test concentration) at test start.
[a] There were no sublethal effects other than immobility observed at any time.
[b] Immobility is defined as the inability of a daphnid to swim within 15 seconds of gentle agitation of the test vessel

A summary of the findings is presented in Table 33.

Table 33 Smmary of observed immobility of unfed *Daphnia magna* exposed to Oxamyl for 48 hours in an unaerated, static-renewal, acute test

Test substance	TG a.i.
Test object	*Daphnia magna*
Exposure	48 h, static
24 EC_{50} mg a.i./l	0.529 (0.379 – 0.634)
48 EC_{50} mg a.i./l	0.319 (0.282 – 0.357)
Lowest observed effect concentration (LOEC) mg a.i./l	0.227
Highest tested conc. without toxic effect (NOEC) mg a.i./l	0.0

Conclusion:

The 48-hour $EC_{50}$of Oxamyl to *Daphnia magna* was 0.319 mg a.s./L (c.i., 0.282 to 0.357 mg a.s./L), based on mean measured concentrations.

The acute toxicity to *Daphnia magna* study DuPont-2553, originally submitted under EU Rev8 Point IIA 8.2.4 and conducted with test material pure oxamyl (PAI), was conducted under guidelines EEC Method C.2. (1992), OECD 202 (1984), and U.S. EPA 72-2 (1988). A review of this study indicates that it fully meets the current guideline OECD 202 (2004). Therefore this study is relied upon.

RMS comments and conclusion

The acute toxicity to *Daphnia magna* study DuPont-2553, originally submitted under EU Rev8 Point IIA 8.2.4 and conducted with test material pure oxamyl (PAI), was conducted under guidelines EEC Method C.2. (1992), OECD 202 (1984), and U.S. EPA 72-2 (1988). A review of this study indicates that it fully meets the current guideline OECD 202 (2004), although no mention is made to a reference toxicant. The RMS added additional information to the study summary.

The validity criteria are met:

- No more than 10% immobilisation or other sign of disease/stress in the control (actual 0%);
- DO at the end of test ≥ 3 mg/L in the control and test vessels (actual 8..7 in all test vessels after 48 h or at test termination).

Conclusion: the study is acceptable and relied upon.

Metabolites

Study submitted in the EU Dossier in 2001 and included in the first EU approval review.

B.9.2.4.1/02

Reference: --	**Report:**	Ward, T.J., Magazu, J.P., Boeri, R.L. (1999a); IN-A2213: Static, acute, 48-hour limit test to *Daphnia magna* **DuPont Report No.:** DuPont-2502 **Guidelines:** EEC Method C.2. (1992), OECD 202 (1984), U.S. EPA 72-2 (1988)

1. Test material: IN-A2213 technical metabolite
   Lot/Batch #: A2213-11
   Purity: 100%

Materials and methods:

The acute toxicity of IN-A2213 to unfed *Daphnia magna* neonates (<24-hour old and collected 18 days after the first appearance of neonates in the parent culture) was determined in an unaerated, static, 48-hour test. Treatments consisted of a dilution water control and one nominal concentration of 130 mg/L IN-A2213. The dilution water control met OECD and ASTM dilution water criteria and specifications and contained no solvent. Ten daphnids were used per replicate with three replicates per test concentration and control. Glass beakers (300-mL) containing 200 mL of test solution were used as test chambers (three replicate chambers per concentration). A photoperiod of 16 hours light (approximately 490 LUX) and 8 hours darkness was employed which included approximately 15 minutes of transitional light preceding and following the 16-hour light interval. Actual concentrations of oxamyl to which the Daphnia were exposed were determined by chemical analysis at 0 and 48 hours using a Hewlett Packard Series 1100 HPLC equipped with a UV detector. Daphnia were assessed for effects in terms of signs of toxicity and immobility at 0, 24 and 48 hours. Water temperature, pH and dissolved oxygen were monitored as required during the test.

Findings:

During the test, water temperature was in the range 19.5 – 20.4°C, dissolved oxygen ranged between 8.4 and 8.8 mg/l and pH was between 7.3 and 7.8. Conductivity ranged from 570 – 600 µmhos/cm during the study. Total alkalinity and EDTA hardness of the dilution water control were 101 and 176 mg/l as CaCO3 mg/l respectively.

The mean, measured concentration of IN-A2213 was 125 mg/L. This concentration was 96% of the nominal concentration. During the 48h test, the test solution concentrations were maintained within 20% of nominals. No sublethal effects were observed during the period of the definitive study.

Immobility and Abnormal Effects in *Daphnia magna* at 24 and 48 Hours in an Unaerated Static, Acute Limit Test with IN-A2213

Mean, Measured IN-A2213 Concentration (mg/L)	Immobility (%)		
	0 Hours	24 Hours	48 Hours
Water Control A†	0	0	0
Water Control B†	0	10	10
Water Control C†	0	0	10
125 A†	0	0	0
125 B†	0	0	0
125 C†	0	0	0[a]

† A, B, and C represent replicates; each replicate contained 10 daphnids (total 30 daphnids per test concentration) at test start.
[a] One surviving daphnid was observed to be floating at 48 hours.

A summary of the findings is presented in Table 35.

Table 34 Summary of observed immobility of unfed *Daphnia magna* exposed to IN- A2213 for 48 hours in an unaerated, static, acute test

Test substance	Metabolite IN-A2213
Test object	*Daphnia magna*
Exposure	48 h, static
48 EC_{50} mg a.i./l	>125
Lowest observed effect concentration (LOEC) mg a.i./l	>125
Highest tested conc. without toxic effect (NOEC) mg a.i./l	125

Conclusion:

The 48-hour $EC_{50}$for IN-A2213 to *Daphnia magna* was >125 mg/L, based on mean measured concentrations.

RMS comments and conclusion

The acute toxicity to *Daphnia magna* study DuPont-2502, originally submitted under EU Rev8 Point IIA 8.2.4 and conducted with test material IN-A2213 technical metabolite, was conducted under guidelines EEC Method

C.2. (1992), OECD 202 (1984), and U.S. EPA 72-2 (1988). A review of this study according the current guideline OECD 202 (2004) indicates that the test design (30 daphnids divided in 3 replicates) is somewhat different from the recommended one (20 daphnids preferably divided in 3 replicates). This deviation is not considered to affect the reliability of the results. The RMS added additional information to the study summary.

The validity criteria are met:

- No more than 10% immobilisation or other sign of disease/stress in the control (actual 7% in the control and 3% in the test item concentration);

- DO at the end of test ≥ 3 mg/L in the control and test vessels (actual 8.7 in all test vessels after 48 h or at test termination).

Conclusion: the study is acceptable and relied upon.

Study submitted in the EU Dossier in 2001 and included in the first EU approval review

B.9.2.4.1/03

Reference: --	**Report:**	Boeri, R.L., Magazu, J.P., Ward, T.J. (1999a); IN-D2708: Static, acute, 48-hour limit test to *Daphnia magna* **DuPont Report No.:** DuPont-2510 **Guidelines:** EEC Method C.2. (1992), OECD 202 (1984), U.S. EPA 72-2 (1988)

1. Test material: IN-D2708 technical metabolite
   Lot/Batch #: D2708-6
   Purity: 99.87%

Materials and methods:

The acute toxicity of IN-D2708 to unfed *Daphnia magna* neonates (<24-hour old and collected 20 days after the first appearance of neonates in the parent culture) was determined in an unaerated, static, 48-hour test. Treatments consisted of a dilution water control and one nominal concentration of 130 mg/L IN-D2708. The dilution water control met OECD and ASTM dilution water criteria and specifications and contained no solvent. Ten daphnids were used per replicate with three replicates per test concentration and control. Glass beakers (300-mL) containing 250 mL of test solution were used as test chambers (three replicate chambers per concentration). A photoperiod of 16 hours light (approximately 490 LUX) and 8 hours darkness was employed which included approximately 15 minutes of transitional light preceding and following the 16-hour light interval. Actual concentrations of oxamyl to which the Daphnia were exposed were determined by chemical analysis at 0 and 48 hours using a Hewlett Packard Series 1100 HPLC equipped with a UV detector. Daphnia were assessed for effects in terms of signs of toxicity and immobility at 0, 24 and 48 hours. Water temperature, pH and dissolved oxygen were monitored as required during the test.

Findings:

The mean, measured concentration of IN-D2708 was 134 mg/L. This concentration was 103% of the nominal concentration. During the 48h test, the test solution concentrations were maintained within 20% of nominals. No abnormal effects were seen in surviving daphnids at 134 mg/L at the end of 48 hours, but three of the 30 surviving daphnids were floating on the surface at 24 hours (no floating daphnids were observed at 48 hours).No immobility or abnormal effects were seen in the dilution water control and no control daphnids were floating. During the test, water temperature was in the range 19.6 – 21.0°C, dissolved oxygen ranged between 7.5 and 8.5 mg/l and pH was between 6.3 and 7.7. Conductivity was 610 µmhos/cm during the study. Total alkalinity and EDTA hardness of the dilution water control were 92 and 180 mg/l as CaCO3 mg/l resepectively.

Immobility and Abnormal Effects in *Daphnia magna* at 24 and 48 Hours in an Unaerated Stat
Acute Limit Test with IN-D2708

Mean, Measured IN-D2708 Concentration (mg/L)	Immobility (%)		
	0 Hours	24 Hours	48 Hours
Water Control A†	0	0	0
Water Control B†	0	0	0
Water Control C†	0	0	0
134 A†	0	0	0
134 B†	0	0	0
134 C†	0	0	0

† A, B, and C represent replicates; each replicate contained 10 daphnids (total 30 daphnids per test concentration) at test start.

A summary of the findings is presented in Table 36.

Table 35 Summary of observed immobility of unfed *Daphnia magna* exposed to IN-D2708 for 48 hours in an unaerated, static, acute test

Test substance	Metabolite IN-D2708
Test object	*Daphnia magna*
Exposure	48 h, static
48 EC_{50} mg a.i./l	>134
Lowest observed effect concentration (LOEC) mg a.i./l	>134
Highest tested conc. without toxic effect (NOEC) mg a.i./l	134

Conclusion:

The 48-hour $EC_{50}$for IN-D2708 to *Daphnia magna* was >134 mg/L, based on mean measured concentrations.

RMS comments and conclusion

The acute toxicity to *Daphnia magna* study DuPont-2502, originally submitted under EU Rev8 Point IIA 8.2.4 and conducted with test material IN-A2213 technical metabolite, was conducted under guidelines EEC Method C.2. (1992), OECD 202 (1984), and U.S. EPA 72-2 (1988). A review of this study according the current guideline OECD 202 (2004) indicates that the test design (30 daphnids divided in 3 replicates) is somewhat different from the recommended one (20 daphnids preferably divided in 3 replicates). This deviation is not considered to affect the reliability of the results. The RMS added additional information to the study summary.

The validity criteria are met:

- No more than 10% immobilisation or other sign of disease/stress in the control (actual 7% trapped daphnids at 24h only in the test item concentration);

- DO at the end of test ≥ 3 mg/L in the control and test vessels (actual 7.5 as minimum).

Conclusion: the study is acceptable and relied upon.

Study submitted in the EU Dossier in 2001 and included in the first EU approval review.

B.9.2.4.1/04

Reference: --	**Report:**	Ward, T.J., Magazu, J.P., Boeri, R.L. (1999b); IN-N0079: Acute, static, 48-hour toxicity (EC_{50}) test to *Daphnia magna* **DuPont Report No.:** DuPont-2513 **Guidelines:** EEC Method C.2. (1992), OECD 202 (1984), U.S. EPA 72-2 (1988)

1. Test material: IN-N0079 technical metabolite
   Lot/Batch #: N0079-8
   Purity: 99.57%

Materials and methods:

The acute toxicity of IN-N0079 to unfed *Daphnia magna* neonates (<24-hour old and collected 25 days after the first appearance of neonates in the parent culture) was determined in an unaerated, static, 48-hour test. Treatments consisted of a dilution water control, and nominal concentrations of 17, 28, 47, 78, and 130 mg/L IN-N0079. The dilution water control met OECD and ASTM dilution water criteria and specifications and contained no solvent. Ten daphnids were used per replicate with two replicates per test concentration and control. Glass beakers (300-mL) containing 200 mL of test solution were used as test chambers. A photoperiod of 16 hours light (approximately 490 LUX) and 8 hours darkness was employed which included approximately 15 minutes of transitional light preceding and following the 16-hour light interval. Actual concentrations of oxamyl to which the Daphnia were exposed were determined by chemical analysis at 0 and 48 hours using a Hewlett Packard Series 1100 HPLC equipped with a UV detector. Daphnia were assessed for effects in terms of signs of toxicity and immobility at 0, 24 and 48 hours. Water temperature, pH and dissolved oxygen were monitored as required during the test. The 24- and 48-hour EC50s and their 95% confidence intervals could not be calculated because >50% survival of mobile daphnids occurred at all tested concentrations.

Findings:

During the test, water temperature was in the range 19.0 – 20.6°C, dissolved oxygen ranged between 8.5 and 8.7 mg/l and pH was between 7.2 and 7.6. Conductivity ranged from 550 - 570 µmhos/cm during the study. Total alkalinity and EDTA hardness of the dilution water control were 70 and 168 mg/l as CaCO3 mg/l respectively.

Temperature (°C) of IN-N0079 Test Solutions

Mean, Measured IN-N0079 Concentration (mg/L)	0 Hours	24 Hours	48 Hours
Water Control A†	20.6	19.0	19.7
Water Control B†	20.6	19.0	19.8
16.8 A†	20.6	19.0	19.6
16.8 B†	20.6	19.0	19.6
28.2 A†	20.5	19.1	19.8
28.2 B†	20.5	19.1	19.8
50.6 A†	20.5	19.0	19.7
50.6 B†	20.5	19.1	19.7
76.9 A†	20.5	19.1	19.7
76.9 B†	20.5	19.1	19.7
128 A†	20.2	19.0	19.8
128 B†	20.2	19.1	19.8

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

Mean, measured concentrations of IN-N0079 were 16.8, 28.2, 50.6, 76.9, and 128 mg/L and ranged from 98 to 108% of nominal concentrations. During the 48h test, the test solution concentrations were maintained within 20% of nominals. No immobility was observed below 50.6 mg/L IN-N0079 and total immobility was not reached at any test concentration. Immobile *Daphnia magna* were observed in test vessels containing 50.6, 76.9, and 128 mg/L IN-N0079 at 24 and 48 hours.

Immobility and Sublethal Effects in *Daphnia magna* at 24 and 48 Hours in an Unaerated Static, Acute Toxicity (EC_{50}) Test with IN-N0079

Mean, Measured IN-N0079 Concentration (mg/L)	Immobility (%)[a]		
	0 Hours	24 Hours	48 Hours
Water Control A[†]	0	0	0
Water Control B[†]	0	0	0
16.8 A[†]	0	0	0
16.8 B[†]	0	0	0
28.2 A[†]	0	0	0
28.2 B[†]	0	0	0
50.6 A[†]	0	10	10
50.6 B[†]	0	0	0
76.9 A[†]	0	0	0
76.9 B[†]	0	0	10
128 A[†]	0	0	30
128 B[†]	0	0	40

[†] A and B represent replicates; each replicate contained 10 daphnids (total 20 daphnids per test concentration) at test start.
[a] There were no sublethal effects other than immobility observed at any time.

A summary of the findings is presented in Table 37.

Table 36 Summary of observed immobility of unfed *Daphnia magna* exposed to IN- N0079 for 48 hours in an unaerated, static, acute test

Test substance	Metabolite IN-N0079
Test object	*Daphnia magna*
Exposure	48 h, static
48 EC_{50} mg a.i./l	>128
Lowest observed effect concentration (LOEC) mg a.i./l	50.6
Highest tested conc. without toxic effect (NOEC) mg a.i./l	28.2

Conclusion:

The 48-hour $EC_{50}$for IN-N0079 to *Daphnia magna* was >128 mg/L, based on mean measured concentrations.

RMS comments and conclusion

The acute toxicity to Daphnia magna study DuPont-2553, originally submitted under EU Rev8 Point IIA 8.2.4 and conducted with test material pure oxamyl (PAI), was conducted under guidelines EEC Method C.2. (1992), OECD 202 (1984), and U.S. EPA 72-2 (1988). The RMS added additional information to the study summary.

A review of this study indicates that the validity criteria are met:
- No more than 10% immobilisation or other sign of disease/stress in the control (actual 0%);
- DO at the end of test ≥ 3 mg/L in the control and test vessels (actual 8.5 as a minimum)

A deviation was noted for the temperature during the test, which was not maintained within ±1°C (actual deviation – 1.6°C).

Conclusion**: considering the low toxicity of the substance and that the endpoint is unbounded (>128 mg a.i./l), the deviation in temperature is not considered severe enough to reject the study. In addition the risk assessment produced a very high TER. The study is considered acceptable and relied upon. Study submitted in the EU Dossier in 2001 and included in the first EU approval review.**

B.9.2.4.1/05

Reference: --	**Report:**	Boeri, R.L., Ward, T.J. (2000); IN-T2921: Acute, 48-hour, EC_{50} to *Daphnia magna* **DuPont Report No.:** DuPont-4441 **Guidelines:** OECD 202 (1984), EEC Method C.2. (1992), U.S. EPA 72-2 (1988)

1. Test material: IN-T2921 technical metabolite
   Lot/Batch #: T2921-2
   Purity: 98.7%

Materials and methods:

The acute toxicity of IN-T2921 to unfed *Daphnia magna* (<24-hour old and collected 3 days after the first appearance of neonates in the parent culture) was determined in an unaerated, static, 48-hour limit test. Treatments consisted of a dilution water control and a single nominal concentration of 120 mg IN-T2921/L. The dilution water control met OECD and ASTM dilution water criteria and specifications and contained no solvent. Ten daphnids were used per replicate with three replicates per single test concentration and control. Glass beakers (300-mL) containing 250 mL of test solution were used as test chambers. A photoperiod of 16 hours light (approximately 490 LUX) and 8 hours darkness was employed which included approximately 15 minutes of transitional light preceding and following the 16-hour light interval. Actual concentrations of oxamyl to which the Daphnia were exposed were determined by chemical analysis at 0 and 48 hours using a Hewlett Packard Series 1100 HPLC equipped with a UV detector. Daphnia were assessed for effects in terms of signs of toxicity and immobility at 0, 24 and 48 hours. Water temperature, pH and dissolved oxygen were monitored as required during the test.

Findings:

No immobility nor abnormal effects were seen in daphnids at 134 mg/L and in the controls. A summary of the findings is presented in Table 37. During the 48h test, the test solution concentrations were maintained within 20% of nominals. The mean, measured concentration of IN-T2921 was 123 mg/L. There was no test concentration-related immobility or sublethal effects at the single tested concentration.

During the test, water temperature was in the range 20.3 – 20.6°C, dissolved oxygen ranged between 8.3 and 9.0 mg/l and pH was between 7.3 and 7.5. Conductivity was 570 µmhos/cm during the study. Total alkalinity and EDTA hardness of the dilution water control were 61 and 180 mg/l as CaCO3 mg/l respectively.

Table 37 Summary of observed immobility of unfed *Daphnia magna* exposed to IN- T2921 for 48 hours in an unaerated, static, acute test

Test substance	Metabolite IN-T2921
Test object	*Daphnia magna*
Exposure	48 h, static
48 EC_{50} mg a.i./l	>123
Lowest observed effect concentration (LOEC) mg a.i./l	>123
Highest tested conc. without toxic effect (NOEC) mg a.i./l	123

Conclusion:

The 48-hour $EC_{50}$of IN-T2921 to *Daphnia magna* was ≥123 mg/L, based on mean, measured concentrations.

RMS comments and conclusion

The acute toxicity to *Daphnia magna* study DuPont-4441, originally submitted under EU Rev8 Point IIA 8.2.4 and conducted with test material IN-T2921 technical metabolite, was conducted under guidelines OECD 202 (1984), EEC Method C.2. (1992), and U.S. EPA 72-2 (1988). A review of this study indicates that it fully meets the current guideline OECD 202 (2004). The validity criteria are met:

- No more than 10% immobilisation or other sign of disease/stress in the control (actual 0%);
- DO at the end of test ≥ 3 mg/L in the control and test vessels (actual 8.3 mg/L as a minimum)

Conclusion: The study is acceptable study is relied upon.

B.9.2.4.2 Acute toxicity to additional aquatic invertebrate species

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

B.9.2.4.2/01

Reference: CA 8.2.4.2/08	**Report:**	Rebstock, M. (2012); Oxamyl (DPX-D1410) technical (98% w/w): Acute toxicity with the mysid shrimp, *Americamysis bahia*, determined under flow-through test conditions **DuPont Report No.:** DuPont-34271 **Guidelines:** OPPTS 850.1035 (1996), EPA 712-C-96-136 **Deviations:** None **Testing Facility:** ABC Laboratories, Inc. (Missouri), Columbia, Missouri, USA **Testing Facility Report No.:** 68031 **GLP:** Yes

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

Executive summary:

The acute toxicity of oxamyl with the mysid shrimp, *Americamysis bahia*, was determined in a 96-hour flow-thorugh test. The test was conducted in accordance with the U.S. EPA Ecological Effects Test guidelines, OPPTS 850.1035.

The test substance, oxamyl, contains 98.0% a.s. by analysis. The study was conducted with five concentrations of oxamyl (0.0050, 0.010, 0.020, 0.040, and 0.080 mg a.s./L) and a dilution water control at a temperature range of 24.0 to 24.6°C. Ten mysids were used per test substance concentration and dilution water control replicate, for a total of 20 mysids per treatment. The measured concentrations of oxamyl in the replicate solution samples collected ranged from 68 to 91% of the nominal concentrations at initiation and from 78 to 92% of nominal at termination. The treatment mean measured concentrations of oxamyl during the 96 hour exposure were 0.00371, 0.00805, 0.0173, 0.0349, and 0.0716 mg a.s./L or 74 to 90% of the nominal concentrations. After 96 hours of exposure, mortality was 0, 0, 0, 5, 5, and 100% in the 0 (control), 0.00371, 0.00805, 0.0173, 0.0349, and 0.0716 mg a.s./L treatments. The highest mean measured concentration causing no mortality at test end was 0.00805 mg a.s./L. The lowest mean measured concentration causing 100% mortality at test end was 0.0716 mg a.s./L. The 96-hour LC_{50} was estimated to be 0.0465 mg a.s./L, with 95% confidence intervals of 0.0421 and 0.0514 mg a.s./L. The NOEC based on mean measured concentrations, mortality, and sublethal effects, was 0.0349 mg a.s./L.I. MATERIALS AND METHODS

A. MATERIALS

1.	Test material:	Pure Oxamyl (PAI)
	Lot/Batch #:	D1410-196
	Purity:	98.0% by analysis
	Description:	White crystalline solid
	CAS#:	23135-22-0
	Stability of test compound:	Stable at normal temperatures and storage conditions
2.	Control:	Dilution (artificial see water) water
	Solvent control:	None
	Test vehicle:	Dilution water
	Toxic reference:	None
3.	Test organism:	Mysid Shrimp
	Species:	*Americamysis bahia*
	Age at dosing:	5-6 days
	Initial population:	10 mysids per test chamber, 2 replicates per treatment for a total of 20 mysids per treatment
	Source:	ABC Laboratories, in-house culture
	Diet:	Fed ad libitum during test with newly hatched brine shrimp nauplii at least once daily
	Test chambers:	Glass aquaria measuring 14 cm × 22.6 cm × 16.9 cm There were two replicate chambers per treatment.
4.	Environmental conditions (in-life period)	
	Temperature:	24.0 to 24.6°C (of test chambers)
	Photoperiod:	14 hr photoperiod (443 lux) and 10 hr darkness which included two-30 min transitional light periods.
	Dissolved oxygen	6.4 to 7.4 mg/L (89 to 103% saturation)
	pH	8.1 to 8.4
	Salinity	(19.5 to 20.0‰

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed

05-March-2012 to 09-March-2012

2. Experimental treatments

The acute toxicity of oxamyl to *Americamysis bahia* was determined in a flow-thorugh, 96-hour test. Treatments consisted of a dilution water control and five nominal concentrations of 0.0050, 0.010, 0.020, 0.040, and 0.080 mg/L. Ten young adult mysids (based on greater sensitivity to the test substance observed during the range-finding test.) were used per test concentration and control replicate for a total of 20 mysids per treatment.A diluter system with a FMI metering pump was used. During the course of the definitive test, approximately 38 L of dilution-water control and test solution were delivered to each chamber each day. This rate was sufficient to provide approximately 9.6 volume additions in a 24-hour period.

The laboratory saltwater used as culture and dilution water in this study was prepared at a salinity of 20 ± 3‰ by adding a commercial sea salt mix to laboratory freshwater. The laboratory freshwater was well water that was demineralized by reverse osmosis. Prior to use, the dilution water was passed through a sediment filter,heated, and UV irradiated.

3. Observations and measurement

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

The dissolved oxygen concentration, temperature, salinity, and pH were measured at daily intervals in each chamber

The concentration of the test substance in the chambers was measured two days prior the start of the test, at the stard and at end of the test in all replicates.

4. Statistics

All statistical analyses were performed with SAS software (version 9.1). Estimates of LC_{50} values and their 95% confidence limits were calculated using the probit method and Trimmed Spearman-Karber 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-Karber method was selected for reporting. The no-observed-effect concentration (NOEC) was determined by using Fisher's one-tailed exact test.

II. RESULTS AND DISCUSSION

A. FINDINGS

Total nominal formulation concentrations were 0.0050, 0.010, 0.020, 0.040, and 0.080 mg oxamyl/L. The measured concentrations of oxamyl in the replicate solution samples collected ranged from 68 to 91% of the nominal concentrations at initiation and from 78 to 92% of nominal at termination. The mean measured concentrations of oxamyl during the 96 hour exposure were <LOD (dilution water control), 0.00371, 0.00805, 0.0173, 0.0349, and 0.0716 mg a.s./L, or 74 to 90% of the nominal concentrations. Recoveries from the Oxamyl QC samples ranged from 90 to 110% of the nominal concentrations throughout the test. All results from biological responses were based on mean measured concentrations of oxamyl.

Among replicate test chambers of a treatment concentration, the measured concentration of the test substance did not vary more than 20 percent.After 96 hours of exposure, mortality was 0, 0, 0, 5, 5, and 100% in the 0 (control), 0.00371, 0.00805, 0.0173, 0.0349, and 0.0716 mg a.s./L nominal treatments. The highest mean measured concentration causing no mortality at test end was 0.00805 mg a.s./L. The lowest mean measured concentration causing 100% mortality at test end was 0.0716 mg a.s./L. The 96-hour LC50

was estimated to be 0.0465 mg a.s./L, with 95% confidence intervals of 0.0421 and 0.0514 mg a.s./L. The NOEC based on mean measured concentrations, mortality, and sublethal effects, was 0.0349 mg a.s./L.

A summary of cumulative mortality and sublethal effects are presented in Table 38.

Table 38 Observed mortality of Mysid Shrimp, *Americamysis bahia*, exposed to oxamyl for 96 hours in a static acute test

Mean, measured oxamyl concentration (mg a.s./L)	Cumulative mortality/ Number at test start								Mean % Mortality
	24 Hours		48 Hours		72 Hours		96 Hours		
	A	B	A	B	A	B	A	B	
0 (Control)	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0
0.00371	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0
0.00805	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0
0.0173	1/10	0/10	1/10	0/10	1/10	0/10	1/10	0/10	5
0.0349	0/10[a]	0/10[b]	1/10[c]	0/10[c]	1/10[c]	0/10[a]	1/10[c]	0/10[c]	5
0.0716	6/10[a]	6/10[c]	10/10	9/10[c]	10/10	10/10	10/10	10/10	100[d]

Note: Test chambers contained ten mysid shrimp each at test initiation.

[a] Two mysids appeared lethargic.

[b] Three mysids appeared lethargic.

[c] One mysid appeared lethargic.

[d] Indicates a statistically significant reduction in survival or normal behaviors as compared to the control after the 96-hour exposure (one-tailed Fisher's Exact Test, p <0.05)

III. CONCLUSION

Oxamyl was assessed for acute toxicity with the mysid shrimp, *Americamysis bahia*, in a 96-hour static test. The 96-hour LC_{50} was estimated to be 0.0465 mg a.s./L, with 95% confidence intervals of 0.0421 and 0.0514 mg a.s./L. The NOEC based on mean measured concentrations, mortality, and sublethal effects, was 0.0349 mg a.s./L.

(Rebstock, M., 2012)

RMS: The RMS revised the summary also adding additional information. The test is valid (no more than 10 percent of the control organisms died or exhibited abnormal behavior during the 96–h test period). The study meets the requirement of OPPTS 850.1035 (1996), except that the conductivity of the demineralized well water used for preparing th artificial seawater is not reported (it should be less than 1 µohm/cm at 12 °C). Anyow, the dilution water allowed satisfactory survival in controls and the requirement for conductivity is not given in the OECD dratft guideline for the Mysid Two-Generation Test".Conclousion: the study is acceptable.

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

B.9.2.4.2/02

Reference: **CA 8.2.4.2/05**	**Report:**	Brougher, D.S., Martin, K.H., Gallagher, S.P., Krueger, H.O. (2013a); Oxamyl (DPX-D1410) technical (98% w/w): A 48-hour static acute toxicity test with the mayfly (*Centroptilum triangulifer*) **DuPont Report No.:** DuPont-37401 **Guidelines:** OECD 202 (2004), OPPTS 850.1010 (1996) **Deviations:** None **Testing Facility:** Wildlife International Ltd. (USA), Easton, Maryland, USA **Testing Facility Report No.:** 112A-458A **GLP:** Yes **Certifying Authority:** Laboratories in the USA are not certified by any government agency, but are subject to regular inspections.

Executive summary:

The acute toxicity of oxamyl to unfed larval stage *Centroptilum triangulifer* was determined in an unaerated, static, 48-hour test. The test was conducted in accordance with the OECD Guideline for Testing of Chemicals, 202, *Daphnis sp. Acute Immobilization Test*, U.S. Environmental Protection Agency Series 850 – Ecological Effects Test Guidelines (draft) and OPPTS Number 850.1010, *Aquatic Invertebrate Acute Toxicity Test, Freshwater Daphnids*. Treatments consisted of a dilution water control and six nominal concentrations 0.016, 0.030, 0.063, 0.13, 0.25 and 0.50 mg oxamyl/L (equivalent to 0.013, 0.026, 0.055, 0.12, 0.22 and 0.50 mg oxamyl/L, based on mean, measured oxamyl concentrations). The 48-hour LC_{50}, based on mean measured concentrations and mortality data, was determined to be 0.067 mg a.s./L, with a 95% confidence interval of 0.055 to 0.081 mg a.s./L. The highest mean measured test concentration causing no mortality at test end was 0.026 mg a.s./L. The lowest mean measured test concentration causing 100% mortality at test end was 0.22 mg a.s./L.

I. MATERIALS AND METHODS

A. MATERIALS

1.	Test material:	Pure Oxamyl (PAI)
	Lot/Batch #:	D1410-532
	Purity:	99.1%
	Description:	Solid
	CAS#:	23135-22-0
	Stability of test compound:	Shown to be stable under the conditions of the test.
2.	Controls:	Dilution water (laboratory well water) control.
	Test vehicle:	None
	Toxic reference:	Not applicable
3.	Test organism:	Mayfly
	Species:	*Centroptilum triangulifer*
	Age/life stage at dosing:	Larval stage
	Initial population:	Four replicate test chambers with 5 mayflies per test chamber
	Source:	Stroud Water Research Center Avondale, PA 19311-9514
	Diet:	Unfed during test
	Test chamber:	250-mL glass beaker containing approximately 200 mL of test solution (7.3-cm test solution depth)
4.	Environmental conditions:	Dissolved oxygen: ≥7.7 mg/L (≥86% of saturation) pH: 7.3 to 7.9
	Temperature:	19.9 to 20.5°C in test chambers; 20.0 to 20.0°C measured continuously in an adjacent container of water.
	Photoperiod:	16 hr light (453 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

27-June-2013 to 29-June-2013

2. Experimental treatments

The acute toxicity of oxamyl to unfed larval stage *Centroptilum triangulifer* was determined in an unaerated, static, 48-hour test. Late instar larval stage mayflies used in the test were cultured and placed in Whirlpack plastic bags by Stroud Water Research Center in Avondale, Pennsylvania. Upon arrival, the mayflies were acclimated in an environmental test chamber for approximately 1.5 hours prior to test initiation, without feeding.

Treatments consisted of a dilution water control and six nominal concentrations of 0.016, 0.030, 0.063, 0.13, 0.25 and 0.50 mg a.s./L (equivalent to 0.013, 0.026, 0.055, 0.12, 0.22 and 0.50 mg a.s./L, based on mean, measured oxamyl concentrations). Prior to use, the dilution well water was filtered to 0.45 µm and passed through an ultraviolet sterilizer to remove fine particles and microorganisms. The dilution water was well water with conductance of 400 µS/cm, alkalinity of 172 mg/L as CaCO3, and hardness of 128 mg/L as CaCO3, OC = 1.045 mg C/L. The water was adjusted to about pH of about 7. The primary stock solution was sonicated for approximately 10 minutes, and was then stirred on a magnetic stir plate for 30 minutes to mix. The test solutions were stirred on a magnetic stir plate for 30 minutes to mix, and appeared clear and colorless at test initiation and termination. Five daphnids were used per replicate with four replicates per test concentration and control.

Samples were collected from the batches of test solution prepared for each treatment and control group at the beginning of the test and from two of the four replicate test chambers at 48 hours (± 1 hour) to measure concentrations of the test substance. Two sets of samples were also collected at 24 hours from two of the four replicate test chambers of the 0.16, 0.40 and 1.0 mg a.s./L treatment groups due to

100% mortality. Samples were analyzed by high performance liquid chromatography with UV detector.

Temperature, DO, and pH were measured in each test chamber at the beginning and end of the test and at approximately 24 hours. Temperature also was measured continuously in a extra container

Five mayflies were used per replicate with four replicates per test concentration and control.

3. Observations

Mortality and behavioral observations were made at approximately 3 hours, and at 24 and 48 hours (± 1 hour) following initiation of exposure. Mortality was defined as a lack of movement by the test organism except for minor activity of the appendages.

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, probit analysis was used to calculate the 24 and 48-hour LC_{50} values and the 95% confidence intervals. The highest test concentration causing no mortality at test end and the lowest 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

Dissolved oxygen concentrations remained ≥7.7 mg/L (≥86% of saturation) throughout the test. Measurements of pH ranged from 7.3 to 7.9. Mean, measured concentrations of oxamyl 0.013, 0.026, 0.055, 0.12, 0.22 and 0.50 mg a.s./L and ranged from 81 to 100% of nominal concentrations.

Mayflies in the negative control appeared normal throughout the test. Mayflies in the 0.013 and 0.026 mg a.s./L treatment groups also appeared normal throughout the test, with no mortality or overt signs of toxicity observed. Lethargy was observed for mayflies in the 0.12, 0.22 and 0.50 mg a.s./L treatment groups. Percent mortality at test termination in the 0.013, 0.026, 0.055, 0.12, 0.22 and 0.50 mg a.s./L treatment groups was 0, 0, 30, 95, 100 and 100%, respectively. At test termination, one mayfly in the 0.013 mg a.s./L treatment group and two mayflies in the 0.026 mg a.s./L treatment group were missing from the test chambers and the molts were found floating on the surface. It appears the missing organisms emerged prior to termination and were excluded from calculation of the LC50 value.

Summaries of cumulative mortality and sublethal effects are presented in Table 39 and Table 40, respectively.

Table 39 Summary of cumulative mortality of unfed *Centroptilum triangulifer* exposed to oxamyl for 48 hours in an unaerated, static, acute test

Mean measured oxamyl concentration (mg a.s./L)	Mortality (Cumulative No. dead/No. at test start) [a]											
	~3 Hours				24 Hours				48 Hours			
	A	B	C	D	A	B	C	D	A	B	C	D
Dilution water control (0.0)	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
0.013	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/4 [b]
0.026	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/4 [b]	0/4 [b]	0/5
0.055	0/5	0/5	0/5	0/5	1/5	0/5	0/5	0/5	2/5	1/5	0/5	3/5
0.12	0/5	0/5	0/5	0/5	4/5	3/5	4/5	4/5	5/5	5/5	4/5	5/5
0.22	0/5	2/5	1/5	1/5	5/5	5/5	3/5	5/5	5/5	5/5	5/5	5/5
0.50	0/5	2/5	4/5	0/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5

[a] A–D represent replicate test chambers containing five mayflies each at test start.

[b] mayfly missing from test chamber, with molt floating on surface of solution - mayfly assumed to have emerged. These organisms were excluded from statistical analysis.

Table 40 Summary of sublethal effects of unfed *Centroptilum triangulifer* exposed to oxamyl for 48 hours in an unaerated, static, acute test

Mean measured oxamyl concentration (mg a.s./L)	Number affected/ Number alive [a]											
	~2 Hours				24 Hours				48 Hours			
	A	B	C	D	A	B	C	D	A	B	C	D
Dilution water control (0.0)	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
0.013	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	1[b]/4
0.026	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	1[b]/4	1[b]/4	0/5
0.055	0/5	0/5	0/5	0/5	0/4	0/5	0/5	0/5	0/3	0/4	0/5	0/2
0.12	0/5	0/5	0/5	0/5	1[c]/1	2[c]/2	1[c]/1	1[c]/1	-	-	1[c]/1	-
0.22	0/5	0/3	0/4	1[c]/4	-	-	2[c]/2	-	-	-	-	-
0.50	5[c]/5	3[c]/3	1[c]/1	5[c]/5	-	-	-	-	-	-	-	-

[a] A–D represent replicate test chambers containing five mayflies each at test start

[b] V = mayfly missing from test chamber; found molt floating on surface of solution. Not included in statistical analysis.

[c] C = lethargic

- = 100% mortality

III. CONCLUSION

The 48-hour LC_{50} value was 0.067 mg a.s./L with a 95% confidence interval of 0.055 to 0.081 mg a.s./L, based on mean measured concentrations. The highest mean measured test concentration causing no mortality at test end was 0.026 mg a.s./L. The lowest mean measured test concentration causing 100% mortality at test end was 0.22 mg a.s./L

(Brougher, D.S.; Martin, K.H.; Gallagher, S.P.; Krueger, H.O., 2013a)

RMS: Additional information were added by the RMS to the study summary.

There is no standard method for mayflies. The OECD 202 (2004) validity criteria are met:

- No more than 10% effect or sign of disease/stress in the control (actual 0%);
- DO at the end of test ≥ 3 mg/L in the control and test vessels (actual 7.7 mg/L as a minimum).

The acclimation of the testing organisms was only 1.5h and no mention is made to the culturing conditions of the test organisms in the sending Lab and the water temperature at arrival to the testing Lab. Nevertheless, during the test period the control animals performed well.

Conclusion: The study is acceptable and relied upon.

0.320

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

B.9.2.4.2/03

Reference: CA 8.2.4.2/04	**Report:**	Brougher, D.S., Martin, K.H., Gallagher, S.P., Bodle, E.S. (2013d); Oxamyl (DPX-D1410) technical (98% w/w): A 48-hour static acute toxicity test with the Cladoceran (*Ceriodaphnia dubia*) **DuPont Report No.:** DuPont-37399 **Guidelines:** OECD 202 (2004), OPPTS 850.1010 (1996) **Deviations:** None **Testing Facility:** Wildlife International Ltd. (USA), Easton, Maryland, USA **Testing Facility Report No.:** 112A-456 **GLP:** Yes **Certifying Authority:** Laboratories in the USA are not certified by any government agency, but are subject to regular inspections.

Executive summary:

The acute toxicity of oxamyl to unfed *Ceriodaphnia dubia* was determined in an unaerated, static, 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 (draft), OPPTS Number 850.1010, *Aquatic Invertebrate Acute Toxicity Test, Freshwater Daphnids*. Treatments consisted of a dilution water control and six mean, measured concentrations of 0.009, 0.023, 0.055, 0.16, 0.38 and 0.97 mg oxamyl (a.s.)/L. The 48-hour EC_{50}, based on mean, measured concentrations of oxamyl and immobility data, was estimated to be 0.094 mg a.s./L. The highest mean, measured concentration causing no immobility at test end was 0.055 mg a.s./L. The lowest mean, measured test concentration causing 100% immobility at test end was 0.16 mg a.s./L.

I. MATERIALS AND METHODS

A. MATERIALS

1.	Test material:	Pure Oxamyl (PAI)
	Lot/Batch #:	D1410-532
	Purity:	99.1%
	Description:	Solid
	CAS#:	23135-22-0
	Stability of test compound:	Data not provided.
2.	Controls:	Dilution water (UV-sterilized laboratory well water) control
	Test vehicle:	None
	Toxic reference:	Not applicable
3.	Test organism:	Cladoceran
	Species:	*Ceriodaphnia dubia*
	Age/life stage at dosing:	<24 hours
	Initial population:	Four replicate test chambers with 5 daphnids per test chamber
	Source:	Wildlife International in-house culture
	Diet:	Unfed during test
	Test chamber:	250-mL glass beaker containing approximately 220 mL of test solution (6.2-cm test solution depth)
4.	Environmental conditions:	Dissolved oxygen: ≥7.9 mg/L (≥88% of saturation) pH: 7.2 to 7.8
	Temperature:	19.1 to 20.2°C in test chambers; ranged from 20 to 21°C measured continuously in an adjacent container of water.
	Photoperiod:	16 hr light (356 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

14-May-2013 to 16-May-2013

2. Experimental treatments

The acute toxicity of oxamyl to unfed *Ceriodaphnia dubia* (<24-hour old) was determined in an unaerated, static, 48-hour test. Treatments consisted of a dilution water control and nominal test concentrations of oxamyl were 0.01, 0.025, 0.063, 0.16, 0.4 and 1.0 mg a.s./L (mean, measured test concentrations of 0.009, 0.023, 0.055, 0.16, 0.38, and 0.97 mg a.s./L). Prior to use, the dilution well water was filtered to 0.45 µm and passed through an ultraviolet sterilizer to remove fine particles and microorganisms. The dilution water was well water with conductance of 367 µS/cm, alkalinity of 178 mg/L as CaCO3, and hardness of 140 mg/L as CaCO3, OC <2 mg C/L. The water was adjusted to about pH=7. The primary stock solution was sonicated for approximately 10 minutes, and was then stirred on a magnetic stir plate for 660 minutes to mix. The test solutions were stirred on a magnetic stir plate for 60 minutes to mix, and appeared clear and colorless at test initiation and termination. Five daphnids were used per replicate with four replicates per test concentration and control.

Samples were collected from the batches of test solution prepared for each treatment and control group at the beginning of the test and from two of the four replicate test chambers at 48 hours (± 1 hour) to measure concentrations of the test substance. Two sets of samples were also collected at 24 hours from two of the four replicate test chambers of the 0.16, 0.40 and 1.0 mg a.s./L treatment groups due to 100% mortality. Samples were analyzed by high performance liquid chromatography with tandem mass spectrometric detection (LC/MS/MS).

Temperature, DO, and pH were measured in each test chamber at the beginning and end of the test and at approximately 24 hours. Temperature also was measured continuously in a extra container

Five daphnids were used per replicate with four replicates per test concentration and control.

3. Observations

Immobility and behavioral observations were made at approximately 2 hours, and at 24 and 48 hours (± 1 hour) following initiation of exposure.

4. Statistics

The 48-hour mortality data were analyzed using the computer program of C. E. Stephan. The program was designed to calculate the EC_{50} value and the 95% fiducial interval by probit analysis, the moving average method, and binomial probability with nonlinear interpolation. In this study, nonlinear interpolation was used to calculate the 24 and 48-hour EC_{50} values. 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 oxamyl were 0.009, 0.023, 0.055, 0.16, 0.38 and 0.97 mg a.s./L and ranged from 87 to 100% of nominal concentrations. Daphnids in the negative control, 0.009 and 0.023 mg a.s./L treatment groups appeared normal throughout the test, with no immobility or sublethal effects observed. There were a few observations of floating daphnids in the 0.023 mg a.s./L treatment group, however, these daphnids appeared normal after being gently submerged. The 0.055 mg a.s./L treatment group had no immobility, however, lethargy was observed at test termination. Summaries of cumulative immobility and sublethal effects are presented in Table 41 and Table 42, respectively.

Table 41 Summary of cumulative immobility of unfed *Ceriodaphnia dubia* exposed to oxamyl for 48 hours in an unaerated, static, acute test

Mean, measured oxamyl concentration (mg a.s./L)	Immobility (Cumulative No. immobile/No. at test start) [a]											
	~2 Hours				24 Hours				48 Hours			
	A	B	C	D	A	B	C	D	A	B	C	D
Dilution water control (0.0)	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
0.009	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
0.023	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
0.055	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
0.16	0/5	0/5	0/5	0/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
0.38	4/5	1/5	3/5	4/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
0.97	5/5	4/5	4/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5

[a] A–D represent replicate test chambers containing 5 daphnids each at test start.

Table 42 Summary of sublethal effects of unfed *Ceriodaphnia dubia* exposed to oxamyl for 48 hours in an unaerated, static, acute test

Mean, measured oxamyl concentration (mg a.s./L)	Number affected/ Number alive [a, b]											
	~3 Hours				24 Hours				48 Hours			
	A	B	C	D	A	B	C	D	A	B	C	D
Dilution water control (0.0)	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
0.009	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
0.023	0/5	1Q,AN/5	0/5	0/5	0/5	1Q,AN/5	0/5	0/5	0/5	0/5	0/5	0/5
0.055	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	1 Q,AN/5	1 C/5	1 C/5	2 C/5
0.16	0/5	0/5	0/5	1 C/5	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
0.38	1 C/1	4 C/4	2 C/2	1 C/1	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
0.97	0/0	1 C/1	1 C/1	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0

[a] A–D represent replicate test chambers containing 5 daphnids each at test start.

[b] C = lethargic; Q,AN = organism trapped at water surface but appear normal after gentle submersion below the water's surface.

III. CONCLUSION

The 48-hour EC_{50} value, based on the mean, measured test concentrations of oxamyl and immobility, was estimated to be 0.094 mg a.s./L. The 95% fiducial limits determined by binomial probability were 0.055 – 0.16 mg a.s./L. The highest mean, measured test concentration causing no immobility at test end was 0.055 mg a.s./L. The lowest mean, measured test concentration causing 100% immobility at test end was 0.16 mg a.s./L.

(Brougher, D.S.; Martin, K.H.; Gallagher, S.P.; Bodle, E.S., 2013d)

RMS: Additional information were added by the RMS to the study summary.

The OECD 202 (2004) validity criteria are met:

- No more than 10% effect or sign of disease/stress in the control (actual 5%);
- DO at the end of test ≥ 3 mg/L in the control and test vessels (actual 8.1 mg/L as a minimum).

Conclusion: The study is acceptable and relied upon.

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

B.9.2.4.2/04

Reference: CA 8.2.4.2/01	**Report:**	Brougher, D.S., Martin, K.H., Gallagher, S.P., Bodle, E.S. (2013a); Oxamyl (DPX-D1410) technical (98% w/w): A 48-hour static acute toxicity test with *Hyalella azteca* **DuPont Report No.:** DuPont-37397 **Guidelines:** OECD 202 (2004), OPPTS 850.1010 (1996) **Deviations:** None **Testing Facility:** Wildlife International Ltd. (USA), Easton, Maryland, USA **Testing Facility Report No.:** 112A-454 **GLP:** Yes **Certifying Authority:** Laboratories in the USA are not certified by any government agency, but are subject to regular inspections.

Executive summary:

The acute toxicity of oxamyl to unfed *Hyalella azteca* was determined in an unaerated, static, 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 (draft), OPPTS Number 850.1010, *Aquatic Invertebrate Acute Toxicity Test, Freshwater Daphnids*. Treatments consisted of a dilution water control and mean measured test concentrations of 0.058, 0.13, 0.29, 0.63, 1.32 and 3.5 mg a.s./L. The 48-hour LC_{50} in *Hyalella azteca* was 0.32 mg a.s./L, with a 95% fiducial interval of 0.24 to 0.44 mg a.s./L, based on mean measured of oxamyl test concentrations and mortality data.

I. MATERIALS AND METHODS

A. MATERIALS

1.	Test material:	Pure Oxamyl (PAI)
	Lot/Batch #:	D1410-532
	Purity:	99.1%
	Description:	Solid
	CAS#:	23135-22-0
	Stability of test compound:	Shown to be stable under the conditions of the test.
2.	Controls:	Dilution water (laboratory well water) control
	Test vehicle:	None
	Toxic reference:	Not applicable
3.	Test organism:	Amphipod
	Species:	*Hyalella azteca*
	Age/life stage at dosing:	10 days
	Initial population:	Four replicate test chambers with 5 amphipods per test chamber
	Source:	Wildlife International in-house culture
	Diet:	Unfed during test
	Test chamber:	250-mL glass beaker containing approximately 220 mL of test solution (6.3-cm test solution depth)
4.	Environmental conditions:	Dissolved oxygen: ≥8.0 mg/L (≥94% of saturation) pH: 7.1 to 8.0
	Temperature:	22.1 to 23.1°C in test chambers; 22.9 to 23.3°C measured continuously in an adjacent container of water.
	Photoperiod:	16 hr light (799 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

   04-June-2013 to 06-June 2013

2. Experimental treatments

The acute toxicity of oxamyl to unfed *Hyalella azteca* (10 days old) was determined in an unaerated, static, 48-hour test. Treatments consisted of a dilution water control and mean, measured test concentrations of 0.058, 0.13, 0.29, 0.63, 1.32, and 3.5 mg a.s./L. Prior to use, the dilution well water was filtered to 0.45 µm and passed through an ultraviolet sterilizer to remove fine particles and microorganisms. The dilution water was well water with conductance of 387 µS/cm, alkalinity of 178 mg/L as CaCO3, and hardness of 124 mg/L as CaCO3, OC <1 mg C/L. The primary stock solution was sonicated for approximately 10 minutes, and was then stirred on a magnetic stir plate for 660 minutes to mix. The test solutions were stirred on a magnetic stir plate for 60 minutes to mix, and appeared clear and colorless at test initiation and termination. Five daphnids were used per replicate with four replicates per test concentration and control.

Samples were collected from the batches of test solution prepared for each treatment and control group at the beginning of the test and from two of the four replicate test chambers at 48 hours (± 1 hour) to measure concentrations of the test substance. Two sets of samples were also collected at 24 hours from two of the four replicate test chambers. Samples were analyzed by high performance liquid chromatography with UV detector.

Temperature, DO, and pH were measured in each test chamber at the beginning and end of the test and at approximately 24 hours. Temperature also was measured continuously in a extra container

Five amphipods were used per replicate with four replicates per test concentration and control.

3. Observations

Mortality and behavioral observations were made at approximately 4 hours, and at 24 and 48 hours (± 1 hour) following initiation of exposure. Mortality was defined as a lack of reaction by the test organism to application of a gentle stimulus.

4. Statistics

The 48-hour mortality data were analyzed using the computer program of C. E. Stephan. The program was designed to calculate the LC_{50} value and the 95% fiducial interval by probit analysis, the moving average method, and binomial probability with nonlinear interpolation. In this study, probit analysis was used to calculate the 24 and 48-hour LC_{50} values and the 95% fiducial intervals. 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

Nominal test concentrations of oxamyl were 0.078, 0.17. 0.38, 0.83, 1.8 and 4.0 mg a.s./L. Mean, measured concentrations of oxamyl were 0.058, 0.13, 0.29, 0.63, 1.32 and 3.5 mg a.s./L and ranged from 73 to 88% of nominal concentrations. Amphipods in the negative control and the 0.058 mg a.s./L treatment group appeared normal throughout the test, with no mortality or sublethal effects observed. There were a few observations of amphipods floating in the negative control, but all amphipods appeared normal after being gently submerged. Percent mortality at test termination in the 0.13, 0.29, 0.63, 1.32 and 3.5 mg a.s./L treatment groups was 25, 50, 70, 90 and 100%, respectively. Signs of toxicity included lethargy and floating amphipods that appeared lethargic after being gently submerged. The frequency of these observations increased with increasing concentrations. Summaries of cumulative immobility and sublethal effects are presented in Table 44 and Table 45, respectively.

Table 43 Summary of observed mortality of unfed *Hyalella azteca* exposed to oxamyl for 48 hours in an unaerated, static, acute test

Mean, measured oxamyl concentration (mg a.s./L)	Mortality (Cumulative No. dead/No. at test start)[a]											
	~4 Hours				24 Hours				48 Hours			
	A	B	C	D	A	B	C	D	A	B	C	D
Dilution water control (0.0)	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
0.058	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	0/5	0/5	0/5	0/5	4/5	0/5	0/5	0/5	4/5	0/5	0/5	1/5
0.29	0/5	0/5	0/5	0/5	5/5	5/5	0/5	0/5	5/5	5/5	0/5	0/5
0.63	0/5	0/5	0/5	0/5	3/5	0/5	5/5	5/5	4/5	0/5	5/5	5/5
1.32	0/5	0/5	0/5	0/5	4/5	1/5	5/5	4/5	4/5	4/5	5/5	5/5
3.5	0/5	0/5	0/5	0/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5

[a] A–D represent replicate test chambers containing 5 amphipods each at test start.

Table 44 Summary of sublethal effects of unfed *Hyalella azteca* exposed to oxamyl for 48 hours in an unaerated, static, acute test

Mean, measured oxamyl concentration (mg a.s./L)	Number affected/ Number alive [a, b]											
	~ 4 Hours				24 Hours				48 Hours			
	A	B	C	D	A	B	C	D	A	B	C	D
Dilution water control (0.0)	0/5	0/5	0/5	0/5	0/5	0/5	0/5	1Q,AN/5	0/5	0/5	1Q,AN/5	1Q,AN/5
0.058	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	5C/5	0/5	0/5	0/5	0/1	0/5	2C/5	0/5	0/1	0/5	1C/5	0/4
0.29	4C/5	4Q,C/5	1C/5	1C/5	0/0	0/0	5C/5	2Q,C/ 5	0/0	0/0	5C/5	1Q,C; 4C/5
0.63	3Q,C; 2C/5	1C/5	5Q,C/5	5Q,C/5	2C/2	1C/5	0/0	0/0	1C/1	1C/5	0/0	0/0
1.32	2Q,C/5	2Q,C/5	2Q,C/5	3Q,C/5	1Q,C/1	4C/4	0/0	1Q,C/1	1Q,C/1	1C/1	0/0	0/0
3.5	3 C/5	2Q,C/5	4Q,C/5	1Q,C; 2C/5	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0

[a] A–D represent replicate test chambers containing 5 amphipods each at test start.

[b] C = lethargy; Q,AN = floating on the surface, appeared normal after being gently submerged; Q,C = floating on the surface, appeared lethargic after being gently submerged.

III. CONCLUSION

The 48-hour LC_{50} value, based on the mean, measured test concentrations of oxamyl and mortality, was estimated to be 0.32 mg a.s./L, with a 95% fiducial interval of 0.24 to 0.44 mg a.s./L. The highest mean, measured test concentration causing no mortality at test end was 0.058 mg a.s./L. The lowest mean, measured test concentration causing 100% mortality at test end was 3.5 mg a.s./L.

(Brougher, D.S.; Martin, K.H.; Gallagher, S.P.; Bodle, E.S., 2013a)

RMS: Additional information were added by the RMS to the study summary.

The OECD 202 (2004) validity criteria are met:

- No more than 10% effect or sign of disease/stress in the control (actual 5% floating individuals);
- DO at the end of test ≥ 3 mg/L in the control and test vessels (actual 8.1 mg/L as a minimum).

Conclusion: The study is acceptable and relied upon.

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

B.9.2.4.2/05

Reference: CA 8.2.4.2/03	**Report:**	Brougher, D.S., Martin, K.H., Gallagher, S.P., Bodle, E.S. (2013c); Oxamyl (DPX-D1410) technical (98% w/w): A 48-hour static acute toxicity test with the cladoceran (*Daphnia pulex*) **DuPont Report No.:** DuPont-37398 **Guidelines:** OECD 202 (2004), OPPTS 850.1010 (1996) **Deviations:** None

		Testing Facility: Wildlife International Ltd. (USA), Easton, Maryland, USA **Testing Facility Report No.:** 112A-455 **GLP:** Yes **Certifying Authority:** Laboratories in the USA are not certified by any government agency, but are subject to regular inspections.

Executive summary:

The acute toxicity of oxamyl to unfed *Daphnia pulex* was determined in an unaerated, static, 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 (draft), OPPTS Number 850.1010, *Aquatic Invertebrate Acute Toxicity Test, Freshwater Daphnids*. Treatments consisted of a dilution water control and six mean, measured concentrations of 0.00427, 0.0125, 0.038, 0.13, 0.38 and 1.1 mg active substance (a.s.)/L. The 48-hour EC_{50}, based on mean, measured concentrations of oxamyl and immobility data, was estimated to be 0.08 mg a.s./L. The highest mean, measured concentration causing no immobility at test end was 0.038 mg a.s./L. The lowest mean, measured test concentration causing 100% immobility at test end was 0.38 mg a.s./L.

I. MATERIALS AND METHODS

A. MATERIALS

1.	Test material:	Pure Oxamyl (PAI)
	Lot/Batch #:	D1410-532
	Purity:	99.1%
	Description:	Solid
	CAS#:	23135-22-0
	Stability of test compound:	Shown to be stable under the conditions of the test.
2.	Controls:	Dilution water (UV-sterilized laboratory well water) control
	Test vehicle:	None
	Toxic reference:	Not applicable
3.	Test organism:	Cladoceran
	Species:	*Daphnia pulex*
	Age/life stage at dosing:	<24 hours
	Initial population:	Four replicate test chambers with 5 daphnids per test chamber
	Source:	Wildlife International in-house culture
	Diet:	Unfed during test
	Test chamber:	250-mL glass beaker containing approximately 220 mL of test solution (6.5-cm test solution depth)
4.	Environmental conditions:	Dissolved oxygen: ≥8.0 mg/L (≥88% of saturation) pH: 7.3 to 7.9
	Temperature:	20.2 to 20.9°C in test chambers; ranged from 20 to 21°C measured continuously in an adjacent container of water.
	Photoperiod:	16 hr light (414 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

07-August-2013 to 09-August-2013

2. Experimental treatments

The acute toxicity of oxamyl to unfed *Daphnia pulex* (<24-hour old) was determined in an unaerated, static, 48-hour test. Treatments consisted of a dilution water control and nominal test concentrations of oxamyl were 0.005, 0.015, 0.045, 0.14, 0.41 and 1.2 mg a.s./L. Prior to use, the dilution well water was filtered to 0.45 µm and passed through an ultraviolet sterilizer to remove fine particles and microorganisms. The primary stock solution was sonicated for approximately 10 minutes, and was then stirred on a magnetic stir plate for 30 minutes to mix. The test solutions were stirred on a magnetic stir plate for 30 minutes to mix, and appeared clear and colorless at test initiation and termination. Five daphnids were used per replicate with four replicates per test concentration and control.

Samples were collected from the batches of test solution prepared for each treatment and control group at the beginning of the test and from two of the four replicate test chambers at 48 hours (± 1 hour) to measure concentrations of the test substance.Samples were analyzed by high performance liquid chromatography with tandem mass spectrometric detection (LC/MS/MS).

Temperature, DO, and pH were measured in each test chamber at the beginning and end of the test and at approximately 24 hours. Temperature also was measured continuously in a extra container

3. Observations

Immobility and behavioral observations were made at approximately 3 hours, and at 24 and 48 hours (± 1 hour) following initiation of exposure.

4. Statistics

The 48-hour mortality data were analyzed using the computer program of C. E. Stephan. The program was designed to calculate the EC_{50} value and the 95% fiducial limits by probit analysis, the moving average method, and binomial probability with nonlinear interpolation. In this study, nonlinear interpolation was used to calculate the 24 and 48-hour EC_{50} values. 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

Dissolved oxygen ranged from 8.6 – 8.9 mg/L.

Mean, measured concentrations of oxamyl were 0.00427, 0.0125, 0.038, 0.13, 0.38 and 1.1 mg a.s./L and ranged from 83 to 93% of nominal concentrations. Summaries of cumulative immobility and sublethal effects are presented in Table 45 and Table 46, respectively. The LC50 value was estimated using non-linear interpolation between 0.038 and 0.13 mg a.s./L; the 95% Fiducial Limits were determined by binomial probability.

Table 45 Summary of cumulative immobility of unfed *Daphnia pulex* exposed to oxamyl for 48 hours in an unaerated, static, acute test

Mean, measured oxamyl concentration (mg a.s./L)	Immobility (Cumulative No. immobile/No. at test start)[a]											
	~3 Hours				24 Hours				48 Hours			
	A	B	C	D	A	B	C	D	A	B	C	D
Dilution water control (0.0)	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
0.00427	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
0.0125	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
0.038	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	0/5	0/5	0/5	0/5	4/5	3/5	4/5	3/5	5/5	4/5	4/5	5/5
0.38	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
1.1	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5

[a] A–D represent replicate test chambers containing 5 daphnids each at test start.

Table 46 Summary of sublethal effects of unfed *Daphnia pulex* exposed to oxamyl for 48 hours in an unaerated, static, acute test

Mean, measured oxamyl concentration (mg a.s./L)	Number affected/ Number alive [a, b]											
	~3 Hours				24 Hours				48 Hours			
	A	B	C	D	A	B	C	D	A	B	C	D
Dilution water control (0.0)	0/5	0/5	0/5	1Q,AN/5	0/5	0/5	0/5	0/5	0/5	2Q,AN/5	1Q,AN/5	2Q,AN/5
0.00427	0/5	0/5	0/5	0/5	0/5	1Q,AN/5	3Q,AN/5	1Q,AN/5	0/5	1Q,AN/5	1Q,AN/5	2Q,AN/5
0.0125	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	2Q,AN/5	0/5	1Q,AN/5
0.038	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	4Q,AN/5	5Q,AN/5	4Q,AN/5	5Q,AN/5
0.13	0/5	0/5	0/5	0/5	1C/1	1C/2	0/1	2 C/2	--	0/1	1Q,AN/1	--
0.38	--	--	--	--	--	--	--	--	--	--	--	--
1.1	--	--	--	--	--	--	--	--	--	--	--	--

[a] A–D represent replicate test chambers containing 5 daphnids each at test start.

[b] C = lethargic; Q,AN = organism trapped at water surface but appear normal after gentle submersion below the water's surface.

III. CONCLUSION

The 48-hour EC_{50} value, based on the mean, measured test concentrations of oxamyl and immobility, was estimated to be 0.08 mg a.s./L. The highest mean, measured test concentration causing no immobility at test end was 0.038 mg a.s./L (0.038 and 0.13 mg a.s./L). The lowest mean, measured test concentration causing 100% immobility at test end was 0.38 mg a.s./L.

(Brougher, D.S.; Martin, K.H.; Gallagher, S.P.; Bodle, E.S., 2013c)

RMS: Additional information were added by the RMS to the study summary. Daphnids in the negative control appeared normal throughout the test, with no immobility or sublethal effects observed. There were observations of floating daphnids throughout the control and all treatment groups. The report states that the floating daphnids appeared normal after being gently submerged.

A review of this study indicates that the validity criterion for DO is met:

- DO at the end of test ≥ 3 mg/L in the control and test vessels (actual 8.2 mg/L as a minimum)

The criterion:

"No more than 10% immobilisation or other sign of disease/stress in the control" is not fulfilled because 25% daphnids were trapped at water surface.

Conclusion: the test is **not valid** because of the high percentage of floating daphnids in the control. Floating was observed also in all the test concentrations.

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

B.9.2.4.2/06

Reference: CA 8.2.4.2/06	**Report:**	Brougher, D.S., Martin, K.H., Gallagher, S.P., Krueger, H.O. (2013b); Oxamyl (DPX-D1410) technical (98% w/w): A 48-hour static acute toxicity test with the caddisfly (*Chimarra atterima*) **DuPont Report No.:** DuPont-37402 **Guidelines:** OECD 202 (2004), OPPTS 850.1010 (1996) **Deviations:** None **Testing Facility:** Wildlife International Ltd. (USA), Easton, Maryland, USA **Testing Facility Report No.:** 112A-459 **GLP:** Yes **Certifying Authority:** Laboratories in the USA are not certified by any government agency, but are subject to regular inspections.

Executive summary:

The acute toxicity of oxamyl to unfed larval stage *Chimarra atterima* was determined in an unaerated, static, 48-hour test. The test was conducted in accordance with the OECD Guideline for Testing of Chemicals, 202, *Daphnis sp. Acute Immobilization Test*, U.S. Environmental Protection Agency Series 850 – Ecological Effects Test Guidelines (draft) and OPPTS Number 850.1010, *Aquatic Invertebrate Acute Toxicity Test, Freshwater Daphnids*. Treatments consisted of a dilution water control and six nominal concentrations of 0.0016, 0.008, 0.04, 0.20, 1.0 and 5.0 mg oxamyl (a.s.)/L (equivalent to 0.0016*, 0.0091, 0.041, 0.19, 0.96 and 4.9 mg oxamyl (a.s.)/L, based on mean, measured oxamyl concentrations). The 48-hour LC_{50}, based on mean measured

concentrations and mortality data, was determined to be 0.096 mg a.s./L, with a 95% confidence interval of 0.059 to 0.156 mg a.s./L. The highest mean measured test concentration causing no mortality at test end was 0.0091 mg a.s./L. The lowest mean measured test concentration causing 100% mortality at test end was 0.96 mg a.s./L.

* Samples for the 0.0016 mg a.s./L test concentration were collected and analyzed, however, due to limitations of the method employed to analyze the samples, concentrations below 0.005 mg a.s./L cannot be detected. The 0.0016 mg a.s./L concentration represents the nominal concentration for this test solution.

I. MATERIALS AND METHODS

A. MATERIALS

1.	Test material:	Pure Oxamyl (PAI))
	Lot/Batch #:	D1410-532
	Purity:	99.1%
	Description:	Solid
	CAS#:	23135-22-0
	Stability of test compound:	Shown to be stable under the conditions of the test.
2.	Controls:	Dilution water (laboratory well water) control.
	Test vehicle:	None
	Toxic reference:	Not applicable
3.	Test organism:	Caddisfly
	Species:	*Chimarra atterima*
	Age/life stage at dosing:	Larval stage
	Initial population:	Four replicate test chambers with 5 caddisflies per test chamber
	Source:	Field collected by Stroud Water Research Center Avondale, PA 19311-9514
	Diet:	Unfed during test
	Test chamber:	250-mL glass beaker containing approximately 220 mL of test solution (6.5-cm test solution depth)
4.	Environmental conditions:	Dissolved oxygen: ≥8.0 mg/L (≥70% of saturation) pH: 7.3 to 7.7
	Temperature:	10.2 to 11.0°C in test chambers; 9.1 to 10.3°C measured continuously in an adjacent container of water.
	Photoperiod:	16 hr reduced light intensity (299 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

   01-May-2013 to 03-May-2013

2. Experimental treatments

   The acute toxicity of oxamyl to unfed larval stage *Chimarra atterima* was determined in an unaerated, static, 48-hour test. Treatments consisted of a dilution water control and six nominal concentrations of 0.0016, 0.008, 0.04, 0.20, 1.0, and 5.0 mg a.s./L (equivalent to 0.0016*, 0.0091, 0.041, 0.19, 0.96, and 4.9 mg a.s./L, based on mean, measured oxamyl concentrations). Larval stage caddisflies used in the test were field collected and placed in Whirlpack plastic bags by Stroud Water Research in Avondale, Pennsylvania. Upon receipt, the bags containing caddisflies were transferred to 2-L glass beakers containing dilution water and allowed to float while acclimating to the test temperature as required by the protocol. The temperature of the water was measured as 10°C. The caddisflies were acclimated in an environmental test chamber (incubator) for approximately 3 hours prior to test initiation.

The dilution water was well water with conductance of 324 µS/cm, alkalinity of 180 mg/L as CaCO3, and hardness of 136 mg/L as CaCO3, OC <1 mg C/L. Prior to use, the dilution well water was filtered to 0.45 µm and passed through an ultraviolet sterilizer to remove fine particles and microorganisms. The primary stock solution was sonicated for approximately 10 minutes, and was then stirred on a magnetic stir plate for 30 minutes to mix. The test solutions were stirred on a magnetic stir plate for 90 minutes to mix, and appeared clear and colorless at test initiation and termination. Five caddisflies were used per replicate with four replicates per test concentration and control.

* Samples for the 0.0016 mg a.s./L test concentration were collected and analyzed, however, due to limitations of the method employed to analyze the samples, concentrations below 0.005 mg a.s./L cannot be detected. The 0.0016 mg a.s./L concentration represents the nominal concentration for this test solution.

Samples were collected from the batches of test solution prepared for each treatment and control group at the beginning of the test and from two of the four replicate test chambers at 48 hours (± 1 hour) to measure concentrations of the test substance.Samples were analyzed by high performance liquid chromatography with UV detector.

Temperature, DO, and pH were measured in each test chamber at the beginning and end of the test and at approximately 24 hours. Temperature also was measured continuously in a extra container

3. Observations

Mortality and behavioral observations were made at approximately 2 hours, and at 24 and 48 hours (± 1 hour) following initiation of exposure.

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, probit analysis was used to calculate the 24 and 48-hour LC_{50} values and the 95% confidence intervals. The highest test concentration causing no mortality at test end and the lowest 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 oxamyl were 0.0016*, 0.0091, 0.041, 0.19, 0.96 and 4.9 mg a.s./L and ranged from 95 to 114% of nominal concentrations for the five highest test concentrations. Test concentration could not be accurately measured at the lowest test concentration due to limits of the analytical methodology. While observation results for the lowest test concentration are reported, the LC_{50} values and results of the test are based upon the five test concentrations where concentration could be measured. With the exception of a single mortality observed at 48 hours, caddisflies in the negative control appeared normal throughout the test. Caddisflies in the 0.0016 and 0.0091 mg a.s./L treatment groups also appeared normal throughout the test, with no mortality or overt signs of toxicity observed. Caddisflies that contracted their body when probed with a pipette, but had little to no motion, and appeared curled were noted in the 0.19, 0.96 and 4.9 mg a.s./L treatment groups at initial observations and at 24-hours. Summaries of cumulative mortality and sublethal effects are presented in Table 47 and Table 48, respectively.

* Samples for the 0.0016 mg a.s./L test concentration were collected and analyzed, however, due to limitations of the method employed to analyze the samples, concentrations below 0.005 mg a.s./L cannot be detected. The 0.0016 mg a.s./L concentration represents the nominal concentration for this test solution.

Table 47 Summary of cumulative mortality of unfed *Chimarra atterima* exposed to oxamyl for 48 hours in an unaerated, static, acute test

Mean measured oxamyl concentration (mg a.s./L)	Mortality (Cumulative No. dead/No. at test start)[a]											
	~2 Hours				24 Hours				48 Hours			
	A	B	C	D	A	B	C	D	A	B	C	D
Dilution water control (0.0)	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5	0/5
0.0016[b]	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
0.0091	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
0.041	0/5	0/5	0/5	0/5	0/5	1/5	0/5	1/5	2/5	2/5	2/5	2/5
0.19	0/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5	3/5	2/5	3/5	3/5
0.96	0/5	0/5	0/5	0/5	2/5	2/5	4/5	3/5	5/5	5/5	5/5	5/5
4.9	0/5	0/5	0/5	0/5	3/5	2/5	2/5	2/5	5/5	5/5	5/5	5/5

[a] A–D represent replicate test chambers containing 5 caddisflies each at test start.

[b] The 0.0016 m a.s./L test concentration could not be quantified, therefore 0.0016 mg a.s./L is the nominal test concentration for this group; all other concentrations are mean measured.

Table 48 Summary of sublethal effects of unfed *Chimarra atterima* exposed to Oxamyl for 48 hours in an unaerated, static, acute test

Mean measured oxamyl concentration (mg a.s./L)	Number affected/ Number alive [a]											
	~2 Hours				24 Hours				48 Hours			
	A	B	C	D	A	B	C	D	A	B	C	D
Dilution water control (0.0)	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/4	0/5	0/5
0.0016[b]	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
0.0091	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
0.041	0/5	0/5	0/5	0/5	0/5	0/4	0/5	0/4	0/3	0/3	0/3	0/3
0.19	1[c]/5	0/5	0/5	0/5	1[c]/5	0/5	1[c]/4	0/5	0/2	0/3	0/2	0/2
0.96	1[c]/5	1[c]/5	1[c]/5	0/5	3[c]/3	2[c]/3	1[c]/1	2[c]/2	-	-	-	-
4.9	2[c]/5	2[c]/5	2[c]/5	2[c]/5	2[c]/2	3[c]/3	3[c]/3	3[c]/3	-	-	-	-

[a] A–D represent replicate test chambers containing 5 caddisflies each at test start.

[b] The 0.0016 mg a.s./L test concentration could not be quantified, therefore 0.0016 mg a.s./L is the nominal test concentration for this group; all other concentrations are mean measured.

[c] C = organism contracts body when probed with a pipette, but has little to no motion, and has a curled appearance.

- = 100% mortality.

III. CONCLUSION

The 48-hour LC_{50} value for Caddisflies (*Chimarra atterima*) exposed to pure oxamyl (PAI) was 0.096 mg a.s./L (c.i., 0.059 - 0.156 mg a.s./L) based on mean measured concentrations. The highest mean measured test concentration causing no mortality at test end was 0.0091 mg a.s./L. The lowest mean measured test concentration causing 100% mortality at test end was 0.96 mg a.s./L.

(Brougher, D.S.; Martin, K.H.; Gallagher, S.P.; Krueger, H.O., 2013b)

RMS: Additional information were added by the RMS to the study summary.

There is no standard method for caddisflies. The OECD 202 (2004) validity criteria are met:

- No more than 10% effect or sign of disease/stress in the control (actual 5%);
- DO at the end of test ≥ 3 mg/L in the control and test vessels (actual 8.0 mg/L as a minimum).

Conclusion: The study is acceptable and relied upon.

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

B.9.2.4.2/07

Reference: CA 8.2.4.2/02	**Report:**	Brougher, D.S., Martin, K.H., Gallagher, S.P., Bodle, E.S. (2013b); Oxamyl (DPX-D1410) technical (98% w/w): A 48-hour static acute toxicity test with *Chironomus tentans* **DuPont Report No.:** DuPont-37400 **Guidelines:** OECD 202 (2004), OPPTS 850.1010 (1996) **Deviations:** None **Testing Facility:** Wildlife International Ltd. (USA), Easton, Maryland, USA **Testing Facility Report No.:** 112A-457 **GLP:** Yes **Certifying Authority:** Laboratories in the USA are not certified by any government agency, but are subject to regular inspections.

Executive summary:

The acute toxicity of oxamyl to unfed *Chironomus tentans* was determined in an unaerated, static, 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 (draft), OPPTS Number 850.1010, *Aquatic Invertebrate Acute Toxicity Test, Freshwater Daphnids*. Treatments consisted of a dilution water control and mean measured test concentrations of 0.053, 0.11, 0.21, 0.42 and 0.82 mg a.s./L. The 48-hour LC_{50} in *Chironomus tentans* was 0.35 mg a.s./L, with a 95% fiducial interval of 0.28 to 0.44 mg a.s./L, based on mean measured of oxamyl test concentrations and mortality data.

I. MATERIALS AND METHODS

A. MATERIALS

1.	Test material:	Pure Oxamyl (PAI)
	Lot/Batch #:	D1410-532
	Purity:	99.1%
	Description:	Solid
	CAS#:	23135-22-0
	Stability of test compound:	Shown to be stable under the conditions of the test.
2.	Controls:	Dilution water (laboratory well water) control at pH ~ 7.
	Test vehicle:	None
	Toxic reference:	Not applicable
3.	Test organism:	Midge
	Species:	*Chironomus tentans*
	Age/life stage at dosing:	First instar (1-4 days)
	Initial population:	Four replicate test chambers with 5 midges per test chamber
	Source:	Environmental Consulting and Testing, Superior, Wisconsin 54880
	Diet:	Unfed during test
	Test chamber:	250-mL glass beaker containing approximately 200 mL of test solution (6.0-cm test solution depth)
4.	Environmental conditions:	Dissolved oxygen: ≥8.2 mg/L (≥91% of saturation) pH: 7.2 to 7.9
	Temperature:	19.9 to 20.8°C in test chambers; 19 to 20°C measured continuously in an adjacent container of water.
	Photoperiod:	16 hr light (540 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-June-2013 to 28-June-2013

2. Experimental treatments

The acute toxicity of oxamyl to unfed *Chironomus tentans* (1-4 days old) was determined in an unaerated, static, 48-hour test. Midge larvae used in the test were first instar larvae (approximately 1 – 4 days after hatching) at test initiation and were obtained from Environmental Consulting and Testing, Superior, Wisconsin. Larvae were collected from three separate egg masses to start the test and were hatched in water from the same source and at approximately the same temperature as was used in the test. Midges larvae were fed prior to test initiation.

Treatments consisted of a dilution water control and nominal test concentrations of oxamyl were 0.063, 0.13, 0.25, 0.50 and 1.0 mg a.s./L. Five midges were used per replicate with four replicates per test concentration and control. The dilution water was well water with conductance of 324 µS/cm, alkalinity of 180 mg/L as CaCO3, and hardness of 136 mg/L as CaCO3, OC <1 mg C/L. Prior to use, the dilution well water was filtered to 0.45 µm and passed through an ultraviolet sterilizer to remove fine particles and microorganisms, and the pH was adjusted. The primary stock solution was sonicated for approximately 10 minutes, and was then stirred on a magnetic stir plate for 30 minutes to mix. The test solutions were stirred on a magnetic stir plate for 30 minutes to mix, and appeared clear and colorless at test initiation and termination.

Measured test concentrations for oxamyl were determined from samples of test water collected from each treatment and control group at the beginning and end of the test using HPLC with a UV detector.

3. Observations

Mortality and behavioral observations were made at approximately 4.5, 24, and 48 hours (± 1 hour) following initiation of exposure. Mortality was defined as a lack of reaction by the test organism to application of a gentle stimulus.

4. Statistics

The 48-hour mortality data were analyzed using the computer program of C. E. Stephan. The program was designed to calculate the LC_{50} value and the 95% fiducial interval by probit analysis, the moving average method, and binomial probability with nonlinear interpolation. In this study, probit analysis was used to calculate the 48-hour LC_{50} value and the 95% fiducial intervals. There was less than 50% mortality in any of the treatment groups at the 24-hour interval, which precluded the statistical calculation of an LC_{50} value at 24 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 oxamyl were 0.053, 0.11, 0.21, 0.42 and 0.82 mg a.s./L and ranged from 82 to 85% of nominal concentrations. Midges in the negative control and the 0.053 mg a.s./L treatment group appeared normal throughout the test, with no mortality or sublethal effects observed. Percent mortality at test termination in the 0.053, 0.11, 0.21, 0.42 and 0.82 mg a.s./L treatment groups was 0, 5, 30, 40 and 100%, respectively. Lethargy was observed for midges in the 0.42 and 0.82 mg a.s./L treatment groups. Results are summarized below.

Table 49 Summary of observed mortality of unfed *Chironomus tentans* exposed to oxamyl for 48 hours in an unaerated, static, acute test

Mean, measured oxamyl concentration (mg a.s./L)	Mortality (Cumulative No. dead/No. at test start)[a]											
	~4.5 Hours				24 Hours				48 Hours			
	A	B	C	D	A	B	C	D	A	B	C	D
Dilution water control (0.0)	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
0.053	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
0.11	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5
0.21	0/5	0/5	0/5	0/5	0/5	1/5	0/5	0/5	1/5	3/5	0/5	2/5
0.42	0/5	0/5	0/5	0/5	0/5	2/5	1/5	1/5	1/5	3/5	3/5	1/5
0.82	0/5	0/5	0/5	0/5	3/5	1/5	2/5	3/5	5/5	5/5	5/5	5/5

[a] A–D represent replicate test chambers containing 5 midges each at test start.

Table 50 Summary of sublethal effects of unfed *Chironomus tentans* exposed to oxamyl for 48 hours in an unaerated, static, acute test

Mean, measured oxamyl concentration (mg a.s./L)	Number affected/ Number alive [a, b]											
	~ 4.5 Hours				24 Hours				48 Hours			
	A	B	C	D	A	B	C	D	A	B	C	D
Dilution water control (0.0)	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
0.053	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
0.11	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/4	0/5
0.21	0/5	0/5	0/5	0/5	0/5	0/4	0/5	0/5	0/4	0/2	0/5	0/3
0.42	0/5	0/5	0/5	0/5	0/5	0/3	0/4	0/4	2 C/4	0/2	0/2	1 C/4
0.82	2 C/5	0/5	1 C/5	0/5	2 C/2	4 C/4	3 C/3	2 C/2	-	-	-	-

[a] A–D represent replicate test chambers containing 5 midges each at test start.

[b] C = lethargy.

III. CONCLUSION

The 48-hour LC_{50} value, based on the mean, measured test concentrations of oxamyl and mortality, was estimated to be 0.35 mg a.s./L, with a 95% fiducial interval of 0.28 to 0.44 mg a.s./L. The highest mean, measured test concentration causing no mortality at test end was 0.053 mg a.s./L. The lowest mean, measured test concentration causing 100% mortality at test end was 0.82 mg a.s./L.

(Brougher, D.S.; Martin, K.H.; Gallagher, S.P.; Bodle, E.S., 2013b)

RMS: Additional information were added by the RMS to the study summary.

The OECD 202 (2004) validity criteria are met:

- No more than 10% effect or sign of disease/stress in the control (actual 0%);
- DO at the end of test ≥ 3 mg/L in the control and test vessels (actual 8.2 mg/L as a minimum).

Conclusion: The study is acceptable and relied upon.

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

B.9.2.4.2/08

Reference: CA 8.2.4.2/07	**Report:**	Hicks, S.L. (2012); Oxamyl (DPX-D1410) technical (98% w/w): Effect on new shell growth of the eastern oyster (*Crassostrea virginica*) **DuPont Report No.:** DuPont-34273 **Guidelines:** OPPTS 850.1025 (1996) **Deviations:** None **Testing Facility:** ABC Laboratories, Inc. (Missouri), Columbia, Missouri, USA **Testing Facility Report No.:** 68030 **GLP:** Yes **Certifying Authority:** Laboratories in the USA are not certified by any government agency, but are subject to regular inspections.

Executive summary:

The effect of oxamyl on new shell growth by the Eastern oyster, *Crassostrea virginica*, was determined in an intermittent flow-through, 96-hour test. The test was conducted in accordance with the U.S. EPA, Office of Prevention, Pesticides and Toxic Substance (OPPTS), Ecological Effects Test Guideline 850.1025 and was intended to comply with U.S. FIFRA Subdivision E, Section 72-3. Treatments consisted of a dilution water control and six nominal concentrations of 3.3, 6.5, 13, 25, 50, and 100 mg a.s./L. The 96-hour LC_{50}, based on mortality was estimated to be greater than 94.4 mg a.s./L, the highest concentration tested. The 96-hour EC_{50}, based on new shell growth, was estimated to be 27.5 mg a.s./L with 95% confidence limits of 24.3 and 30.7 mg a.s./L. The NOEC was 5.28 mg a.s./L after 96 hours of exposure, based on lack of statistical significance in inhibition in new shell growth.

I. MATERIALS AND METHODS

A. MATERIALS

1.	Test material:	Oxamyl
	Lot/Batch #:	D1410-196
	Purity:	98.0% by analysis
	Description:	Technical material
	CAS#:	23135-22-0
	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:	Eastern oyster
	Species:	*Crassostrea virginica*
	Age at dosing:	Juvenile
	Valve height at dosing:	30.1 to 37.9 mm
	Initial population:	10 oysters per test chamber; 20 oysters per treatment
	Source:	Circle C Oyster Ranchers Assoc. Inc. of Dameron, Maryland.
	Acclimation period:	3 days
	Diet:	Pre-test: marine microalgae concentrate Test period: marine microalgae concentrate
	Test chamber:	Glass aquaria measuring approximately 21.7 cm in width, 37.0 cm in length, and 17.8 cm in height. The depth of the test solution was maintained at approximately 10.5 cm. The volume in each test chamber was approximately 8.4 L. There were two replicate chambers per treatment.
	Test medium:	Laboratory saltwater prepared at a salinity of approximately 20‰ by adding a commercial sea salt mix to laboratory freshwater.
4.	Environmental conditions (in-life period)	
	Temperature:	19.6 to 21.0°C (in test chambers)
	Salinity	18.1 to 19.8‰ during the test
	Dissolved oxygen	92 to 96% of saturation) at test initiation, 5.5 to 7.4 mg/L (70 to 94% of saturation) for the remainder of the test
	Photoperiod:	16 hr photoperiod (481 to 653 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

23-July-2012 to 27-July-2012

2. Experimental treatments

The effect of oxamyl on new shell growth by the Eastern oyster, *Crassostrea virginica*, was determined in an intermittent flow-through, 96-hour test. Treatments consisted of a dilution water control and six nominal concentrations of 3.3, 6.5, 13, 25, 50, and 100 mg a.s./L. Ten oysters were used per replicate with two replicates per test substance treatment and control.

3. Observations

Observations for mortality and other signs of test substance effect (e.g., slow valve closure and lack of feeding activity as evident from lack of fecal deposits) were made at 24, 48, 72, and 96 hours. New shell growth at test termination was measured to the nearest 0.1 mm with a vernier caliper.

4. Statistics

All statistical analyses were performed with SAS software (version 9.1). An estimate of 96-hour LC_{50} value was calculated using the probit method The NOEC, based on new shell growth, was estimated using a one-way analysis of variance (ANOVA) procedure and one-tailed Dunnett's test at the 0.05 level of significance. The alternate hypothesis was that the mean new shell growth for the treatment group had been reduced in comparison to the control mean new shell growth. Prior to the Dunnett's test, a Shapiro-Wilk test for normality and Bartlett's test for homogeneity of variance over treatments were conducted. The assumptions of normality and homogeneity of variance were met for the raw data values; therefore, a parametric analysis was performed on the raw data.

Percent change in new shell growth was determined using the following equation:

$$\%\,\text{Change} = \frac{\text{Mean Shell Growth of Treated Oysters - Mean Shell Growth of Control Oysters}}{\text{Mean Shell Growth of Control Oysters}} \text{ x } 100$$

The EC_{50} for new shell growth was estimated by a four-parameter logistic (sigmoid-shaped) model, two parameters fixed (100 and 0% inhibition), fit to the data with percent inhibition as the dependent variable and log concentration as the independent variable.

II. RESULTS AND DISCUSSION

Mean measured concentrations (*i.e.*, mean of the 0 and 96 hour measured concentrations) were 3.37, 5.28, 11.6, 23.0, 47.3, and 94.4 mg a.s./L, which represented recoveries of 81 to 102% of the nominal test substance treatment concentrations. No residues of oxamyl were detected in the dilution water control above the LOD of 0.0111 mg a.s./L.

Measured Concentrations of Oxamyl During a 96-Hour Exposure of the Eastern Oyster, *Crassostrea virginica*, Under Flow-Through Conditions

Sample	Nominal Concentration [a] (mg a.s./L)	Oxamyl Measured Concentration as mg a.s./L							
		Day -3		Day 0		Day 4		Mean [b]	
		mg/L	% of Nominal	mg/L	% of Nominal	mg/L	% of Nominal	mg/L	% of Nominal
Control	0	ND [c]	---	ND [c]	---	ND [c]	---	ND [c]	---
Level-1	3.30	3.24	98	3.52	107	3.21	97	3.37	102
Level-2	6.50	5.08	78	5.18	80	5.38	83	5.28	81
Level-3	13.0	11.1	85	11.4	88	11.8	91	11.6	89
Level-4	25.0	22.6	90	23.7	95	22.3	89	23.0	92
Level-5	50.0	43.5	87	45.8	92	48.7	97	47.3	95
Level-6	100	89.8	90	96.0	96	92.8	93	94.4	94
Stock	2,000	1,950	98	2,040	102	2,200	110	2,120	106
Low Spike	0.0532	0.0554 [d]	104	0.0469 [e]	88	0.0563	106	---	---
High Spike	143	145 [d]	101	145	101	152	106	---	---

[a] Nominal concentrations were corrected for 98.0% oxamyl purity.
[b] Mean value does not include Day -3.
[c] ND denotes not detected. The LOD was 0.0111 mg a.s./L.
[d] Average of triplicate injections
[e] Average of duplicate re-injections

All validation criteria were met for the study. A summary of cumulative mortality and new shell growth are presented the tables below, respectively.

Table 51 Observed mortality of eastern oyster, *Crassostrea virginica*, exposed to oxamyl for 96 hours in a flow-though, acute test

Mean measured oxamyl concentration (mg a.s./L)	Cumulative mortality (No. dead/No. at test start)[a]							
	24 Hour		48 Hour		72 Hour		96 Hour	
	A	B	A	B	A	B	A	B
0 (control)	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
3.37	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
5.28	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
11.6	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
23.0	0/10	1/10	0/10	2/10	0/10	2/10	0/10	2/10
47.3	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
94.4	0/10	0/10	1/10	0/10	1/10	0/10	1/10	5/10

[a] A and B represent replicates; each replicate contained 10 oysters (total 20 oysters per treatment) at test start.

Table 52 New shell growth by the eastern oyster, *Crassostrea virginica*, exposed to oxamyl for 96 hours in a flow–though, acute test

Mean measured oxamyl concentration (mg a.s./L)	Shell growth statistics	
	Mean length ± SD (mm)	Percent difference from control
0 (control)	3.8 ± 0.68 (range: 1.6 to 5.9)	NA
3.37	3.6 ± 0.51 (range: 2.1 to 5.5)	-5
5.28	3.2 ± 0.18 (range: 1.7 to 5.3)	-16
11.6	2.7 ± 0.03 (range: 1.1 to 4.5)	-28[a]
23.0	2.4 ± 0.06 (range: 1.2 to 3.8)	-37[a]
47.3	1.0 ± 0.12 (range: 0.2 to 2.0)	-73[a]
94.4	0.40 ± 0.10 (range: 0.1 to 0.9)	-90[a]

[a] Indicates a statistically significant reduction as compared to the negative control (p = 0.05). The 96-hour EC_{50} was estimated to be 27.5 mg a.s./L.

III. CONCLUSION

The 24-, 48-, 72-, and 96-hour LC_{50}, based on mortality was estimated to be greater than 94.4 mg a.s./L, the highest concentration tested. Based on mean measured concentrations of oxamyl and new shell growth, the 96-hour EC_{50} was estimated to be 27.5 mg a.s./L with 95% confidence limits of 24.3 and 30.7 mg a.s./L. The NOEC was 5.28 mg a.s./L after 96 hours of exposure, based on the lack of statistical significance in inhibition in new shell growth at this and all lower concentrations.

(Hicks, S.L., 2012)

RMS comments and conclusion

The validity criteria were checked:

(A) The mortality in the controls should not exceed 10 percent at the end of the test. Fulfilled.
(B) The dissolved oxygen concentration should be at least 60 percent of air saturation throughout the test (actual 92 to 96% of saturation). Fulfilled.

C) If evidence of spawning is observed, the test should be repeated. None of the animals were in spawning condition. Fulfilled.
(D) There should be evidence that the concentration of the substance being tested has been satisfactorily maintained over the test period. Fulfilled.
(E) Dissolved oxygen, temperature, salinity, and pH measurements should be made at the beginning and end of the test in each chamber. Fulfilled.
(F) A minimum of 2 mm of new shell growth should be observed in control oysters (solvent and dilution water). Actual 3.8 mm. Fulfilled.

Conclusion: the study is acceptable. 96-hour EC_{50} = 27.5 mg a.s./L.

B.9.2.5 Long-term and chronic toxicity to aquatic invertebrates

B.9.2.5.1 Reproductive and development toxicity to *Daphnia magna*

Active substance

Study submitted in the EU Dossier in 2001 and included in the first EU approval review.

B.9.2.5.1/01

Reference: --	**Report:**	Boeri, R.L., Ward, T.J. (2000a); Oxamyl technical: 21-day chronic, flowthrough toxicity to *Daphnia magna* **DuPont Report No.:** DuPont-2554 **Guidelines:** OECD 202 Part II (1984), U.S. EPA 72-4 (1988)

1. Test material: Pure Oxamyl (PAI)
   Lot/Batch #: D1410-196
   Purity: 96.9%

Material and Methods:

The chronic toxicity of ZA1963 to *Daphnia magna* (in-house culture of the T.R. Wilbury Laboratories) was determined in a 21-day study. Daphnia (<24 hours old) were exposed to oxamyl in water in an unaerated flow-through system for 21 days. A total of 20 neonates per concentration (4 replicates/concentration with 5 daphnids each) was used. Dilution water was deionized water added to salts to achieve the hardness of 160 to 180 mg/L as CaCO3. The water was aerated and filtered through a 5-µm filter The pH of the dilution water was continuously adjusted to 7.2 to 7.5 by the addition of 5% H_3PO_4. An intermittent flow proportional diluter was used, resulting in an average of 17.3 volume additions per 24 hours in each test vessel. Test solutions were maintained between 19.1 and 20.9°C (mean = 20.0°C). Daphnia were fed a mixture of yeast-ceraphyl-trout chow (approx. 5mg/l) and the green algae *Selenastrum capricornutum* (approx. 10^8 cells/l) three times per day throughout the test (except during the last day of testing when they were fed only once). The nominal test concentrations were 0.028, 0.052, 0.10, 0.20, and 0.40 mg a.s./L. One control group of animals were exposed to dilution water only. A photoperiod of 16 hours light and 8 hours darkness was employed with a 15 minutes transition period between dark and light intervals. Observations on immobilisation, abnormal effects and production of young were made daily (production of young observed daily following day of first brood). The number of live young per surviving daphnid was calculated as the sum of the live young produced each day divided by the number of surviving adults on the respective day. Length and dry weight of surviving adult Daphnids were determined at test end.

Dissolved oxygen, pH, temperature, and conductivity were measured in each replicate of the water control and test substance concentrations at the beginning of the test, and daily thereafter. Temperature in a water control chamber without daphnids was also measured continuously. Actual concentrations of oxamyl to which the Daphnia were exposed were determined by chemical analysis on Day 0 and every 7 days during the study, using an HPLC equipped with a UV detector..

Findings:

Table 53 Effects of Oxamyl Technical to *Daphnia magna* following chronic exposure (21 days).

Mean Concentration (mg a.i./l)	Mean No. of young/surviving adult *Daphnia*	% Parent Survival [b]	1st Day of Reproduction [c]	Adult dry weight	Mean *Daphnia* length on Day
Untreated Control	67	90	10	0.53	4.8
0.0268	66	85	9	0.47	4.8
0.0502	37	65	15	0.36	4.5
0.0935	37	80	13	0.35	4.5
0.192	24	50	12	0.37	4.1
0.405	--[a]	0	--	--	--

a Indicates that no measurements were taken due to 100% immobility in all replicates at that concentration.
b Percent of adult daphnids alive at the end of the test (immobility was synonymous with death)
c First day that reproduction was observed in the replicates

Observations:

Analytical verification of Oxamyl concentrations was made on test solutions sampled on day 0 and at regular intervals during the study. Mean, measured concentrations of Oxamyl were 0.0268, 0.0502, 0.0935, 0.192, and 0.405 mg a.s./L and ranged from 94 to 101% of nominal concentrations. All chemical and physical parameters for the 21-day study were within acceptable ranges. Temperature ranged between 19.1 and 20.9°C; pH ranged between 7.4 and 7.8; dissolved oxygen concentrations were between 8.6 and 9.3 mg/l (mean = 9.0 mg/l). The conductivity of the test solutions, including the control solution ranged from 520 – 610 µmshos/cm (mean = 570 µmhos/cm). Total alkalinity and EDTA hardness ranged 90-105 and 168-180 mg/L as CaCO3, respectively.

The effects of oxamyl exposure to Daphnia are presented in Table 54. No aborted eggs, ephippia or immobilised young were seen at any test substance concentration or in the control.

Table 54 Chronic toxicity of Oxamyl to the waterflea *Daphnia magna*

Test substance	TG a.i.		
Test object	*Daphnia magna*		
Exposure	21 day Flow-through		
Parameters	Reproduction	Length	Overall
Highest concentration without toxic effect (NOEC) mg a.i./l	0.0268	0.0268	0.0268
Lowest test concentration with effect (LOEC) mg a.i./l	0.0502	0.0502	0.0502
Maximum allowable toxicant concentration (MATC) mg a.i./l	0.0367	0.0367	0.0367

Conclusion:

The 21-day NOEC and MATC (maximum acceptable toxicant concentration) for Oxamyl, based on young per surviving adult daphnid, time to first brood and, mean length and dry weight of surviving adult daphnids were 0.0268 and 0.0367 mg a.s./L, respectively, for *Daphnia magna* .

RMS comments and conclusion

The reproductive and development toxicity to Daphnia magna study DuPont-2554, originally submitted under EU Rev8 Point IIA 8.2.5 and conducted with test material pure oxamyl (PAI), was conducted under guidelines OECD 202 Part II (1984), and U.S. EPA 72-4 (1988). A review of this study indicates that it partially meets the current guideline, OECD 211 (2012).

The following deviations were noted:

The C content of the diet was not measured.
The total number living offspring produced at the end of the test per parent daphnia at the start of the test was not calculated.

The following comments are made:

- The mortality of the parent animals does not follow a concentration-response pattern.
- The results should be expressed as ECx rather than (on in addition to) NOEC.

The RMS has recalculated the endpoints using ToxRAT 3.2.1, providing:

Per introduced parent daphnids:
Cumulative offspring per introduced parent
EC10= 15,626 µg/L (6,477-37,695); EC20 =25,676 µg/L (11,101-58,947); NOEC =26,800 µg/L
Cumulative offspring per surviving parent:
EC10= 22,562 (20,741-24,543); EC20= 36,995 (34,100-40,165); NOEC =26,800 µg/L
Immobility
LC10 = 26,323 µg/L (n.d.); EC20= 44,520 µg/L (n.d.); NOEC=93,500 µg/L

The validity criteria are met: the mortality of the parent animals (female Daphnia) does not exceed 20% at the end of the test; the mean number of living offspring produced per parent animal surviving at the end of the test is > 60 (actual 66.5).

Conclusion: Due to the lack of a clear concentration/response curve for parent mortality, the LCx could not be valid and as consequence also the ECx for the reproduction parameters could not be reliable. Taking into account all the above it is concluded that the NOEC =26,800 µg/L can be retained.

Metabolites

Study submitted in the EU Dossier in 2001 and included in the first EU approval review.

B.9.2.5.1/02

Reference: --	**Report:**	Boeri, R.L., Ward, T.J. (2000b); IN-D2708: Chronic, static-renewal toxicity to the daphnid, *Daphnia magna* **DuPont Report No.:** DuPont-3909 **Guidelines:** OECD 211 (1998)

1. Test material: IN-D2708 technical metabolite
   Lot/Batch #: D2708-6
   Purity: 99.87%

Materials and methods:

The effects of IN-D2708 on the growth and reproduction of *Daphnia magna* (<24-hour old, in-house culture of the testing T.R. Wilbury Laboratories) were assessed in an unaerated, static-renewal, 21-day test. Treatments consisted of a dilution water control, and nominal concentrations of 8.5, 17, 33, 65, and 130 mg IN-D2708/L. Dilution water was deionized water added to salts to achieve the hardness of 160 to 180 mg/L as CaCO3. The water was aerated and filtered through a 5-µm filter and then UV sterilized. The pH of the dilution water was continuously adjusted to 7.2 to 7.3 by the addition of 5% H3PO4. Ten replicates were used per treatment, with one daphnid per replicate. Beakers (250-mL) containing 200 mL of test solution (7-cm depth) were used. Test concentrations were renewed every Monday, Wednesday and Friday. The daphnids were fed YCT (yeast-ceraphyl-trout chow (approximately 5 mg/L dry weight)) and *Selenastrum capricornutum* (about 10^8 cells/L) daily (about0.2 mg total organic carbon per daphnid daily). Test solutions were maintained between 18.6 and 20.0°C. Dissolved oxygen, pH, and temperature were measured in two randomly selected replicates of the water control and all test concentrations before daphnids were added at the beginning of the test, at test solution renewal (from both old and new solutions), and at the end of the test. The temperature was continuously recorded in a representative beaker of water (without daphnids).

A photoperiod of 16 hours light and 8 hours darkness was employed with a 15 minutes transition period between dark and light intervals.

Analytical verification of IN-D2708 concentrations was made by HPLC with DAD/UV detector on test solutions sampled on day 0 and from newly-prepared test solutions on days 7 and 14. The old (aged) solution was collected from two randomly selected replicates of the control and each test concentration before media renewal on days 3, 10, 17, and 21. Observations on immobilisation, abnormal effects and production of young were made daily (production of young observed daily following day of first brood). Length and dry weight (after approximately 92 hours at 60°C) of surviving adult Daphnids were determined at test end.

A Chi-squared test was used to determine that all data were normally distributed except the immobilization of first generation daphnids. Bartlett's test was used to determine that variances were homogeneous for day of first brood, length, dry weight, and young per daphnid data and heteroscedastic for immobilization. A parametric ANOVA and Bonferroni's test were used to compare treatments to the control for day of first brood, length, dry weight, and young per daphnid data. A nonparametric Williams test was used to compare treatments to the control mean for immobilization data.

Findings:

Mean, measured concentrations of IN-D2708 were 8.39, 17.1, 33.1, 66.1, and 130 mg a.s./L and ranged from 99 to 102% of nominal concentrations. All chemical and physical parameters for the 21-day study were within acceptable ranges. Temperature ranged between 18.6 and 20.0°C (mean = 19.6); pH ranged between 7.3 and 7.8; dissolved oxygen concentrations were between 5.5 and 9.3 mg/l (mean = 7.7 mg/l). The conductivity of the test solutions, including the control solution ranged from 520 – 550 µmshos/cm (mean = 570 µmhos/cm).

The 21-day NOEC (no observed effect concentration) for IN-D2708 was 130 mg/L IN-D2708, based on mean, measured concentrations, and adult immobilization, the number of neonates per surviving adult, the day of first brood, and dry weight of surviving adult daphnids. For length, the most sensitive endpoint, the NOEC was 66.1 mg/L, and the MATC (maximum acceptable toxicant concentration), and LOEC (lowest observ d effect concentration) were 92.7 and 130 mg/L, respectively. T e 21 day EC50 (median effective concentration), based on adult daphnid immobility, was greater than 130 mg/L IN-D2708. A summary of percent adult survival, first day of reproduction, total live young produced per surviving female, total immobile young produced per surviving female, and length and dry weight of surviving adults is presented in Table 55.

Table 55 Summary of test endpoints following exposure of *Daphnia magna* to IN-D2708 for 21 days

Mean, measured concentrations of IN-D2708 (mg/L)	Mean % adult survivala	Mean first day of reproductionb	Mean total live youngc	Mean total immobile youngd	Mean adult length (mm)	Mean adult dry weight (mg)
Water Control	100	12	84	0	5.4	1.04
8.39	80	11	91	0	5.3	1.02

17.1	90	11	77	0	5.4	0.99
33.1	90	11	66	0	5.2	0.95
66.1e	100	11	81	0	5.3	1.06
130f	100	11	79	0	5.1	0.98

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 mean total length of surviving daphnids
f LOEC based on mean total length of surviving daphnids

Conclusion:

The 21-day NOEC and MATC for IN-D2708 were 66.1 and 92.7 mg/L, respectively, for *Daphnia magna* , based on mean, measured concentrations and the mean total length of surviving daphnids.

RMS comments and conclusion

The reproductive and development toxicity to Daphnia magna study DuPont-3909, originally submitted under EU Rev8 Point IIA 8.2.5 and conducted with test material IN-D2708 technical metabolite, was conducted under guideline OECD 211 (1998). A review of this study indicates that it partially meets the current guideline, OECD 211 (2012).

The validity criteria are met: the mortality of the parent animals (female Daphnia) does not exceed 20% at the end of the test (actual 0%); the mean number of living offspring produced per parent animal surviving at the end of the test is > 60 (actual 84).

The replicates with parental mortality have been excluded from the analysis of the test result, as recommended by the OECD guideline in case the mortality does not follow a concentration-response pattern.

The following deviations were noted:

Adult daphnids in the water control produced an average of 84 ± 23 total live young at the end of 21-days (the CV was 27% instead of ≤25%).

The total number living offspring produced at the end of the test per parent daphnia at the start of the test was not calculated . Anyhow, considering the 100% survival of parent animals in the two highest concentrations, is not going to change the NOEC.

Conclusion: Acceptable. A dose response curve is not available due to the low toxicity observed, hence any ECx can be calculated. The NOEC =66.1 mg/L is retained.

B.9.2.5.2 Reproductive and development toxicity to an additional aquatic invertebrate species

Oxamyl is not applied directly to water bodies or rivers. Acute toxicity testing with three insect species (*Chironomus tentans*, *Centroptilum triangulifer*, and *Chimarra atterima*) and four crustacean species (*Hyalella*

azteca, *Ceriodaphnia dubia*, *Daphnia pulex*, and *Americamysis bahia*) revealed that *A. bahia* was the most sensitive. Thus additional chronic testing was conducted with this most sensitive species.

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

B.9.2.5.2/01

Reference: CA 8.2.5.2/01	**Report:**	Hicks, S.L. (2013); Oxamyl (DPX-D1410) technical (98% w/w): Life-cycle toxicity test of the saltwater mysid, *Americamysis bahia*, conducted under flow-through conditions **DuPont Report No.:** DuPont-34269 **Guidelines:** OPPTS 850.1350 (1996) **Deviations:** None **Testing Facility:** ABC Laboratories, Inc. (Missouri), Columbia, Missouri, USA **Testing Facility Report No.:** 68032 **GLP:** No **Certifying Authority:** Laboratories in the USA are not certified by any government agency, but are subject to regular inspections.

Executive summary:

The life-cycle toxicity of oxamyl to the estuarine/marine mysid, *Americamysis bahia* was determined in 28-day flow-through test. The test was conducted in accordance with U.S. Environmental Protection Agency, 1996 Ecological Effects Test Guidelines, OPPTS 850.1350, Mysid Chronic Toxicity Test. The purpose of this test was to determine the potential for acute and chronic effects of the test substance of mysids exposed during a 28-day life-cycle test. The toxicological endpoints of concern were survival (*i.e.*, 7-, 13-, 14-, 21-, and 28-day parental mysid, *i.e.*, F_0 or G1 and 4 and 7-day juvenile, *i.e.*, F_1 or G2 survival), growth (*i.e.*, total length of males and females for parental and juvenile mysids), and reproduction (*i.e.*, young per female for parental mysids). Results of the study were used to estimate the no-observed-effect concentration (NOEC) and the lowest-observed effect concentration (LOEC) of oxamyl during the life-cycle of the mysid conducted under flow-through test conditions. Treatments consisted of a saltwater control and six nominal test concentrations of 0 (Control), 1.30, 2.5, 5.0, 10, 20, 40 µg a.s./L. Based on mean measured concentrations of oxamyl, survival of F_0 mysids was the most sensitive endpoint when compared to the control. The NOEC and LOEC values for this parameter were 18.9 and 37.8 µg a.s./L, respectively, and the MATC was 26.7 µg a.s./L.

I. MATERIALS AND METHODS

A. MATERIALS

1.	Test material:	Pure Oxamyl (PAI)
	Lot/Batch #:	D1410-196
	Purity:	98.0% by analysis
	Description:	Solid, powder
	CAS#:	23135-22-0
	Stability of test compound:	Stable at normal temperatures and storage conditions
2.	Control:	Dilution (laboratory salt water) water salinity 20 ± 3‰
	Solvent control:	None
	Test vehicle:	Dilution (laboratory salt water) water
	Toxic reference:	None
3.	Test organism:	Mysid Shrimp
	Species:	*Americamysis bahia*
	Age at dosing:	<24 hours
	Initial population:	30 mysids per test chamber, 3 replicates per treatment for a total of 90 mysids per treatment
	Source:	ABC Laboratories, in-house culture
	Diet:	Fed at least twice daily during test
	Test chambers:	Glass aquaria measuring 19 cm wide × 76.5 cm long (maximum) or 38 cm long (minimum) × 21 cm high. Each chamber had a glass pane with in the middle of the chamber with two holes near the bottom of the chamber. This partition effectively cut the test chamber volume in half when the hoses were stoppered during the first 13 days of the study. There were three replicate chambers per treatment.
4.	Environmental conditions (in-life period)	
	Temperature:	23.9 to 25.9°C (of test chambers)
	Photoperiod:	14 hr photoperiod (438 to 689 lux) and 10 hr darkness which included two-30 min transitional light periods.

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed

02-October-2012 to 30-October-2012

2. Experimental treatments

The life-cycle toxicity of oxamyl to the estuarine/marine mysid, *Americamysis bahia* was determined in 28-day flow-through test. Treatments consisted of a saltwater control and six nominal test concentrations of 1.3, 2.5, 5.0, 10, 20, and 40 µg a.s./L. Thirty mysids were used per replicate with three replicates per test concentration and control (total of 90 mysids per treatment group).

3. Observations

Observations of mortality and sublethal responses were made daily for the duration of the testing period. Dead mysids were counted, identified by gender (if mature), and removed at the time of observation. Any missing mysids at the time of observation were noted as not found and were considered dead if not found on following observations days; that mortality was subsequently reflected back to the time that the mysid was first observed missing. Any mysids that were inadvertently damaged or died as a result of becoming impinged onto the retention- or brood-basket mesh were removed from the initial number of mysids for those replicates and were not considered to be treatment-related mortality.

After 13 days of exposure, the adult males and females from the reproduction retention chamber were isolated and paired with each pair being placed into a brood cup. Where possible a total of seven

breeding pairs were set up from these animals. If there were insufficient numbers of males or females to achieve this total, adults were removed from those in the growth retention chamber until a total of seven breeding pairs were achieved. Once paired, the mysids in the brood cups were observed for mortality and reproduction (*i.e.*, young per female). The first day young were observed was considered the day of first brood, although release of these young may have occurred over a period of up to 2 or 3 days. The F_0 mysids were terminated on Day 28 of the exposure, and no further data were collected for this generation. The F1-mysid exposure phase of the test was initiated with at least the first 15 post-larval F1 mysids, or fewer when 15 young were not available. The post-larval F1 mysids were assigned to retention baskets within the same test chambers as the F0-mysid exposure. The F1 mysids were terminated and body length measured when they reached 7 days of age, with the exception of groups inadvertently measured at 8 days of age, because this was the maximum achievable age for all F1 mysids at termination of the F0-mysid exposure (day 28). However, the statistical results reported were for only 7-day-old mysids.

Daily observation and enumeration of young produced per reproductive pair were made after test Day 14 until test termination.

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. Salinity was measured daily.

4. Statistics

The NOECs, based on percent survival, survival of second generation offspring, reproduction (*i.e.*, young per female), and adult length, were estimated using a one-way analysis of variance (ANOVA) procedure and either one-tailed Fisher's test with Hochberg's familywise adjustment for significance or a one-tailed Dunnett's test. Estimates of LC_{50} values and their 95% confidence limits were calculated using the probit method and Trimmed Spearman-Karber 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-Karber method was selected for reporting.

II. RESULTS AND DISCUSSION

A. FINDINGS

Mean measured concentrations of oxamyl ranged from 75 to 100%. All validation criteria were met for the study. A summary of cumulative mortality and sublethal effects is presented in the table below.

Table 56 A summary of cumulative mortality and sublethal effects for *Americamysis bahia*

Mean measured concentration (µg a.s./L)	0-28-Day survival of F_0 Mysids (%)	7-Day survival of F_1 Mysids (%)	Day 28 F_0-Male Mysid mean Total length (mm)	Day 28 F_0-Female Mysid mean Total length (mm)	Mean total Young per F_0-female Mysid
Control	100	98	6.29	6.32	8.33
1.06	100	100	6.33	6.39	7.86
1.88	91	98	6.27	6.40	8.19
4.04	93	93	6.19	6.23	6.52
9.07	96	98	6.21	6.32	5.95
18.9	96	96	6.27	6.29	5.19
37.8	33	100 [a]	6.48	6.47	5.33

[a] Represents survival for one of the three replicates. The remaining two replicates did not reach 7 days of age before test termination (*i.e.*, study day 28)

III. CONCLUSION

Based on mean measured concentrations of oxamyl, survival of the F_0 mysids was the most sensitive endpoint when compared to the control. The NOEC and LOEC values for this parameter was 18.9 and 37.8 µg a.s./L, respectively, and the MATC was 26.7 µg a.s./L.

(Hicks, SL., 2013)

RMS comments and conclusion
The study is acceptable. The EC10, and EC20 are not reliably calculable due to the data set.

B.9.2.5.3 Development and emergence in Chironomus species

Oxamyl is not an insect growth regulator, thus no additional study on chronic toxicity was carried out using relevant non-crustacean species such as *Chironomus* spp.

B.9.2.5.4 Sediment dwelling organisms

A study was not conducted since oxamyl is not expected to move to or persist in aquatic sediments. The average K_{oc} = 17 mL/g for oxamyl. Oxamyl is not a persistent substance in water/sediment systems. Persistence endpoints for the total water sediment system in two streams were: Red Oak Stream DT_{50} = 0.82, DT_{90} = 8.31 days, and for Town Park Pond, DT_{50} = 0.69, DT_{90} = 2.28 days (DuPont-42129). An acute toxicity test was carried out with first instar (2–3 days old) *Chironomus tentans* (48-h water-only study). The onset of effects is rapid. Oxamyl is of similar toxicity to *Chironomus tentans* compared to *Daphnia magna* , thus no chronic study was triggered with *Chironomus* sp.

B.9.2.6 Effects on algal growth

B.9.2.6.1 Effects on growth of green algae

Active substance

Study submitted in the EU Dossier in 2001 and included in the first EU approval review

B.9.2.6.1/01

Reference: -99-	**Report:**	Boeri, R.L., Magazu, J.P., Ward, T.J. (2000); Oxamyl technical: Influence on growth and growth rate of the alga, *Selenastrum capricornutum* **DuPont Report No.:** DuPont-2909 **Guidelines:** OECD 201 (1984)

1. Test material: Pure Oxamyl (PAI)
   Lot/Batch #: D1410-196
   Purity: 96.9%

Material and Methods:

The effect of Oxamyl on *Selenastrum capricornutum* was determined using algal cultures with sterile synthetic medium (T.R. Wilbury Laboratories). The test was performed under static conditions with five concentrations of test substance and a test media control at 24 ±2°C. Three replicates at 0.517, 1.01, 1.91, 3.99, and 8.01 mg a.s./L, measured at test initiation, were incubated for 120 hours and cell counts were taken at 24-hour intervals.

One control group (3 replicates) of cultures were exposed to growth medium only. Growth medium met the characteristic of the AAP medium indicated in the OECD guideline andcontained less than 10 mg/L particulate matter and had a total organic carbon concentration of 1.4 mg/L. The pH was 7.5 at test start. The test was performed in 250 mL glass flasks) that contained 50 mL of test solution arranged in a rotary shaker adjusted to 100 rpm. An incubator was used which mainained the temperature in a range of 23.9 to 24.0 °C. A photoperiod of 24 hours light (approximately 3,800 to 4,000 lux) was employed. The occurrence of cell size differences, unusual cell shapes, colours, flocculations, adherence of cells to test containers or aggregation of cells was determined. Recovery after a further 120 hours was assessed at 8.01 mg a.s./L.

Actual concentrations of oxamyl were determined by chemical analysis at 0 and after 72 and 120 hours in control and test solutions, using a Hewlett Packard Series 1100 HPLC equipped with a UV detector. Algal cell numbers were determined visually by means of direct microscopic examination with a haemacytometer at 24, 48, 72, 96 and 120 hours. Temperature and pH were determined at the beginning and at the end of the test.

The 24-hour EC25 and EC50 values could not be calculated because there was identical growth in the control and all tested concentrations. The 48, 72, 96, and 120 hour EC25 and EC50 values were calculated using the number of cells per mL, using the average specific growth rate, and using the area under the growth curve by a weighted least squares non-linear regression technique described by Bruce and Versteeg. The slope of the concentration-response curve is not calculated by this method. The no observed effect concentration (NOEC) was determined using a one-way analysis of variance (ANOVA) and Dunnett's test (TOXSTAT 3.3).

Findings:

In the control medium the PH increased from 7.5 at 0 h to a mean of 8.8 (ranging from 7.5 tpo 10.1) at 120h.

The initial measured concentrations of Oxamyl ranged from 96 to 103% of the targeted nominal concentrations after correction for test substance purity of 96.9%. Because not all measured concentrations of Oxamyl were greater than 70% of nominal at 72 and 120 hours, initial measured concentrations were used for all calculations.

Measured Concentrations of Oxamyl in Oxamyl Technical in Test Solution Samples

Nominal Concentration (mg/L)	Corrected Nominal Concentration[a] (mg/L)	Measured Oxamyl Concentration (mg/L) 0 Hour	72 Hours	120 Hours	0 Hour Percent Recovery (%) [a]
Test Media					
Water Control		ND [b]	ND	ND	---
0.52	0.50	0.517	0.224	ND[c]	103
1.0	1.0	1.01	0.529	ND[c]	101
2.1	2.0	1.91	1.04	0.141	96
4.1	4.0	3.99	2.37	1.93	100
8.3	8.0	8.01	4.72	3.65	100
Laboratory Control Sample					
2.1	2.0	1.96	2.03	2.02	98
Matrix Spike Sample					
2.1	2.0	--	1.98	1.24	
		--	1.90	1.29	--
Stability Blank					
8.3 (light)	8.0	8.42	4.84	3.85	105
8.3 (dark)	8.0[d]	---	5.16	3.87	---
Blank					
0		ND	ND	ND	---

[a] Based on nominal concentrations corrected for Oxamyl Technical purity of 96.9%.
[b] ND denoted the limit of detection was 0.000370 mg/L.
[c] ND denoted the limit of quantitation was 0.00123 mg/L.
[d] This sample was shielded from the light for 120 hours.

Cell Growth Data from the Toxicity Test with the Freshwater Alga, *Selenastrum capricornutum*, and Oxamyl Technical

Initial Measured Concentration of Oxamyl (mg/L)	Rep.	Number of Cells per Milliliter					
		Hour of Exposure					
		0	24	48	72	96	120
Water Control	1	3,000	<10,000	56,000	600,000	2,620,000	4,740,000
	2	3,000	10,000	55,000	622,000	2,680,000	4,340,000
	3	3,000	10,000	60,000	482,000	2,220,000	3,700,000
	mean	3,000	<10,000	57,000	568,000	2,507,000	4,260,000
0.517	1	3,000	<10,000	40,000	380,000	1,740,000	4,540,000
	2	3,000	<10,000	41,000	476,000	2,420,000	3,600,000
	3	3,000	<10,000	39,000	422,000	1,740,000	4,120,000
	mean	3,000	<10,000	40,000	426,000	1,967,000	4,087,000
	% of control	100	100	70	75	78	96
1.01	1	3,000	<10,000	31,000	356,000	1,800,000	3,060,000
	2	3,000	<10,000	30,000	314,000	1,480,000	2,740,000
	3	3,000	<10,000	22,000	280,000	1,240,000	3,520,000
	mean	3,000	<10,000	28,000	317,000	1,507,000	3,107,000
	% of control	100	100	49	56	60	73
1.91	1	3,000	<10,000	11,000	118,000	584,000	1,940,000
	2	3,000	<10,000	15,000	114,000	780,000	1,780,000
	3	3,000	<10,000	<10,000	102,000	508,000	1,820,000
	mean	3,000	<10,000	<12,000	111,000	624,000	1,847,000
	% of control	100	100	<21	20	25	43
3.99	1	3,000	<10,000	<10,000	<10,000	28,000	86,000
	2	3,000	<10,000	<10,000	<10,000	52,000	124,000
	3	3,000	<10,000	12,000	<10,000	56,000	188,000
	mean	3,000	<10,000	<11,000	<10,000	45,000	133,000
	% of control	100	100	<19	<2	2	3
8.01	1	3,000	<10,000	<10,000	<10,000	<10,000	<10,000
	2	3,000	<10,000	<10,000	<10,000	<10,000	<10,000
	3	3,000	<10,000	<10,000	<10,000	<10,000	<10,000
	mean	3,000	<10,000	<10,000	<10,000	<10,000	<10,000
	% of control	100	100	<18	<2	<1	<1

Average Specific Growth Rate and Percent of Control from the Toxicity Test with the Freshwater Alga, *Selenastrum capricornutum,* and Oxamyl Technical

Initial Measured Concentration of Oxamyl (mg/L)	Average Specific Growth Rate				
	24 hour	48 hour	72 hour	96 hour	120 hour
Water Control	0.050	0.061	0.073	0.070	0.060
0.517	0.050	0.054	0.069	0.068	0.060
1.01	0.050	0.047	0.065	0.065	0.058
1.91	0.050	0.029	0.050	0.056	0.054
3.99	0.050	0.027	0.017	0.028	0.032
8.01	0.050	0.025	0.017	0.013	0.010
Initial Measured Concentration of Oxamyl (mg/L)	Percent of Control				
	24 hour	48 hour	72 hour	96 hour	120 hour
Water Control	--	--	--	--	--
0.517	100	89	95	97	100
1.01	100	77	89	93	97
1.91	100	48	68	80	90
3.99	100	44	23	40	53
8.01	100	41	23	19	17

The effects of Oxamyl on the growth of *Selenastrum capricornutum* are shown in Table 57. The algal population grew well and was in the log phase of growth resulting in an average of 4,260,000 cells/ml in the control after 120 hours. No effects (size differences, flocculations, unusual cell shapes, colours, adherence of cells to test containers or aggregation of cells) were observed during the test. Recovery was observed as an increase in cell number by >300 cells/ml in the highest concentration tested (8.01 mg/l) indicated that oxamyl was algistatic rather than algicidal.

Table 57 Summary of algal growth inhibition following exposure of Selenastrum capricornutum to Oxamyl for 120 hours

Initial measured concentrations of Oxamyl (mg a.s./L)	Mean cell density (cells/mL)	% Inhibition	
		Growth rate	Area under the growth curve
Water control	4,260,000	--	--
0.517	4,087,000	0	15*
1.01	3,107,000*	3	35*
1.91	1,847,000*	10*	68*
3.99	133,000*	47*	98*

8.01	<10,000*	83*	99*

*Significantly different from control by one way analysis of variance (ANOVA) and Dunnett's test.

Conclusion:

Growth inhibition data obtained with Oxamyl on *Selenastrum capricornutum* are given below, based on initial measured concentrations The effects of Oxamyl on *Selenastrum capricornutum* are expected to be reversible at 8.01 mg a.s./L.

Parameter	Endpoint (mg a.s./l)			
	72h EC_{50}	72h Calculated NOEC	120h EC_{50}	120h NOEC
Cell Density	1.0	<0.517	1.68	0.517
Area under the growth curve	0.931	<0.517	1.37	<0.517
Growth Rate	2.61	1.01	4.16	1.01

RMS comments and conclusion

Additional information and tables have been added by the RMS to the original summary.

The effects on growth of green algae study, DuPont-2909, originally submitted under EU Rev8 Point IIA 8.2.6 and conducted with test material pure oxamyl (PAI), was conducted under guideline OECD 201 dated 1984 which was the adopted version at the time of testing. The RMS re-evaluated the study according to current version of the guideline (2006-2011) and added some information to the study summary..

The following deviations were noted:

-initial population was $1x10^3$ cell/mL instead of $5x10^3$- 10^4 cell/mL.

-In one control vessel the pH increased of 2.5 unit at the end of test (120h).

- The nominal concentrations of the active ingredient, oxamyl, were not maintained during the test, hence the results at 72h should be expressed as geomean measured concentrations. In support of this, it is noted that a decrease of growth inhibition was observed during the course of the test. At 120h, the calculation of the mean concentration is not possible because two <LOQ values were recorded.

Not all the validity criteria (analyzed with Toxrat 3.2 Professional) can be checked:

1)"The biomass in the control cultures have increased exponentially by a factor of >16 within the 72-hour test period" is fulfilled (actual 189.3).

2) "The mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3, for 72-hour tests) in the control cultures is lower than 35%" cannot be verified because lack of adequate raw data (see table of cell growth data in the table above).

3) "The coefficient of variation of average specific growth rates during the 72h test period in replicate control cultures was ≤ 7%" is fulfilled (actual 2.6%).

Conclusion: **the study is not acceptable.**

Study submitted in the EU Dossier in 2001 and included in the first EU approval review.

B.9.2.6.1/02

Reference: --	**Report:**	Ward, T.J., Magazu, J.P., Boeri, R.L. (1999); IN-A2213: Influence on growth and growth rate of the alga, *Selenastrum capricornutum* **DuPont Report No.:** DuPont-2505 **Guidelines:** OECD 201 (1984)

1. Test material: IN-A2213 technical metabolite
   Lot/Batch #: A2213-11
   Purity: 100%

Materials and methods:

The effect of IN-A2213 on *Selenastrum capricornutum* was determined using algal cultures with sterile synthetic medium. Three replicates at 0, 7.58, 15.7, 31.5, 60.4, and 122 mg/L IN-A2213, measured at test initiation, were incubated for 120 hours and cell counts were taken at 24-hour intervals. Recovery after a further 48 hours was assessed at 130 mg/L IN- A2213. Growth medium met the characteristic of the AAP medium indicated in the OECD guideline. The pH was 7.4 at test start. The test was performed in 250 mL glass flasks that contained 50 mL of test solution arranged in a rotary shaker adjusted to 100 rpm. An incubator was used which mainained the temperature in a range of 23.5 to 24.0 °C. A photoperiod of 24 hours light (approximately 3,700 to 4,000lux) was employed.

The occurrence of cell size differences, unusual cell shapes, colours, flocculations, adherence of cells to test containers or aggregationof cells was determined. Actual concentrations of IN-A2213 were determined by chemical analysis at 0 and after 72 and 120 hours in control and test solutions, using HPLC with UV detector. Algal cell numbers were determined visually by means of direct microscopic examination with a haemacytometer at 24, 48, 72, 96 and 120 hours. Temperature and pH were determined at the beginning and at the end of the test.

Findings:

In the three control replicates the pH at 0h was 7.4 and at 120h ranged 8.5-10.0. Considering all test replicates, the pH ranged from 7.3 to 10.

Mean Measured Concentration of IN-A2213 (mg/L)	Rep.	Number of Cells per Milliliter					
		Hour of Exposure					
		0	24	48	72	96	120
ND[a] (control)	1	3,000	<10,000	26,000	250,000	1,674,000	9,900,000
	2	3,000	<10,000	22,000	294,000	2,240,000	10,840,000
	3	3,000	<10,000	40,000	336,000	2,340,000	8,640,000
	mean	3,000	<10,000	29,000	293,000	2,085,000	9,793,000
7.58	1	3,000	<10,000	32,000	238,000	3,100,000	7,420,000
	2	3,000	<10,000	28,000	324,000	2,020,000	6,860,000
	3	3,000	<10,000	34,000	368,000	2,740,000	7,000,000
	mean	3,000	<10,000	31,000	310,000	2,620,000	7,093,000
	% of control	100	100	107	106	126	72
15.7	1	3,000	<10,000	20,000	310,000	1,800,000	6,560,000
	2	3,000	<10,000	38,000	214,000	1,920,000	5,580,000
	3	3,000	<10,000	32,000	346,000	2,780,000	5,460,000
	mean	3,000	<10,000	30,000	290,000	2,167,000	5,867,000
	% of control	100	100	103	99	104	60
31.5	1	3,000	<10,000	24,000	266,000	2,020,000	5,840,000
	2	3,000	<10,000	30,000	414,000	2,620,000	6,260,000
	3	3,000	<10,000	26,000	258,000	2,040,000	5,600,000
	mean	3,000	<10,000	27,000	313,000	2,227,000	5,900,000
	% of control	100	100	93	107	107	60
60.4	1	3,000	<10,000	24,000	382,000	2,260,000	4,300,000
	2	3,000	<10,000	28,000	410,000	1,600,000	4,760,000
	3	3,000	<10,000	22,000	292,000	3,560,000	4,900,000
	mean	3,000	<10,000	25,000	361,000	2,473,000	4,653,000
	% of control	100	100	86	123	119	48
122	1	3,000	<10,000	26,000	442,000	2,240,000	4,920,000
	2	3,000	<10,000	34,000	354,000	2,780,000	4,340,000
	3	3,000	<10,000	28,000	380,000	2,220,000	6,380,000
	mean	3,000	<10,000	29,000	392,000	2,413,000	5,213,000
	% of control	100	100	100	134	116	53

[a] ND denoted the limit of detection was 0.00258 mg/L.

Average Specific Growth Rate and Percent of Control from the Toxicity Test with the Freshwat Alga, *Selenastrum capricornutum,* and IN-A2213

Mean Measured Concentration of IN-A2213 (mg/L)	Average Specific Growth Rate				
	24 hour	48 hour	72 hour	96 hour	120 hour
ND[1] (control)	0.050	0.047	0.064	0.068	0.067
7.58	0.050	0.049	0.064	0.071	0.065
15.7	0.050	0.048	0.063	0.069	0.063
31.5	0.050	0.046	0.065	0.069	0.063
60.4	0.050	0.044	0.067	0.070	0.061
122	0.050	0.047	0.068	0.070	0.062

Mean Measured Concentration of IN-A2213 (mg/L)	Percent of Control				
	24 hour	48 hour	72 hour	96 hour	120 hour
ND (control)	--	--	--	--	--
7.58	100	104	100	104	97
15.7	100	102	98	101	94
31.5	100	98	102	101	94
60.4	100	94	105	103	91
122	100	100	106	103	93

[a] ND denoted the limit of detection was 0.00258 mg/L.

The effects of IN-A2213 on the growth of *Selenastrum capricornutum* are shown in Table 58. The mean measured concentrations of IN-A2213 ranged from 89 – 94% of the targeted nominal concentrations after correction for test substance purity of 100%. The algal population was all in the log phase of growth, resulting in an average of 9,793,000 cells/ml in the control water after 120 hours. No effects (size differences, flocculations, unusual cell shapes, colours, adherence of cells to test containers or aggregation of cells) were observed during the test. Recovery was observed as an increase in cell number from a calculated cell count of 156,000 cells/ml to 1,390,000 cells/ml in the highest concentration tested (130 mg/l) indicated that oxamyl was algistatic rather than algicidal.

Table 58 Summary of algal growth inhibition following exposure of *Selenastrum capricornutum* to IN-A2213 for 120 hours

Mean measured concentration (mg/L)	Mean cell density (cells/mL)	% Inhibition	
		Growth rate	Area under the growth curve
Water control	9,793,000	--	--
7.58	7,093,000*	3*	11
15.7	5,867,000*	6*	26*
31.5	5,900,000*	6*	24*
60.4	4,653,000*	9*	29*
122	5,213,000*	7*	26*

*Significantly different from control by one way analysis of variance(ANOVA) and Dunnett's test.

Conclusion:

Growth inhibition data obtained with IN-A2213 on *Selenastrum capricornutum* are given below, based on mean concentrations. The effects of IN-A2213 on *Selenastrum capricornutum* are expected to be reversible at 130 mg/L.

Parameter	Endpoint			
	72h EC_{50}	72h Calculated NOEC	120h EC_{50}	120h NOEC
Cell Density	>122	122	>122	<7.58
Area under the growth curve	>122	122	>122	7.58
Growth Rate	>122	122	>122	<7.58

RMS comments and conclusion

Additional information and tables have been added by the RMS to the original summary.

The effects on growth of green algae study, DuPont-2505, originally submitted under EU Rev8 Point IIA 8.2.6 and conducted with test material IN-A2213 technical metabolite, was conducted under guideline OECD 201 (1984). The RMS re-evaluated the study according to current version of the guideline (2006-2011) and added some information to the study summary.

The following deviations were noted:

-initial population was $1x10^3$ cell/mL instead of $5x10^3$- 10^4 cell/mL.

-In one control vessel the pH increased of 2.6 unit at the end of test (120h).

Not all the validity criteria (analyzed with Toxrat 3.2 Professional) can be checked:

1)"The biomass in the control cultures have increased exponentially by a factor of >16 within the 72-hour test period" is fulfilled (actual 97.8)

2) "The mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3, for 72-hour tests) in the control cultures is lower than 35%" cannot be verified because lack of adequate raw data (see table of cell growth data in the table above).

3) "The coefficient of variation of average specific growth rates during the 72 test period in replicate control cultures was ≤ 7%" is fulfilled (actual 3.2).

Both the 72h and the 120h EC50 are based on mean concentrations measured at 0. 72 and 120h.

Conclusion: one validity criterion could not be verified. Taking into account the lack of any effect up to the highest concentration, the 72h/120h EC_{50} can be taken as supportive information of the low toxicity of the metabolite.

Study submitted in the EU Dossier in 2001 and included in the first EU approval review.

B.9.2.6.1/03

Reference: --	**Report:**	Boeri, R.L., Magazu, J.P., Ward, T.J. (1999a); IN-D2708: Influence on growth and growth rate of the alga, *Selenastrum capricornutum* **DuPont Report No.:** DuPont-2511 **Guidelines:** OECD 201 (1984)

1. Test material: IN-D2708 technical metabolite
   Lot/Batch #: D2708-6
   Purity: 99.87%

Materials and methods:

The effect of IN-D2708 on *Selenastrum capricornutum* was determined using algal cultures with sterile synthetic AAP medium. Nominal concentrations of IN-D2709 were 0, 3.8, 7.6, 15, 30, and 60 mg/L,Three replicates at mean, measured concentrations of 3.65, 7.77, 14.5, 29.6, and 59.4 mg/L IN-D2708, measured at test initiation and termination, were incubated for 120 hours and cell counts were taken at 24-hour intervals. Because all measured concentrations were greater than 70% of nominal concentrations, mean measured concentrations were used for all calculations. The test was performed in 250 mL glass flasks that contained 50 mL of test solution arranged in a rotary shaker adjusted to 100 rpm. An incubator was used which mainained the temperature in a range of 23.5 to 23.9 °C. A photoperiod of 24 hours light (approximately 3,900 lux) was employed. Recovery after a further 144 hours was assessed at 59.4 mg/L IN-D2708. The occurrence of cell size differences, unusual cell shapes, colours, flocculations, adherence of cells to test containers or aggregation of cells was determined.

Actual concentrations of IN-D2708 were determined by chemical analysis at 0 and after 72 and 120 hours in control and test solutions using HPLC with UV detector. Algal cell numbers were determined visually by means of direct microscopic examination with a haemacytometer at 24, 48, 72, 96 and 120 hours. Temperature and pH were determined at the beginning and at the end of the test.

Findings:

In the three control replicates the pH at 0h was 7.4 and at 120h ranged 9.2-9.9. Considering all test replicates, the pH ranged from 3.5 (at 0h) to 9.9.

pH of IN-D2708 Test Solutions

Mean Measured Concentration of IN-D2708 (mg/L)	Replicate	pH	
		Initial	Final
<LOQ[a] (water control)	1	7.4	9.8
	2	7.4	9.9
	3	7.4	9.2
3.65	1	7.2	8.7
	2	7.1	8.6
	3	7.1	8.6
7.77	1	6.9	8.6
	2	6.9	9.4
	3	6.8	8.8
14.5	1	6.4	8.6
	2	6.4	8.8
	3	6.4	8.8
29.6	1	4.2	4.6
	2	4.2	4.5
	3	4.2	4.5
59.4	1	3.5	3.7
	2	3.5	3.6
	3	3.5	3.7

Cell Growth Data from the Toxicity Test with the Freshwater Alga, *Selenastrum capricornutum,* and IN-D2708

Mean Measured Concentration of IN-D2708 (mg/L)	Rep.	Number of Cells per Milliliter					
		Hour of Exposure					
		0	24	48	72	96	120
<LOQ[a] (control)	1	3,000	12,000	52,000	250,000	1,482,000	4,280,000
	2	3,000	12,000	22,000	226,000	1,284,000	3,580,000
	3	3,000	10,000	12,000	194,000	1,182,000	4,480,000
	mean	3,000	11,000	29,000	223,000	1,316,000	4,113,000
3.65	1	3,000	<10,000	24,000	238,000	1,562,000	5,600,000
	2	3,000	10,000	30,000	296,000	1,770,000	4,300,000
	3	3,000	<10,000	42,000	208,000	1,464,000	4,800,000
	mean	3,000	<10,000	32,000	247,000	1,599,000	4,900,000
	% of control	100	<91	110	111	122	119
7.77	1	3,000	<10,000	30,000	204,000	1,066,000	5,360,000
	2	3,000	<10,000	26,000	206,000	1,082,000	4,880,000
	3	3,000	<10,000	20,000	212,000	930,000	4,840,000
	mean	3,000	<10,000	25,000	207,000	1,026,000	5,027,000
	% of control	100	91	86	93	78	122
14.5	1	3,000	<10,000	10,000	92,000	396,000	2,500,000
	2	3,000	<10,000	10,000	112,000	348,000	3,100,000
	3	3,000	<10,000	20,000	166,000	534,000	2,880,000
	mean	3,000	<10,000	13,000	123,000	426,000	2,827,000
	% of control	100	91	45	55	32	69
29.6	1	3,000	<10,000	<10,000	11,000	20,000	24,000
	2	3,000	<10,000	<10,000	<10,000	19,000	22,000
	3	3,000	<10,000	<10,000	<10,000	16,000	17,000
	mean	3,000	<10,000	<10,000	<10,000	18,000	21,000
	% of control	100	91	<34	<4	1	1
59.4	1	3,000	<10,000	<10,000	<10,000	<10,000	<10,000
	2	3,000	<10,000	<10,000	<10,000	<10,000	<10,000
	3	3,000	<10,000	<10,000	<10,000	<10,000	<10,000
	mean	3,000	<10,000	<10,000	<10,000	<10,000	<10,000
	% of control	100	91	<34	<4	<1	<1

[a] LOQ denoted the limit of quantitation was 0.0413 mg/L.

Average Specific Growth Rate and Percent of Control from the Toxicity Test with the Freshwater Alga, *Selenastrum capricornutum,* and IN-D2708

Mean Measured Concentration of IN-D2708 (mg/L)	Average Specific Growth Rate				
	24 hour	48 hour	72 hour	96 hour	120 hour
<LOQ[1] (control)	0.054	0.047	0.060	0.063	0.060
3.65	0.050	0.049	0.061	0.065	0.062
7.77	0.050	0.044	0.059	0.061	0.062
14.5	0.050	0.031	0.052	0.052	0.057
29.6	0.050	0.025	0.017	0.019	0.016
59.4	0.050	0.025	0.017	0.013	0.010
Mean Measured Concentration of IN-D2708 (mg/L)	Percent of Control				
	24 hour	48 hour	72 hour	96 hour	120 hour
<LOQ (control)	--	--	--	--	--
3.65	93	104	102	103	103
7.77	93	94	98	97	103
14.5	93	66	87	83	95
29.6	93	53	28	30	27
59.4	93	53	28	21	17

[a] LOQ denoted the limit of quantitation was 0.0413 mg/L.

The effects of IN-D2708 on the growth of *Selenastrum capricornutum* are shown in

Table 59. The mean measured concentrations of IN-A2213 ranged from 96 – 102% of the targeted nominal concentrations after correction for test substance purity of 99.9%. The algal population was all in the log phase of growth, resulting in an average of 4,113,000 cells/ml in the control water after 120 hours. No effects (size differences, flocculations, unusual cell shapes, colours, adherence of cells to test containers or aggregation of cells) were observed during the test. Recovery was observed as an increase in cell number from a calculated cell count of >150 cells/ml to 166,000 cells/ml in the highest concentration tested (59.4 mg/l) indicated that oxamyl was algistatic rather than algicidal.

Table 59 Summary of algal growth inhibition following exposure of *Selenastrum capricornutum* to IN-D2708 for 120 hours

Initial measured concentration (mg/L)	Mean cell density (cells/mL)	% Inhibition	
		Growth rate	Area under the growth curve
Water control	4,113,000	--	--
3.65	4,900,000	-3	-19
7.77	5,027,000	-3	-4
14.5	2,827,000*	5*	46*
29.6	21,000*	73*	99*
59.4	<10,000*	83*	99*

*Significantly different from control by one way analysis of variance (ANOVA) and Dunnett's test.

Conclusion:

Growth inhibition data obtained with IN-D2708 on *Selenastrum capricornutum* are given below, based on mean concentrations. The effects of IN-D2708 on *Selenastrum capricornutum* are expected to be reversible at 59.4 mg/L.

Parameter	Endpoint (mg a.s./l)			
	72h EC_{50}	72h Calculated NOEC	120h EC_{50}	120h NOEC
Cell Density	13.7	7.77	15.5	7.77
Area under the growth curve	14.2	7.77	13.8	7.77
Growth Rate	25.0	7.77	24.1	7.77

RMS comments and conclusion

Additional information and tables have been added by the RMS to the original summary.

The effects on growth of green algae study, DuPont-2511, originally submitted under EU Rev8 Point IIA 8.2.6 and conducted with test material IN-D2708 technical metabolite, was conducted under guideline OECD 201 (1984). The RMS re-evaluated the study according to current version of the guideline (2006-2011) and added some information to the study summary.

The following deviations were noted:

-initial population was 1x103 cell/mL instead of 5x103- 104 cell/mL.

-In the control vessels the pH was 7.4 at 0h and increased of 1.8, 2.4 and 2.5 units at the end of test (120h), i..e. more than 1.5 unit as recommended in OECD 201. Also, the pH of test media was significantly affected by the test substance at the beginning of the test, decreasing with increasing test substance concentration, being as low as 4.2 and 3.5 at 29.6 and 59.4 mg/L, respectively. .

The validity criteria (analyzed with Toxrat 3.2 Professional) have been checked:

1)"The biomass in the control cultures have increased exponentially by a factor of >16 within the 72-hour test period" is fulfilled (actual 74.4)

2) "The mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3, for 72-hour tests) in the control cultures is lower than 35%" is not fulfilled (actual 53.5%).

3) "The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures was ≤ 7%" is fulfilled (actual 3%).

It is noted that both the 72h and the 120h EC50 are based on mean concentrations measured at 0, 72 and 120h. Several data are expressed as "less than" therefore the analysis of data is hampered.

Conclusion: the great variation in pH among the control and the test concentrations might have affected the reliability of the test results. At 72h, one validity criterion is not fulfilled. **The test is not acceptable.**

Study submitted in the EU Dossier in 2001 and included in the first EU approval review.

B.9.2.6.1/04

Reference: --	**Report:**	Boeri, R.L., Magazu, J.P., Ward, T.J. (1999b); IN-N0079: Influence on growth and growth rate of the alga, *Selenastrum capricornutum* **DuPont Report No.:** DuPont-2514 **Guidelines:** OECD 201 (1984)

1. Test material: IN-N0079 technical metabolite
   Lot/Batch #: N0079-8
   Purity: 99.57%

Materials and methods:

The effect of IN-N0079 on *Selenastrum capricornutum* was determined using algal cultures with sterile synthetic AAP medium. Three replicates at 1.26, 2.47, 4.86, 9.74, and 19.6 mg/L IN N0079, measured at test initiation, were incubated for 120 hours and cell counts were taken at 24-hour intervals. The test was performed in 250 mL glass flasks that contained 50 mL of test solution arranged in a rotary shaker adjusted to 100 rpm. An incubator was used which mainained the temperature in a range of 23.5 to 24.0 °C. A photoperiod of 24 hours light (approximately 3,800-3,900 lux) was employed. Recovery after a further 144 hours was assessed at 19.6 mg/L IN-N0079. The occurrence of cell size differences, unusual cell shapes, colours, flocculations, adherence of cells to test containers or aggregation of cells was determined.

Actual concentrations of IN-N0079 were determined by chemical analysis at 0 and after 72 and 120 hours in control and test solutions using HPLC with UV detector. Algal cell numbers were determined visually by means of direct microscopic examination with a haemacytometer at 24, 48, 72, 96 and 120 hours. Temperature and pH were determined at the beginning and at the end of the test.

The 72 hour EC50 value using the number of cells per mL was calculated by the binomial/nonlinear interpolation method. The remaining 48, 72, 96, and 120 hour EC25 and EC50 values were calculated using the number of cells per mL, using the average specific growth rate, and using the area under the growth curve by a weighted least squares non-linear regression technique described by Bruce and Versteeg. The slope of the concentration-response curve is not calculated by this method. The no observed effect concentration (NOEC) was determined using a one-way analysis of variance (ANOVA) and Dunnett's test (TOXSTAT 3.3(7)).

Findings:

In the three control replicates the pH at 0h was 7.4 and at 120h was 9.5, 10.0, and 10.0.

Cell Growth Data from the Toxicity Test with the Freshwater Alga, *Selenastrum capricornutum*, and IN-N0079

Initial Measured Concentration of IN-N0079 (mg/L)	Rep.	Number of Cells per Milliliter					
		Hour of Exposure					
		0	24	48	72	96	120
Media Control	1	3,000	<10,000	70,000	262,000	2,400,000	3,420,000
	2	3,000	<10,000	54,000	234,000	1,600,000	2,440,000
	3	3,000	<10,000	56,000	214,000	1,660,000	2,880,000
	mean	3,000	<10,000	60,000	237,000	1,887,000	2,913,000
1.26	1	3,000	<10,000	58,000	266,000	2,220,000	2,420,000
	2	3,000	<10,000	72,000	274,000	2,240,000	3,340,000
	3	3,000	<10,000	52,000	300,000	2,420,000	3,210,000
	mean	3,000	<10,000	61,000	280,000	2,293,000	2,990,000
	% of control	100	100	102	118	122	103
2.47	1	3,000	<10,000	62,000	248,000	2,180,000	2,920,000
	2	3,000	<10,000	66,000	200,000	2,660,000	2,900,000
	3	3,000	<10,000	60,000	274,000	2,020,000	2,400,000
	mean	3,000	<10,000	63,000	241,000	2,287,000	2,740,000
	% of control	100	100	105	102	121	94
4.86	1	3,000	<10,000	43,000	242,000	1,580,000	2,600,000
	2	3,000	<10,000	54,000	204,000	1,300,000	1,780,000
	3	3,000	<10,000	60,000	254,000	1,120,000	2,160,000
	mean	3,000	<10,000	52,000	233,000	1,333,000	2,180,000
	% of control	100	100	87	98	71	75
9.74	1	3,000	<10,000	39,000	100,000	204,000	476,000
	2	3,000	<10,000	40,000	78,000	160,000	264,000
	3	3,000	<10,000	36,000	94,000	166,000	276,000
	mean	3,000	<10,000	38,000	91,000	177,000	339,000
	% of control	100	100	63	38	9	12
19.6	1	3,000	<10,000	<10,000	32,000	27,000	<10,000
	2	3,000	<10,000	18,000	25,000	44,000	<10,000
	3	3,000	<10,000	15,000	20,000	22,000	<10,000
	mean	3,000	<10,000	<14,000	26,000	31,000	<10,000
	% of control	100	100	<23	11	2	<1

Average Specific Growth Rate and Percent of Control from the Toxicity Test with the Freshwater Alga, *Selenastrum capricornutum,* and IN-N0079

Initial Measured Concentration of IN-N0079 (mg/L)	Average Specific Growth Rate				
	24 hour	48 hour	72 hour	96 hour	120 hour
Media Control	0.050	0.062	0.061	0.067	0.057
1.26	0.050	0.063	0.063	0.069	0.058
2.47	0.050	0.063	0.061	0.069	0.057
4.86	0.050	0.059	0.060	0.064	0.055
9.74	0.050	0.053	0.047	0.042	0.039
19.6	0.050	0.032	0.030	0.024	0.010

Initial Measured Concentration of IN-N0079 (mg/L)	Percent of Control				
	24 hour	48 hour	72 hour	96 hour	120 hour
Media Control	--	--	--	--	--
1.26	100	102	103	103	102
2.47	100	102	100	103	100
4.86	100	95	98	96	96
9.74	100	85	77	63	68
19.6	100	52	49	36	18

The effects of IN-N0079 on the growth of *Selenastrum capricornutum* are shown in Table 60. At 0h, the mean measured concentrations of IN-A2213 ranged from 97 – 99% of the targeted nominal concentrations after correction for test substance purity of 99.6%. Since not all the concentrations were greater than 70% of nominal at 120 hours, initial measured concentrations were used for all calculations.The algal population was all in the log phase of growth, resulting in an average of 2,913,000 cells/ml in the control water after 120 hours. No effects (size differences, flocculations, unusual cell shapes, colours, adherence of cells to test containers or aggregation of cells) were observed during the test. Recovery was observed as an increase in cell number from a calculated cell count of <300cells/ml to 302,000 cells/ml in the highest concentration tested (19.6 mg/l) indicated that oxamyl was algistatic rather than algicidal.

Table 60 Summary of algal growth inhibition following exposure of *Selenastrum capricornutum* to IN-N0079 for 120 hours

Initial measured concentration (mg/L)	Mean cell density (cells/mL)	% Inhibition	
		Growth rate	Area under the growth curve
Water control	2,913,000	--	--
1.26	2,990,000	-2	-13
2.47	2,740,000	0	-9
4.86	2,180,000	4	26*
9.74	339,000*	32*	87*
19.6	<10,000*	82*	98*

* Significantly different from control by one way analysis of variance (ANOVA) and Dunnett's test.

Conclusion:

Growth inhibition data obtained with IN-N0079 on *Selenastrum capricornutum* are given below expressed as initial measured concentrations. The effects of IN-N0079 on *Selenastrum capricornutum* are expected to be reversible at 19.6 mg/L.

Parameter	**Endpoint (mg a.s./l)**			
	72h EC_{50}	**72h Calculated NOEC**	**120h EC_{50}**	**120h NOEC**
Cell Density	8.71 (4.86 to 9.74)		6.20 (5.47 to 7.02)	4.86
Area under the growth curve	9.46 (8.36 to 10.7)		5.89 (5.04 to 6.76)	2.47
Growth Rate	18.5 (17.2 to >19.6)		12.3 (11.9 to 12.7)	4.86

RMS comments and conclusion

Additional information and tables have been added by the RMS to the original summary.

The effects on growth of green algae study, DuPont-2514, originally submitted under EU Rev8 Point IIA 8.2.6 and conducted with test material IN-N0079 technical metabolite, was conducted under guideline OECD 201 (1984). The RMS re-evaluated the study according to current version of the guideline (2006-2011).

At 72h, the concentrations were within 20% the initial measured ones, hence it is acceptable express the results as initial measured concentrations. This would not be the case for data at 120h, hence the 120 EC50 should calculated based on mean measured concentrations.

The following deviations were noted:

-initial population was 1x103 cell/mL instead of 5x103- 104 cell/mL.

-In the control vessels the pH was 7.5 at 0h and increased of 2.1, 2.5 and 2.5 units at the end of test (120h), i..e. more than 1.5 unit as recommended in OECD 201.

The validity criteria (analyzed with Toxrat 3.2 Professional) have been checked:

1)"The biomass in the control cultures have increased exponentially by a factor of >16 within the 72-hour test period" is fulfilled (78.9).

2) "The mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3, for 72-hour tests) in the control cultures is lower than 35%" cannot be verified because the lack of adequate data at 24h.

3) "The coefficient of variation of average specific growth rates during the 72 test period in replicate control cultures was ≤ 7%" is fulfilled (actual 2.3%).It is noted that at 24h the number of cells is expressed as "less than" values.

Conclusion: One validity criterion cannot be verified. The lack of adequate data at 24 h makes also obstacles the analysis of data**. Not acceptable-**

Study submitted in the EU Dossier in 2001 and included in the first EU approval review.

B.9.2.6.1/05

Reference: --	**Report:**	Boeri, R.L., Ward, T.J. (2001); IN-T2921: Influence on growth and growth rate of the alga, *Selenastrum capricornutum* **DuPont Rep** **ort No.:** DuPont-4442 **Guidelines:** OECD 201 (1984)

1. Test material: IN-T2921 technical metabolite
   Lot/Batch #: T2921-2
   Purity: 98.7%

Materials and methods:

The effect of IN-T2921 on *Selenastrum capricornutum* was determined using algal cultures with AAP nutrient AAP medium. Nominal concentrations of IN-T2921 were 0 mg/L (control), 7.7, 15, 30, 60, and 120 mg/L. Three replicates at mean measured concentrations of 7.08, 14.3, 28.7, 57.5, and 113 mg/L were incubated for 72 hours and cell counts were taken at 24-hour intervals. Six replicates were set for the control. The test was performed in 250 mL glass flasks that contained 100 mL of test solution arranged in a rotary shaker adjusted to 100 rpm. An incubator was used which mainained the temperature in a range of 23.6 to 23.7 °C. A photoperiod of 24 hours light (approximately 100 to 110 kEin/m2sec) was employed. The occurrence of cell size differences, unusual cell shapes, colours, flocculations, adherence of cells to test containers or aggregation of cells was determined. Recovery was not determined as there were no effects on the algal population in the highest concentration tested of 113 mg/l.

Actual concentrations of IN-N0079 were determined by chemical analysis at 0 and after 72 hours in control and test solutions using a HPLC equipped with a DAD/UV detector. Mean measured concentrations were used for all calculations. Algal cell numbers were determined visually by means of direct microscopic examination with a haemacytometer at 24, 48, and 72 hours. Temperature and pH were determined at the beginning and at the end of the test. Temperature was also measured continuously in a representative vessel incubated among the test vessels.

The no observed effect concentration (NOEC) was determined using a one-way analysis of variance (ANOVA) and Bonferroni's test (TOXSTAT 3.3).

Findings:

In the six control replicates, at 0h, the pH was 7.3, at 72h the pH increased up to 10.4-10.6.

Cell Growth Data from the Toxicity Test with the Freshwater Alga, *Selenastrum capricornutum*, and IN-T2921

Mean Measured Concentration of IN-T2921 (mg/L)	Rep.	Number of Cells per Milliliter			
		Hour of Exposure			
		0	24	48	72
<LOQ[a] (control)	1	10,000	70,000	606,000	2,120,000
	2	10,000	104,000	586,000	2,460,000
	3	10,000	64,000	404,000	2,360,000
	4	10,000	52,000	506,000	3,380,000
	5	10,000	78,000	490,000	3,040,000
	6	10,000	80,000	458,000	2,940,000
	mean	10,000	75,000	508,000	2,717,000
	st. dev.	0	18,000	77,000	478,000
7.08	1	10,000	92,000	532,000	2,680,000
	2	10,000	100,000	434,000	2,480,000
	3	10,000	106,000	706,000	2,860,000
	mean	10,000	99,000	557,000	2,673,000
	st. dev.	0	7,000	138,000	190,000
	% of control	100	132	110	98
14.3	1	10,000	94,000	494,000	3,940,000
	2	10,000	78,000	394,000	2,780,000
	3	10,000	90,000	386,000	3,080,000
	mean	10,000	87,000	425,000	3,267,000
	st. dev.	0	8,000	60,000	602,000
	% of control	100	116	84	120
28.7	1	10,000	78,000	462,000	2,800,000
	2	10,000	80,000	560,000	3,480,000
	3	10,000	84,000	648,000	2,980,000
	mean	10,000	81,000	557,000	3,087,000
	st. dev.	0	3,000	93,000	352,000
	% of control	100	108	110	114
57.5	1	10,000	66,000	458,000	2,380,000
	2	10,000	52,000	476,000	2,200,000
	3	10,000	78,000	644,000	3,300,000
	mean	10,000	65,000	526,000	2,627,000
	st. dev.	0	13,000	103,000	590,000
	% of control	100	87	104	97
113	1	10,000	90,000	514,000	3,560,000
	2	10,000	76,000	642,000	2,720,000
	3	10,000	82,000	750,000	2,960,000
	mean	10,000	83,000	635,000	3,080,000
	st. dev.	0	7,000	118,000	433,000
	% of control	100	111	125	113

[a] LOQ denotes the limit of quantitation was 0.0359 mg/L.

The effects of IN-T2921 on the growth of *Selenastrum capricornutum* are shown in Table 61. The mean measured concentrations of IN-A2213 ranged from 92 - 96% of the targeted nominal concentrations after

correction for test substance purity of 98.7%. The algal population was all in the log phase of growth, resulting in an average of 2,717,000 cells/ml in the control water after 120 hours. No effects (size differences, flocculations, unusual cell shapes, colours, adherence of cells to test containers or aggregation of cells) were observed during the test.

Table 61 Summary of algal growth inhibition following exposure of *Selenastrum capricornutum* to IN-T2921 for 72 hours

Mean measured concentration (mg/L)	Mean cell density (cells/mL)	% Inhibition		
		Cell density	Growth rate	Area under the growth curve
Water control	2.7×10^6	—	—	—
7.08	2.7×10^6	2	0	0
14.3	3.3×10^6	0	0	0
28.7	3.1×10^6	0	0	0
57.5	2.6×10^6	0	1	2
113	3.1×10^6	0	0	0

Conclusion:

Growth inhibition data obtained with IN-T2921 on *Selenastrum capricornutum* were as follows, calculated based on mean measured concentrations:

Parameter	Endpoint (mg a.s./l)	
	72h EC_{50}	72h Calculated NOEC
Cell Density	>113	113
Area under the growth curve	>113	113
Growth Rate	>113	133

RMS comments and conclusion

Additional information and tables have been added by the RMS to the original summary.

The effects on growth of green algae study, DuPont-4442, originally submitted under EU Rev8 Point IIA 8.2.6 and conducted with test material IN-T2921 technical metabolite, was conducted under guideline OECD 201 (1984). The RMS re-evaluated the study according to current version of the guideline (2006-2011).

The following deviation was noted:

-In the control vessels the pH was 7.3 at 0h and increased of 3.1-3.3 units at the end of test (120h), i..e. more than 1.5 unit as recommended in OECD 201. Practically the same increment was also recorded in all the test concentrations.

The validity criteria (analyzed with Toxrat 3.2 Professional) have been checked:

1)"The biomass in the control cultures have increased exponentially by a factor of >16 within the 72-hour test period" is fulfilled (actual 217.7).
2) "The mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3, for 72-hour tests) in the control cultures is lower than 35%" is fulfilled (14.4%).

3) "The coefficient of variation of average specific growth rates during the 72 test period in replicate control cultures was ≤ 7%" is fulfilled (actual 3.2).

Conclusion: the high increase of pH in the control seems not have affect its performance. Considering the nature of the limit test and the lack of effects at all concentrations, the study is considered acceptable.

B.9.2.6.2 Effects on growth of an additional algae species

Oxamyl is not an herbicide, thus no study of effects on growth of an additional algae species is required.

B.9.2.7 Effects on aquatic macrophytes

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

B.9.2.7/01

Reference: CA 8.2.7/01	**Report:**	Rebstock, M. (2012); Oxamyl (DPX-D1410) technical (98% w/w): 7-day growth inhibition test with the freshwater aquatic plant, duckweed, *Lemna gibba* **DuPont Report No.:** DuPont-34272 **Guidelines:** OPPTS 850.4400 (1996), OECD 221 (2006) **Deviations:** None **Testing Facility:** ABC Laboratories, Inc. (Missouri), Columbia, Missouri, USA **Testing Facility Report No.:** 68028 **GLP:** Yes **Certifying Authority:** Laboratories in the USA are not certified by any government agency, but are subject to regular inspections.

Executive summary:

The acute toxicity of oxamyl to the freshwater aquatic plant, duckweed, *Lemna gibba*, was determined in a 7-day growth inhibition test. The test was conducted in accordance with Organization for Economic Cooperation and Development (OECD) Guidelines for Testing of Chemicals, Guideline No. 221 and U.S. EPA Ecological Effects Test Guidelines, OPPTS 850.4400. The exposure test was conducted with a filter-sterilized 20X AAP nutrient medium (blank) control and 6 concentrations of oxamyl. Three replicates were initiated per test concentration and blank control. A single test vessel was used for the abiotic (stability) control, included in order to demonstrate the stability of the test substance in 20X AAP nutrient medium under the test conditions without the presence of the test organism.

The geometric mean, measured concentrations of oxamyl active substance (a.s.) in the test concentration and the abiotic control solutions ranged from 37 to 60% of nominal oxamyl concentrations. Frond counts increased in the blank control by at least a factor of 7 in 7 days and the doubling time in the blank control based on frond count was 1.6 days, thereby satisfying the appropriate test acceptance criteria.

Geometric mean, measured concentrations of oxamyl were used for calculation of E_bC_{50}, E_yC_{50}, and E_rC_{50} values (effect concentration producing a 50% inhibition of growth based on biomass, yield, and growth rate, respectively, based on frond count and dry weight, relative to the control). The 7-day E_bC_{50} and NOEC based on frond count were 3.57 and 0.640 mg oxamyl/L, respectively. The 7-day E_yC_{50} and NOEC based on frond count yield were 3.16 and 0.640 mg oxamyl/L, respectively. The 7-day E_rC_{50} and NOEC based on frond count growth rate were >7.17 and 0.640 mg oxamyl/L, respectively. The 7-day E_bC_{50} and NOEC based on dry

weight were 2.20 and 0.640 mg oxamyl/L, respectively. The 7-day E_yC_{50} and NOEC based on dry weight yield were 1.67 and 0.640 mg oxamyl/L, respectively. The 7-day E_rC_{50} and NOEC based on dry weight growth rate were 3.30 and 0.640 mg oxamyl/L, respectively.

Recovery data (7-day recovery period) indicated that the effects of oxamyl on *Lemna gibba* G3 are phytostatic at concentrations less than or equal to 3.11 mg oxamyl/L and phytocidal at concentrations ≥7.17 mg oxamyl/L.

I. MATERIALS AND METHODS

A. MATERIALS

1.	Test material:	Pure Oxamyl (PAI)
	Lot/Batch #:	D1410-196
	Purity:	98.0% by analysis
	Description:	White crystalline solid
	CAS#:	23135-22-0
	Stability of test compound:	Not stable
2.	Control:	20X AAP nutrient medium
	Test vehicle:	20X AAP nutrient medium
	Toxic reference:	None
3.	Test organism:	Duckweed
	Species:	*Lemna gibba* G3
	Initial population:	3 plant with 4 fronds each
	Source:	ABC Laboratories, Inc., Columbia, Missouri In house culture, parent culture from USDA/ARS Beltsville Agricultural Research Center, Beltsville, Maryland
	Test chamber:	500-mL Erlenmeyer flask with a foam stopper, containing 200 mL of test solution.
	Growth medium:	20X AAP nutrient medium. pH-adjusted to 7.5 ± 0.1 using 0.1N HCl and 0.1N NaOH and filtered through 0.45-µm Millipore® filters
4.	Environmental conditions (in-life period):	
	Temperature:	23.0 to 26.3ºC
	Photoperiod:	24 hr photoperiod (6368 to 6674 lux)
	pH:	7.5 to 8.8 throughout the exposure period

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed

   13-January-2011 to 20-January-2011

2. Experimental treatments

   The effect of oxamyl to the floating freshwater vascular plant *Lemna gibba* G3 was determined in a static-renewal, 7-day test. The plants were exposed to an untreated control and six nominal concentrations of 0.38, 0.75, 1.5, 3.0, 6.0, and 12 mg oxamyl/L in 20X AAP nutrient medium for 7 days, with daily test medium renewal. An abiotic control was included in the test to determine the stability of oxamyl in 20X AAP nutrient medium under the same environmental conditions without the plants. Each test concentration and the untreated control were tested as three replicates. The abiotic control was tested as a single unit. Three plants with 4 fronds each were used per replicate. Test units were incubated in an environmental chamber for 7 days.

3. Observations

   Test solutions were measured for the active substance, oxamyl on Days 0, 1, 3, 4, 6, and 7 to verify stability of the test item, using a HPLC-UV system.

   Frond counts were made on Days 0, 3, 5, and 7.

Dry weight was determined at the completion of the 7-day test.

Frond count yield and dry weight yield were determined by subtracting the initial frond count or dry weight from the test end values.

Growth rate was determined on Day 7 and was based on frond count and dry weight.

Biomass, yield, and growth rate based on frond count or dry weight after 7 days were expressed as percent inhibition relative to the untreated control following exposure to oxamyl for 7 days.

4. Statistics

Analyses are reported based on mean, measured oxamyl concentrations and were conducted using SAS Version 9.1.3. All statistical tests were calculated at a significance level of p = 0.05.

The NOEC values, based on average specific 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). 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 for biomass, growth rate, and yield based on frond count data on Days 3, 5, and 7. Parametric analyses were performed for biomass, growth rate, and yield based on dry weight data at Day 7.

The 7-day E_bC_{50}, E_yC_{50}, and E_rC_{50} values (and 95% confidence intervals) for biomass, yield, and growth rate, respectively, all based on frond count and dry weight, were calculated using a logistic (sigmoid-shaped) model fit to the data with percent inhibition as the dependent variable and concentration as the independent variable.

II. RESULTS AND DISCUSSION

A. FINDINGS

The geometric mean, measured concentrations of oxamyl over the 7-day exposure period in the test concentrations ranged from 37 to 60% of nominal and was 60% of nominal in the 12 mg/L abiotic control. The untreated control solutions contained no detectable concentrations of oxamyl on Days 0, 1, 3, 4, 6, and 7. The test item was determined to not be stable during the renewal periods (daily). All validation criteria were met for the study.

Data on biomass, yield, and growth rate based on frond count and dry weight after 7 days following exposure of *Lemna gibba* G3 to oxamyl for 7 days are summarised in the tables that follow.

Table 62 Summary of growth inhibition (frond count and dry weight) following exposure of *Lemna gibba* to oxamyl for 7 days

Geometric mean, measured oxamyl concentration (mg a.s./L)	Frond count		Dry weight	
	Mean 7-day frond count	% Inhibition relative to control	Mean 7-day dry weight (mg)	% Inhibition relative to control
Untreated control (0.0)	178	---	0.0258	---
0.141	176	1	0.0229	11
0.291	172	3	0.0260	-1
0.640	156	12	0.0206	20
1.35	146[a]	18	0.0163[a]	37
3.11	92.7[a]	48	0.0101[a]	61
7.17	52.7[a]	70	0.0056[a]	78

[a] Significantly different from the control (Dunnett's, alpha = 0.05)

Table 63 Summary of growth inhibition (frond count yield and dry weight yield) following exposure of *Lemna gibba* to oxamyl for 7 days

Geometric mean, measured oxamyl concentration (mg a.s./L)	Frond count		Dry weight	
	Mean 7-day frond count yield	% Inhibition relative to control	Mean 7-day dry weight yield (mg)	% Inhibition relative to control
Untreated control (0.0)	166	---	0.0224	---
0.141	164	1	0.0195	13
0.291	160	4	0.0226	-1
0.640	144	13	0.0172	23
1.35	134[a]	19	0.0129[a]	42
3.11	80.7[a]	51	0.0067[a]	70
7.17	40.7[a]	75	0.0022[a]	90

[a] Significantly different from the control (Dunnett's, alpha = 0.05)

Table 64 Summary of growth inhibition (growth rate) following exposure of *Lemna gibba* to oxamyl for 7 days

Geometric mean, measured oxamyl concentration (mg a.s./L)	7-Day growth rate based on frond count		7-Day growth rate based on dry weight	
	Mean 7-day growth rate	% Inhibition relative to control	Mean 7-day mean growth rate	% Inhibition relative to control
Untreated control (0.0)	0.385	--	0.289	--
0.141	0.384	0	0.272	6
0.291	0.378	2	0.288	0
0.640	0.367	5	0.257	11
1.35	0.356[a]	8	0.224[a]	22
3.11	0.291[a]	24	0.155[a]	46
7.17	0.211[a]	45	0.068[a]	76

[a] Significantly different from the control (Dunnett's, alpha = 0.05)

Geometric mean, measured test concentrations of 3.11 and 7.17 mg oxamyl/L exhibited ≥50% inhibition based on frond count at the end of the exposure period (7 days). *Lemna gibba* G3 from these concentrations were used to assess recovery. In the recovery test, normal growth and reproduction resumed in geometric mean, measured test concentrations equal to or below 3.11 mg oxamyl/L based on an observed ≥7X

increase in frond count over 7 days. Normal growth and reproduction did not resume in geometric mean, measured test concentrations equal to 7.17 mg oxamyl/L based on an observed <7X increase in frond count over 14 days. Recovery data indicate that the effect of oxamyl at geometric mean, measured concentrations equal to or below 3.11 mg oxamyl/L is phytostatic and phytocidal at geometric mean, measured concentrations ≥7.17 mg oxamyl/L.

III. CONCLUSION

Growth inhibition values based on geometric mean, measured test concentrations obtained with oxamyl on *Lemna gibba* G3 were as follows:

Biomass, Frond Count:	7-day E_bC_{50} 3.57 mg oxamyl/L 7-day NOEC = 0.640 mg oxamyl/L
Yield, Frond Count:	7-day E_yC_{50} 3.16 mg oxamyl/L 7-day NOEC = 0.640 mg oxamyl/L
Growth Rate, Frond Count:	7-day E_rC_{50} >7.17 mg oxamyl/L 7-day NOEC = 0.640 mg oxamyl/L
Biomass, Dry Weight:	7-day E_bC_{50} 2.20 mg oxamyl/L 7-day NOEC = 0.640 mg oxamyl/L
Yield, Dry Weight:	7-day E_yC_{50} 1.67 mg oxamyl/L 7-day NOEC = 0.640 mg oxamyl/L
Growth Rate, Dry Weight:	7-day E_rC_{50} 3.30 mg oxamyl/L 7-day NOEC = 0.640 mg oxamyl/L

The effect of oxamyl on *Lemna gibba* G3 is expected to be reversible at concentrations less than or equal to 3.11 mg oxamyl/L.

(Rebstock, M., 2012)

RMS comments and conclusion

The study fulfilles the validity criteria of OECD 221 and the recommendations herein. The test is acceptable and the results are reliable.

B.9.2.8 Further testing on aquatic organisms

See Section 8.2.4.2 for additional testing with aquatic invertebrates.

B.9.2.9 Summary of toxicity to aquatic organisms

A summary of the aquatic toxicity testing values obtained with oxamyl is included in Table 65.

Table 65 Oxamyl aquatic toxicity endpoint values

Species	Test/duration	Measurement endpoint	Endpoint value (mg a.s./L)	Reference[a]
Rainbow trout	acute (96 h)	LC_{50}	3.13	DuPont-2907
Bluegill sunfish	acute (96 h)	LC_{50}	6.12	DuPont-2908
Daphnia magna	acute (48 h)	EC_{50}	0.319	DuPont-2553
Pseudokirchneriella subcapitata[b]	data gap			
Lemna gibba	chronic (7-d)	E_yC_{50} E_rC_{50}	1.670 3.30	DuPont-34272
Chironomus tentans	acute (48 h)	EC_{50}	0.350	DuPont-37400
Chimarra atterima	acute (48 h)	EC_{50}	0.096	DuPont-37402
Centroptilum triangulifer	acute (48 h)	EC_{50}	0.067	DuPont-37401
Hyalella azteca	acute (48 h)	EC_{50}	0.320	DuPont-37397
Daphnia pulex	acute (48 h) Not valid			DuPont-37398
Ceriodaphnia dubia	acute (48 h)	EC_{50}	0.094	DuPont-37399
Americamysis bahia	acute (48 h)	EC_{50}	0.0465	DuPont-34271
Crassostrea virginica	acute (96 h)	EC_{50}	27.5	DuPont-34273
Fathead minnow	Early life stage (28 d) Supportive information	NOEC	0.500	HLR 877-81
Rainbow trout	early life stage (90 d) Not valid			HLR 468-88
Sheepshead minnow	early life stage (29 d)	NOEC	0.356	DuPont-34270
Daphnia magna	chronic (21 d)	NOEC	0.0268	DuPont-2554
Americamysis bahia	chronic (28 d)	NOEC	0.0189	DuPont-34269

[a] All studies cited or summarised in this document.

[b] Formerly known as *Selenastrum capricornutum*.

A summary of the aquatic toxicity testing values obtained with the metabolites of oxamyl is included in Table 66.

Table 66 Aquatic toxicity endpoint values for the metabolites of oxamyl

Metabolite	Species	Test/duration	Measurement endpoint	Endpoint value (mg met/L)	Reference[a]
IN-A2213	Rainbow trout	acute (96 h)	LC_{50}	>132	DuPont-2500
	Daphnia magna	acute (48 h)	EC_{50}	>125	DuPont-2502
	Pseudokirchneriella subcapitata[b]	acute (72 h) Supportive information	EC_{50}	>122 Supportive information	DuPont-2505
IN-D2708	Rainbow trout	acute (96 h) Supportive information	LC_{50}	93.8 Supportive information	DuPont-2507
	Daphnia magna	acute 48 h)	EC_{50}	>134	DuPont-2510
	Pseudokirchneriella subcapitata[b]		data gap		
	Daphnia magna	chronic (21 d)	NOEC	66.1	DuPont-3909
IN-N0079	Rainbow trout	acute (96 h)	LC_{50}	22.4	DuPont-2512
	Daphnia magna	acute (48 h)	EC_{50}	>128	DuPont-2513
	Pseudokirchneriella subcapitata[b]	acute (72 h)	data gap		
IN-T2921	Rainbow trout	acute (96 h)	LC_{50}	>127	DuPont-4439
	Daphnia magna	acute (48 h)	EC_{50}	>123	DuPont-4441
	Pseudokirchneriella subcapitata[b]	acute (72 h)	EC_{50}	>113	DuPont-4442

[a] All studies cited or summarised in this document.

[b] Formerly known as *Selenastrum capricornutum*.

B.9.3 Effects on arthropods

B.9.3.1 Effects on bees

B.9.3.1.1 Acute toxicity to bees

B.9.3.1.1.1 Acute oral toxicity

Study submitted in the EU Dossier in 2001 and included in the first EU approval review

B.9.3.1.1.1/01

Reference: --	**Report:**	Schur, A. (1999); Oxamyl technical: Acute oral and contact toxicity to the honey bee, *Apis mellifera* L. **DuPont Report No.:** DuPont-2740 **Guidelines:** EPPO 170, U.S. EPA 850.3020 (1996)

A. MATERIALS

1.	Test material:	Pure Oxamyl (PAI)
	Lot/Batch #:	D1410-196
	Purity:	96.9%
	Description:	solid; colourless
	CAS#:	23135-22-0
	Stability of test compound:	Not reported
2.	Vehicle and/or positive control:	tap water; Perfekthion" (containing 395.7 g/L dimethoate)
3.	Test organism:	Honey bees, worker, caught in front of the hive approximately 20 hours before test initiation.
	Species:	*Apis mellifera*
	Strain:	Carnica
	Source:	Bee hives located in Rheinland-Pfalz, Germany
	Acclimation period:	Not stated
	Diet:	50% sucrose solution
	Water:	Not applicable - see Diet
	Test chamber:	High grade steel cage (10 cm wide × 5 cm deep × 8.5 cm high)
4.	Environmental conditions	
	Temperature:	26 to 28°C
	Relative humidity:	49 to 66
	Photoperiod:	Continuous dark

Materials and methods:

Acute oral and contact toxicity of Oxamyl (purity 96.9% w/w) to honeybees (*Apis mellifera* L.) was tested in a laboratory study conducted under EPPO Guideline No. 170. These tests were conducted with five test substance treatment rates (Oral & Contact: 0.1; 0.2; 0.4; 0.8 and 1.6 µg a.s./bee) plus a control (50% sucrose solution) and four toxic standard treatment rates (Oral: 0.09, 0.13, 0.19 and 0.25 µg a.s./bee; Contact: 0.18; 0.26; 0.38 and 0.50 µg a.s./bee) five replicates per treatment; and 10 honeybees per replicate. Dimethoate ("Perfekthion", 395.7 g dimethoate/L) was the toxic standard used in these tests. Before treatment the bees were starved for 2 hours 10 minutes. A quantity of 250 µL of treated sucrose solution was offered to each cage of 10 bees to ensure a sufficient intake of test item. For the contact toxicity test, the bees have been anaesthetized with carbon dioxide they were treated individually by ventral thorax application of test item suspension (2 µL).

Following exposure to test substance, bees were provided with an aqueous 50% sucrose solution in both the oral and contact studies. Bees were observed 2, 4, 24, and 48 hours after treatment for mortality and sublethal effects. Temperature and relative humidity were measured during the test.

Statistics:

Schneider-Orelli (1947): Correction for control mortality.
Easy Assay Critical Values computer program (Ratte, 1995), (□=0.05): LD50
Maximum Likelihood method (Finney, 1971; Schuemer et al., 1990), (□=0.05): Estimation of Probit straight line
Fisher's Exact test, (□=0.05): NOEL

Findings:

Actual test substance intake in the oral test and mortality results for both tests at 24 and 48 hours are reported in, Table 67, Table 68 and Table 69. During the study, the temperature ranged from 26 – 28°C and the relative humidity was between 49 and 66%. Treated bees did not differ from the control bees in behavioural effects at any time. The oral and contact toxicity of the toxic reference standard, dimethoate, to honeybees in these tests fell within the accepted range, indicating the validity of these tests.

Table 67 Acute oral toxicity of Oxamyl to honey bees

Treatment[a] µg a.s./bee	Test Substance Intake[b] µg a.s./bee	Cumulative Mortality (%)[c]	
		24 hours	48 hours
0	N/A	0.0	2.0
0.1	0.08	4.0	2.0
0.2	0.18	10.0	18.4
0.4	0.32	52.0	57.1
0.8	0.61	64.0	69.4
1.6	1.07	72.0	75.5

[a]Treatments are specified as intended uptake, in mean µg a.s./bee per tratment rate.
[b]Test substance intake is specified as actual uptake, in mean µg a.s./bee per treatment rate.
[c]Test mortality for treatments is corrected for control mortality (mortality at 0 µg a.s./bee).

Table 68 Acute contact toxicity of Oxamyl to honey bees

Treatment[a] µg a.s./bee	Cumulative Mortality (%)	
	24 hours	48 hours
0	0.0	0
0.1	0.0	0
0.2	6.0	6.0
0.4	34.0	42.0
0.8	80.0	80.0
1.6	98.0	100.0

[a]Treatments are specified as mean µg a.i./bee per treatment rate, applied ventrally.

Table 69 Acute oral and contact toxicity of Oxamyl to honey bees - Summary of endpoints

Acute Endpoint	mg Oxamyl/bee
Oral LD_{50}	0.38
Oral NOEL	0.08
Contact LD_{50}	0.47
Contact NOEL	0.20

Conclusion:

The 48-hour oral $LD_{50}$was 0.38 µg Oxamyl/bee. The NOEL value was 0.08 µg Oxamyl/bee. In the contact study the 48-hour $LD_{50}$was 0.47 µg Oxamyl/bee. The statistically determined NOEL value was 0.20 µg Oxamyl/bee.

RMS comments and conclusion

The acute oral toxicity study DuPont-2740, originally submitted under EU Rev8 Point IIA 8.3.1.1 and conducted with test material pure oxamyl (PAI), was conducted under guidelines EPPO 170, and U.S. EPA 850.3020 (1996). The study has been reviewed according to EPPO 1/170 (4), 2010 and ECD 213, 1998 and OECD 214, 1998).

In the contact test bees product was applied to ventral thorax as recommended in OECD 214, 1998.

Deviations Maximum temperature was 28 instead of 27°C. Low impact.

For dimethoate, the 48-hour oral LD50 was 0.12 µg a.s./bee (0.11 to 0.13 kg a.s./bee), the 48-hour contact LD50 was calculated 0.22 kg a.s./bee (0.20 to 0.25 kg a.s./bee), which fell within the published acceptable range

(Gough, et al., 1948), ranged 0.100- 0.318 µg a.s./bee and 0.105- 0.237 µg a.s./bee, respectively for oral and contact 48hLD50. The tests are valid.

Conclusion: the study is acceptable.

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

B.9.3.1.1.1/02

Reference: CA 8.3.1.1.1/01	**Report:**	Haupt, S. (2014); Oxamyl (DPX-D1410) technical (98% w/w): Acute oral and contact toxicity to the bumblebee, *Bombus terrestris* L. (Hymenoptera) **DuPont Report No.:** DuPont-39670 **Guidelines:** Van der Steen (2001), OECD 213 (1998), OECD 214 (1998), Current recommendations of the non-Apis ring test group (2014) **Deviations:** None **Testing Facility:** Institut für Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany **Testing Facility Report No.:** 84911105 **GLP:** Yes **Certifying Authority:** Hess. Ministerium fur Umwelt, Energie, Landwirtschaft and Verbraucherschutz

Executive summary:

The aim of this study was to determine the acute contact and oral toxicity of oxamyl to the bumblebee (*Bombus terrestris* L.) in a laboratory study. A contact test with 100, 50, 25, 12.5, and 6.25 µg a.s./bee and an oral test with 0.52, 0.26, 0.13, 0.064, and 0.032 µg a.s./bee were conducted according to van der Steen (2001), OECD 213 and OECD 214 (1998), with modifications and adaptions, and current recommendations of the non-Apis ring test group (2014). The 48 h acute contact LD_{50} and NOED of oxamyl to bumblebees were 39.3 µg a.s./bee and 12.5 µg a.s./bee. The 48 h acute oral LD_{50} and NOED of oxamyl to bumblebees were 0.36 µg a.s./bee and 0.13 µg a.s./bee.

I. MATERIALS AND METHODS

A. MATERIALS

1.	Test material:	Pure Oxamyl (PAI)
	Lot/Batch %:	D1410-532
	Content (analysed);	99.1%, by analysis
	Description:	Solid
	CAS#:	23135-22-0
	Test vehicle:	Oral test: 50% w/v sucrose solution (50 g sucrose/L tap water); Contact test: Tap water with 0.5% Tween80*
2.	Control	Vehicle without test item
	Reference item:	Perfekthion (BAS 152 11 I - dimethoate 400 g/L). One dose (12 µg dimethoate/bee in the contact test, 4 µg dimethoate/bee in the oral test).
3.	Test organism:	Worker bumblebees (Insecta, Hymenoptera)
	Species:	Adult *Bombus terrestris* L.
	Stage and Sex:	Female worker bees
	Source:	Bumblebee colonies, healthy and queen-right, obtained from a commercial bumblebee breeding company (Biobest Belgium N.V., Ilse Velden 18, 2260 Westerlo, Belgium) in a plastic box.
	Acclimatisation:	Contact Test: 20 hours 45 minutes Oral Test: 24 hours 40 minutes
4.	Test Units	
	Type and Size:	Cylindrical, latticed plastic cages with a length of approximately 7 cm and a diameter of 2.2 cm at the large and 1.7 cm at the small opening. The bees were kept in the above mentioned test units. The contact application was conducted outside of the test unit. The test units were laid on a plate, the small opening was closed by a rubber plug holding a syringe which contained the feeding solution. The large opening was closed by a lid.
	No. of Individuals:	1 per test unit
	Replicates:	30 per treatment group/control
5.	Environmental conditions	
	Temperature:	Acclimatisation: 22–24°C Exposure: 21–24°C
	Relative humidity:	Acclimatisation: 0–100% Exposure: 2–100%
	Light:	Darkness (except during observation)

B. STUDY DESIGN AND METHODS

1. Application Information

Application in the Contact Test:	Bumblebees were anaesthetised with CO2 for weighing and application. One single 5 µL droplet of oxamyl in an appropriate carrier (tap water with 0.5% Tween80) was placed on the dorsal bee thorax using a pipette (Multipette©, Eppendorf). For the control one 5 µL droplet of tap water containing 0.5% Tween80 was used. The reference item was also applied in 5 µL tap water (dimethoate made up in tap water containing 0.5% Tween80). * The Tween80 was used to improve the adhesion of the droplet on the bee body. Tween80 is non-toxic to bumblebees.
Application in the Oral Test:	The test item and reference item were dissolved in 50% w/v sucrose solution that was used as carrier in the oral test. For the control group untreated 50% w/v sucrose solution was used. The treated food was offered in syringes, which were weighed before and after introduction into the cages (duration of uptake was ranging from 45 minutes to 4 hours and 40 minutes for the test item treatments). After a maximum of 4 hours and 40 minutes, the uptake of the test item treated food was completed and the syringes containing the treated food were removed, weighed, and replaced by ones containing fresh, untreated food (50% w/v sucrose solution) ad libitum. The mean target dose levels (*e.g.*, 0.5 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. Bumblebees were anaesthetised with CO2 for weighing.

2. 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 LD_{50} values of the reference item were estimated with Probit Analysis (according to Finney 1971).

If necessary, the LD_{50} calculation was carried out taking into account the mortality data corrected by control mortality using Abbott's formula (1925).

The NOED was estimated using Fisher's Exact Test (pairwise comparison, one-sided greater, $\alpha = 0.05$), which is a distribution-free test and does not require testing for normality or homogeneity prior to analysis.

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

Number of dead bees were recorded after 4 (±0.5 h) hours (first day), 24 and 48 (±2 h) hours. Behavioural Abnormalities (moribund, affected, cramps) were recordeare reported after 4 (±0.5 h) hours (first day); 24, 48h.

Weight distribution of the bumblebees among the treatment groups.

Contact toxicity test			Oral toxicity test		
Treatment Group	Mean weight [mg]	SD	Treatment Group	Mean weight [mg]	SD
Test Item [µg a.i./bee]			Test Item [µg a.i./bee]		
100	261	54.3	0.52	278	42.4
50	261	41.7	0.26	270	46.5
25	249	48.7	0.13	262	42.5
12.5	264	49.9	0.064	247	42.0
6.25	261	56.5	0.032	283	53.4
Control	248	50.5	Control	296	46.4
Ref.: 12 µg dimethoate/bee	255	48.2	Ref.: 4 µg dimethoate/bee	263	36.2

Mean weight = mean weight of 30 individuals per treatment group; SD = standard deviation
Contact Test: Control = tap water containing 0.5 % Tween80;
Oral Test: Control = 50 % w/v sucrose solution;
Ref. = Reference Item

II. RESULTS AND DISCUSSION

All study validity criteria were achieved.

In the contact test 30 worker bees per treatment group (30 replicates) were exposed to doses of 100, 50, 25, 12.5, and 6.25 µg a.s./bee by dorsal application of a 5 µL droplet (test item in tap water containing 0.5% Tween80) on the bumblebee thorax.

At test end (48 hours after application) dose levels of 100, 50, 25, 12.5, and 6.25 µg a.s./bee led to mortalities of 90.0, 60.0, 20.0, 6.7, and 10.0%, respectively.

No mortality occurred in the control group (tap water containing 0.5% Tween80).

Four hours after application behavioural abnormalities (affected or moribund) occurred in 56.7, 80.0, 66.7, 40.0, and 36.7% of the bees at dose levels of 100, 50, 25, 12.5, and 6.25 µg a.s./bee. Twenty-four hours after application, 13.3, 43.3, 20.0, 0.0, and 13.3% of the bees at dose levels of 100, 50, 25, 12.5, and 6.25 µg a.s./bee were affected or moribund. At test end (48 hours after application), 10.0, 20.0, 16.7, 0.0, and 3.3% of the bees at dose levels of 100, 50, 25, 12.5, and 6.25 µg a.s./bee were affected.

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

Treatment Group	after 4 hours		after 24 hours		after 48 hours	
	mortality mean %	beh.abnor. mean %	mortality mean %	beh.abnor. mean %	mortality mean %	beh.abnor. mean %
Test Item [µg a.i./bee]						
100	40.0	56.7	86.7	13.3	90.0	10.0
50	20.0	80.0	56.7	43.3	60.0	20.0
25	0.0	66.7	20.0	20.0	20.0	16.7
12.5	0.0	40.0	6.7	0.0	6.7	0.0
6.25	0.0	36.7	10.0	13.3	10.0	3.3
Control	0.0	3.3	0.0	3.3	0.0	3.3
Ref: 12 µg dimethoate/bee	3.3	86.7	76.7	13.3	90.0	10.0

beh. abnor. = behavioural abnormalities; mean = mean of 30 individuals per treatment group
control = tap water containing 0.5 % Tween80; Ref. = Reference Item

In the oral test, the target dose levels of 0.5, 0.25, 0.125, 0.0625, and 0.03125 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 0.52, 0.26, 0.13, 0.064, and 0.032 µg a.s./bee.

At test end (48 hours after application), dose levels of 0.52, 0.26, 0.13, 0.064, and 0.032 µg a.s./bee led to mortalities of 83.3, 23.3, 3.3, 0.0, and 0.0%, respectively.

There was 3.3% mortality in the control group (50% w/v sucrose solution).

Four hours after application, behavioural abnormalities (affected or moribund) occurred in 40.0, 40.0 and 6.7% of the bees at dose levels of 0.52, 0.26, and 0.13 µg a.s./bee. Twenty-four hours after application, 6.7 and 16.7% of the bees at dose levels of 0.52 and 0.26 µg a.s./bee were affected. At test end (48 hours after application), 6.7, 6.7, and 3.3% of the bees at dose levels of 0.52, 0.26, and 0.13 µg a.s./bee were affected.

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

Treatment Group	after 4 hours		after 24 hours		after 48 hours	
	mortality mean %	beh.abnor. mean %	mortality mean %	beh.abnor. mean %	mortality mean %	beh.abnor. mean %
Test Item [µg a.i./bee]						
0.52	50.0	40.0	83.3	6.7	83.3	6.7
0.26	6.7	40.0	23.3	16.7	23.3	6.7
0.13	0.0	6.7	0.0	0.0	3.3	3.3
0.064	0.0	0.0	0.0	0.0	0.0	0.0
0.032	0.0	0.0	0.0	0.0	0.0	0.0
Control	0.0	0.0	0.0	0.0	3.3	0.0
Ref: 4 µg dimethoate/bee	6.7	93.3	93.3	6.7	96.7	3.3

beh. abnor. = behavioural abnormalities; mean = mean of 30 individuals per treatment group
control = 50 % w/v sucrose solution; Ref. = Reference Item

Mortality of the bumblebees treated with reference item (dimethoate, 400 g/L EC) was 90.0% in the contact test (12 µg a.s./bee) and 96.7% in the oral test (4.2 µg a.s./bee) at test end (48 hours after application), respectively.

Table 70 Toxicity of oxamyl to bumblebees (*Bombus terrestris* L.) in an acute contact and oral toxicity test

Test item	Oxamyl	
Test object	*Bombus terrestris* L.	
Exposure	contact (solution in water + 0.5% Tween80)	oral (50% w/v sucrose solution)
LD_{50} (µg a.s./bee)	39.3 (16.6-191.0)(48 h)	0.36 (0.31- 0.42) (48 h)
NOED (µg a.s./bee)	12.5 (48 h)	0.13 (48 h)

III. CONCLUSIONS

The effects of oxamyl on the bumblebee (*Bombus terrestris* L.) were assessed in an acute contact and oral toxicity test, conducted in the laboratory.

The 48 h contact LD_{50}, based on measured concentration, was 39.3 µg a.s./bee and the NOED was 12.5 µg a.s./bee.

The 48 h oral LD_{50}, based on measured concentration, was 0.36 µg a.s./bee and the NOED was 0.13 µg a.s./bee.

(Haupt, S., 2014)

RMS comments and conclusion

The test method is in line with the recommendations of the non-Apis ring test group (2014). Since mortality did not increase between 24 and 48 hours the test was not needed to be prolonged.

The results are expressed as µg a.s./bee. Since the weights of each bumblebee were measured, the mean value per group is reported in the table added to the summary. The weight of bees was slighlyt higher than the criterion indicated by van der Steen (2001) for modal bumblebees, i.e 0.1400 g - 0.2300 g. The weight distribution among test groups was omogeneous.

The deviation recorded for the temperature and relative humidity was explained in the report: "For the recordings data loggers (tiny tags) were used. Recording intervals were 15 minutes and data were read out after the test. During the test the climate conditions were checked visually on the incubator's display. There were no conspicuous values…" The unusual recordings were explained by inappropriate climate data recording position of the data logger (placed in the gutter). After the test, the incubator functioning was tested with 4 data logger set in different positions and confirmed that the data logger placed in the gutter recorded anomalous data…." Mortality results of the definitive test and a climate control check proved that that the climate data produced in the acute toxicity test with oxamyl does not show the prevalent temperature and relative humidity in the incubator as the data-logger did not work correctly due to wrong placement."

The validity criteria are fulfilled:

-Control mortality was 0.0% in the contact test and 3.3% in the oral test and thus did not exceed 10% at test end (after 48 hours).
-For the reference toxicant the 48h mortality exceeded 50%: in the contact test was 90.0% and in the oral test 96.7% .
Conclusion: the study is acceptable.

B.9.3.1.1.2 Acute contact toxicity

Study submitted in the EU Dossier in 2001 and included in the first EU approval review.

B.9.3.1.1.2/01

Reference: --	**Report:**	Schur, A. (1999); Oxamyl technical: Acute oral and contact toxicity to the honey bee, *Apis mellifera* L. **DuPont Report No.:** DuPont-2740 Guidelines: **EPPO 170, U.S. EPA 850.3020 (1996)**

1. Test material: Pure Oxamyl (PAI)
   Lot/Batch #: D1410-196
   Purity: 96.9%

RMS comments and conclusion

The acute contact toxicity study DuPont-2740, originally submitted under EU Rev8 Point IIA 8.3.1.1 and conducted with test material pure oxamyl (PAI), was conducted under guidelines EPPO 170, and U.S. EPA 850.3020 (1996).

See summary and RMS evaluation in B.9.3.1.1.1 above.

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

B.9.3.1.1.2/02

Reference: **CA 8.3.1.1.2/01**	**Report:**	Haupt, S. (2014); Oxamyl (DPX-D1410) technical (98% w/w): Acute oral and contact toxicity to the bumblebee, *Bombus terrestris* L. (Hymenoptera) **DuPont Report No.:** DuPont-39670 **Guidelines:** Van der Steen (2001), OECD 213 (1998), OECD 214 (1998), Current recommendations of the non-Apis ring test group (2014) **Deviations:** None

		Testing Facility: Institut für Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany **Testing Facility Report No.:** 84911105 **GLP:** Yes **Certifying Authority:** Hess. Ministerium fur Umwelt, Energie, Landwirtschaft and Verbraucherschutz

Acute contact toxicity studies on bees are conducted within the acute oral toxicity study (see Point B.9.3.1.1.1/02 in this document for summary).

B.9.3.1.2 Chronic toxicity to bees

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

B.9.3.1.2/01

Reference: CA 8.3.1.2/01	**Report:**	Schmitt, H. (2014); Oxamyl (DPX-D1410) technical (98% w/w): Assessment of effects to the honeybee, *Apis mellifera* L., in a 10 days chronic feeding test under laboratory conditions **DuPont Report No.:** DuPont-39665 **Guidelines:** No specific guideline available **Deviations:** None **Testing Facility:** Eurofins Agroscience Services, Gmbh, Neifern-Oschelbronn, Germany **Testing Facility Report No.:** S14-00409 **GLP:** Yes **Certifying Authority:** Landesanstalt Fur Umwelt, Messungen Und Naturschutz Baden-Wurttemberg

Executive summary:

Chronic effects of the test item oxamyl 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 and oxamyl at the concentration levels of 1.13, 2.25, 4.5, 9, and 18 mg a.s./kg.

The control group was exposed for the same period of time under identical exposure conditions to untreated 50% (w/v) aqueous sucrose feeding solution. The analytical verification of the test item concentrations in stock and feeding solutions was done by analysing the content of oxamyl in the samples taken before first application.

Assessments of mortality, sub-lethal effects, or behavioural differences were carried out daily during the 10-days test period. Furthermore, the daily food consumption was determined.

The actual concentration of oxamyl in the stock solution, determined on day 1, was 108% of nominal. The actual concentration of oxamyl in the feeding solutions, determined for the preparation on day 1, was in the range from 99 to 115% of the nominal concentration. The average actual concentration of oxamyl in the feeding solutions accounted to 109.6% of nominal.

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, 0.0, 17.5, 100, and 100% was observed at the test item concentration levels of 1.13, 2.25, 4.5, 9, and 18 mg a.s./kg at the end of the test. The mortality at the three highest test item

levels of 4.5, 9, and 18 mg a.s./kg were statistically significantly higher compared to the control group. The NOEC was determined to be 2.25 mg a.s./kg, and the NOED was determined to be 0.07 µg a.s./bee/day.

There were no sub-lethal effects recorded at the two lowest treatment levels of 1.13 and 2.25 mg a.s./kg during the entire observation period. At the treatment level of 4.5 mg a.s./kg, some sub-lethal effect were recorded from evaluation E3 to E10. At the two highest treatment levels of 9.0 and 18.0 mg a.s./kg, many sub-lethal effects were recorded, and the bees finally died.

The 10-day LC_{50} was determined to be 4.83 mg a.s./kg (with the 95% confidence limits of 3.44 to 6.90 mg a.s./kg).

The NOEC after 10 days was statistically determined to be 2.25 mg a.s./kg.

The 10-day LD_{50}, based on the test item consumption per bee per day, was determined to be 0.14 µg a.s./bee/day (with the 95% confidence limits of 0.11 to 0.20 µg a.s./bee/day).

The NOED after 10 days was statistically determined to be 0.07 µg a.s./bee/day.

MATERIALS AND METHODS

A. MATERIALS

1.	Test material:	Pure Oxamyl (PAI)
	Lot/Batch #:	D1410-532
	Purity:	99.1%
	Description:	Solid, chrystalline
	CAS Registry Number:	E115827-39
	Stability in Solution:	Sufficient for test purpose
2.	Control C:	50% (w/v) aqueous sucrose feeding solution
	Test vehicle	50% (w/v) aqueous sucrose feeding solution
	Toxic reference:	Perfekthion (dimethoate)
3.	Test organism:	Honey bees
	Species:	*Apis mellifera* L.
	Age at dosing:	Young adult worker bees (newly hatched; 1 to 4 days old)
	Source:	Beekeeper Mr. Hampel, Mühlhausenerstr. 1/1, 75233 Tiefenbronn, Germany
	Test chamber:	Stainless steel cages, base: 8 × 4 cm, height: 6 cm
4.	Environmental conditions (In-life phase)	
	Temperature:	32.4–33.2°C
	Relative humidity:	62.0–70.4%
	Photoperiod:	Continuous dark, except during the assessments

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed

   24-June-2014 to 16-July-2014

2. Experimental treatments

   One control, one toxic reference, and five test item concentrations of 1.13, 2.25, 4.5, 9, and 18 mg a.s./kg were tested. Four replicates per concentration level each with 10 honey bees were used. The feeding solutions were continuously offered to the bees *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 consumption was determined. The daily

consumption of feeding solution per bee was calculated by dividing the total daily consumption per replicate by the number of living bees at start of the corresponding feeding interval.

4. Statistics

The LC_{50} and LD_{50} values with 95% confidence intervals of the test item group were calculated by means of a probit analysis using the statistical program, ToxRat Professional 2.10, (ToxRat Solutions GmbH).

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 and NOED value.

5. Chemical Analysis

The analysis of samples was performed in the analytical laboratories of the test facility with a suitable analytical method. The determination was done in accordance with SANCO/3029/99 rev. 4 from 11/07/2000. The analytical method was validated with regard to specificity, linearity, accuracy (recovery), precision, and limit of quantification.

II. RESULTS AND DISCUSSION

A. FINDINGS

There was no mortality in the control group treated with 50% aqueous sucrose solution during the whole examination phase of 10 days. In the toxic reference group treated with dimethoate, 97.5% mortality was recorded at the end of the 10-day examination phase. Consequently, the validity criterion for the test was met, and the test was deemed valid.

In the test item group, a mortality of 0.0, 0.0, 17.5, 100, and 100% was observed at the test item concentration levels of 1.13, 2.25, 4.5, 9, and 18 mg a.s./kg at the end of the test. The NOEC was determined to be 2.25 mg a.s./kg, and the NOED was determined to be 0.07 µg a.s./bee/day.

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

Table 71 Summary of effects of oxamyl on honey bees in a 10-day chronic feeding test

Oxamyl[a] (mg a.s./kg)	10-Day cumulative mortality (%)	Overall mean consumption of feeding solution (mg/bee/day)	Mean uptake of test item (µg a.s./bee/day)	Accumulated mean uptake of test item (µg a.s./bee)
Control (0.0) 50% w/v aqueous sucrose solution	0.0	35.7	0.0	0.0
Toxic reference item (0.85 mg dimethoate/kg) [a]	97.5	29.9	0.03	0.30
1.13	0.0	33.9	0.04	0.41
2.25	0.0	32.8	0.07	0.75
4.5	17.5[b]	27.2	0.12[b]	1.25
9	100.0[b]	28.7	0.26[b]	0.87
18	100.0[b]	28.2	0.51[b]	3.70
LC_{50} (95% confidence limits)	4.83 mg a.s./kg (3.44–6.90 mg a.s./kg)			
NOEC[c]	2.25 mg a.s./kg			
LD_{50} (95% confidence limits)	0.14 µg a.s./bee/day (0.11–0.20 mg a.s./bee/day)			
NOED[d]	0.07 µg a.s./bee/day			

[a] Based on the analyzed content of a.s

[b] Statistically significantly different compared to the control; Fisher's Exact Test (Bonferroni-Holms corrected, one-sided greater, $\alpha = 0.05$)

[c] No Observed Effect Concentration (NOEC) based on mortality (not significantly different compared to the control; Fisher's Exact Test, Bonferroni-Holms corrected, one-sided greater, $p \leq 0.05$)

[d] No Observed Effect Dose (NOED) based on mortality (not significantly different compared to the control; Fisher's Exact Test, Bonferroni-Holms corrected, one-sided greater, $p \leq 0.05$)

III. CONCLUSIONS

The effects of oxamyl were assessed in a 10-day oral laboratory toxicity test.

The 10-day LC_{50} was determined to be 4.83 mg a.s./kg (with the 95% confidence limits of 3.44 to 6.90 mg a.s./kg).

The 10-day LD_{50}, based on the test item consumption per bee per day, was determined to be 0.14 µg a.s./bee/day (with the 95% confidence limits of 0.11 to 0.20 µg a.s./bee/day).

The NOEC after 10 days was statistically determined to be 2.25 mg a.s./kg.

The NOED after 10 days was statistically determined to be 0.07 µg a.s./bee/day.

(Schmitt, H., 2014)

RMS comments and conclusion

No specific guideline is available, but the study was conducted in line with the recommendations of EFSA (2013). The test is valid (control mortality < 15%). The reference group showed mortality of 97.5%.The test is acceptable.

B.9.3.1.3 Effects on honeybee development and other honeybee life stages

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

B.9.3.1.3/01

Reference: CA 8.3.1.3/01	**Report:**	Klank, C. (2014); Oxamyl (DPX-D1410) technical (98% w/w): Honey bee (*Apis mellifera* L.) larval toxicity test (single feeding exposure) **DuPont Report No.:** DuPont-39678 **Guidelines:** OECD 237 (2013) **Deviations:** None **Testing Facility:** Eurofins Agroscience Services EcoChem GmbH, Niefern-Öschelbronn, Germany **Testing Facility Report No.:** S14-00410 **GLP:** Yes **Certifying Authority:** Landesanstalt Fur Umwelt, Messungen Und Naturschutz Baden-Wurttemberg

Executive summary:

Effects of the test item oxamyl on the honey bee, *Apis mellifera* L., were assessed in a 7-day laboratory test. Synchronised honey bee larvae (first instar, L1) were transferred into well-plates where they were fed a standardized amount of artificial diet. On Day 4 (D4) of the test, five different doses (0.1, 0.18, 0.32, 0.56, and 1.0 µg oxamyl/larva) of the test item were applied to the larvae with the diet. The control group was exposed for the same period of time under identical exposure conditions to untreated artificial diet. Assessments of mortality were carried out on D5, D6, and D7 (24 h, 48 h, and 72 h after application of treated and untreated food, respectively). The dose which causes 50% mortality to larvae of *Apis mellifera* L. (LD_{50}) and the NOED (No Observed Effect Dose) after 72 hours were determined.

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

The 72-hour NOED for oxamyl was determined as 0.18 µg oxamyl/larva.

The 72-hour LD_{50} for oxamyl was calculated as 0.81 µg oxamyl/larva (95% confidence limits: 0.66–1.07 µg oxamyl/larva).

In the reference item treatment group (8.8 µg dimethoate/larva), the adjusted mortality was 91.7% at the final evaluation on D7.

I. MATERIALS AND METHODS

A. MATERIALS

1.	Test material:	Pure Oxamyl (PAI)
	Lot/Batch #:	D1410-532
	Purity:	99.1%, by analysis
	Description:	Solid, crystalline/white
	CAS #:	23135-22-0
	Stability in solution:	Sufficient for test purpose (at least 1 hour)
2.	Control:	Diet C containing autoclaved, deionized water as solvent
	Test vehicle	Diet C (50% weight of fresh royal jelly + 50% weight of an aqueous solution containing 4% weight of yeast extract, 18% weight of glucose and 18% weight of fructose) containing oxamyl diluted with autoclaved, deionized water
	Toxic reference:	Dimethoate 8.8 µg dimethoate/larva
3.	Test organism:	Honey bee larvae
	Species:	*Apis mellifera* L.
	Age at grafting:	First instar larvae (L1)
	Source:	Eurofins Agroscience Services, EcoChem GmbH, Eutinger Straße 24, D-75223 Niefern-Öschelbronn, Germany
	Test chamber:	Crystal polystyrene grafting cells (NICOTPLAST) diameter of 9 mm; cells placed into a well of a 48-well cell culture plate; plates placed into desiccator; desiccator placed into an incubator with forced air circulation
4.	Environmental conditions (In-life phase)	
	Temperature	33.1 to 34.4°C
	Relative humidity	40.1* to 100.0% * Only short term deviations <2 h
	Exposure to light	Constant darkness except during feeding and assessment.

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed

23-June-2014 to 15-July-2014

2. Experimental treatments

One control and five test item concentrations of 0.1, 0.18, 0.32, 0.56 and 1.0 µg oxamyl/larva were tested. In total 48 larvae per treatment group from three different colonies (each colony representing a replicate), each containing 16 test organisms, were used. Four days prior to test start, in order to ensure the production of synchronized larvae of at least three replicate colonies, the queens of six colonies were confined in their own colony in an excluder cage containing a comb with empty cells. Three days before test start (within 30 hours after encaging) the queens were released from the cages. The combs containing eggs were left in the cages during the incubation stage and until hatching. On the day of test start (D1) three out of six colonies were selected containing the highest number of synchronized larvae. The corresponding combs were transferred from the hives to the laboratory using an insulated container in order to avoid temperature variation. On D1 the test was initiated with larvae in excess. Therefore two reserve plates were prepared containing larvae of the same replicate hives. Before application of the test item on day 4 (D4), it was assured that all larvae used were of the same size and alive. For each treatment group 3 to 8 non-suitable larvae were replaced with individuals from the reserve plates, using larvae from the same hive to maintain 16 larvae per replicate. On Day 4 (D4) of the test, the diet C containing the application solutions was applied to the larvae.

3. Observations

Assessments of mortality were carried out after 24 h, 48 h and 72 h (D5, D6 and D7) after feeding with treated diet.

4. Statistics

Fisher's Exact Test with Bonferroni Correction (one-sided greater, $\alpha = 0.05$) was used to evaluate whether there were significant differences between the mortality data of the test item group and the control group and to determine the NOED value. For calculation of the LD_{50} with 95% confidence limits the Probit analysis using linear max. likelihood regression was used. Fisher's Exact Test (one-sided greater, $\alpha = 0.05$) was used to evaluate whether there were significant differences between the mortality data of the reference item group and the control group.

II. RESULTS AND DISCUSSION

A. FINDINGS

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

Treatment group	Dose level (µg/larva)	Mortality on D7 (72h after feeding) (%)	Adjusted mortality on D7 (72h after feeding) (%)
Control	0	0.0	-
Test item (oxamyl)	0.1	0.0	0.0
	0.18	4.2	4.2
	0.32	12.5[a]	12.5
	0.56	45.8[a]	45.8
	1.0	52.1[a]	52.1
Reference item (dimethoate)	8.8	91.7[b]	91.7
		Endpoints (µg oxamyl/larva)	
72-hour NOED		0.18	
72-hour LD_{50} (95% confidence limits)		0.81 (0.66–1.07)	

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

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

III. CONCLUSIONS

The 72-hour NOED for larvae of *Apis mellifera* L. was determined as 0.18 µg oxamyl/larva.

The 72-hour LD_{50} for larvae of *Apis mellifera* L. was calculated as 0.81 µg oxamyl/larva (95% confidence limits: 0.66–1.07 µg oxamyl/larva).

(Klank, C., 2014)

RMS comments and conclusion

The study is valid:

Control mortality: <15% from D4 to D7 across all replicates.

Reference item mortality: >50% at D7 across all replicates.

The study is acceptable.

B.9.3.1.4 Sub-lethal effects

Chronic and sublethal effects of the test item oxamyl on the honey bee, *Apis mellifera* L., were assessed in a 10-day laboratory feeding test, which is summarized under Point B.9.3.1.2 in this document (DuPont-40935 EU).

B.9.3.2 Effects on non-target arthropods other than bees

B.9.3.2.1 Effects on *Aphidius rhopalosiphi*

Study submitted in the EU Dossier in 2001 and included in the first EU approval review.

B.9.3.2.1/01

Reference: --	**Report:**	Austin, H. (1999); Oxamyl 10L (10% w/w): A laboratory study to evaluate the effects on the aphid parasitoid *Aphidius rhopalosiphi* (Hymenoptera: Braconidae) **DuPont Report No.:** DuPont-2609 **Guidelines:** SETAC-ESCORT (1994) "A laboratory method to evaluate the side-effects of pesticides on the cereal aphid parasitoid, Aphidius rhopalosiphi" (Mead Briggs 1992); "Guidelines for testing the effects of pesticide on Aphidius matricariae HAL" (Polgar, 1988).

1. Test material: Oxamyl 10SL
   Lot/Batch #: D1410-381
   Purity: 100 g a.s./L

Testing Facility: Ecotox Ltd., Tavistock, Devon, UK

GLP: Yes.

Materials and methods:

Adult mortality and reproductive effects (parasitism rate) to *Aphidius rhopalosiphi* were evaluated following a 48-hour exposure period to freshly dried spray deposits of Oxamyl 10 SL on treated glass plates. Based on results of initial range-finding tests, the definitive rates of the study were set at 0.01, 0.05, 0.1, 0.5, 1 and 2 g Oxamyl/ha. Each treatment consisted of three replicates with ten adult *A. rhopalosiphi* wasps (5 males and 5 females <48h okd) each. An untreated control and toxic standard (0.1 g a.s./ha Dimethoate) were also tested. The test substance was applied to glass plates in a nominal volume of 200 L water/ha (equivalent to 2 mg/cm^2 ±10%).. Test units consisted of two glass plates (11.7 x 11.7 em) fitted to a square frame (13 x 13 em) made from aluminium casing.

Mortality was assessed 1, 2, 4, 24 and 48 hours after introduction of wasps into the test system. Fecundity was also assessed using surviving females from the control and 0.01 g/ha treatments. Females from these test units were transferred into aphid (Rhopalosiphum padi) infested barley seedlings for a period of 24 hours. Each chamber contained one female parasitoid. Each chamber consisted of a 12.5 cm diameter, 20.5 cm high clear perspex cylinder with a solid bottom and a gauze lid. Once removed, the seedlings were left for 12 days for aphid mummies to develop. The number of mummies produced per female was then counted for each treatment to determine any effect of the test substance on fecundity.

The mortality results obtained at the 48 hour assessment were corrected for control mortality using Abbott's formula (1925) and tabulated. The results for percentage mortality at each concentration were transformed to probit values (Finney, 1978) and plotted in order to obtain an estimated LCso value. To determine the effect of the test substance on fecundity, one way analysis of variance (Parker, 1973) was carried out on the number of mummies produced per female in the test substance concentration tested compared with the control.

Findings:

Temperature ranged from 19.8 to 23.4°C, humidity from 53.1 to 69.1%, lighti intensity was 752 lux at the test start and 920 lux at the test end.

Adult mortality in the control after 48 hours was 16.7%. The results for the toxic standard fell within the expected range, demonstrating validity of the test. Oxamyl 10 SL, tested at 0.01, 0.05, 0.1, 0.5, 1, and 2 g Oxamyl/ha, showed corrected mortality (using Abbott's correction) of 40%, 60%, 64%, 100%, 100% and 100% for the six respective test concentrations (Table 73). The results for percentage mortality at each concentration were transformed to probit values and plotted to obtain an estimated LCso value of 0.03 g ai ha. The numbers of offspring per female were 4.6 for the control group and 5.3 for treatment group of 0.01 g Oxamyl/ha. Female wasps exposed to Oxamyl 10 SL at a rate of 0.01 g Oxamyl/ha experienced no statistically significant effect on fecundity when compared to the control treatment (ANOVA, p>0.05).

Table 73 Effects on mortality and reproduction in the aphid parasitoid, *A. rhopalosiphi,* after 48-hours exposure to freshly dried spray deposits of Oxamyl 10 SL on treated glass plates

Test substance	Oxamyl 10 SL
Test organism	*A. rhopalosiphi*
Exposure	48 hour, dose response, freshly dried spray deposits on glass plates
Control Mortality	16.7%
Treatment Mortality (using Abbott's correction)	
0.01gOxamyl/ha	40%
0.05gOxamyl/ha	60%
0.1gOxamyl/ha	64%
0.5gOxamyl/ha	100%
1.0gOxamyl/ha	100%
2.0 g Oxamyl/ha	100%
0.1 g Dimethoate/ha (Toxic Reference Standard)	70 – 100% (actual 100%)
LR_{50}	0.03 g Oxamyl/ha
LR_{30}	0.005 g Oxamyl/ha
Mean Number of Mummies per Female	
Control	4.6
0.01 g Oxamyl/ha	5.3

Conclusion:

The $LR_{50}$for 48-hour exposure of adult *A. rhopalosiphi* wasps to Oxamyl 10 SL was 0.03 g Oxamyl/ha. The LR30 was 0.005 g Oxamyl/ha. Female wasps exposed to freshly dried spray deposits of Oxamyl 10 SL at a rate of 0.01 g Oxamyl/ha experienced no statistically significant reduction in fecundity when compared to the control treatment.

RMS comments and conclusion

The effects on *Aphidius rhopalosiphi* study DuPont-2609, originally submitted under EU Rev8 Point IIA 8.3.2.1 and conducted with test material Oxamyl 10SL, was conducted under guideline SETAC-ESCORT (1994). A re-evaluation of the study according to the current guideline (Mead-Briggs et al. 2000) was carried out, and the following deviations were noted:

- Range of temperature and RU fell outside the recommended 20± 2°C and 75±15%, respectively but the report do not specifies for how long.
-The replicates were 3 in the control and treatments instead of a minimum of 4-5.
- The number of individual replicates in the reproduction phase where 11 in the control, and 9 in the treatment.

Two validity criteria are not met:
- The mortality in the control was 16.7% (higher than the required ≤ 13%.
- More than 4 mummies were produced by the control (actual 4.6) but 3 control females did not produce anu mummy.

Also it is noted that the LC50 is an extrapolated value.

Conclusion: **the test is not valid.** It is noted that for *Aphidius rhopalosiphi*, an extended laboratory test is available, which mimicks the proposed exposure via drip irrigation.

B.9.3.2.2 CA 8.3.2.2 Effects on *Typhlodromus pyri*

Study submitted in the EU Dossier in 2001 and included in the first EU approval review.

B.9.3.2.2/01

Reference: --	**Report:**	Walker, H. (2000); Oxamyl (DPX-D1410) 10L: A laboratory study to determine the LC_{50} and evaluate the sublethal effects on the predatory mite *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae) **DuPont Report No.:** DuPont-4037 **Guidelines:** Overmeer (1988), Louis and Ufer (1995)

1. Test material: Oxamyl 10SL
   Lot/Batch #: D1410-381
   Purity: 100 g a.s./L

Testing Facility: Ecotox Ltd., Tavistock, Devon, UK

GLP: Yes.

Materials and methods:

Adult mortality and reproductive effects (expressed as fertile egg production/female) were evaluated following exposure of *Typhlodromus pyri* to freshly dried spray deposits of Oxamyl 10 SL on treated glass slides. Oxamyl 10 SL was applied at 0.2, 0.4, 0.8, 1.2, 1.6, 2.0 and 2.4 g Oxamyl/ha in a volume equivalent to 200 L water/ha. An untreated control and toxic standard Meothrin (0.03 g fenpropathrin /ha) were also tested. The untreated control had six replicates, and the toxic standard and treatment groups had three replicates. Each replicate contained 20 mites. Juglans pollen was added as food and renewed 1,3,5d after treatment.

Fecundity was also assessed in the control, 0.4 and 0.8g a.i./ha test units. After 7 days, the numbers of males and females in each test unit were counted to determine sex ratio. Fecundity assessments were carried out 10, 12 and 14 days after treatment by counting the number of juveniles and eggs laid per female.

Findings:

Temperature ranged from 24-1 to 26.3°C, humidity from 44.5 to 93.5%, lighti intensity was 903 lux at the test start and 1320 lux at the test end.

After 7 d, mortality in the control group was 5.8%. Results for the toxic reference substance fell within acceptable ranges, indicating validity of the study. The corrected mortality for Oxamyl 10 SL tested at 0.2, 0.4, 0.8, 1.2, 1.6, 2.0, and 2.4 g Oxamyl/ha were 4.5, 13.3, 23.9, 32.8, 45.1, 55.7, and 62.8%, respectively (Table 74). The numbers of offspring per female were 6.7, 5.3, and 4.8 for the control, 0.4 and 0.8 g Oxamyl/ha, respectively (Table 74). There were no statistically significant differences in the number of eggs per female in the 0.4 and 0.8 g Oxamyl/ha treatment groups as compared with the control (ANOVA at $p< 0.05$).

Table 74 Effects on mortality and reproduction of the predatory mite, *Typhlodromus pyri*, exposed to freshly dried spray deposits of Oxamyl 10 SL on glass plates

Test substance	Oxamyl 10 SL
Test organism	*Typhlodromus pyri*
Exposure	Dose response, freshly dried spray deposits on glass plates.
Control Mortality Treatment Mortality (using Abbott's correction) 0.2 g Oxamyl/ha 0.4 g Oxamyl/ha 0.8 g Oxamyl/ha 1.2 g Oxamyl/ha 1.6 g Oxamyl/ha 2.0 g Oxamyl/ha 2.4 g Oxamyl/ha	5.8% 4.5% 13.3% 23.9% 32.8% 45.1% 55.7% 62.8%
0.03 g Fenpropathrin/ha (Toxic Reference Standard)	70 – 100% (98.2% , corrected)
LR_{50}	1.8 g Oxamyl/ha
LR_{30}	1.0 g Oxamyl/ha
Mean Reproduction Rate Control 0.4 g Oxamyl/ha 0.8 g Oxamyl/ha	 6.7 ± 1.3 5.3 ± 0.5 4.8± 1.7

Conclusion:

The 7-day LR_{50} for *T. pyri* exposed to freshly dried spray deposits of Oxamyl 10 SL was 1.8 g Oxamyl/ha. The LR30 was 1.0 g Oxamyl/ha. Female mites exposed to Oxamyl 10 SL at rates of 0.4 and 0.8 g Oxamyl/ha experienced no statistically significant reduction in fecundity compared to the control.

RMS comments and conclusion

Additional information has been added to the original summary by the RMS.

The effects on Typhlodromus pyri study DuPont-4037, originally submitted under EU Rev8 Point IIA 8.3.2.1 and conducted with test material Oxamyl 10SL, was conducted under guidelines Overmeer (1988), and the methodological improvements by Louis and Ufer (1995). During the re-eavaluation of the study, the following deviations from the current guideline (Blumel et al. 2000) were noted:

-The replicates were 3 in the treatments instead of a minimum of 4-5.
- Range of RU fell outside the recommend 75±15%, but it is not mentioned for how long.
- The reference substance was different from the recommended dimethoate.

Conclusion: the test can be considered as **supportive information**. It is noted that for T. pyri an extended laboratory test is available, which mimicks the proposed exposure via drip irrigation.

Oxamyl and Oxamyl 10SL toxicity endpoint values on beneficial arthropods are summarised in Table 75.

Table 75 Oxamyl and Oxamyl 10SL toxicity endpoint values on beneficial arthropods including other terrestrial invertebrates

Species	Test (test substance)	Measurement endpoint	Endpoint value	Reference[a]
Apis mellifera	48-hr Oral (oxamyl)	LD_{50}	0.38 µg a.s./bee	DuPont-2740
Apis mellifera	48-hr Contact (oxamyl)	LD_{50}	0.47 µg a.s./bee	DuPont-2740
Apis mellifera	10-d adult oral (oxamyl)	LD_{50} NOED	0.14 µg a.s./bee 0.07µg a.s./bee	DuPont-39665
Apis mellifera	72-hr larvae (oxamyl)	LD_{50} NOED	0.81 µg a.s./bee 0.18µg a.s./bee	DuPont-39678
Bombus terrestris	48-hr Oral (oxamyl)	LD_{50}	0.36 µg a.s./bee	DuPont-39670
Bombus terrestris	48-hr Contact (oxamyl)	LD_{50}	39.3 µg a.s./bee	DuPont-39670
Aphidius rhopalosiphis	Not valid			
Typhlodromus pyri Supportive information	Oxamyl 10SL Glass plate dose response (14 d)	7 d LR_{50} 7 d LR_{30} 30% effect on reproduction	1.8 g oxamyl/ha 1.0 g oxamyl/ha ≥0.8 g a.s/ha	DuPont-4037

[a] All studies cited or summarised in this document

B.9.4 Effects on non-target soil meso- and macrofaunaMacrofauna

NOTE of the RMS: The summaries of the acute toxicity studies in earthworms on the active substance (ref. AMR 3068) and on the metabolites (ref. DuPont-4130, DuPont-4617, DuPont-4132, DuPont-4134) have not been submitted by the Applicant. In Document M-CA, Section 8, for the above references, it is stated that "Acute toxicity in earthworm studies are no longer a requirement. Therefore this study is not relied upon.". The RMS acknowledges that according to the commission Regulation (EU) No 283/2013, a study on sub-lethal effects on earthworms should be required when the active substance can contaminate soil. Nevertheless, since the acute data are available, the RMS has included the relative studies with their summaries in the present document (see below, sections B.9.4.1/06 to B.9.4.1/09).

B.9.4.1 Earthworm – sub-lethal effects

Study submitted in the EU Dossier in 2001 and included in the first EU approval review.

B.9.4.1/01

A chronic earthworm study was conducted with Oxamyl 10GR (Luhrs, 2001; DuPont-4296). The summary of this study was submitted in the Oxamyl EU Renewal Dossier, Document M-CA, Section 8, DuPont-40935 EU. Since the test item is the representative formulation, the RMS moved the study summary to Vol.3 (Ozamyl 10GR).

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

B.9.4.1/02

Reference: **CA 8.4.1/02**	**Report:**	Pavić, B. (2014); IN-A2213: Effects on reproduction and growth of the earthworm, *Eisenia fetida*, in artificial soil **DuPont Report No.:** DuPont-39672

		Guidelines: OECD 222 (2004), ISO 11268-2 (2012) **Deviations:** None **Testing Facility:** Institut für Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany **Testing Facility Report No.:** 89591022 **GLP:** Yes **Certifying Authority:** Hess. Ministerium fur Umwelt, Energie, Landwirtschaft and Verbraucherschutz

Executive summary:

The sublethal toxicity of IN-A2213 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 6.25, 12.5, 25.0, 50.0 and 100 mg IN-A2213/kg dry artificial soil and to an untreated control (deionized water only). Mortality and growth (body weight) of the earthworms were assessed after 28 days and the effect on reproduction (number of juveniles produced) was assessed after 56 days. The LC_{50} after 28 days was estimated to be greater than 100 mg IN-A2213/kg dry artificial soil. The NOEC (No-Observed-Effect Concentration) for earthworms based on mortality, growth, and nominal concentrations was 100 mg test item/kg dry artificial soil, the highest concentration tested. The NOEC for earthworms based on reproduction was 25.0 mg test item/kg dry artificial soil.

The EC_{10} and EC_{20} for reproduction were 26.60 mg IN-A2213/kg dry artificial soil (with 95% confidence limits of 1.27 and 53.77 mg/kg soil) and 130.33 mg IN-A2213/kg dry artificial soil (with 95% confidence limits of 62.33 and 26402.12 mg/kg soil). The EC_{50} for reproduction could not be determined by statistical analysis but was estimated to be greater than 100.0 mg IN-A2213/kg dry artificial soil.

I. MATERIALS AND METHODS

A. MATERIALS

1.	Test material	IN-A2213 technical metabolite
	Lot/Batch #:	A2213-011
	Purity:	100%
	Description:	Solid
	CAS Registry Number:	66344-33-0
	Stability of test compound:	Not determined in the test system
2.	Control:	Untreated (and moistened with deionized water)
	Test vehicle:	Deionized water
3.	Test organism	Earthworm
	Species:	*Eisenia fetida*
	Age at dosing:	11 to 12 months
	Weight at dosing:	300 to 600 mg
	Source:	In-house laboratory culture (Laboratory: IBACON, Rossdorf, Germany)
	Acclimatization period:	1 day and none for the worms taken directly
	Test chamber:	Plastic boxes with perforated transparent lids (volume: 1 L), filled with ca. 500 g artificial soil dry weight
	Test medium:	Artificial soil prepared according to OECD 222, maximum water holding capacity of the artificial soil, as measured: 59%; 10% sphagnum peat.
	Diet:	Finely ground cattle manure
	Water content of soil:	Initiation: 31.5 to 33.3% (equivalent to 53.5 to 56.4% of the maximum water holding capacity) Termination: 32.6 to 34.5% (equivalent to 55.3 to 58.5% of the maximum water holding capacity)
	Soil pH:	5.7 to 5.8 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

10-July-2014 to 05-September-2014

2. Experimental treatments

The sublethal toxicity of IN-A2213 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 6.25, 12.5, 25.0, 50.0, and 100 mg IN-A2213/kg dry artificial soil and to an untreated control soil (deionized water only). One day after application of IN-A2213, 5 g/container of finely ground and moistened cattle manure was scattered uniformly on the soil surface. Food was added the same way each week for the first four weeks of the experiment, when the food of the previous week was almost completely consumed. If the food was not quite fully consumed, the added amount of food was adjusted to account for visually estimated consumption. After removing the adult worms on day 28, the food was mixed into the substrate. The reference item, Luxan Carbendazim 500 FC, 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 (behavioral) 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 Williams t-test (multiple comparison, alpha = 0.05, two-sided for weight changes) and for reproduction was performed using Bonferroni-Welch t-test (multiple comparison, alpha = 0.05, one-sided smaller for reproduction). The software used to perform the statistical analysis was ToxRat Professional, Version 2.10.05.

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 100 mg IN-A2213/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 behavioral 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. A statistically significant effect on reproduction (56-day assessment) compared to the control was observed at 50.0 mg IN-A2213/kg dry soil and 100 mg IN-A2213/kg dry soil.

Cumulative mortality and weight change (of adults) at 28 days, and reproduction at 56 days are reported in the summary table below.

Table 76 Sublethal toxicity of IN-A2213 to earthworms

Nominal IN-A2213 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	38.8	387
6.25	0	43.9	381
12.5	0	38.8	368
25.0	0	39.6	363
50.0	0	44.8	333[a]
100	0	40.0	314[a]

Weight change: there were no significant differences from the control (Williams t-test, two sided, alpha = 0.05)

[a] Statistically significantly different from the control (Reproduction: Bonferroni-Welch t-test, one-sided smaller, alpha = 0.05)

III. CONCLUSIONS

IN-A2213 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 100 mg IN-A2213/kg dry artificial soil. IN-A2213 had no significant effects on reproduction of the earthworm *Eisenia fetida* when exposed to concentrations up to and including 25.0 mg IN-A2213/kg dry artificial soil.

The overall NOEC (No-Observed-Effect Concentration) was determined to be 25.0 mg IN-A2213/kg dry artificial soil and the overall LOEC (Lowest-Observed-Effect Concentration) was determined to be 50.0 mg IN-A2213/kg dry artificial soil.

The LC_{50} after 28 days was estimated to be greater than 100 mg IN-A2213/kg dry artificial soil, the highest concentration tested.

The EC_{10} and EC_{20} for reproduction were 26.60 mg IN-A2213/kg dry artificial soil (with 95% confidence limits of 1.27 and 53.77 mg/kg soil) and 130.33 mg IN-A2213/kg dry artificial soil (with 95% confidence limits of 62.33 and 26402.12 mg/kg soil). The EC_{50} for reproduction could not be determined by statistical analysis but was estimated to be greater than 100.0 mg IN-A2213/kg dry artificial soil.

(Pavić, B., 2014)

RMS comments and conclusion: A deviation from the guideline was reported: only for 50% of the test organisms there acclimatization was for 1 day, while the remaining 50% of the earthworms were taken out of the breeding box at the day of application. This deviation is considered acceptable as adapted and not adapted worms were distributed evenly amongst control and all test item concentrations.

The test is valid: the mean control mortality was 0%, there were more than 30 juveniles per control unit after the 8 week testing period (was 345 to 437 per replicate), the coefficient of variance for the mean number of juveniles in the untreated control did not exceed 30% (was 8.3%).

The study is acceptable.

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

B.9.4.1/03

Reference: CA 8.4.1/03	**Report:**	Pavić, B. (2015a); IN-D2708: Effects on reproduction and growth of the earthworm, *Eisenia fetida*, in artificial soil **DuPont Report No.:** DuPont-41042 **Guidelines:** OECD 222 (2004), ISO 11268-2 (2012) **Deviations:** None **Testing Facility:** Institut für Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany **Testing Facility Report No.:** 92291022 **GLP:** Yes **Certifying Authority:** Hess. Ministerium fur Umwelt, Energie, Landwirtschaft and Verbraucherschutz

Executive summary:

The sublethal toxicity of IN-D2708 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 6.43, 12.86, 25.72, 51.44 and 102.9 mg IN-D2708/kg dry artificial soil (corresponding to 6.25, 12.5, 25.0, 50.0 and 100 mg IN-D2708/kg dry artificial soil, adjusted for purity) and to an untreated control(deionized water only). Mortality and growth (body weight) of the earthworms were assessed after 28 days and the effect on reproduction (number of juveniles produced) was assessed after 56 days. The LC_{50} after 28 days was estimated to be greater than 100 mg IN-D2708/kg dry artificial soil. The NOEC (No-Observed-Effect Concentration) for earthworms based on mortality, reproduction, growth and nominal concentrations was 100 mg test item/kg dry artificial soil, the highest concentration tested.

I. MATERIALS AND METHODS

A. MATERIALS

1.	Test material	IN-D2708 technical metabolite
	Lot/Batch #:	D2708-007
	Purity:	97.2%, by analysis
	Description:	Solid
	CAS Registry Number:	32833-96-8
	Stability of test compound:	Not analysed in the test system
2.	Control:	Untreated (moistened with deionized water)
	Test vehicle:	Deionized water
3.	Test organism	Earthworm
	Species:	*Eisenia fetida*
	Age at dosing:	10 to 11 months
	Weight at dosing:	306 to 596 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 ca. 500 g artificial soil dry weight (664.7 wet weight).
	Test medium:	Artificial soil prepared according to OECD 222, maximum water holding capacity of the artificial soil, as measured: 64%
	Diet:	Finely ground cattle manure
	Water content of soil:	Initiation: 29.0 to 37.3% (equivalent to 45.4 to 58.2% of the maximum water holding capacity) Termination: 34.5 to 39.7% (equivalent to 53.9 to 62.0% of the maximum water holding capacity)
	Soil pH:	6.0 to 6.4 at test start and 6.2 at test termination
4.	Environmental conditions (in-life period)	
	Temperature:	Within the range of 18 to 22°C
	Photoperiod:	16 hour light, 8 hour dark, photoperiod within the range of 400 to 800 lux

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed

13-November-2014 to 19-January-2015

2. Experimental treatments

The sublethal toxicity of IN-D2708 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 6.43 12.86, 25.72, 51.44 and 102.9 mg IN-D2708/kg dry artificial soil (corresponding to 6.25, 12.5, 25.0, 50.0, and 100 mg IN-D2708/kg dry artificial soil, adjusted for purity) and to an untreated control (moistened with deionized water only). At theday of application of IN-A2213, 5 g/container of finely ground and moistened cattle manure was scattered uniformly on the soil surface. Food was added the same way each week for the first four weeks of the experiment, when the food of the previous week was almost

completely consumed. If the food was not quite fully consumed, the added amount of food was adjusted to account for visually estimated consumption. After removing the adult worms on day 28, the food was mixed into the substrate. 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 July 2014 to September 2014.

3. Observations

Worms were assessed for mortality and sublethal (behavioral) 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 software used to perform the statistical analysis was ToxRat Professional, Version 2.10.05.

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 1.87 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 100 mg IN-D2708/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 behavioral 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 77 Sublethal toxicity of IN-D2708 to earthworms

Nominal IN-D2708 concentration, adjusted for purity (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	38.8	287
6.25	0	42.0	286
12.5	0	42.2	261
25.0	0	41.5	266
50.0	0	39.5	265
100	0	42.0	270

There were no significant differences from the control, Williams t-test, alpha = 0.05,
two sided for weight changes and one-sided smaller for reproduction

III. CONCLUSIONS

IN-D2708 had no statistically significant lethal effects or effects on reproduction, growth or feeding activity of the earthworm *Eisenia fetida* when exposed to concentrations up to and including 100 mg/kg dry artificial soil.

The overall NOEC (No-Observed-Effect Concentration) was determined to be 100 mg IN-D2708/kg dry artificial soil and the overall LOEC (Lowest-Observed-Effect Concentration) was estimated to be greater than 100 mg IN-D2708/kg dry artificial soil.

The LC_{50} after 28 days was estimated to be greater than 100 mg IN-D2708/kg dry artificial soil, the highest concentration tested.

(Pavić, B., 2015a)

RMS comments and conclusion:

Food was first provided on the day of application and not one day after applying the test substance to the soil as recommended. Considering the good control performance and the lack of effects, this deviation is acceptable.

The test is valid: the mean control mortality was 0%, there were more than 30 juveniles per control unit after the 8 week testing period (was 240 to 327 per replicate), the coefficient of variance for the mean number of juveniles in the untreated control did not exceed 30% (was 9.4%).

ECx values cannot be calculated due to the data set, hence the results are expressed as NOEC.

The study is acceptable.

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

B.9.4.1/04

Reference: CA 8.4.1/04	**Report:**	Pavić, B. (2015b); IN-N0079: Effects on reproduction and growth of the earthworm, *Eisenia fetida*, in artificial soil **DuPont Report No.:** DuPont-41045 **Guidelines:** OECD 222 (2004), ISO 11268-2 (2012) **Deviations:** None **Testing Facility:** Institut für Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany **Testing Facility Report No.:** 92271022 **GLP:** Yes **Certifying Authority:** Hess. Ministerium fur Umwelt, Energie, Landwirtschaft and Verbraucherschutz

Executive summary:

The sublethal toxicity of IN-N0079 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 6.38, 12.76, 25.51, 51.02, and 102.0 mg IN-N0079/kg dry artificial soil (corresponding to 6.25, 12.5, 25.0, 50.0 and 100 mg IN-N0079/kg dry artificial soil, adjusted for purity) and to an untreated control (deionized water only). Mortality and growth (body weight) of the earthworms were assessed after 28 days and the effect on reproduction (number of juveniles produced) was assessed after 56 days. The LC_{50} after 28 days was estimated to be greater than 100 mg IN-N0079/kg dry artificial soil. The NOEC (No-Observed-Effect Concentration) for earthworms based on mortality, growth and nominal concentrations was 100 mg test item/kg dry artificial soil, the highest concentration tested. The NOEC for earthworms based on reproduction was 50.0 mg test item/kg dry artificial soil.

I. MATERIALS AND METHODS

A. MATERIALS

1.	Test material	IN-N0079 technical metabolite
	Lot/Batch #:	N0079-010
	Purity:	98.0%, by analysis
	Description:	Liquid
	CAS Registry Number:	16703-51-8
2.	Control:	Untreated (moistened with deionized water)
	Test vehicle:	Deionized water
3.	Test organism	Earthworm
	Species:	*Eisenia fetida*
	Age at dosing:	Approximately 8 months
	Weight at dosing:	303 to 600 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 ca. 500 g artificial soil dry weight (657.8 g wet weight)
	Test medium:	Artificial soil prepared according to OECD 222, maximum water holding capacity of the artificial soil, as measured: 61%
	Diet:	Finely ground cattle manure
	Water content of soil:	Initiation: 32.7 to 33.4% (equivalent to 53.6 to 54.7% of the maximum water holding capacity) Termination: 33.3 to 36.3% (equivalent to 54.5 to 59.5% of the maximum water holding capacity)
	Soil pH:	5.5 at test start and 5.9 to 6.2 at test termination
4.	Environmental conditions (in-life period)	
	Temperature:	Within the range of 18 to 22°C
	Photoperiod:	16 hour light, 8 hour dark, photoperiod within the range of 400 to 800 lux

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed

   02-October-2014 to 28-November-2014

2. Experimental treatments

   The sublethal toxicity of IN-N0079 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 6.38, 12.76, 25.51, 51.02,

and 102 mg IN-N0079/kg dry artificial soil (corresponding to 6.25, 12.5, 25.0, 50.0, and 100 mg IN-N0079/kg dry artificial soil, adjusted for purity) and to an untreated control (moistened with deionized water only). At the day of application of IN-A2213, 5 g/container of finely ground and moistened cattle manure was scattered uniformly on the soil surface. Food was added the same way each week for the first four weeks of the experiment, when the food of the previous week was almost completely consumed. If the food was not quite fully consumed, the added amount of food was adjusted to account for visually estimated consumption. After removing the adult worms on day 28, the food was mixed into the substrate. 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 July 2014 to September 2014.

3. Observations

Worms were assessed for mortality and sublethal (behavioral) 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 NOEC for weight changes and reproduction was performed using Williams t-test, multiple comparison, alpha = 0.05, two-sided for weight changes and Bonferroni-Welch t-test, multiple comparison, one-sided smaller for reproduction. The software used to perform the statistical analysis was ToxRat Professional, Version 2.10.05

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 1.87 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 100 mg IN-N0079/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 behavioral 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 50.0 mg IN-N0079/kg dry artificial soil. At the concentration of 100 mg IN-N0079/kg dry artificial soil 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 78 Sublethal toxicity of IN-N0079 to earthworms

Nominal IN-N0079 concentration, adjusted for purity (mg test item/kg dry soil)	28-Day mortality (%) mean	28-Day weight change (%) mean	56-Day reproduction (# of juveniles) mean
Control (0.0)	1	37.1	214
6.25	0	39.0 n.s.[a]	249 n.s.
12.5	0	39.9 n.s.	221 n.s.
25.0	0	44.9 n.s.	216 n.s.
50.0	0	45.7 n.s.	254 n.s.
100	0	43.1 n.s.	142[b]

[a] n.s. = not statistically significant

[b] Statistically significant

Mortality: Fisher's Exact Test, multiple comparison, one-sided greater, alpha = 0.05

Weight change: Williams t-test, two-sided, alpha = 0.05

Reproduction: Bonferroni-Welch t-test, one-sided smaller, alpha = 0.05

III. CONCLUSIONS

IN-N0079 had no statistically significant lethal effects or effects on growth or feeding activity of the earthworm *Eisenia fetida* when exposed to concentrations up to and including 100 mg/kg dry artificial soil. IN-N0079 had no statistically significant effects on reproduction of the earthworm *Eisenia fetida* when exposed to concentrations up to and including 50.0 mg/kg dry artificial soil.

The overall NOEC (No-Observed-Effect Concentration) was determined to be 50.0 mg IN-N0079/kg dry artificial soil and the overall LOEC (Lowest-Observed-Effect Concentration) was determined to be 100 mg IN–N0079/kg dry artificial soil.

The LC_{50} after 28 days was estimated to be greater than 100 mg IN-N0079/kg dry artificial soil, the highest concentration tested.

RMS comments and conclusion:

Food was first provided on the day of application and not one day after applying the test substance to the soil as recommended in the guideline. Considering the good control performance and the lack of effects, this deviation is acceptable.

The test is valid: the mean control mortality was 1%, there were more than 30 juveniles per control unit after the 8 week testing period (was 172 to 257 per replicate), the coefficient of variance for the mean number of juveniles in the untreated control did not exceed 30% (was 14.5%).

Because of the data set, it was not possible to calculate meaningful EC10 or EC20 values, hence the results expressed as NOEC are acceptable.

Conclusion: The study is acceptable.

(Pavić, B., 2015b)

Bio-accumulation

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

B.9.4.1/05

Reference: CA 8.4.1/01	**Report:**	Meinerling, M. (2014); Oxamyl (DPX-D1410) technical (98% w/w): Accumulation and elimination in earthworms (*Eisenia fetida*) in artificial soil **DuPont Report No.:** DuPont-38477 **Guidelines:** OECD 317 (2010) **Deviations:** None **Testing Facility:** Institut für Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany **Testing Facility Report No.:** 84911119 **GLP:** Yes **Certifying Authority:** Hess. Ministerium fur Umwelt, Energie, Landwirtschaft and Verbraucherschutz

Executive summary:

The residues of oxamyl in earthworms, *Eisenia fetida* Savigny, were determined at intervals up to 21 days exposure in artificial soil in a GLP-compliant laboratory study based on OECD 317 (2010). The elimination of residues was investigated over a further 21-day period. Twenty-two replicates for the control and 54 replicates for the test item groups of five clitellated adult earthworms each were exposed to artificial soil (prepared according to OECD 222 with 10% organic matter) treated with the test item to obtain the nominal concentrations of 1 mg oxamyl/kg dry artificial soil and to an untreated control moistened with deionized water (deionized water only).

Adult earthworms were sorted from soil and assessed for mortality, weight loss, and concentration of oxamyl after different exposure time. After 21 days exposure the worm were transferred to untreated soil. During for the 21 days elimination phase worms were removed after 4-6 h, and after 1, 2, 4, 7, 10, 14, 17, and 21 days.

No mortality was observed in any treatment group. During the uptake phase, the measured concentration of oxamyl in soil decreased from 1 mg/kg soil dry weight to 0.2 mg/kg soil dry weight. The concentration of oxamyl in worm tissues was below the limit of quantification (0.01 µg/g wet weight) during the exposure period. A conservative bioaccumulation factor was calculated to be 0.03.

I. MATERIALS AND METHODS

A. MATERIALS

1.	Test material	Pure Oxamyl (PAI)
	Lot/Batch #:	D1410-532
	Purity:	99.1% by analysis
	CAS #:	23135-22-0
	Stability of test compound:	
2.	Control:	Untreated and moistened with deionized water
	Test vehicle:	Deionized water
3.	Test organism	Earthworm
	Species:	*Eisenia fetida*
	Age at dosing:	Approximately 12 months
	Weight at dosing:	350 to 550 mg
	Source:	In-house laboratory culture (Laboratory: IBACON, Rossdorf, Germany)
	Acclimation period:	3 days
	Test chamber:	Plastic boxes with perforated transparent lids (volume: 0.5 L), filled with ca. 250 g artificial soil dry weight
	Test medium:	Artificial soil prepared according to OECD 222, maximum water holding capacity of the artificial soil, as measured: 55%
	Diet:	Finely ground cattle manure
	Water content of soil:	Initiation: 26.8% to 27.0% (equivalent to 48.7% to 49.1% of the maximum water holding capacity) Termination of Uptake Phase: 28.2 to 30.9% (equivalent to 51.3% to 56.2% of the maximum water holding capacity)
	Soil pH:	5.2 to 5.3 at test start and 5.7 to 5.8 at end of the uptake phase
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-October-2013 to 23-December-2013

2. Experimental treatments

The residues of oxamyl in earthworms, *Eisenia fetida* Savigny, were determined at intervals up to 21 days exposure in artificial soil in a GLP-compliant laboratory study based on OECD 317 (2010). The elimination of residues was investigated over a further 21-day period. Twenty-two replicates for the control and 54 replicates for the test item groups of five clitellated adult earthworms each were exposed to artificial soil (prepared according to OECD 222 with 10% organic matter) treated with the test item to obtain the nominal concentrations of 1 mg oxamyl/kg dry artificial soil and to an untreated control moistened with deionized water (deionized water only). Adult earthworms were sorted from soil and assessed for mortality, weight loss, and concentration of oxamyl after different exposure time. After 21 days exposure, the worms were transferred to untreated soil. During the 21 days elimination phase, worms were removed after 4-6 h, and after 1, 2, 4, 7, 10, 14, 17, and 21 days.

3. Observations

Worms were assessed for mortality, body weight change and test item concentration after different times of exposure during the uptake phase.

4. Statistics

No statistical analysis was performed.

II. RESULTS AND DISCUSSION

A. FINDINGS

No mortality was observed in any of the treatment groups.

The concentration of oxamyl in soil at the start of the study was determined to be 1.0 mg/kg soil dry weight. During the uptake phase, the concentration of oxamyl in the soil decreased continuously, being 0.197 mg/kg soil dry weight at the end of the uptake phase.

No oxamyl was detected in worm samples taken from the control. Concentrations in worm of the oxamyl treated group were low throughout the uptake phase, ranging all below the limit of quantification (0.1 or 0.05 µg/g tissue wet weight) (Table 79).

No worm samples of the elimination phase were analysed since the uptake was insignificant.

It was evident from the study that oxamyl does not bioaccumulate in earthworms. Concentrations were below the limit of quantification during the uptake.

Table 79 DuPont-38477, Summary of results

Test Item:	Oxamyl	
Test Species:	*Eisenia fetida*	
Exposure:	Test item mixed into the soil	
	Control	Oxamyl
Concentration [mg per kg dry soil]	-	1.0 mg/kg
Mortality [%]	0	0
Body weight change up-take [%]	23	10
Body weight change elimination [%]	21	25
Oxamyl concentration in worm tissue [mg per kg wet weight]	<LOQ[a]	<LOQ

[a] LOQ worm tissues = 0.01 mg/kg wet weight

However a conservative bioaccumulation factor may be calculated with the estimate of the oxamyl concentration in worms to be ½ the LOQ of 0.01 mg/kg.

$$BAF_{ss} = \frac{0.005 \text{ mg/kg fresh worms}}{0.153 \text{ mg/kg wet soil}}$$

$$BAF_{ss} = 0.03$$

III. CONCLUSIONS

In conclusion oxamyl does not bioaccumulate in earthworms. Concentrations were below the limit of quantification during the uptake. A conservative bioaccumulation factor was calculated to be 0.03.

(Meinerling, M., 2013)

RMS: the study is acceptable.

Study submitted in the EU Dossier in 2001 and included in the first EU approval review.

B.9.4.1/06 Acute toxicity of the active substance

Report: Knight, B., Boyle, J. (1995); Oxamyl technical determination of acute toxicity (LC50) in earthworms. DuPont Report No. AMR 3068-94.

Published: No.

Guidelines: OECD 207 (1984). Deviations: None that affect the validity of the study.

Testing Facility: Inveresk Research International (IRI) Limited (Scotland), Tranent, Scotland, U.K.

GLP: Yes.

Certified Laboratory: Yes. Department of Health (U.K.).

Test substance: DPX-D1410 Technical. Batch: D1410-167. Purity: 96.4%. Materials and methods:

Four replicates of ten worms per treatment were allocated to soil treated with Oxamyl at nominal concentrations of 0 (control), 12.5, 25, 50, 100 or 200 mg a.s./kg soil. A control soil was also used. No reference compound was tested or reported by the notifier. Illumination (614 lux) was continuous throughout the study, with a temperature of 20 - 22°C, and soil pH 5.2 - 5.5. Weights of worms were recorded in treatment replicates immediately prior to treatment and on Day 14. Observations of any visible worms for behavioural or pathological signs (absence of burrowing, lethargy or colour changes) were made daily and mortality was assessed on Days 7 and 14.

Findings:

All worms at test concentrations of 0, 12.5, 25 and 50 mg a.s./kg soil burrowed into the soil within a few minutes of addition to the test vessels. Worms in the 100 and 200 mg a.s./kg soil treatments took longer to burrow, with worms still present on the soil surface one hour after addition. Percentage mortality and mean individual weights of worms are presented in Table below.

Table 80: Acute toxicity of Oxamyl to earthworms

Nominal Concentration (mg a.s./kg)	% Mortality		Mean individual weight (mg)		Mean % Weight Change
	Day 7	Day 14	Day 0	Day 14	
0	7.5	7.5	341	362	+6
12.5	2.5	5	356	333	-6
25	2.5	2.5	345	298	-14
50	12.5	15	331	256	-23
100	25	30	354	256	-28
200	80	87.5	334	124	-63

Conclusion:

The 14-day LC50 value for Oxamyl to the earthworm *Eisenia foetida* was 112 mg a.s./kg soil dry weight. However, the data for 14 days does not fit the probit model and therefore no Confidence limits could be calculated. The 7d LC50 was 129 mg a.s./kg soil (95% CI: 105 – 168 mg a.s./kg soil). The NOEC is 25 mg a.s./kg soil based on bodyweight changes of greater than <20% at that concentration.

RMS comments and conclusion

The study has been evaluated for the first Annex I inclusion and carried out according to OECD 207. Since the OECD guideline has not been updated since then, the study is still considered acceptable and relied upon.

Acute toxicity of the Metabolites

Study submitted in the EU Dossier in 2001 and included in the first EU approval review.

Report: Wachter, S. (2000c); IN-A2213: Acute toxicity to the earthworm (*Eisenia foetida*) in artificial soil. DuPont Report No. DuPont-4130.

Published: No.

Guidelines: OECD 207 (1984). Deviations: None that affect the validity of the study.

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

GLP: Yes.

Certified Laboratory: Yes. Ministerium fur Umwelt und Verkehr (Baden-Wurttemberg, Germany).

Test substance: IN-A2213 Technical Metabolite. Batch: A2213-11. Purity: 100%.

Materials and methods:

Acute toxicity of IN-A2213 to earthworms, *Eisenia foetida*, was determined in a 14-day soil exposure laboratory study conducted under OECD Guideline 207. Four replicates of 10 clitellated adult earthworms were each exposed to a nominal concentration of 1000 mg IN- A2213/kg dry soil weight (ppm in dry soil). Controls were replicated four times, with 10 earthworms in each replicate. The toxic reference standard, 2-chloroacetamide, was tested at 5 rates (0, 10, 18, 32, 56 and 100 mg/kg dry artificial soil). Worms were assessed for mortality and sublethal effects after 7 and 14 days of exposure and earthworm body weights were assessed at day 0 and day 14.

Findings:

Cumulative mortality results at 7 and 14 days and weight loss at 14 days are reported in Table B.9.6.2-1. No significant sublethal behavioural effects were observed. The LC50 for the toxic reference standard was 10 – 18 mg/kg; this is within accepted limits, indicating the validity of this test.

Table: Acute toxicity of IN-A2213 to earthworms

Treatment (mg IN-A2213/kg dry soil)	Cumulative Mortality (%)a		Cumulative Weight Loss (%)b
	7 days	14 days	14 days
0	0	0	N/A
1000.0	0	0	0

aTest mortality for treatments is corrected for control mortality (mortality at 0 mg IN-A2213/kg dry soil). bCumulative weight loss (%) is corrected for control weight loss at 0 mg IN-A2213/kg dry soil.

Conclusion:

The acute earthworm LC50 of IN-A2213 was > 1000 mg/kg soil dry weight.

RMS comments and conclusion

The study has been evaluated for the first Annex I inclusion and carried out according to OECD 207. Since the OECD guideline has not been updated since then, the study is still considered acceptable and relied upon.

Study submitted in the EU Dossier in 2001 and included in the first EU approval review.

Report: Wachter, S. (2000b); IN-N0079: Acute toxicity to the earthworm (Eisenia foetida) in artificial soil. DuPont Report No. DuPont-4134.

Published: No.

Guidelines: OECD 207 (1984). Deviations: None that affect the validity of the study.

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

GLP: Yes.

Certified Laboratory: Yes. Ministerium fur Umwelt und Verkehr (Baden-Wurttemberg, Germany).

Test substance: IN-N0079 Technical Metabolite. Batch: N0079-8. Purity: 99.6%.

Materials and methods:

Acute toxicity of IN-N0079 to earthworms, Eisenia foetida, was determined in a 14-day soil exposure laboratory study conducted under OECD Guideline 207. Four replicates of 10 clitellated adult earthworms were each exposed to nominal concentrations of 100, 178, 316, 562 and 1000 mg IN-N0079/kg dry soil weight (ppm in dry soil). Controls were replicated four times, with 10 earthworms in each replicate. The toxic reference standard, 2-chloroacetamide, was tested at 5 rates (0, 10, 18, 32, 56 and 100 mg/kg dry artificial soil). Worms were assessed for mortality and sublethal effects after 7 and 14 days of exposure and earthworm body weights were assessed at day 0 and day 14.

Findings:

Cumulative mortality results at 7 and 14 days and weight loss at 14 days are reported in the summary Table B.9.6.2-3 below. Significant lethal effects were observed. The NOEC was 100 mg/kg, the LOEC was 178 mg/kg. The LC50 was 640 mg/kg. Significant differences ($P<0.05$) between body weights of the control group and the test substance groups were observed at 100, 178, 316 and 563 mg IN-N0079/kg dry soil. The NOEC for the loss of body weight was < 100 mg/kg, the lowest concentration tested. The LD50 for the toxic reference standard was 10 – 18 mg/kg; this is within accepted limits, indicating the validity of this test.

Table 81: Acute toxicity of IN-N0079 to earthworms

Treatment (mg IN-N0079/kg dry soil)	Cumulative Mortality (%)[a]		Cumulative Weight Loss (%)[b]
	7 days	14 days	14 days
0	0	0	N/A
100	0	0	-9.9
178	0	2.5	-9.7
316	0	2.5	-13.5
562	7.5	25	-22.0
1000	100	100	N/A

aTest mortality for treatments is corrected for control mortality (mortality at 0 mg IN-N0079/kg dry soil). bCumulative weight loss (%) is corrected for control weight loss at 0 mg IN-N0079/kg dry soil.

Conclusion:

The acute earthworm LC50 of IN-N0079 was 640mg/kg dry soil (95% CI: 589 – 695 mg/kg dry soil). The NOEC for mortality was 100 mg IN-N0079/kg dry soil.

RMS comments and conclusion

The study has been evaluated for the first Annex I inclusion and carried out according to OECD 207. Since the OECD guideline has not been updated since then, the study is still considered acceptable and relied upon.

Study submitted in the EU Dossier in 2001 and included in the first EU approval review.

Report: Wachter, S. (2000a); IN-D2708: Acute toxicity to the earthworm (Eisenia foetida) in artificial soil. DuPont Report No. DuPont-4132.

Published: No.

Guidelines: OECD 207 (1984). Deviations: None that affect the validity of the study.

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

GLP: Yes.

Certified Laboratory: Yes. Ministerium fur Umwelt und Verkehr (Baden-Wurttemberg, Germany). Test substance: IN-D2708 Technical Metabolite. Batch: D2708-6. Purity: 99.9%.

Materials and methods:

Acute toxicity of IN-D2708 to earthworms, Eisenia foetida, was determined in a 14-day soil exposure laboratory study conducted under OECD Guideline 207. Four replicates of 10 clitellated adult earthworms were each exposed to a nominal concentration of 1000 mg IN- D2708/kg dry soil weight (ppm in dry soil). Controls were replicated four times, with 10 earthworms in each replicate. The toxic reference standard, 2-chloroacetamide, was tested at 5 rates (0, 10, 18, 32, 56 and 100 mg/kg dry artificial soil). Worms were assessed for mortality and sublethal effects after 7 and 14 days of exposure and earthworm body weights were assessed at day 0 and day 14.

Findings:

Cumulative mortality results at 7 and 14 days and weight loss at 14 days are reported in the summary Table B.9.6.2-2. No significant sublethal behavioural effects were observed. The LC50 for the toxic reference standard was 10 – 18 mg/kg; this is within accepted limits, indicating the validity of this test.

Table: Acute toxicity of IN-D2708 to earthworms

Treatment (mg IN-D2708/kg dry soil)	Cumulative Mortality (%)[a]		Cumulative Weight Loss (%)[b]
	7 days	14 days	14 days
0	0	0	N/A
1000.0	0	0	-3.1

a Test mortality for treatments is corrected for control mortality (mortality at 0 mg IN-D2708/kg dry soil). b Cumulative weight loss (%) is corrected for control weight loss at 0 mg IN-D2708/kg dry soil.

Conclusion:

The acute earthworm LC50 of IN-D2708 was > 1000 mg/kg soil dry weight.

RMS comments and conclusion

The study has been evaluated for the first Annex I inclusion and carried out according to OECD 207. Since the OECD guideline has not been updated since then, the study is still considered acceptable and relied upon.

Study submitted in the EU Dossier in 2001 and included in the first EU approval review.

Report: Luhrs, U. (2001); IN-T2921: acute toxicity to the earthworm (Eisenia foetida) in artificial soil. DuPont Report No. DuPont-4617.

Published: No.

Guidelines: OECD 207 (1984). Deviations: None that affect the validity of the study.

Testing Facility: IBACON, Rossdorf, Germany

GLP: Yes.

Certified Laboratory: Yes. Hessisches Ministerium fur Umwelt, Landwirtschaft und Forsten.

Test substance: IN-T2921 Technical Metabolite. Batch: T2921-2. Purity: 98.7%.

Materials and methods:

Acute toxicity of IN-T2921 to earthworms, Eisenia foetida, was determined in a 14-day soil exposure laboratory study conducted under OECD Guideline 207. Four replicates of 10 clitellated adult earthworms were each exposed to nominal concentrations of 198 mg, 296 mg, 444 mg, 667 mg and 1000 mg IN-T2921/kg dry soil weight (ppm in dry soil). Controls were replicated 4 times, with 10 earthworms in each replicate. The toxic reference standard, 2- Chloroacetamide, is tested once a year. Worms were assessed for mortality and sublethal effects after 7 and 14 days of exposure and earthworm body weights were assessed at day 0 and day 14.

Findings:

Cumulative mortality results at 14 days and weight loss at 14 days are reported in the summary table below (Table B.9.6.2-4). No significant sublethal behavioural effects were observed. The LC50 for the toxic reference standard was 21.5 mg 2-Chloroacetamide/kg dry soil. This is within accepted limits, indicating the validity of this test.

Table B.9.6.2-4: Acute toxicity of IN-T2921 to earthworms

Treatment (mg IN-T2921 ai/kg dry soil)	Cumulative Mortality (%)[a]	Cumulative Weight Loss (%)[b]
	14 days	14 days
0	0	-10.2
198	0	-10.7
296	0	-8.7
444	0	-5.7
667	0	-8.6

1000.0	0	-6.1

a Test mortality for treatments is corrected for control mortality (mortality at 0 mg IN-T2921 ai/kg dry soil). b Cumulative weight loss (%) is not corrected for control weight loss at 0 mg IN-T2921 ai/kg dry soil.

Conclusion:

The acute earthworm LC50 of IN-T2921 was >1000 mg/kg dry soil. The NOEC was determined to be 1000 mg/kg dry soil.

RMS comments and conclusion

The study has been evaluated for the first Annex I inclusion and carried out according to OECD 207. Since the OECD guideline has not been updated since then, the study is still considered acceptable and relied upon.

B.9.4.2 Effects on non-target soil meso- and macrofauna (other than earthworms)

B.9.4.2.1 Species level testing

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

B.9.4.2.1/01

Reference: CA 8.4.2.1/01	**Report:**	Meli, M. (2015); Oxamyl - Population level risk assessment for collembolans **DuPont Report No.:** DuPont-41996 EU **Guidelines:** Not applicable **Deviations:** Not applicable **Testing Facility:** RIFCON GmbH **Testing Facility Report No.:** R 1420505 **GLP:** No **Certifying Authority:** Not applicable

Executive summary:

The objective of this study was to evaluate potential effects of oxamyl on Collembola following applications to potato and tobacco. For this purpose the SpringSim (SPRINGtails SIMulation) 2.1 model was used to simulate population dynamics of *Folsomia candida* over the course of 16 years after exposure to applications in potatoes or tobacco. Years 1 to 3 simulated baseline population dynamics in the absence of oxamyl applications. Years 4 to 13 developed population responses after a single annual application of oxamyl. Years 14 to 16 developed population recovery responses in the absence of oxamyl applications. To identify possible effects of oxamyl exposures, comparisons were made between the abundance of *F. candida* before and during treatments, and during and after treatments.

Simulations were conducted using initial PEC_{soil} values calculated from field applications according to the GAP for uses on potato and tobacco. Realistic spatial distributions of the toxicant were used to simulate the different application patterns (in furrow and broadcast).

After comparison of population density it the overall landscape, it appears that in-furrow applications of oxamyl, both on potatoes and tobacco, have a moderate effect on populations of *F. candida* during the application years. Populations then recover within the first year (Year 14) after applications stop. Broadcast applications on tobacco elicit a higher impact on populations living inside the field, as unlike the case of in-furrow applications, in this case 100% of the landscape is treated and there are no "sources" to help the population to grow.

Population-level effects of oxamyl appear to be stronger for broadcast applications on tobacco. When the model landscape is located entirely within a treated field, the population is not able to recover completely for at least 3 years. However, when recolonization from outside the field is taken into account, population density inside the field recovers more quickly, and in simulation Year 16 is almost entirely within the confidence limits of control year. Even in this scenario the untreated area in the simulated landscape is only 18% of the total surface. It is thus likely that in a more realistic scenario, in which the contribution of the off-fields areas as source habitats is more considerable, the population inside the field would recover more quickly.

Therefore, while applications of oxamyl elicit responses by collembolan populations living within treated fields, these effects appear to be transient and do not pose a long-term risk for populations of *F. candida*.

I. METHODS

A. THE MODEL

The present risk assessment is based on a higher-tier modelling approach using the SpringSim 2.1 model, a modified version of the population model published by Meli et al. (2013). This approach is in agreement with the EFSA Opinion on Good Modelling Practice (EFSA, 2014). The model description follows the ODD (Overview, Design concepts, and Details) protocol for describing individual-based models (Grimm et al., 2006; 2010). The SpringSim model is an individual-based population model of *Folsomia candida*. The purpose of the model is to realistically reproduce population dynamics of *F. candida* with special focus on the dynamic spatial distribution of the individuals and on density dependent population regulation. The model was specifically developed for conducting population-level risk assessments of plant protection products.

The model comprises the entities juvenile and adult females, and grid cells. The model world is a 2–dimensional grid of square cells, each representing 10 cm^2 of soil. As a conservative assumption, no vertical movement is assumed. The global environment is characterized by a temperature profile, determined for the central and southern EU zones, using temperature data from the relevant FOCUS groundwater scenarios, and which regulates the temperature-dependent life-cycle parameters of the springtails. The model proceeds in daily time steps comprising the following processes: updating the concentration of the toxicant, updating the season; foraging; regrowth of food (grid cells); aging and growth; reproduction; density dependence on fecundity and survival; and mortality. Values of almost all parameters are drawn from uniform or normal probability distributions to reflect variability among individuals. Stochasticity is also used for initializing springtails' starting positions, as well as causing individual behaviours (movement, reproduction, mortality) to occur with specified frequencies depending on the values of the parameters.

A simulation starts the first day of the year, and therefore in the winter season. At the beginning of a model run, food resources are also randomly assigned to grid cells that are initialized to be food sources. Usually, 10% of the grid cells, which are randomly chosen, are made food cells. Simulations start with 1000 juvenile springtails randomly distributed over the grid cells.

B. EXPOSURE AND EFFECTS CALCULATIONS

Survival and fecundity of *F. candida* are reduced by exposure to oxamyl, as shown by the results of two 28-day soil exposure GLP-compliant laboratory studies. Based on the laboratory data, log-logistic dose-response equations were calculated for both reduction of survival and reduction of fecundity using the

R package drc. Effects based on the dose response equations are applied as coefficients which reduce the fecundity and survival rates of the individuals. The toxicity data used for these regressions are the result of 28 days of exposure. In the model, however, to ensure a higher conservatism they are entirely applied at each time-step, thus after only 1 day of exposure.

C. SIMULATION SCENARIOS

Simulations were conducted using PEC_{soil} values calculated from field applications according to the GAP for uses on potatoes and tobacco. For in-furrow applications the PEs act values are multiplied by appropriate factors accounting for the percentage of the total field area covered by the furrows. For instance, in the case of in-furrow applications to tobacco, oxamyl is applied on *ca*. 20% of the field area, therefore in simulations for this use the PECs are multiplied by a factor of 5. A total of five exposure scenarios were simulated (Table). The simulations were run over a period of 16 years, composed of 3 years with no application, 10 years with annual applications, and 3 years with no application.

Table 82 Overview of the simulated exposure scenarios

Scenario	Crop	Zone	AR (g/ha)	Soil depth (cm)	Application date (julian day)	$PEC_{ini,}$ (mg/kg)	Factor to account for in-furrow	Initial in-furrow exposure concentration (mg/kg)
1	Potato	Central	1000	10	23 Apr (113)	0.667	3.5	2.335
2	Potato	South	1000	10	22 Feb (53)	0.667	3.5	2.335
3	Tobacco furrows	South	3000	5	11 May (131)	4	5	20.000
4	Tobacco broadcast in-field	South	5500	10	11 May (131)	3.667	1	3.667
5	Tobacco broadcast recolonization	South	5500	10	11 May (131)	3.667	1	3.667

II. RESULTS AND DISCUSSION

A. FINDINGS

Toxicity inputs:

Dose-response curves were generated for reproduction and survival of Collembola after a 28-day exposure to oxamyl in a laboratory study (Figure 1 and Table).

Figure 1 Dose-response curves for survival and reduction of fecundity. Points represent results of the 28-day reproduction test, used to fit the curves

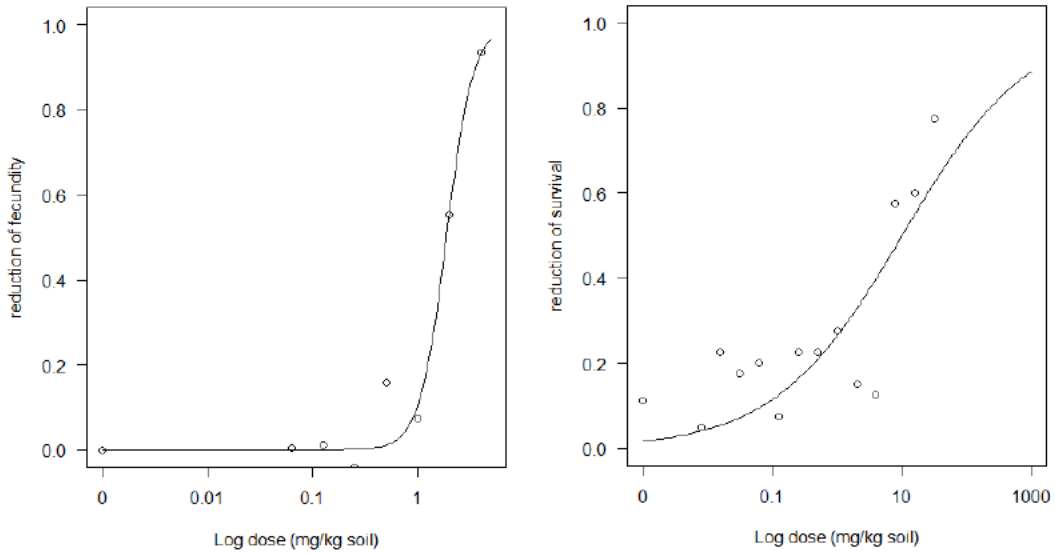


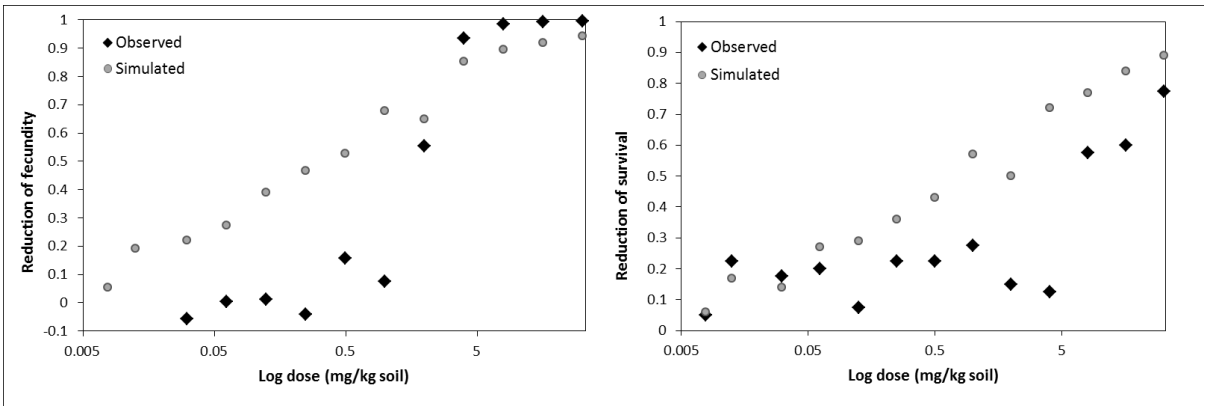
Table 83 Parameters of the log-logistic regressions

Endpoint	Parameter	Estimate	Std error	p-value
Survival	EC_{50}	15.26 ppm	15.34	0.353
	Slope	-0.307	0.077	0.005
	Upper limit	1 (fixed)	-	-
	Lower limit	0 (fixed)	-	-
Reproduction	EC_{50}	8.869 ppm	6.739	0.23
	Slope	-0.466	0.106	0.003
	Upper limit	1 (fixed)	-	-
	Lower limit	0 (fixed)	-	-

To test whether the implementation of individual-level toxic effects leads to realistic predictions of impacts at the population-level, a series of simulations has been conducted with the model set up with the same conditions as the laboratory study:

- constant temperature of 20±1°C,
- optimal food,
- initial population composed of 10 juveniles of 10-12 days of age,
- and same toxicant concentrations as tested in the lab.

Results of testing of the toxic effects implementation: reduction of survival and fecundity after 28 days of exposure in the model and in the lab.



As shown in the above graphs, the implementation of survival and fecundity reductions calculated from the dose–response equations and applied at each time step leads to an overestimation in the model, especially at low concentrations. This is due to the fact that effects that in the lab were observed after 28-day exposure are attributed to 1-day exposures in the model. Therefore it can be assumed that the model is conservative in estimating population-level effects of oxamyl on *F. candida*.

Model outputs:

To make interpretation of model results easier, for all scenarios the most relevant simulation years have been compared. Year 3 is considered as the "control", Year 13 as the "worst-case" over the entire simulation period, while Year 14 is used to investigate population recovery. These years have been chosen because during Year 3 the population has stabilized and has not yet been exposed to oxamyl. Year 13 is the last of the exposure period; therefore if toxic effects are carried over from the previous years, it should represent the worst conditions for the population. On the other hand, risk for soil invertebrate populations living inside the field can be considered acceptable only if possible effects are shown to be transient, and the population fully recovers within a growing season. Therefore population abundance in Year 14 was chosen to assess recovery. As a way of measuring whether differences in population abundance are statistically significant, it has been tested whether mean population abundance in treatment or recovery years is outside the 95% confidence limits of the control year.

For in-furrow applications, population abundance within the furrows, outside the furrows and overall are recorded and analysed separately. It appears that in-furrow applications of oxamyl, both on potatoes and tobacco, have a moderate effect on populations of *F. candida*, which does not impair the capacity for recovering. In potatoes, population density in year 14 is always within confidence limits of year 3, with the exception of a few short periods, due to the stochastic nature of the model rather than to effects of oxamyl. Population-level effects are more visible in simulations with the southern EU temperature profile. This can be attributed to the early application date used in these simulations (February 22), when the survival and reproductive rate rate of F. candida is reduced due to the low temperatures. Also in this case, however, a full recovery of the population is observed.

On the contrary, the application date used for applications of oxamyl on tobacco (May 11) falls in late spring, when population density is at a peak and the simulated environmental conditions are optimal. Furthermore, the area covered by the furrows is lower than for applications on potatoes (20% vs. 31.3%). For these reasons, even though the PECs,ini in this scenario is maximum (20 mg/kg), the corresponding population-level effect is lower than for applications on potatoes.

Broadcast applications on tobacco cause a higher impact on populations living inside the field, as unlike the case of in-furrow applications, in this case 100% of the landscape is treated and there are no "sources" to help the population to grow. Even though the PECs,ini for broadcast applications on tobacco is more than 5 times lower than for in-furrow applications (3.667 vs 20 mg/kg), the population is not able to recover completely for at least 3 years. However, when recolonization from outside the field is taken into account (exposure scenario 5), population density inside the field recovers more quickly, and in simulation year 16 is almost entirely within the confidence limits of control year.

Figure 2 Scenario 1: Potato, central in-furrows. Population density over the entire model landscape (inside and outside the furrows). Population densities in year 13 (worst case year) and 14 (recovery year) are normalized to those in year 3(control year). Dotted lines represent 95% confidence limits of control year.

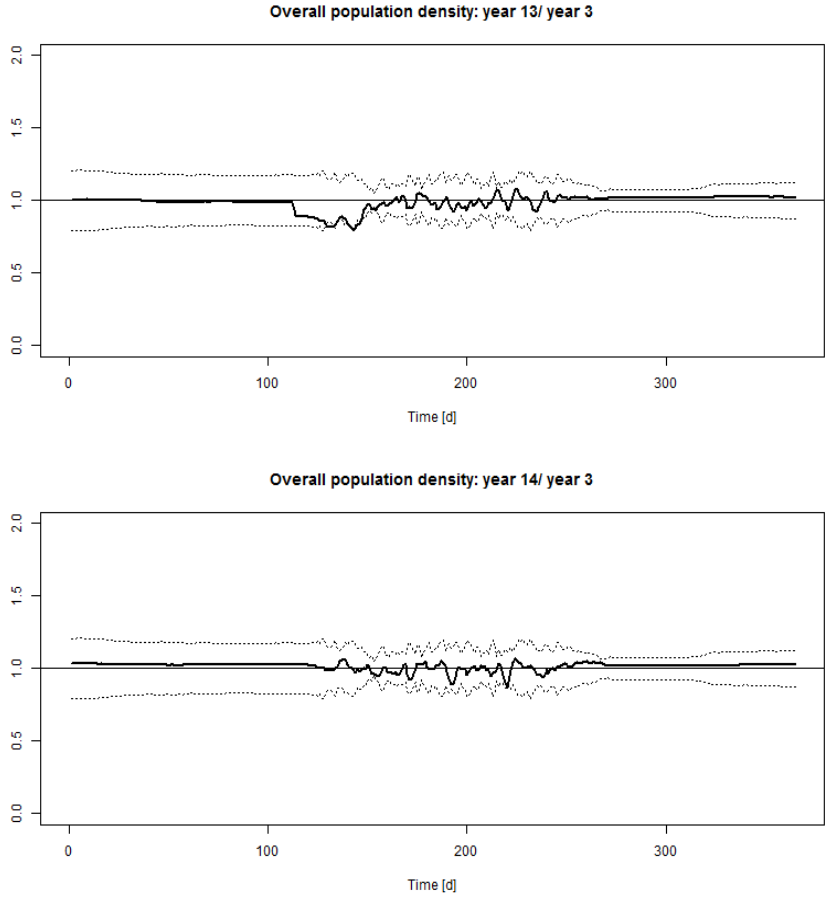


Figure 3 Scenario 2: Potato, south in-furrows. Population density over the entire model landscape (inside and outside the furrows). Population densities in year 13 (worst case year) and 14 (recovery year)

are normalized to those in year 3 (control year). Dotted lines represent 95% confidence limits of control year.

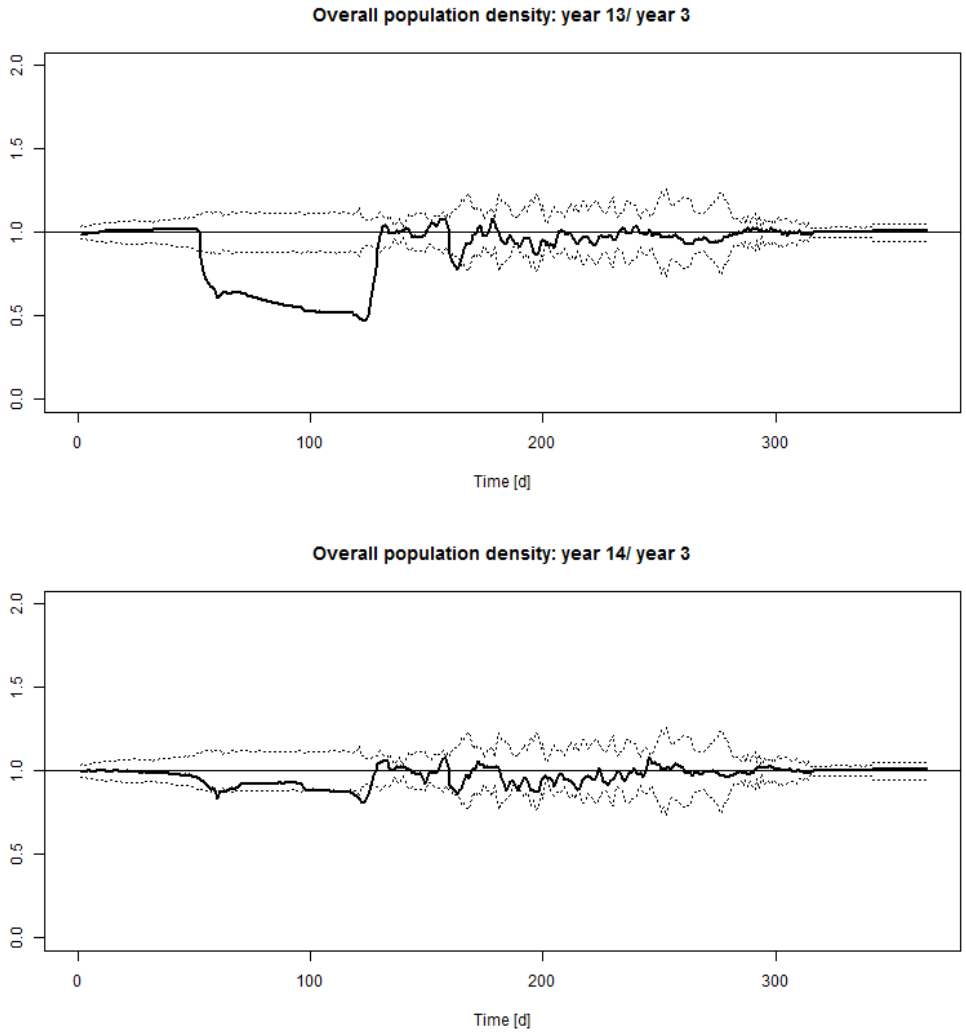


Figure 4 Scenario 3: Tobacco, in-furrow. **Population density over the entire model landscape (inside and outside the furrows). Population densities in year 13 (worst case year) and 14 (recovery year) are normalized to those in year 3 (control year). Dotted lines represent 95% confidence limits of control year.**

Figure 5 Scenario 4: Tobacco, broadcast, in-field. Populationdensity over the entire model landscape. Population densities in year 13 (worst case year) and 14 (recovery year) are normalized to those in year 3(control year). Dotted lines represent 95% confidence limits of control year.

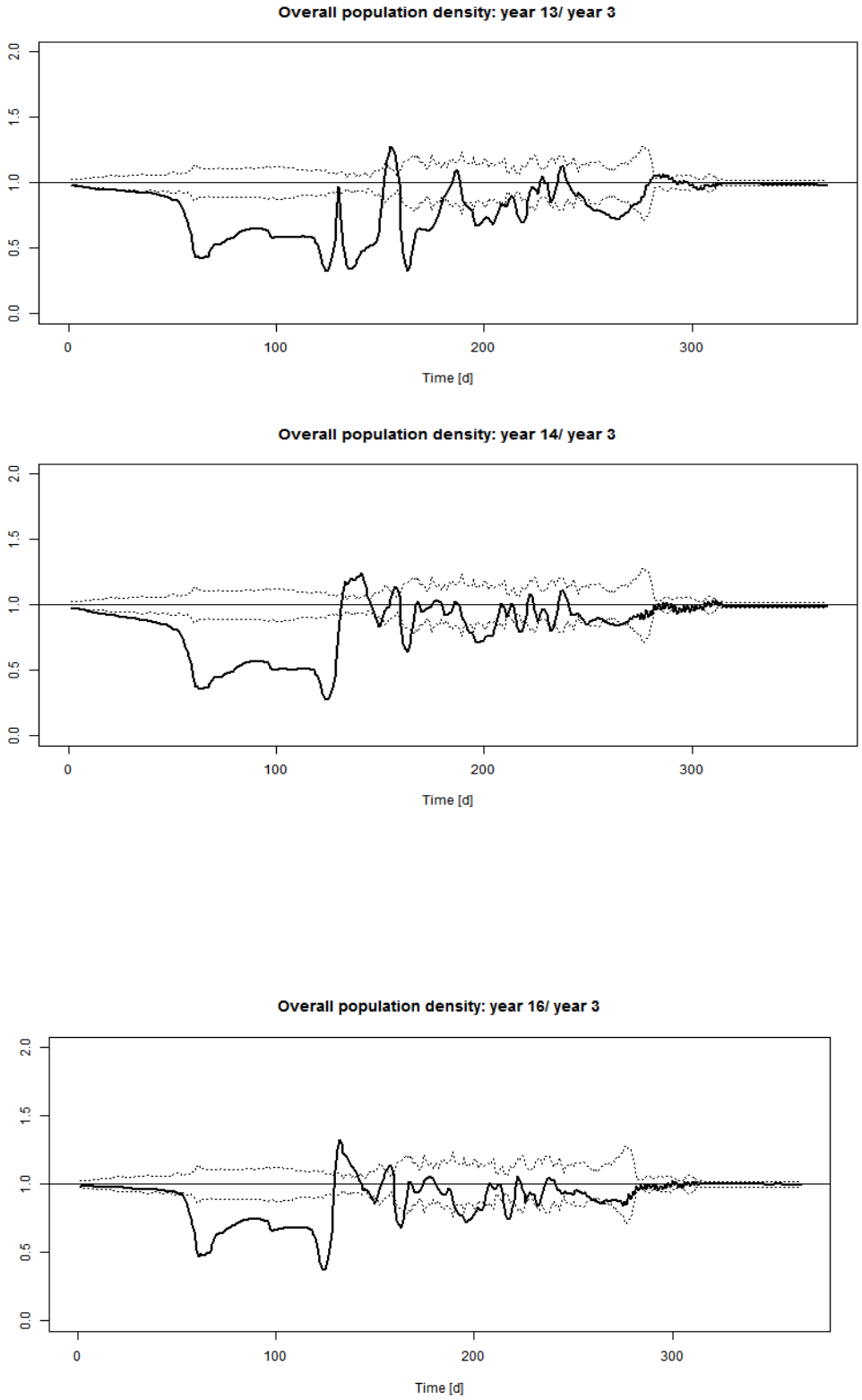
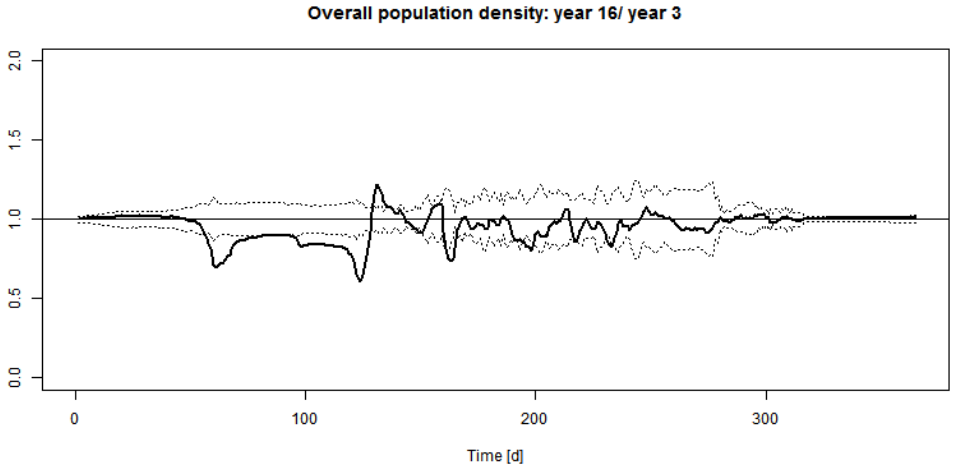


Figure 6 Scenario 5: Tobacco, broadcast. Recolonization, Population density over the entire model landscape. Population densities in year 13 (worst case year), 14 (recovery year) and 16 (third recolonization year) are normalized to those in year 3 (control year). Dotted lines represent 95% confidence limits of control year.



III. CONCLUSION

Applications in-furrows, on both potato and tobacco appear to cause a moderate population-level effect during the application period, as in Year 13 population density is, for a period, below confidence limits of Year 3 both inside the furrows and over the entire landscape. However, these effects do not last beyond the end of the planting season of Year 13: a full recovery is observed in Year 14 for both scenarios, as population density is within confidence limits of the control year in all parts of the landscape.

Population-level effects of oxamyl appear to be stronger for broadcast applications on tobacco. Population-level effects of oxamyl appear to be stronger for broadcast applications on tobacco. When the model landscape is located entirely within a treated field, the population is not able to recover completely for at least 3 years. However, when recolonization from outside the field is taken into account, population density inside the field recovers more quickly, and in simulation Year 16 is almost entirely within the confidence limits of control year. Even in this scenario the untreated area in the simulated landscape is only 18% of the total surface. It is thus likely that in a more realistic scenario, in which the contribution of the off-fields areas as source habitats is more considerable, the population inside the field would recover more quickly.

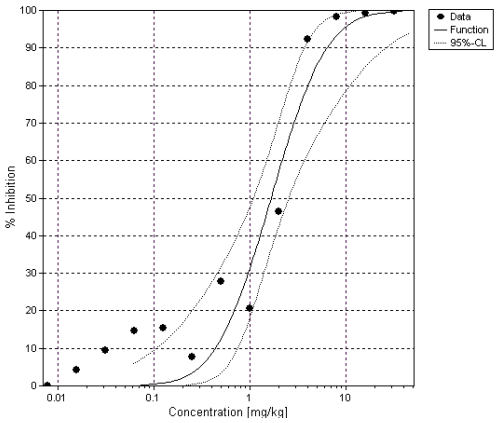
Therefore, while applications of oxamyl are predicted to affect collembolan populations living within treated fields, these effects appear to be transient and do not pose a long-term risk for populations of *F. candida*.

(Meli, M., 2015)

RMS comment and conclusion

1) There seems to be a contraddiction on the food cells: at page 30 of the report, it reads: “All landscapes were initialized in each model run with food resources randomly distributed on 20% of the grid cells.”, while at page 14 it reads “Usually, 10% of the grid cells, which are randomly chosen, are made food cells”.
The Notifier should clarify.

2) The reproduction study with F. candida used as input in the model is by Pavić (2014, DuPont-39676), which is summarized above and judged reliable. In the modelling study, the experimental data are reported in graphs, but the RMS could not match the points in fig 1 (left graph) with the actual results for inhibition of reproduction reported in the Pavić (2014) study (see figure below).



It is also noted that in the Pavić (2014) study an EC50 = 1.663 mg/kg was calculated for reproduction while in the Meli (2'15) study, the EC50 for reproduction was calculated as EC50 = 8.7 mg/kg.

The Applicant should clarify the inconsistencies and check the toxicity inputs used in the model.

3) For scenario 5, tobacco - broadcast application, the conclusion of "almost" complete recovery at year 16 (i.e., 3 years after the last treatment) is not in line with the requirement of demonstrating recovery within one season. Therefore, conclusion of recovery should be referred to Year 14 (as made for the other scenarios).

Also, the simulated untreated area of 18% of the total surface may represent a realistic situation in certain agricultural landscape.

4) Uncertainty.

As for model validation, the following can be highlighted:

- Due to the wrong laboratory toxicity data input (see RMS comment above), the comparison of experimental data with the model predictions and the consequent conclusion that the model overestimates the toxicity cannot be verified.

-Confidence intervals of the model estimates are not reported

- In the EFSA PPR Panel "Scientific Opinion on good modelling practice in the context of mechanistic effect models for risk assessment of plant protection products (2014), it is recommended that the model is run with a control, several tests of substance at application rates and a toxic standard, in order to demonstrate that the model used is able to show negative effects. The Meli (2015) model was based on one toxicity study (two complementary experiments) and no run was made with a toxic standard was made.

-An evaluation of the model performance (validation) based on comparison of model results with field data is not presented.

Conclusion: **the toxicity parameters used as input to the model seems not correct, hence the outputs of the models would not be not reliable. The uncertainty issues should be addressed.**

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

B.9.4.2.1/02

Reference: CA 8.4.2.1/04	**Report:**	Pavić, B. (2014c); Oxamyl (DPX-D1410) technical (98% w/w): Effects on the collembola *Folsomia candida* in artificial soil with 5% peat **DuPont Report No.:** DuPont-39676

		Guidelines: OECD 232 (2009), ISO 11267 (1999) **Deviations:** None **Testing Facility:** Institut für Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany **Testing Facility Report No.:** 84911016 **GLP:** Yes **Certifying Authority:** Hess. Ministerium fur Umwelt, Energie, Landwirtschaft and Verbraucherschutz

Executive summary:

The effects of oxamyl on the mortality and reproduction of Collembola (*Folsomia candida*) were determined in two 28-day soil exposure laboratory studies according to OECD 232, 2009 and ISO 11267, 1999. In both experiments eleven to twelve and ten to twelve days old Collembola, respectively were exposed for 28 days to artificial soil (prepared according to OECD 232) treated with different concentrations of oxamyl and to an untreated control (deionized water only). In the first experiment the nominal concentrations of 0.00788, 0.01577, 0.03153, 0.06307, 0.1261, 0.2523, 0.5045 and 1.0091 mg oxamyl/kg dry artificial soil (corresponding to 0.00781, 0.01563, 0.03125, 0.0625, 0.125, 0.250, 0.500 and 1.000 mg oxamyl/kg dry artificial soil, adjusted for purity) were tested. In the second experiment the nominal concentrations of 2.0182, 4.0363, 8.0727, 16.1453, and 32.2906 mg oxamyl/kg dry artificial soil (corresponding to 2.000, 4.000, 8.000, 16.000, and 32.000 mg oxamyl/kg dry artificial soil, adjusted for purity) were tested.

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 0.250 mg oxamyl/kg soil dry weight.

I. MATERIALS AND METHODS

A. MATERIALS

1.	Test material:	Pure Oxamyl (PAI)
	Lot/Batch #:	D1410-532
	Purity:	99.1%, by analysis
	Description:	Solid
	CAS Registry Number:	23135-22-0
	Stability of test compound:	Not analysed in the test system
2.	Control:	Untreated (and moistened with deionized water)
	Test vehicle:	Deionized water
3.	Test System:	Collembola
	Species:	*Folsomia candida*, Willem (Collembola: Isotomidae)
	Age at dosing:	11–12 days in the first experiment 10–12 days in the second experiment
	Weight at dosing:	Not determined
	Source:	In-house laboratory culture
	Acclimation period:	11, respectively 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: 46% (both experiments)
	Diet:	Granulated dry yeast
	Water content of soil:	First experiment: Initiation: 24.0 to 24.9% equivalent to 52.2 to 54.0% of the maximum water holding capacity Termination: 22.0 to 23.6% equivalent to 47.9 to 51.3% of the

		maximum water holding capacity Second experiment: Initiation: 23.3 to 23.6% equivalent to 50.6 to 51.4% of the maximum water holding capacity Termination: 20.7 to 22.7% equivalent to 44.9 to 49.3% of the maximum water holding capacity
	Soil pH:	First experiment: 5.8 to 6.4 at test start; 6.2 to 6.3 at test termination Second experiment: 6.3 to 6.5 at test start; 6.1 at test termination
4.	Environmental conditions	
	Temperature:	Within the range of 18 to 22°C (both experiments)
	Photoperiod:	16 h light, 8 h dark, photoperiod within the range of 400 to 800 lux (both experiments)

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed

13-August-2014 to 16-December-2014

2. Experimental treatments

Two studies were conducted to determine the effects of oxamyl 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 oxamyl of 0.00788, 0.01577, 0.03153, 0.06307, 0.1261, 0.2523, 0.5045, and 1.0091 mg oxamyl/kg dry artificial soil (corresponding to 0.00781, 0.01563, 0.03125, 0.0625, 0.125, 0.250, 0.500, and 1.000 mg oxamyl/kg dry artificial soil, adjusted for purity) in the first experiment and to 2.0182, 4.0363, 8.0727, 16.1453, and 32.2906 mg oxamyl/kg dry artificial soil (corresponding to 2.000, 4.000, 8.000, 16.000, and 32.000 mg oxamyl/kg dry artificial soil, adjusted for purity) in the second experiment and an untreated control (deionized water only). One additional container per treatment was set to check the pH and water content of the test substrate after 28 days. A reference item (boric acid, at a concentration range of 30.5 to 200 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 November/December 2014.

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 content of the test containers was suspended in water, the suspension was tinted with dark ink and stirred with a fine brush. 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. Surviving Collembola were observed for any abnormal behaviour or conditions on day 28. 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 homoscedasticity 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 in the first experiment and Bonferroni-Welch t-test in the second experiment (multiple comparison, alpha = 0.05, one-sided smaller). EC values were determined by 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 145.1 mg boric acid/kg dry artificial soil.

A summary of the results is provided in the table below.

Table 84 The effects on mortality and reproduction of Collembola, *Folsomia candida*, exposed to oxamyl in artificial soil for 28 days

Nominal oxamyl concentration, adjusted for purity (mg/kg soil)	Mean % mortality	Reproduction	
		Mean juveniles per replicate	% of control
	First experiment		
Untreated control (0.0)	10	662	-
0.00781	5.0 n.s.[a]	679 n.s.	102
0.01563	23 n.s.	634 n.s.	95.7
0.03125	18 n.s.	600 n.s.	90.6
0.0625	20 n.s.	565 n.s.	85.3
0.125	7.5 n.s.	560 n.s.	84.6
0.250	20 n.s.	612 n.s.	92.3
0.500	23 n.s.	478[b]	72.1
1.000	28 n.s.	525[b]	79.3
	Second experiment		
Untreated control (0.0)	13	472	-
2.000	15 n.s.	253[b]	53.6
4.000	13 n.s.	37[b]	7.8
8.000	58 *	8[b]	1.6
16.000	60 *	4[b]	0.85
32.000	78 *	2[b]	0.37

[a] n.s. = There were no significant differences from the control

[b] Statistically significant different from the control

Mortality: Fisher's Exact Test, alpha = 0.05, one-sided greater;

Number of juveniles: Dunnett's t-test (1st experiment), Bonferroni-Welch t-test (2nd experiment), alpha = 0.05, one-sided smaller

III. CONCLUSIONS

The overall 28-day No-Observed-Effect Concentration (NOEC) based on mortality and reproduction was determined to be 0.250 mg oxamyl/kg dry artificial soil.

The 28-day EC_{10}, EC_{20}, and EC_{50}, based on reproduction reduction, were 0.435 (0.105 to 0.747), 0.689 (0.252 to 1.060), and 1.633 (1.089 to 2.540) mg/kg dry artificial soil, respectively. The 28−day overall Lowest-Observed-Effect Concentration (LOEC) for oxamyl was determined to be 0.500 mg oxamyl/kg dry artificial soil.

(Pavić, B., 2014c)

RMS comments and conclusion The reference substance boric acid should reduce reproduction by 50% at about 100 mg/kg dry weight soil; in this study the % of effect were somewhat lower (EC50 = 145.1 mg boric acid/kg).

The validity criteria are fulfilled:

- Mean adult mortality should not exceed 20% at the end of the test (actual 10%).
- The mean number of juveniles per vessel should be at least 100 at the end of the test (actual 662 and 472, in the first and second experiment, respectively);
- The coefficient of variation calculated for the number of juveniles should be less than 30% at the end of the definitive test (actual 14.5% and 12.5% in the first and second experiment, respectively).

Conclusion: the study is acceptable. NOEC = 0.25 mg a.s/kg dry wt soil, EC10= 0.435 mg a.s/kg dry wt soil.

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

B.9.4.2.1/03

Reference: CA 8.4.2.1/02	**Report:**	Pavić, B. (2014a); IN-A2213: Effects on the collembola *Folsomia candida* in artificial soil with 5% peat **DuPont Report No.:** DuPont-39673 **Guidelines:** OECD 232 (2009), ISO 11267 (1999) **Deviations:** None **Testing Facility:** Institut für Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany **Testing Facility Report No.:** 89591016 **GLP:** Yes **Certifying Authority:** Hess. Ministerium fur Umwelt, Energie, Landwirtschaft and Verbraucherschutz

Executive summary:

The effects of IN-A2213 on the mortality and reproduction of Collembola (*Folsomia candida*) were determined in a 28-day soil exposure laboratory study according to OECD 232, 2009 and ISO 11267, 1999. Ten to twelve days old Collembola were exposed for 28 days to artificial soil (prepared according to OECD 232) treated with five nominal concentrations of IN-A2213 of 6.25, 12.5, 25.0, 50.0, and 100 mg test item/kg dry artificial soil and to an untreated control (deionized water only). Mortality and reproduction (number of juveniles produced) were assessed after 28 days. The overall 28-day NOEC (No-Observed-Effect Concentration) based on mortality and reproduction was determined to be 100 mg IN-A2213/kg soil dry weight.

I. MATERIALS AND METHODS

A. MATERIALS

1.	Test material:	IN-A2213 technical metabolite
	Lot/Batch #:	A2213-011
	Purity:	100%, according to Certificate of Analysis Revision 2
	Description:	Solid
	CAS#:	66344-33-0
	Stability of test compound:	Not analysed in the test system
2.	Control:	Untreated (and moistened with deionized water)
	Test vehicle:	Deionized water
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: 46%; 5% sphagnum peat.
	Diet:	Granulated dry yeast
	Water content of soil:	Initiation: 23.2 to 24.4%, equivalent to 50.5 to 53.1% of the maximum water holding capacity Termination: 22.9 to 23.9% equivalent to 49.9 to 52.0% of the maximum water holding capacity
	Soil pH:	6.1 to 6.2 at test start; 6.2 to 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

07-July-2014 to 05-August-2014

2. Experimental treatments

A study was conducted to determine the effects of IN-A2213 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-A2213 of 6.25, 12.5, 25.0, 50.0, and 100 mg test item/kg dry artificial soil and an untreated control (deionized water only). One additional container per treatment was set to check the pH and water content of the test substrate after 28 days.A reference item (boric acid, at a concentration range of 30.5 to 200 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 July/August 2014.

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 content of the test containers was suspended in water, the suspension was tinted with dark ink and stirred with a fine brush. 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. Surviving Collembola were observed for any abnormal behaviour or conditions on day 28.

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 homoscedasticity 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 160 mg boric acid/kg dry artificial soil.

A summary of the results is provided in the table below.

Table 805 The effects on mortality and reproduction of Collembola, *Folsomia candida*, exposed to IN-A2213 in artificial soil for 28 days

Nominal IN-A2213 concentration (mg/kg soil)	Mean % mortality[a]	Reproduction	
		Mean juveniles per replicate[a]	% of control
Untreated control (0.0)	8	567	-
6.25	15	541	95
12.5	15	563	99
25.0	8	562	99
50.0	15	560	99
100	13	583	103

[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

Since the 28-day EC_{10}, EC_{20}, EC_{50} based on reproduction could not be determined by statistical analysis they were estimated based on the overall 28-day No-Observed-Effect Concentration (NOEC) to be greater than 100 mg IN-A2213/kg dry artificial soil. The overall NOEC based on mortality and reproduction was determined to be 100 mg IN-A2213/kg dry artificial soil and the overall Lowest-Observed-Effect Concentration (LOEC) for IN-A2213 was estimated to be greater than 100 mg/kg dry artificial soil.

(Pavić, B., 2014a)

RMS comments and conclusion

The reference substance boric acid should reduce reproduction by 50% at about 100 mg/kg dry weight soil; in this study the % of effect were somewhat lower (EC50 = 160 mg boric acid/kg).

The validity criteria are fulfilled:

- Mean adult mortality should not exceed 20% at the end of the test (actual 8%).

- The mean number of juveniles per vessel should be at least 100 at the end of the test (actual 567);

- The coefficient of variation calculated for the number of juveniles should be less than 30% at the

end of the definitive test (actual 8%).

Conclusion: the study is acceptable. NOEC= 100 mg met/kg dry wt soil, EC10 >100 mg met/kg dry wt soil

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

B.9.4.2.1/04

Reference: CA 8.4.2.1/06	**Report:**	Pavić, B. (2015a); IN-D2708: Effects on the collembola *Folsomia candida* in artificial soil with 5% peat **DuPont Report No.:** DuPont-41043 **Guidelines:** OECD 232 (2009), ISO 11267 (1999) **Deviations:** None **Testing Facility:** Institut für Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany **Testing Facility Report No.:** 92291016 **GLP:** Yes **Certifying Authority:** Hess. Ministerium fur Umwelt, Energie, Landwirtschaft and Verbraucherschutz

Executive summary:

The effects of IN-D2708 on the mortality and reproduction of Collembola (*Folsomia candida*) were determined in a 28-day soil exposure laboratory study according to OECD 232, 2009 and ISO 11267, 1999. Ten to twelve days old Collembola were exposed for 28 days to artificial soil (prepared according to OECD 232) treated with five nominal concentrations of 6.43, 12.86, 25.72, 51.44, and 102.9 mg IN-D2708/kg dry artificial soil (corresponding to 6.25, 12.5, 25.0, 50.0, and 100 mg IN-D2708/kg dry artificial soil, adjusted for purity) and an untreated control (deionized water only). Mortality and reproduction (number of juveniles produced) were assessed after 28 days. The overall 28-day NOEC (No-Observed-Effect Concentration) based on mortality and reproduction was determined to be 100 mg IN-D2708/kg soil dry weight.

I. MATERIALS AND METHODS

A. MATERIALS

1.	Test material:	IN-D2708 technical metabolite
	Lot/Batch #:	D2708-007
	Purity:	97.2%, by analysis
	Description:	Solid
	CAS#:	32833-96-8
	Stability of test compound:	Not analysed in the test system
2.	Control:	Untreated (moistened with deionized water)
	Test vehicle:	Deionized water
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: 42%
Diet:	Granulated dry yeast
Water content of soil:	Initiation: 21.2 to 22.3%, equivalent to 50.6 to 53.1% of the maximum water holding capacity Termination: 19.6 to 21.9% equivalent to 46.7 to 52.0% of the maximum water holding capacity
Soil pH:	6.0 to 6.1 at test start; 6.0 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

08-December-2014 to 06-January-2015

2. Experimental treatments

A study was conducted to determine the effects of IN-D2708 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 6.43, 12.86, 25.72, 51.44, and 102.9 mg IN-D2708/kg dry artificial soil (corresponding to 6.25, 12.5, 25.0, 50.0, and 100 mg IN−D2708/kg dry artificial soil, adjusted for purity) and an untreated control (deionized water only). A reference item (boric acid, at a concentration range of 30.5 to 200 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 November/December 2014.

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 homoscedasticity 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 145.1 mg boric acid/kg dry artificial soil. The EC_{10}, EC_{20}, and EC_{50} for survival and reproduction of the test item, IN-D2708, could not be calculated.

A summary of the results is provided in the table below.

Table 86 The effects on mortality and reproduction of Collembola, *Folsomia candida*, exposed to IN-D2708 in artificial soil for 28 days

Nominal IN-D2708 concentration (mg/kg soil), adjusted for purity	Mean % mortality[a]	Reproduction	
		Mean juveniles per replicate[a]	% of control
Untreated control (0.0)	6	436	-[b]
6.25	3	445	102
12.5	3	419	96
25.0	0	455	104
50.0	5	497	114
100	13	402	92

[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)

[b] - = not applicable

III. CONCLUSIONS

The 28-day EC_{50} for reproduction and the Lowest-Observed-Effect Concentration (LOEC) for IN-D2708 were estimated to be greater than 100 mg IN-D2708/kg dry artificial soil, the highest concentration tested. The overall 28-day No–Observed-Effect Concentration (NOEC) based on mortality and reproduction was determined to be 100 mg IN-D2708/kg dry artificial soil.

(Pavić, B., 2015a)

RMS comments and conclusion

The reference substance boric acid should reduce reproduction by 50% at about 100 mg/kg dry weight soil; in this study the % of effect were somewhat lower (EC50 = 145.1 mg boric acid/kg).

The validity criteria are fulfilled:
- Mean adult mortality should not exceed 20% at the end of the test (actual 6%).
- The mean number of juveniles per vessel should be at least 100 at the end of the test (actual 436);
- The coefficient of variation calculated for the number of juveniles should be less than 30% at the end of the definitive test (actual 14.2%).

Due to the data set the EC10/20 cannot be calculated.

Conclusion: the study is acceptable. NOEC =100 mg IN-D2708/kg dry artificial soil. EC10/20 not calculable.

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

B.9.4.2.1/05

Reference: CA 8.4.2.1/08	**Report:**	Pavić, B. (2015c); IN-N0079: Effects on the collembola *Folsomia candida* in artificial soil with 5% peat **DuPont Report No.:** DuPont-41046 **Guidelines:** OECD 232 (2009), ISO 11267 (1999) **Deviations:** None **Testing Facility:** Institut für Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany

		Testing Facility Report No.: 92271016 **GLP:** Yes **Certifying Authority:** Hess. Ministerium fur Umwelt, Energie, Landwirtschaft and Verbraucherschutz

Executive summary:

The effects of IN-N0079 on the mortality and reproduction of Collembola (*Folsomia candida*) were determined in a 28-day soil exposure laboratory study according to OECD 232, 2009 and ISO 11267, 1999. Ten to twelve days old Collembola were exposed for 28 days to artificial soil (prepared according to OECD 232) treated with eight nominal concentrations of IN-N0079 of 0.797, 1.594, 3.189, 6.378, 12.76, 25.51, 51.02, and 102.0 mg IN-N0079/kg dry artificial soil (corresponding to 0.781, 1.563, 3.125, 6.25, 12.5, 25.0, 50.0, and 100 mg IN-N0079/kg dry artificial soil, adjusted for purity) and an untreated control (deionized water only). Mortality and reproduction (number of juveniles produced) were assessed after 28 days. The overall 28-day NOEC (No-Observed-Effect Concentration) based on mortality and reproduction was determined to be 12.5 mg IN-N0079/kg soil dry weight.

I. MATERIALS AND METHODS

A. MATERIALS

1.	Test material:	IN-N0079 technical metabolite
	Lot/Batch #:	N0079-010
	Purity:	98.0%, by analysis
	Description:	Liquid
	CAS Registry Number:	16703-51-8
	Stability of test compound:	Not analysed in the test system
2.	Control:	Untreated (moistened with deionized water)
	Test vehicle:	Deionized water
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: 42%
	Diet:	Granulated dry yeast
	Water content of soil:	Initiation: 21.2 to 21.8%, equivalent to 50.4 to 51.8% of the maximum water holding capacity Termination: 18.1 to 21.0% equivalent to 43.0 to 50.0% of the maximum water holding capacity
	Soil pH:	6.1 at test start; 5.9 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

10-December-2014 to 09-January-2015

2. Experimental treatments

A study was conducted to determine the effects of IN-N0079 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-N0079 of 0.797, 1.594, 3.189, 6.378, 12.76, 25.51, 51.02, and 102.0 mg IN-N0079/kg dry artificial soil (corresponding to 0.781, 1.563, 3.125, 6.25, 12.5, 25.0, 50.0, and 100 mg IN-N0079/kg dry artificial soil, adjusted for purity) and an untreated control (deionized water only). A reference item (boric acid, at a concentration range of 30.5 to 200 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 November/December 2014.

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 content of the test containers was suspended in water, the suspension was tinted with dark ink and stirred with a fine brush. 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. Surviving Collembola were observed for any abnormal behaviour or conditions on day 28.

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 homoscedasticity 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 values were determined by 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 145.1 mg boric acid/kg dry artificial soil.

A summary of the results is provided in the table below. The EC_{10}, EC_{20}, and EC_{50} for reproduction were 18.33, 24.63, and 43.37 mg of the test item IN-N0079/kg dry artificial soil.

Table 817 The effects on mortality and reproduction of Collembola, *Folsomia candida*, exposed to IN-N0079 in artificial soil for 28 days

Nominal IN-N0079 concentration (mg/kg soil), adjusted for purity	Mean % mortality[a]	Reproduction	
		Mean juveniles per replicate	% of control
Untreated control (0.0)	8	497	-
0.781	18	462 n.s.[b]	93
1.563	8	524 n.s.	106
3.125	5	457 n.s.	92
6.25	8	480 n.s.	97
12.5	8	434 n.s.	87
25.0	18	385[c]	77
50.0	13	248[c]	50
100	15	0[c]	0

[a] There were no significant differences from the control (Fisher's Exact Test, alpha = 0.05, one-sided greater)

[b] n.s. = There were no significant differences from the control

[c] Statistically significant (Williams t-test, alpha = 0.05, one-sided smaller)

III. CONCLUSIONS

IN-N0079 had no statistically significant lethal or reproductive effects on the Collembola *Folsomia candida* when exposed to concentrations up to and including 12.5 mg/kg dry artificial soil for 28 days.

The 28-day EC_{50} for reproduction was determined to be 43.37 mg IN-N0079/kg dry artificial soil and the overall Lowest–Observed-Effect Concentration (LOEC) for IN-N0079 was determined to be 25.0 mg/kg dry artificial soil. The overall 28-day No-Observed-Effect Concentration (NOEC) based on mortality and reproduction was determined to be 12.5 mg/kg dry artificial soil.

(Pavić, B., 2015c)

RMS comments and conclusion

The reference substance boric acid should reduce reproduction by 50% at about 100 mg/kg dry weight soil; in this study the % of effect were somewhat lower (EC50 = 145.1 mg boric acid/kg).

The validity criteria are fulfilled:

- Mean adult mortality should not exceed 20% at the end of the test (actual 8%).
- The mean number of juveniles per vessel should be at least 100 at the end of the test (actual 497);
- The coefficient of variation calculated for the number of juveniles should be less than 30% at the end of the definitive test (actual 14.7%).

Conclusion: the study is acceptable. NOEC= 12.5 mg/kg dry artificial soil. The EC10 should be calculated.

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

B.9.4.2.1/06

Reference: CA 8.4.2.1/05	**Report:**	Pavić, B. (2014d); Oxamyl (DPX-D1410) technical (98% w/w): Effects on reproduction of the predatory mite *Hypoaspis aculeifer* in artificial soil with 5% peat **DuPont Report No.:** DuPont-39677 **Guidelines:** OECD 226 (2008) **Deviations:** None **Testing Facility:** Institut für Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany **Testing Facility Report No.:** 84911089 **GLP:** Yes **Certifying Authority:** Hess. Ministerium fur Umwelt, Energie, Landwirtschaft and Verbraucherschutz

Executive summary:

Two studies were conducted to determine the effect of oxamyl 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 different concentrations of oxamyl and to an untreated control (deionised water only). In the first experiment, the nominal concentrations of 0.00788, 0.01577, 0.03153, 0.06307, 0.1261, 0.2523, 0.5045, and 1.0091 mg oxamyl/kg dry artificial soil (corresponding to 0.00781, 0.01563, 0.03125, 0.0625, 0.125, 0.250, 0.500, and 1.000 mg oxamyl/kg dry artificial soil, adjusted for purity) were tested. In the second experiment, the nominal concentrations of 2.0182, 4.0363, 8.0727, 16.1453, and 32.2906 mg oxamyl/kg dry artificial soil (corresponding to 2.000, 4.000, 8.000, 16.000, and 32.000 mg oxamyl/kg dry artificial soil, adjusted for purity) were tested.

Mortality and reproduction (number of juveniles produced) were assessed after 14 days. The overall 14-day NOEC (No-Observed-Effect Concentration) based on mortality and reproduction was determined to be 16.000 mg oxamyl/kg soil dry weight.

I. MATERIALS AND METHODS

A. MATERIALS

1.	Test material:	Pure Oxamyl (PAI)
	Lot/Batch #:	D1410-532
	Purity:	99.1%, by analysis
	Description:	Solid
	CAS#:	23135-22-0
	Stability of test compound:	Not analysed in the test system
2.	Control:	Untreated (and moistened with deionised water)
	Test vehicle:	Deionised water
	Toxic reference:	Dimethoate
3.	Test System:	Predatory soil mites (adult females)
	Species:	*Hypoaspis aculeifer*
	Age at dosing:	Adults, approximately 9 days after reaching the adult stage (30 days after placing adult females in clean rearing vessels over a period of 3 days) in the first experiment 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) in the second experiment
	Source:	In-house laboratory culture

Acclimation period:	30 days (first experiment) and 31 days (second experiment)
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: 46%
Diet:	Cheese mites (*Tyrophagus putrescentiae*)
Water content of soil:	First experiment: Initiation: 24.0% to 24.9% equivalent to 52.2% to 54.0% of the maximum water holding capacity Termination: 23.3% to 24.2% equivalent to 50.7% to 52.5% of the maximum water holding capacity Second experiment: Initiation: 23.3% to 23.6% equivalent to 50.6% to 51.4% of the maximum water holding capacity Termination: 21.5% to 22.9% equivalent to 46.7% to 49.8% of the maximum water holding capacity
Soil pH:	First experiment: 5.8 to 6.4 at test start; 5.9 to 6.3 at test termination Second experiment: 6.3 to 6.5 at test start; 6.1 to 6.2 at test termination

4. Environmental conditions

Temperature:	Within a range of 18 to 22°C (both experiments)
Photoperiod:	16 hour light, 8 hour dark, photoperiod within a range of 400 to 800 lux (both experiments)

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed

13-August-2014 to 03-December-2014

2. Experimental treatments

Two studies were conducted to determine the effect of oxamyl 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 oxamyl of 0.00788, 0.01577, 0.03153, 0.06307, 0.1261, 0.2523, 0.5045, and 1.0091 mg oxamyl/kg dry artificial soil (corresponding to 0.00781, 0.01563, 0.03125, 0.0625, 0.125, 0.250, 0.500, and 1.000 mg oxamyl/kg dry artificial soil, adjusted for purity) in the first experiment and to 2.0182, 4.0363, 8.0727, 16.1453, and 32.2906 mg oxamyl/kg dry artificial soil (corresponding to 2.000, 4.000, 8.000, 16.000, and 32.000 mg oxamyl/kg dry artificial soil, adjusted for purity) in the second experiment and an untreated control (deionised water only).

A reference item (dimethoate) is tested at least once a year to ensure sensitivity of the test system at five concentrations. The most recent test relevant to this study was conducted in June 2014.

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 homoscedasticity using Shapiro-Wilk's test and Levene's test (second experiment) and Shapiro-Wilk's test and Cochran´s (first experiment), respectively ($\alpha = 0.05$). Further statistical evaluation of the NOEC for reproduction was

performed using Williams t-test (multiple comparison, alpha = 0.05, one-sided smaller). EC values were determined by Probit Analysis.

II. RESULTS AND DISCUSSION

A. FINDINGS

All validity criteria were met. The reference item caused statistically significant effects on reproduction at a concentration of 4.5 mg dimethoate/kg dry artificial soil and above; the EC_{50} for reproduction was 5.5 mg dimethoate/kg dry artificial soil.

A summary of the results is provided in the table below.

Table 828 The effects on mortality and reproduction of the soil mite, *Hypoaspis aculeifer*, exposed to oxamyl in artificial soil for 14 days

Nominal oxamyl concentration, adjusted for purity (mg/kg soil)	Mean % mortality[a]	Reproduction	
		Mean juveniles per replicate[b]	% of control
	First experiment		
Untreated control (0.0)	10	243	—
0.00781	13 n.s.[c]	241 n.s.	99
0.01563	20 n.s.	251 n.s.	103
0.03125	5 n.s.	244 n.s.	100
0.0625	13 n.s.	226 n.s.	93
0.125	13 n.s.	241 n.s.	99
0.250	10 n.s.	245 n.s.	101
0.500	8 n.s.	224 n.s.	92
1.000	15 n.s.	216 n.s.	89
	Second experiment		
Untreated control (0.0)	1	213	—
2.000	0 n.s.	201 n.s.	94
4.000	5 n.s.	200 n.s.	94
8.000	3 n.s.	196 n.s.	92
16.000	3 n.s.	198 n.s.	93
32.000	8 n.s.	94[d]	44

[a] mortality: Fisher's Exact Test, alpha = 0.05, one-sided greater

[b] number of juveniles: Williams t-test, alpha = 0.05, one-sided smaller

[c] n.s. = there were no significant differences from the control

[d] Statistically significant

III. CONCLUSIONS

Oxamyl had no statistically significant lethal effects on the soil mite Hypoaspis aculeifer when exposed to concentrations up to and including 32.000 mg/kg.

The overall 14-day NOEC based on mortality and reproduction was determined to be 16.000 mg oxamyl/kg dry artificial soil (based on nominal concentrations).

The 14-day overall Lowest-Observed-Effect Concentration (LOEC) based on mortality and reproduction for oxamyl was determined to be 32.000 mg oxamyl/kg dry artificial soil.

(Pavić, B., 2014d)

RMS comments and conclusion:

For the referece dimethoate, a response of 10 - 90 % effect was not achieved (actual 0-77%), but the EC50 based on the number of juveniles fell in the recommended range of 3.0-7.0 mg a.s./kg soil (dw).

The validity criteria in the controls are fulfilled:

- Mean adult mortality should not exceed 20% at the end of the test (actual 10% in the 1st experiment and 1% in the 2nd experiment).
- The mean number of juveniles per per replicate (with 10 adult females introduced) should be at least 50 at the end of the test; (actual 243);
- The coefficient of variation calculated for the number of juvenile mites per replicate should not be higher than 30% at the end of the definitive test (actual 7.0% in the 1st experiment 10.3% in the 2nd experiment).

The EC10 (8.55 mg a.s./kg) and EC20 (13.98 mg a.s./kg) for reproduction have been calculated in the test report, but to the data set the RMS does not consider them reliable. Also, it is considered that the EC50 should be better expressed as > 32 mg a.s./kg.

Conclusion: the study is acceptable. 14-day NOEC =16.000 mg oxamyl/kg dry artificial soil (based on nominal concentrations).

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

B.9.4.2.1/07

Reference: **CA 8.4.2.1/03**	**Report:**	Pavić, B. (2014b); IN-A2213: Effects on reproduction of the predatory mite *Hypoaspis aculeifer* in artificial soil with 5% peat **DuPont Report No.:** DuPont-39674 **Guidelines:** OECD 226 (2008) **Deviations:** None **Testing Facility:** Institut für Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany **Testing Facility Report No.:** 89591089 **GLP:** Yes **Certifying Authority:** Hess. Ministerium fur Umwelt, Energie, Landwirtschaft and Verbraucherschutz

Executive summary:

A study was conducted to determine the effect of IN-A2213 on the mortality and reproduction of the soil mite (*Hypoaspis aculeifer*) according to OECD 226. The soil mites were exposed for 14 days to artificial soil (prepared according to OECD 226) treated with the test item to obtain the nominal concentrations of 6.25, 12.5, 25.0, 50.0, and 100 mg IN-A2213/kg dry artificial soil and to an untreated control (deionized water only). IN-A2213 had no statistically significant lethal or reproductive effects on the predatory mite *Hypoaspis aculeifer* when exposed to concentrations up to and including 100 mg/kg dry artificial soil for 14 days, the highest dose tested.

Since the 14-day EC_{10}, EC_{20}, and EC_{50} based on reproduction could not be determined by statistical analysis, they were estimated based on the overall 14-day No-Observed-Effect Concentration (NOEC) to be greater than

100 mg IN-A2213/kg dry artificial soil. The overall NOEC was determined to be 100 mg IN-A2213/kg dry artificial soil and the overall Lowest-Observed-Effect Concentration (LOEC) for IN-A2213 was estimated to be greater than 100 mg/kg dry artificial soil.

I. MATERIALS AND METHODS

A. MATERIALS

1.	Test material:	IN-A2213 technical metabolite
	Lot/Batch #:	A2213-011
	Purity:	100%
	Description:	Solid
	CAS#:	66344-33-0
	Stability of test compound:	Not analysed in the test system
2.	Control:	Untreated (and moistened with deionized water)
	Test vehicle:	Deionized water
	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)
	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: 46%; 5% sphagnum peat.
	Diet:	Cheese mites (*Tyrophagus putrescentiae*)
	Water content of soil:	Initiation: 23.2 to 24.4%, equivalent to 50.5 to 53.1% of the maximum water holding capacity Termination: 22.7 to 23.8% equivalent to 49.3 to 51.7% of the maximum water holding capacity
	Soil pH:	6.1 to 6.2 at test start; 6.0 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

   07-July-2014 to 23-July-2014

2. Experimental treatments

   A study was conducted to determine the effect of IN-A2213 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 6.25, 12.5, 25.0, 50.0, and 100 mg IN-A2213/kg dry artificial soil and to an untreated control (deionized water only). A reference item (dimethoate) is tested at least once a year to ensure sensitivity of the test system at five concentrations. The most recent test relevant to this study was conducted in June 2014.

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 homoscedasticity 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 4.5 mg dimethoate/kg dry artificial soil and above; the EC_{50} for reproduction was 5.5 mg dimethoate/kg dry artificial soil.

A summary of the results is provided in the table below.

Table 839 The effects on mortality and reproduction of the soil mite, *Hypoaspis aculeifer*, exposed to IN-A2213 in artificial soil for 14 days

Nominal IN-A2213 concentration (mg/kg soil dry weight adjusted for purity)	Mean % mortality[a]	Reproduction	
		Mean juveniles per replicate[a]	% of control
Untreated control (0.0)	20	232	-
6.25	13	217	94
12.5	8	230	99
25.0	10	192	83
50.0	15	220	95
100	8	238	103

[a] There were no significant differences compared to 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

Since the 14-day EC_{10}, EC_{20}, EC_{50} based on reproduction could not be determined by statistical analysis they were estimated based on the overall 14-day No-Observed-Effect Concentration (NOEC) to be greater than 100 mg IN-A2213/kg dry artificial soil. The overall NOEC was determined to be 100 mg IN-A2213/kg dry artificial soil, based on nominal concentrations,and the overall Lowest-Observed-Effect Concentration (LOEC) for IN-A2213 was estimated to be greater than 100 mg/kg dry artificial soil.

(Pavić, B., 2014b)

RMS comments and conclusion:

For the referece dimethoate, a response of 10 - 90 % effect was not achieved (actual 0-77%), but the EC50 based on the number of juveniles fell in the recommended range of 3.0-7.0 mg a.s./kg soil (dw).

The validity criteria in the controls are fulfilled:

- Mean adult mortality should not exceed 20% at the end of the test (actual 20%).
- The mean number of juveniles per per replicate (with 10 adult females introduced) should be at least 50 at the end of the test; (actual 232);
- The coefficient of variation calculated for the number of juvenile mites per replicate should not be higher than 30% at the end of the definitive test (actual 11.2%).

The EC_{10} (8.55 mg a.s./kg) and EC20 (13.98 mg a.s./kg) for reproductionhave been calculated in the test report, but to the data set the RMS does not consider them reliable.

Conclusion: the study is acceptable. NOEC =100 mg IN-A2213/kg dry artificial soil, based on nominal concentrations. EC_{10} not calculable.

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

B.9.4.2.1/08

Reference: **CA 8.4.2.1/07**	**Report:**	Pavić, B. (2015b); IN-D2708: Effects on reproduction of the predatory mite *Hypoaspis aculeifer* in artificial soil with 5% peat **DuPont Report No.:** DuPont-41044 **Guidelines:** OECD 226 (2008) **Deviations:** None **Testing Facility:** Institut für Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany **Testing Facility Report No.:** 92291089 **GLP:** Yes **Certifying Authority:** Hess. Ministerium fur Umwelt, Energie, Landwirtschaft and Verbraucherschutz

Executive summary:

A study was conducted to determine the effect of IN-D2708 on the mortality and reproduction of the soil mite (*Hypoaspis aculeifer*) according to OECD 226. The soil mites were exposed for 14 days to artificial soil (prepared according to OECD 226) treated with the test item to obtain the nominal concentrations of 6.43, 12.86, 25.72, 51.44, and 102.9 mg IN-D2708/kg dry artificial soil (corresponding to 6.25, 12.5, 25.0, 50.0, and 100 mg IN-D2708/kg dry artificial soil, adjusted for purity) and to an untreated control (deionized water only). IN-D2708 had no statistically significant lethal or reproductive effects on the predatory mite *Hypoaspis aculeifer* when exposed to concentrations up to and including 100 mg/kg dry artificial soil for 14 days, the highest dose tested.

The 14-day EC_{50} and the overall Lowest-Observed-Effect Concentration (LOEC) for IN-D2708 were estimated to be greater than 100 mg/kg dry artificial soil. The overall 14-day No-Observed-Effect Concentration (NOEC) based on mortality and reproduction was determined to be 100 mg IN-D2708/kg dry artificial soil.

I. MATERIALS AND METHODS

A. MATERIALS

1.	Test material:	IN-D2708 technical metabolite
	Lot/Batch #:	D2708-007
	Purity:	97.2%, by analysis
	Description:	Solid
	CAS#:	32833-96-8
	Stability of test compound:	Not analysed in the test system
2.	Control:	Untreated (moistened with deionized water)
	Test vehicle:	Deionized water
	Toxic reference:	Dimethoate
3.	Test System:	Predatory soil mites (adult females)
	Species:	*Hypoaspis aculeifer*
	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)
	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: 42%
	Diet:	Cheese mites (*Tyrophagus putrescentiae*)
	Water content of soil:	Initiation: 21.6 to 22.4%, equivalent to 51.4 to 53.4% of the maximum water holding capacity Termination: 19.6 to 22.0% equivalent to 46.8 to 52.4% of the maximum water holding capacity
	Soil pH:	5.9 to 6.0 at test start; 5.7 to 6.0 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-November-2014 to 10-December-2014

2. Experimental treatments

A study was conducted to determine the effect of IN-D2708 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 6.43, 12.86, 25.72, 51.44, and 102.9 mg IN-D2708/kg dry artificial soil (corresponding to 6.25, 12.5, 25.0, 50.0, and 100 mg IN-D2708/kg dry artificial soil, adjusted for purity) and to an untreated control (deionized water only). A reference item (dimethoate) is tested at least once a year to ensure sensitivity of the test system at five concentrations. The most recent test relevant to this study was conducted in June 2014.

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 homoscedasticity 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 4.5 mg dimethoate/kg dry artificial soil and above; the EC_{50} for reproduction was 5.5 mg dimethoate/kg dry artificial soil. The EC_{10}, EC_{20}, and EC_{50} for survival and reproduction of the test item, IN-D2708, could not be calculated.

A summary of the results is provided in the table below.

Table 90 The effects on mortality and reproduction of the soil mite, *Hypoaspis aculeifer*, exposed to IN-D2708 in artificial soil for 14 days

Nominal IN-D2708 concentration (mg/kg soil dry weight adjusted for purity)	Mean % mortality[a]	Reproduction	
		Mean juveniles per replicate[a]	% of control
Untreated control (0.0)	1	228	-[b]
6.25	3	231	101
12.5	0	227	99
25.0	3	200	87
50.0	0	232	101
100	0	214	94

[a] There were no significant differences compared to 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)

[b] - = not applicable

III. CONCLUSIONS

The 14-day EC_{50} based on reproduction and the overall Lowest-Observed-Effect Concentration (LOEC) for IN-D2708 were estimated to be greater than 100 mg/kg dry artificial soil. The overall 14-day No-Observed-Effect Concentration (NOEC) was determined to be 100 mg/kg dry artificial soil.

(Pavić, B., 2015b)

RMS comments and conclusion:

For the referece dimethoate, a response of 10 - 90 % effect was not achieved (actual 0-77%), but the EC50 based on the number of juveniles fell in the recommended range of 3.0-7.0 mg a.s./kg soil (dw).

The validity criteria in the controls are fulfilled:

- Mean adult mortality should not exceed 20% at the end of the test (actual 1%).
- The mean number of juveniles per per replicate (with 10 adult females introduced) should be at least 50 at the end of the test; (actual 228);
- The coefficient of variation calculated for the number of juvenile mites per replicate should not be higher than 30% at the end of the definitive test (actual 6.6%).

Conclusion: the study is acceptable. NOEC = 100 mg/kg dry artificial soil.The EC10 cannot be calculated due to the data set.

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

B.9.4.2.1/09

Reference: CA 8.4.2.1/09	**Report:**	Pavić, B. (2015d); IN-N0079: Effects on reproduction of the predatory mite *Hypoaspis aculeifer* in artificial soil with 5% peat **DuPont Report No.:** DuPont-41047 **Guidelines:** OECD 226 (2008) **Deviations:** None **Testing Facility:** Institut für Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany **Testing Facility Report No.:** 92271089 **GLP:** Yes **Certifying Authority:** Hess. Ministerium fur Umwelt, Energie, Landwirtschaft and Verbraucherschutz

Executive summary:

A study was conducted to determine the effect of IN-N0079 on the mortality and reproduction of the soil mite (*Hypoaspis aculeifer*) according to OECD 226. The soil mites were exposed for 14 days to artificial soil (prepared according to OECD 226) treated with the test item to obtain the nominal concentrations of 3.189, 6.378, 12.76, 25.51, 51.02, and 102.0 mg IN-N0079/kg dry artificial soil (corresponding to 3.125, 6.25, 12.5, 25.0, 50.0, and 100 mg IN-N0079/kg dry artificial soil, adjusted for purity) and an untreated control (deionized water only). IN-N0079 had no statistically significant lethal or reproductive effects on the predatory mite *Hypoaspis aculeifer* when exposed to concentrations up to and including 25.0 mg/kg dry artificial soil for 14 days.

The 14-day EC_{50} was determined to be 60.49 mg IN-N0079/kg dry artificial soil and the overall Lowest–Observed-Effect Concentration (LOEC) for IN-N0079 was determined to be 50.0 mg/kg dry artificial soil. The overall 14-day No-Observed-Effect Concentration (NOEC) based on mortality and reproduction was determined to be 25.0 mg IN-N0079/kg dry artificial soil.

I. MATERIALS AND METHODS

A. MATERIALS

1.	Test material:	IN-N0079 technical metabolite
	Lot/Batch #:	N0079-010
	Purity:	98.0%, by analysis
	Description:	Liquid
	CAS Registry Number:	16703-51-8
2.	Control:	Untreated (moistened with deionized water)
	Test vehicle:	Deionized water
	Toxic reference:	Dimethoate
3.	Test System:	Predatory soil mites (adult females)
	Species:	*Hypoaspis aculeifer*
	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)
	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: 42%
	Diet:	Cheese mites (*Tyrophagus putrescentiae*)
	Water content of soil:	Initiation: 21.3 to 22.1%, equivalent to 50.8 to 52.6% of the maximum water holding capacity Termination: 20.0 to 20.5% equivalent to 47.6 to 48.8% of the maximum water holding capacity
	Soil pH:	5.8 to 6.3 at test start; 5.9 to 6.0 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

01-December-2014 to 17-December-2014

2. Experimental treatments

A study was conducted to determine the effect of IN-N0079 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 3.189, 6.378, 12.76, 25.51, 51.02, and 102.0 mg IN-N0079/kg dry artificial soil (corresponding to 3.125, 6.25, 12.5, 25.0, 50.0, and 100 mg IN-N0079/kg dry artificial soil, adjusted for purity) and to an untreated control (deionized water only). A reference item (dimethoate) is tested at least once a year to ensure sensitivity of the test system at five concentrations. The most recent test relevant to this study was conducted in June 2014.

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 homoscedasticity 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 values were determined by Probit Analysis.

II. RESULTS AND DISCUSSION

A. FINDINGS

All validity criteria were met. The reference item caused statistically significant effects on reproduction at a concentration of 4.5 mg dimethoate/kg dry artificial soil and above; the EC_{50} for reproduction was 5.5 mg dimethoate/kg dry artificial soil.

A summary of the results is provided in the table below. The EC_{10}, EC_{20}, and EC_{50} for reproduction were 38.71, 45.12, and 60.49 mg of the test item IN-N0079/kg dry artificial soil.

Table 84 The effects on mortality and reproduction of the soil mite, *Hypoaspis aculeifer*, exposed to IN-N0079 in artificial soil for 14 days

Nominal IN-N0079 concentration (mg/kg soil dry weight adjusted for purity)	Mean % mortality[a]	Reproduction	
		Mean juveniles per replicate	% of control
Untreated control (0.0)	6	240	-
3.125	15	225 n.s.[b]	94
6.25	13	211 n.s.	88
12.5	8	234 n.s.	97
25.0	3	222 n.s.	92
50.0	5	172[c]	71
100	18	16[c]	7

[a] There were no significant differences from the control (mortality: Fisher's Exact Test, alpha = 0.05, one-sided greater)

[b] n.s. = There were no significant differences from the control

[c] Statistically significant Williams t-test, (alpha = 0.05, one-sided smaller)

III. CONCLUSIONS

IN-N0079 had no statistically significant lethal or reproductive effects on the soil mite *Hypoaspis aculeifer* when exposed to concentrations up to and including 25.0 mg/kg dry artificial soil.

The 14-day EC_{50} based on reproduction was determined to be 60.49 (47.93 to 112.36) mg IN-N0079/kg dry artificial soil and the overall Lowest-Observed-Effect Concentration (LOEC) for IN-N0079 were determined to be 50.0 mg/kg dry artificial soil. The overall 14-day No-Observed-Effect Concentration (NOEC) was determined to be 25.0 mg/kg dry artificial soil.

(Pavić, B., 2015d)

RMS comments and conclusion

For the reference dimethoate, a response of 10 - 90 % effect was not achieved (actual 0-77%), but the EC50 based on the number of juveniles fell in the recommended range of 3.0-7.0 mg a.s./kg soil (dw).

The validity criteria in the controls are fulfilled:
- Mean adult mortality should not exceed 20% at the end of the test (actual 6%).

- The mean number of juveniles per per replicate (with 10 adult females introduced) should be at least 50 at the end of the test; (actual 240);
- The coefficient of variation calculated for the number of juvenile mites per replicate should not be higher than 30% at the end of the definitive test (actual 9.2%).

Conclusion: the study is acceptable. The following were also calculated: EC10= 38.71 (6.98 to 48.59) mg IN-N0079/kg soil dry weight, EC20 = 45.12 (15.87 to 55.21) mg IN-N0079/kg soil dry weight.

B.9.4.3 Summary of toxicity to non-target soil meso- and macrofauna

Toxicity endpoint values for earthworms and soil macro organisms are summarised in Table .

Table 92 Toxicity endpoint values for non-target soil meso- and macrofauna

Test item	Species	Test/duration	Endpoint	Endpoint value (mg a.s. or met./kg dry wt soil)[a]	Reference[b]
Oxamyl 10GR	*Eisenia fetida*	14 d	LC_{50}	>100	DuPont-3850[c]
Oxamyl 10GR	*Eisenia fetida*	Sub-lethal, 56 d	EC_{50} NOEC	>6.4 mg 6.4 mg	DuPont-4296
Oxamyl	14 d	LC_{50}	112	AMR 3068	
Oxamyl	*Eisenia fetida*	Bioconcentration, 42 d	BCF	0.03	DuPont-38477
IN-A2213	*Eisenia fetida*	14 d	LC_{50}	>1000	DuPont-4130
IN-A2213	*Eisenia fetida*	Sub-lethal, 56 d	EC_{50} NOEC EC10	>100 25 26.6	DuPont-39672
IN-D2708	*Eisenia fetida*	14 d	LC_{50}	>1000	DuPont-4132
IN-D2708	*Eisenia fetida*	Sub-lethal, 56 d	EC_{50} NOEC	>100 100	DuPont-41042
IN-N0079	*Eisenia fetida*	14 d	LC_{50}	640	DuPont-4134
IN-N0079	*Eisenia fetida*	Sub-lethal, 56 d	EC_{50} NOEC	>100 50	DuPont-41045
IN-T2921	*Eisenia fetida*	14 d	LC_{50}	>1000	DuPont-4617
Oxamyl	*Folsomia candida*	Sub-lethal, 28 d	EC_{50} NOEC EC_{10}	1.663 0.25 0.435	DuPont-39676
IN-A2213	*Folsomia candida*	Sub-lethal, 28 d	EC_{50} NOEC	>100 100	DuPont-39673
IN-D2708	*Folsomia candida*	Sub-lethal, 28 d	EC_{50} NOEC	>100 100	DuPont-41043
IN-N0079	*Folsomia candida*	Sub-lethal, 28 d	EC_{50} NOEC	43.37 12.5	DuPont-41046
Oxamyl	*Hypoaspis aculeifer*	Sub-lethal, 14 d	EC_{50} NOEC	>32 16	DuPont-39677
IN-A2213	*Hypoaspis aculeifer*	Sub-lethal, 14 d	EC_{50} NOEC	>100 100	DuPont-39674
IN-D2708	*Hypoaspis aculeifer*	Sub-lethal, 14 d	EC_{50} NOEC	>100 100	DuPont-41044
IN-N0079	*Hypoaspis aculeifer*	Sub-lethal, 14 d	EC_{50} NOEC EC_{10}	60.49 25 38.71	DuPont-41047

[a] Represents the highest concentration tested.

[b] All studies cited or summarised in this document.

[c] Summarized in dRAR Vol 3 Oxamyl GR 10 B9.

B.9.5 Effects on soil nitrogen transformation

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

B.9.5/01

Reference: CA 8.5/01	**Report:**	Cardinali, V.C.B. (2008); Effects of OXAMIL TÉCNICO on soil microorganisms: Nitrogen transformation test **DuPont Report No.:** RF-0014.218.286.07 **Guidelines:** OECD 216 (2000) **Deviations:** None. **Testing Facility:** BIOAGRI Laboratorios Ltda., Piracicaba/SP, Brazil **Testing Facility Report No.:** 0014.218.286.07 **GLP:** Yes **Certifying Authority:** Food and Consumer Product Safety Authority (Den Haag, The Netherlands), Republica Federativa do Brasil Ministerio do Desenvolvimento, Industria e Comercio Exterior Instituto Nacional de Metrologia, Normalizacao e Qualidade Industrial - Inmetro

Executive summary:

The effects of oxamyl on nitrogen transformation were investigated in a Rhodic Hapludox (LVdf) and a Typic Hapludalf (PVAe) soil in a laboratory study. The study was conducted according to OECD guideline 216 (2000). Both soils were treated with oxamyl at rates of 12.0 and 60.0 µg a.s./g soil (1 and 5 times the field application rate of 9 kg a.s./ha). A control (untreated soil) was also tested. At the end of 28 days, deviations in nitrate concentration at nominal concentrations up to and including 60.0 µg a.s./g soil compared to the control were <25%, the effect threshold specified by the OECD test guideline. It can be concluded, therefore, that oxamyl, at concentrations up to and including 60.0 µg oxamyl/g soil, corresponding to 5× the maximum field application rate of 9 kg oxamyl/ha, can be categorised as having low risk to soil microflora.

I. MATERIALS AND METHODS

A. MATERIALS

1.	Test material:	Oxamyl technical	
	Lot/Batch#:	D1410-490	
	Purity:	420 g a.s./kg (nominal), 426.7 g a.s./kg (analysed)	
	Description:	Liquid	
	Stability of test compound:	Not determined in the test system	
2.	Control:	Untreated soil	
	Test vehicle:	Distilled water	
3.	Test organism:	Soil microorganisms	
	Source:	Two soils were collected in a forest tableland area (subcaducifolia stational forest)	
	Test chamber	Nitrogen transformation test: 500 mL glass flasks, closed with a plastic cover, containing 130 g dw soil	
	Substrates:	Leuceana (*Leucaena leucicephala*), ratio C/N 14/1 (5g/kg dw soil)	
	Soil:	Natural soils	
		LVdf	PVAe
	Soil type:	Rhodic Hapludox	Typic Hapludalf
	Soil pH:	4.9	6.0
		5.09–5.17 (during the study)	6.10–6.16 (during the study)
	% Total organic carbon:	1.5	1.6
	CEC (meg/100 g):	23	86
	Water holding capacity (%):	50	50
	Clay (%)	53	22
	Sand (%)	45	58
	Silt (%)	2	20
	Microbial biomass (% of total soil organic carbon):	20.00	26.25
4.	Environmental conditions		
	Temperature:	20–21°C	
	Photoperiod:	Continuous dark	

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed

08-February-2008 to 18-April-2008

2. Experimental treatments

The effects of oxamyl on nitrogen transformation were investigated in two soils (Rhodic Hapludox [LVdf] and Typic Hapludalf [PVAe]) in a laboratory study. Oxamyl was applied to two soil at nominal application rates of 12.0 µg a.s./g soil and 60.0 µg a.s./g soil (1 and 5 times the maximum use rate of 9 kg a.s./ha). The control consisted of an untreated soil. Three replicats per treatment group were set. The water content was adjusted to 50 % of the maximum capacity of water retention for both soils. Samples for nitrogen determination were incubated for 28 days.

3. Observations

Samples were collected for determination of nitrogen transformation at Days 0, 7, 14, and 28 following application of the test item.

II. RESULTS AND DISCUSSION

A. FINDINGS

Mean values of NO_3^-N concentrations in LVdf soil on days o,7,14,28.

Treatment	Day of the study				Mean of the 28th day	%D
	0	7	14	28		
	Concentration of NO_3^- ($\mu g\ g^{-1}$ of dry soil)					
	6.93	10.55	13.82	20.37		
C + OM	7.18	11.26	14.47	20.23	**20.21**	
	7.15	10.53	14.43	20.05		
Mean	**7.09**	**10.78**	**14.24**	**20.21**		
SD	**0.14**	**0.42**	**0.36**	**0.16**		
VC (%)	**1.96**	**3.89**	**2.54**	**0.79**		
	7.15	9.87	14.24	20.44		
MUR	6.91	10.36	14.40	19.79	**20.05**	**-0.79**
	7.42	10.33	14.25	19.93		
Mean	**7.16**	**10.19**	**14.30**	**20.05**		
SD	**0.26**	**0.27**	**0.09**	**0.34**		
VC (%)	**3.58**	**2.67**	**0.63**	**1.69**		
%D	**1.06**	**-5.50**	**0.39**	**-0.79**		
	6.46	9.61	13.32	19.27		
5MUR	6.47	9.64	13.81	19.15	**19.16**	**-5.23**
	6.01	9.44	13.33	19.06		
Mean	**6.31**	**9.56**	**13.49**	**19.16**		
SD	**0.26**	**0.11**	**0.28**	**0.10**		
VC (%)	**4.16**	**1.17**	**2.07**	**0.54**		
%D	**-10.91**	**-11.29**	**-5.30**	**-5.23**		

Extraction 10 g humid soil/50 mL of $CaSO_4$
C + OM = Control + organic matter
SD = Standard deviation
VC = variation coefficient (%)
%D = Deviation percentage between treated samples and control

Variation between the control samples were <15%. The validation criterion was met indicating the validity of this study.

Nitrate concentration (NO_3^-) at the end of the study (Day 28) and the percent deviation from the control are reported in Table 85.

Table 853 Summary of effects of oxamyl on nitrate concentration and nitrate formation rate in control and treatments of the two soils

Soil	Nominal oxamyl concentration	Mean NO_3^--Nconcentration (Day 28)		Mean Formation rate NO_3^-N (Day 28)
		µg/g dry soil	% Deviation from control[a]	mg /kg/d (%deviation from the control)
Rhodic Hapludox (LVdf)	Control	40.62	--	
	12.0 mg a.s./kg	40.78	0.39	
	60.0 mg a.s./kg	40.64	0.06	
Typic Hapludalf (PVAe)	Control	20.21		0.47
	12.0 mg a.s./kg	20.05	-0.79	0.46 (-2%)
	60.0 mg a.s./kg	19.16	-5.23	0.46 (-2%)

[a] Negative value = % inhibition, positive value = % simulation

III. CONCLUSION

At the end of 28 days, deviations in nitrate concentration compared to the control were <25%, the effect threshold specified by the OECD test guideline. It can be concluded, therefore, that oxamyl, at concentrations up to and including 60.0 µg oxamyl/g soil, corresponding to 5× the maximum field application rate of 9 kg oxamyl/ha, can be categorised as having low risk to soil microflora.

(Cardinali, V.C.B., 2008)

RMS comments and conclusion

Only the PVAe soil fully meets the characteristic recommended in the OECD216 guideline, hence the evaluation is focussed on this soil.

Leucaena leucicephala is used as substrate instead of Lucerne but the C/N ratio and food rate is in line with the OECD recommendations, hence it is acceptable.

In the study the results were reported only as NO3- concentration. The RMS calculated the nitrate formation rate as requested in OECD 216. In the PVAe soil the reduction in nitrate rate was <25% (actual - 2%) up to 60.0 mg a.s./kg.

Validity criterion:

The guideline requires a CV of control replicates <= 15% to be valid. In the present test with **PVAe** soil the CV for the nitrate formation rate at 28d was 1.7%. Fulfilled.

Conclusion: the study is acceptable. Reduction in nitrate rate was <25% (actual - 2%) up to 60.0 mg a.s./kg (PVAe soil).

Study submitted in the EU Dossier in 2001 and included in the first EU approval review.

B.9.5/02

Reference: --	**Report:**	Wachter, S. (2000b); IN-A2213: Assessment of the effects on soil microflora **DuPont Report No.:** DuPont-4131 **Guidelines:** BBA VI 1-1 (1990), OECD 217 (2000), OECD 216 (2000)

1.	Test material:	IN-A2213 technical metabolite
	Lot/Batch #:	A2213-11
	Purity:	100%

Materials and methods:

The effects of IN-A2213 (purity 100%) on soil microbial respiration (carbon mineralisation) and nitrogen transformations (ammonification and nitrification) were examined in a typical agricultural sandy loam soil in a laboratory study. The test was conducted in accordance with BBA-Guideline and OECD-Guideline for the testing of chemicals, using one soil type (loamy sand, BBA type 2.3). The soil was characaterized as follows: pH = 6.9, TOC = 0.90%, CEC (meg/100 g):9.2, Water holding capacity (%): 41.1%, Microbial biomass (% of total soil organic carbon): 8.1, Clay (%) < 2 µm = 11.3, Silt (%) 63 µm to ≥ 2 µm = 24.9, Sand (%) ≥ 63 - 2000 µm = 63.8, $N0_3$ ⁻·N (mg/100 g dry weight) = 0.90. Prior to the application of the test materials, the soil was thoroughly mixed with ground lucerne meal. The concentration of the dried lucerne meal was 0.5 % ofthe soil dry weight Before adding the sample the soil moisture was adjusted to 40% WHC.

The test contained 3 replicates of 4 treatment groups: untreated soil amended with Lucerne meal, soil amended with Lucerne meal and treated with IN-A2213 at a rates equivalent to 4.9 and 49 mg IN-A2213/kg soil dry weight (1x and 10x the maximum recommended seasonal application rate of 7.5 kg Oxamyl/ha in drip irrigation); and soil amended with Lucerne meal and treated with the reference substance Herbogil liquid (a.s. Dinoterb) applied at 20 L/ha (5x the maximum field application rate.

For nitrogen turnover, for each replicate, 800 g soil sub-samples were placed in 1000 mL glass bottles. Short term respiration was measured in soil after the addition of 300 mg glucose/100g soil weight with the OxiTop System". The tests were carried out at 20 ± 2 °C in the dark.

Observations

Samples were collected for determination of nitrogen transformation at 3h, and at days 14, 28, and 56 following application of the test item.

Short-term respiration was measured in soil samples taken 3 hours, 14 days and 28 days after applying test material. The rate of oxygen uptake was was determined within the first 12 hours,following the addition of glucose.

Statistics

The results ofthe nitrification and short-term respiration measurements were submitted to a statistical analysis procedure for a pairwise comparison of samples using the multiple-ttest according to Dunnett (computer program EASY ASSAY, Multiple Testing, Ratte 1995) for significant and non-significant differences between the control and the study groups. The statistical program included a pre-test for homogeneities ofvariance according to Cochran.

Findings:

Summary of N03- -N, ~+ -N, NO2—N and Nmin(sum of N03- -N, NH/ -N, NO2--N) content of soil (mg/ 100 g dry weight) and the deviation from the control for the loamy sand soil for the assessments on day 1 (3 h), day 14, day 28 and day 56.

Time	3 h	14 d	28 d	56 d
Control				
NH_4^+-N	b.q.	0.22	b.q.	0.14
NO_3^--N	0.90	1.72	2.76	4.52
NO_2^--N	b.q.	b.q.	b.q.	0.12
$N_{min.}$	0.90	1.94	2.76	4.78
IN-A2213 1X PECsoil				
NH_4^+-N	0.11	0.18	b.q.	0.11
NO_3^--N	0.89	1.91	2.30	4.68
NO_2^--N	b.q.	b.q.	b.q.	0.10
$N_{min.}$	1.00	2.09	2.30	4.89
Deviation from the control (%)				
NH_4^+-N	-	-18.18	-	-21.43
NO_3^--N	-1.11	11.05	-16.67	3.54
NO_2^--N	-	-	-	-16.67
$N_{min.}$	11.11	7.73	-16.67	2.30
IN-A2213 10 X PECsoil				
NH_4^+-N	0.13	0.17	b.q.	b.q.
NO_3^--N	0.92	2.73	3.68	5.58
NO_2^--N	b.q.	b.q.	b.q.	0.10
$N_{min.}$	1.05	2.9	3.68	5.68
Deviation from the control (%)				
NH_4^+-N	-	-22.73	-	-
NO_3^--N	2.22	58.72	33.33	23.45
NO_2^--N	-	-	-	-16.67
$N_{min.}$	16.67	49.5	33.33	18.83
Reference substance				
NH_4^+-N	0.24	0.17	b.q.	b.q.
NO_3^--N	0.89	1.79	3.54	6.03
NO_2^--N	b.q.	1.93	b.q.	0.31
$N_{min.}$	1.13	3.89	3.54	6.34
Deviation from the control (%)				
NH_4^+-N	-	-22.73	-	-
NO_3^--N	-1.11		28.26	33.41
NO_2^--N	-26.32	+4.07	-	158.33
$N_{min.}$	+25.56	100.52	28.26	32.64

b.q. = below the limit of quantitation ,
0 no deviation from the control was calculated because the measured values were below the limit of quantitation

A summary of the findings is presented in Table 86. Deviations in the ammonium and nitrate levels and respiration rate in control soil and soil treated with IN-A2213 were <25% at the end of the study.

IN-A2213 at concentrations of 1x and 10x the maximum PECsoil had no biologically significant effect on short-term respiration after 28 days and at 1x the maximum PECsoil of IN-A2213 on nitrogen turnover after 56 days.

Statistically significant effects were calculated at 10x the maximum PECsoil of IN-A2213. The rate of short-term respiration (after 28 days) and the nitrogen turnover in soil treated with IN-A2213 deviated < 25% from the controls (after 56 days).

Table 864 Summary of effects of IN-A2213 on ammonium and nitrate levels and respiration in soil amended with Lucerne meal

	Effect on ammonium levels (%)[a]		Effect on nitrate levels (%)[a]		Effect on nitrite levels (%)[a]		Effect on respiration (%)[a]	
Day	4.9 mg/kg	49mg/kg	4.9mg/kg	49mg/kg	4.9mg/kg	49mg/kg	4.9mg/kg	49mg/kg
0	_[b]	-	-1.11	2.22	-	-	6.82	6.82
14	-18.18	22.73	11.05	58.72	-	-	0.0	-13.95
28	-	-	-16.67	33.33	-	-	-2.78	-13.95
56	-21.43	-	3.54	23.45	-16.67	-16.67	No data	No data

[a] % Effect: [(measured parameter in control soil / measured parameter in treated soil)-1] x100
[b] A dash (-) indicates the measure values were. below limit of quantitation, thus a % effect cannot be calculated.

Effects on nitrate formation concentration and nitrate formation rate at the end of the study (56d).

Nominal IN-A2213 concentration	Mean NO_3^--N Levels (Day 56)		Mean Formation rate NO_3^--N (Day 56)	
	mg/100g dry soil	% Deviation from control[a]	mg /kg/d	% Deviation from control
Control (0.0)	4.52	--	0.62	--
4.9 mg a.s/kg	4.68	3.54	0.68	9.7
49 mg a.s/kg	5.58	23.45	0.84	35
Reference toxicant	6.03	33.41	0.92	48

Negative value = % inhibition, positive value = % stimulation

Conclusion:

IN-A2213, at soil concentrations equivalent to 1x and 10x the maximum conditions of Oxamyl field use had no significant effect on soil nitrogen transformation (23.45%) or carbon mineralisation (-13.95%). IN-A2213 therefore can be categorised as having low risk to soil microflora.

RMS comments and conclusion

The effects on soil nitrogen transformation study DuPont-4131, originally submitted under EU Rev8 Point IIA 8.5 and conducted with test material IN-A2213 technical metabolite, was conducted under guidelines BBA VI 1-1 (1990), OECD 217 (2000), and OECD 216 (2000). A review of this study was made according to the current guideline (OECD 216, 2000) and the summary has been integrated with additional information and tables. In the study the results were reported only as NO3- concentration. The RMS calculated the nitrate formation rate as

requested in OECD 216 (see table included in the summary). After 56d, at 4.9 kg a.s./ha, the effects on nitrate concentration and nitrate formation rate (mg /kg/d) were <25%. At 49 kg a.s./ha, the effect on nitrate concentration was <25% but the effects on nitrate formation rate was >25% (actual 35%).

Validity criterion:

The guideline requires a CV of control replicates <= 15% to be valid. In this study .the CV for the nitrate formation rate at 56d was 3.5 %. Fulfilled.

Conclusion: the study is acceptable. After 56 days, at 4.9 kg a.s./ha effects on nitrate formation rate (mg /kg/d) <25%.

Study submitted in the EU Dossier in 2001 and included in the first EU approval review.

B.9.5/03

Reference: --	**Report:**	Wachter, S. (2000a); IN-D2708: Assessment of the effects on soil microflora **DuPont Report No.:** DuPont-4133 **Guidelines:** BBA VI 1-1 (1990)

1. Test material: IN-D2708 technical metabolite
   Lot/Batch #: D2708-6
   Purity: 99.87%

Materials and methods:

The effects of IN-D2708 (purity 99.9%) on soil microbial respiration (carbon mineralisation) and nitrogen transformations (ammonification and nitrification) were examined in a typical agricultural sandy loam soil in a laboratory study. The test was conducted in accordance with BBA-Guideline and OECD-Guideline for the testing of chemicals, using one soil type (loamy sand, BBA type 2.3). The soil was characaterized as follows: pH = 7.0, TOC = 0.90%, CEC (meg/100 g):9.2, Water holding capacity (%): 41.1%, Microbial biomass (% of total soil organic carbon): 8.1, Clay (%) < 2 µm = 11.3, Silt (%) 63 µm to ≥ 2 µm = 24.9, Sand (%) ≥ 63 - 2000 µm = 63.8, N03 -·N (mg/100 g dry weight) = 0.90. Prior to the application of the test materials, the soil was thoroughly mixed with ground lucerne meal. The concentration of the dried lucerne meal was 0.5 % ofthe soil dry weight Before adding the sample the soil moisture was adjusted to 40% WHC. The test contained 3 replicates of 4 treatment groups: untreated soil amended with Lucerne meal; soil amended with Lucerne meal and treated with IN-D2708 at a rates equivalent to 3.6 and 36 mg IN-D2708/kg soil dry weight (1x and 10x the maximum recommended seasonal application rate of 7.5 kg Oxamyl/ha in drip irrigation); and soil amended with Lucerne meal and treated with the reference substance, dinoterb, at 5x the maximum field application rate. Production of carbon dioxide by soil microbes and concentrations of ammonium, nitrate, and nitrite were determined periodically, up to 28 days.

For nitrogen turnover, for each replicate, 800 g soil sub-samples were placed in 1000 mL glass bottles. Short term respiration was measured in soil after the addition of 300 mg glucose/100g soil weight with the OxiTop System". The tests were carried out at 20 ± 2 °C in the dark.

Observations

Samples were collected for determination of nitrogen transformation at 3h, and at days 14, and 28 following application of the test item.

Short-term respiration was measured in soil samples taken 3 hours, 14 days and 28 days after applying test material. The rate of oxygen uptake was was determined within the first 12 hours,following the addition of glucose.

Statistics

The results ofthe nitrification and short-term respiration measurements were submitted to a statistical analysis procedure for a pairwise comparison of samples using the multiple-ttest according to Dunnett (computer program EASY ASSAY, Multiple Testing, Ratte 1995) for significant and non-significant differences between the control and the study groups. The statistical program included a pre-test for homogeneities ofvariance according to Cochran.

Findings:

Summary of $N0_3^-$ -NH_4^+-N, NO_2^-—*N* and N_{min} (sum of $N0_3^-$ -N, NH_4^+ -N, NO_2^--*N)* content of soil (mg/ 100 g dry weight) and the deviation from the control for the assessments on day 1 (3 h), day 14, and day 28.

Time	3 h	14 d	28 d
Control			
NH_4^+-N	b.q.	0.22	b.q.
NO_3^--N	0.90	1.72	2.76
NO_2^--N	b.q.	b.q.	b.q.
$N_{min.}$	0.90	1.94	2.76
IN-D2708 1X field rate			
NH_4^+-N	0.17	0.15	b.q.
NO_3^--N	0.94	2.14	2.92
NO_2^--N	b.q.	b.q.	b.q.
$N_{min.}$	1.11	2.29	2.92
Deviation from the control (%)			
NH_4^+-N	-	-31.82	-
NO_3^--N	+4.44	+24.44	+5.80
NO_2^--N	-	-	-
$N_{min.}$	23.33	+18.04	+5.80
IN-D2708 10 X field rate			
NH_4^+-N	0.21	0.18	b.q.
NO_3^--N	0.91	2.39	3.13
NO_2^--N	b.q.	b.q.	b.q.
$N_{min.}$	1.12	2.57	3.13
Deviation from the control (%)			
NH_4^+-N	-	-18.18	-
NO_3^--N	1.11	+38.95	+13.41
NO_2^--N	-	-	-
$N_{min.}$	+24.44	32.47	+13.41
Reference substance			
NH_4^+-N	0.24	0.17	b.q.
NO_3^--N	0.89	1.79	3.54
NO_2^--N	b.q.	1.93	b.q.
$N_{min.}$	1.13	3.89	3.54
Deviation from the control (%)			
NH_4^+-N	-	-22.73	-
NO_3^--N	-1.11	+4.07	28.26
NO_2^--N	-	-	-
$N_{min.}$	+25.56	100.52	28.26

b.q. = below the limit of quantitation ,
- no deviation from the control was calculated because the measured values were below the limit of quantitation

Effects on nitrate formation concentration and nitrate formation rate at the end of the study (56d).

Nominal IN-D2708 concentration	Mean NO_3^--N Levels (Day 28)		Mean Formation rate NO_3^--N (Day 28)	
	mg/100g dry soil	% Deviation from control[a]	mg /kg/d	% Deviation from control
Control (0.0)	2.76	---	0.66	--
3.6 mg a.s/kg	2.92	5.80	0.72	9
36 mg a.s/kg	3.13	13.4	0.80	21
Reference toxicant	3.54	28.6	0.94	42

Negative value = % inhibition, positive value = % stimulation

A summary of the findings is presented in Table 87. Deviations in the ammonium and nitrate levels and respiration rate in control soil and soil treated with IN-D2708 were <25% at the end of the study.

Table 875 Summary of effects of IN-D2708 on ammonium and nitrate levels and respiration in soil amended with Lucerne meal

	Effect on ammonium levels (%)[a]		Effect on nitrate levels (%)[a]		Effect on nitrite levels (%)[a]		Effect on respiration (%)[a]	
Day	1x	10x	1x	10x	1x	10x	1x	10x
0	-[b]	-	4.44	1.11	-	-	6.82	6.82
14	-31.82	-18.18	24.44	38.95	-	-	0.00	-2.33
28	-	-	5.80	13.41	-	-	8.33	2.78

[a] % Effect: [(measured parameter in control soil / measured parameter in treated soil)-1] x100
[b] A dash (-) indicates the effect was below quantitation, thus a % effect cannot be calculated

Conclusion:

IN-D2708, at soil concentrations equivalent to 1x and 10x the maximum conditions of Oxamyl field use had no significant effect on soil nitrogen transformation (13.41%) or carbon mineralisation (2.78%). IN-D2708 therefore can be categorised as having low risk to soil microflora.

RMS comments and conclusion

The effects on soil nitrogen transformation study DuPont-4133, originally submitted under EU Rev8 Point IIA 8.5 and conducted with test material IN-D2708 technical metabolite, was conducted under guideline BBA VI 1-1 (1990).

A review of this study was made according to the current guideline (OECD 216, 2000) and the summary has been integrated with additional information and tables.

In the study the results were reported only as NO3- concentration. The RMS calculated the nitrate formation rate as requested in OECD 216 (see table included in the summary). After 28d, at 3.6 and 36 mg /kg/d, the effects on nitrate concentration and nitrate formation rate (mg /kg/d) were <25%.

Validity criterion:

The guideline requires a CV of control replicates <= 15% to be valid. In this study, the CV for the nitrate formation rate at 28d was 22 %. Not fulfilled.

Conclusion: the study is not valid.

Study submitted in the EU Dossier in 2001 and included in the first EU approval review.

B.9.5/04

Reference: --	**Report:**	Wachter, S. (2000c); IN-N0079: Assessment of the effects on soil microflora **DuPont Report No.:** DuPont-4135 **Guidelines:** BBA VI 1-1 (1990), OECD 216 (2000), OECD 217 (2000)

1. Test material: IN-N0079 technical metabolite
   Lot/Batch #: N0079-8
   Purity: 99.57%

Materials and methods:

The effects of IN-N0079 (purity 99.6%) on soil microbial respiration (carbon mineralisation) and nitrogen transformations (ammonification and nitrification) were examined in a typical agricultural sandy loam soil in a laboratory study. The test was conducted in accordance with BBA-Guideline and OECD-Guideline for the testing of chemicals, using one soil type (loamy sand, BBA type 2.3). The soil was characaterized as follows: pH = 7.0, TOC = 0.90%, CEC (meg/100 g):9.2, Water holding capacity (%): 41.1%, Microbial biomass (% of total soil organic carbon): 8.1, Clay (%) < 2 µm = 11.3, Silt (%) 63 µm to ≥ 2 µm = 24.9, Sand (%) ≥ 63 - 2000 µm = 63.8, N03 -·N (mg/100 g dry weight) = 0.90. Prior to the application of the test materials, the soil was thoroughly mixed with ground lucerne meal. The concentration of the dried lucerne meal was 0.5 % ofthe soil dry weight Before adding the sample the soil moisture was adjusted to 40% WHC. The test contained 3 replicates of 5 treatment groups: untreated soil amended with Lucerne meal; soil amended with Lucerne meal and treated with IN-N0079 at a rates equivalent to 3, 15 and 30 mg IN-N0079/kg soil dry weight (1x, 5x and 10x the maximum recommended seasonal application rate of 7.5 kg Oxamyl/ha in drip irrigation); and soil amended with Lucerne meal and treated with the reference substance, dinoterb, at 5x the maximum field application rate. Production of carbon dioxide by soil microbes and concentrations of ammonium, nitrate, and nitrite were determined periodically, up to 90 days (only nitrogen turnover).

For nitrogen turnover, for each replicate, 800 g soil sub-samples were placed in 1000 mL glass bottles. Short term respiration was measured in soil after the addition of 300 mg glucose/100g soil weight with the OxiTop System". The tests were carried out at 20 ± 2 °C in the dark.

Observations

Samples were collected for determination of nitrogen transformation at 3h, and at days 14, 28. 56 amd 90 following application of the test item.

Short-term respiration was measured in soil samples taken 3 hours, 14 days, 28 days and 56 days after applying test material. The rate of oxygen uptake was was determined within the first 12 hours,following the addition of glucose.

Statistics

The results ofthe nitrification and short-term respiration measurements were submitted to a statistical analysis procedure for a pairwise comparison of samples using the multiple-ttest according to Dunnett (computer program EASY ASSAY, Multiple Testing, Ratte 1995) for significant and non-significant differences between the control and the study groups. The statistical program included a pre-test for homogeneities of variance according to Cochran.

Findings:

Summary of $N0_3^-$ -NH_4^+-N, NO_2^-—*N* and N_{min} (sum of $N0_3^-$-N, NH_4^+ -N, NO_2^--*N)* content of soil (mg/ 100 g dry weight) and the deviation from the control for the assessments on day 1 (3 h), day 14, day 28, day 56 and day 90.

N) content of soil (mg/100g dry weight) and the deviation from the control

Time	3 h	14 d	28 d	56 d	90 d
Control					
NH_4^+-N	b.q.	0.22	b.q.	0.14	b.q.
NO_3^--N	0.90	1.72	2.76	4.52	5.14
NO_2^--N	b.q.	b.q.	b.q.	0.12	b.q.
$N_{min.}$	0.90	1.94	2.76	4.78	5.14
IN-N0079 1X field rate					
NH_4^+-N	0.27	0.20	b.q.	0.12	b.q.
NO_3^--N	1.23	2.14	3.38	5.09	5.41
NO_2^--N	b.q.	b.q.	b.q.	0.10	b.q.
$N_{min.}$	1.50	2.34	3.38	5.31	5.41
Deviation from the control (%)					
NH_4^+-N	-	-9.09	-	-14.29	-
NO_3^--N	36.67	24.42	22.46	12.61	5.25
NO_2^--N	-	-	-	-16.67	-
$N_{min.}$	66.67	20.6	22.46	11.09	5.25
IN-N0079 5X field rate					
NH_4^+-N	0.33	0.20	b.q.	0.12	b.q.
NO_3^--N	0.98	2.00	3.50	5.15	5.77
NO_2^--N	b.q.	b.q.	b.q.	0.10	b.q.
$N_{min.}$	1.31	2.20	3.50	5.27	5.77
Deviation from the control (%)					
NH_4^+-N	-	-9.09	-	-14.29	-
NO_3^--N	8.89	16.28	26.81	11.73	12.26
NO_2^--N	-	-	-	-16.67	-
$N_{min.}$	45.56	13.40	26.81	10.25	12.26
IN-N0079 10X field rate					
NH_4^+-N	0.32	0.17	b.q.	b.q.	b.q.
NO_3^--N	1.11	2.29	3.69	6.02	6.35
NO_2^--N	b.q.	b.q.	b.q.	0.096	b.q.
$N_{min.}$	1.43	2.46	3.69	6.08	6.35
Deviation from the control (%)					
NH_4^+-N	-	-22.73	-	-	-
NO_3^--N	23.33	33.14	33.70	33.19	23.54
NO_2^--N	-	-	-	–20.00	-
$N_{min.}$	58.89	26.80	33.70	27.20	23.54
Reference Substance					
NH_4^+-N	0.24	0.17	b.q.	b.q.	b.q.
NO_3^--N	0.89	1.78	3.54	6.03	7.08
NO_2^--N	b.q.	1.92	b.q.	0.31	b.q.
$N_{min.}$	1.13	3.87	3.54	6.34	7.36
Deviation from the control (%)					
NH_4^+-N	-	-22.73	-	-	-
NO_3^--N	-1.11	3.49	28.26	33.41	37.74
NO_2^--N	-	-	-	158.33	-
$N_{min.}$	25.56	99.48	28.26	32.64	43.19

b.q. = below the limit of quantitation , - no deviation from the control was calculated because the measured values were below the limit of quantitation

A summary of the findings is presented in Table 88. Deviations in the ammonium and nitrate levels and respiration rate in control soil and soil treated with IN-N0079 were <25% at the end of the study.

Table 886 Summary of effects of IN-N0079 on ammonium and nitrate levels and respiration in soil amended with Lucerne meal

	Effect on ammonium levels (%)[a]			Effect on nitrate levels (%)[a]			Effect on nitrite levels (%)[a]			Effect on respiration (%)[a]		
Day	**1X**	**5X**	**10X**	**1X**	**5X**	**10X**	**1X**	**5X**	**10X**	**1X**	**5X**	**10X**
0	-[b]	-	-	36.7	8.9	23.3	-	-	-	31.8	22.7	25.0
14	-9.1	-9.1	-22.7	24.4	16.3	33.1	-	-	-	39.5	30.2	25.6
28	-	-	-	22.5	26.8	33.7	-	-	-	16.7	22.2	19.4
56	-14.3	-14.3	-	12.6	11.7	33.2	-16.7	-16.7	-20.0	2.9	5.7	-8.6
90		-	-	5.3	12.3	23.5	-	-	-	No data	No data	No data

a% Effect: [(measured parameter in control soil -/ measured parameter in treated soil)/ measured parameter in control soil] x100
[b]A dash (-) indicates the effect was below quantitation, thus a % effect cannot be calculated

Effects on nitrate formation concentration and nitrate formation rate at the end of the study (56d).

Nominal IN-N0079 concentration	Mean NO_3^--N Levels (Day 90)		Mean Formation rate NO_3^--N (Day 90)	
	mg/100g dry soil	% Deviation from control[a]	mg /kg/d	% Deviation from control
Control (0.0)	5.14	…	0.47	--
3 mg a.s/kg	5.41	5.25	0.50	6
15 mg a.s/kg	5.77	13.04	0.54	15
30 mg a.s/kg	6.35	23.54	0.60	28
Reference toxicant	7.08	37.74	0.69	47

Negative value = % inhibition, positive value = % stimulation

Conclusion:

IN-N0079, at soil concentrations equivalent to 1x, 5x and 10x the maximum conditions of Oxamyl field use had no significant effect on soil nitrogen transformation (23.5%) or carbon mineralisation (-8.6%). IN-N0079 therefore can be categorised as having low risk to soil microflora.

RMS comments and conclusion

The effects on soil nitrogen transformation study DuPont-4135, originally submitted under EU Rev8 Point IIA 8.5 and conducted with test material IN-N0079 technical metabolite, was conducted under guidelines BBA VI 1-1 (1990), OECD 216 (2000), and OECD 217 (2000). A review of this study was made according to the current guideline (OECD 216, 2000) and the summary has been integrated with additional information and tables.

In the study the results were reported only as NO3- concentration. The RMS calculated the nitrate formation rate as requested in OECD 216 (see table included in the summary). After 90d, at 3 and 15 mg/kg soil dw the effects on nitrate concentration and nitrate formation rate (mg /kg/d) were <25%, at 30 mg/kg soil dw the nitrate quantity formed was <25% different from the control, but the nitrate formation rate (mg /kg/d) was >25% different (actual 28%).

Validity criterion:

The guideline requires a CV of control replicates <= 15% to be valid. In this study, the CV for the nitrate formation rate at 28d was 3.5 %. Fulfilled.

Conclusion: the study is acceptable. After 90d xposure at 3 and 15 mg/kg soil dw the effects on nitrate formation rate (mg /kg/d) were <25% (actual +6% and +15%, respectively).

Study submitted in the EU Dossier in 2001 and included in the first EU approval review.

B.9.5/05

Reference: --	**Report:**	Carter, J. (2001); IN-T2921: Assessment of the effects on soil microflora **DuPont Report No.:** DuPont-4736 **Guidelines:** OECD 216 (2000), OECD 217 (2000), BBA VI 1-1 (1990)

1. Test material: IN-T2921 technical metabolite
   Lot/Batch #: T2921-2
   Purity: 98.7%

Materials and methods:

The effects of IN-T2921 (purity 98.7%) on soil microbial respiration (carbon mineralisation) and nitrogen transformations (ammonification and nitrification) were examined in a typical agricultural sandy loam soil in a laboratory study. The test was conducted in accordance with BBA-Guideline and OECD-Guideline for the testing of chemicals, using one soil type (sandy loam). The soil was characaterized as follows: pH = 7.0, TOC = 1.2%, CEC (meg/100 g): 11.3, Water holding capacity (%): 42.7%, Microbial biomass (% of total soil organic arbon): 2.93, Clay (%) < 2 µm = 9, Silt (%) 63 µm to ≥ 2 µm = 26, Sand (%) ≥ 63 - 2000 µm = 65, N03 -·N (mg/100 g dry weight) = 0.90.

The test contained 3 replicates of 5 treatment groups: untreated soil amended with Lucerne meal; soil amended with Lucerne meal and treated with IN-T2921 at a rates equivalent to 3.53, 17.65 and 35.3 mg IN-T2921/kg soil dry weight (1x, 5x and 10x the maximum recommended seasonal application rate of 7.5 kg Oxamyl/ha in drip irrigation); and soil amended with Lucerne meal and treated with the reference substance, Dinoseb acetate (20 mg /kg soil nitrogen transformation test and 100 mg /kg soil in the carbon transformation test), The concentration of lucerne meal (for nitrogen transformation only) was 0.5 % of the soil dry weight. The moisture content of each soil group was adjusted to 40% of its maximum water holding capacity. Following preparation, the soil groups were incubated in the dark in a temperature controlled room set at 20°C ± 2°C.

The glucose amended soil samples were distributed into respirometer flasks in 100 g quantities. Carbon dioxide measurements were started within 1 to 2 hours following the addition of glucose to the soil samples.

Production of carbon dioxide by soil microbes and concentrations of ammonium, nitrate, and nitrite were determined periodically, up to 90 days (only nitrogen turnover).Observations

Samples were collected for determination of nitrogen transformation at days 0, 7, 14, and 28 following application of the test item.

Short-term respiration was measured in soil samples taken 0, 7, 14, and 28 days after applying test material. The rate of oxygen uptake was was determined within the first 12 hours,following the addition of glucose.

Statistics

Due to the large numbers of treatments in this study, the analysis was performed on subsets of treatments. Each subset consisted of one non-treated control group and one or two treated groups. For the ammonia and nitrate ion levels the treated groups of the first subset were the 1 x PEC, the 5 x PEC and the Dinoseb acetate group. Whilst the treated group for the second subset was the 10 x PEC group. For the carbon transformation levels there were three subsets with respectively following treated groups, the 1 x PEC and the 5 x PEC group, the lOx PEC group, and the Dinoseb acetate group.

For each subset, one-way analyses of variance were applied to the ammonia and the nitrate levels for each day separately. This was followed by Dunnett's test (Dunnett 1955, 1964) to compare the test substance treated groups with the non-treated control group and by Student's z-test to compare the Dinoseb acetate treated group with the non-treated control group. Values of p less than 0.05 would normally be considered significant.

Analysis of nitrate levels in soil - group mean values
(µg N/g dry weight soil)

Treatment Group	Day 0			Day 7		
	n	Mean	sd	n	Mean	sd
Control	3	18.53	1.064	3	32.12	2.133
1 x PEC	3	17.89	1.309	3	38.77	1.536
5 x PEC	3	18.47	0.561	3	42.62**	2.165
Dinoseb	3	17.46	1.885	3	18.77+++	5.111
Sed		1.06			2.51	
Treat1		0.717			<0.001	
	Day 14			Day 28		
Group	n	Mean	sd	n	Mean	sd
Control	3	31.54	7.380	3	48.38	4.856
1 x PEC	3	37.20	13.417	3	57.38	5.736
5 x PEC	3	48.00	4.623	3	56.78	7.978
Dinoseb	3	39.15	6.975	3	78.65+++	3.620
Sed		7.12			4.71	
Treat1		0.219			0.001	

Treatment Group	Day 0			Day 7		
	n	Mean	sd	n	Mean	sd
Control	3	16.33	1.200	3	32.16	0.402
10 x PEC	3	16.76	1.973	3	40.93	6.088
Sed		1.33			3.52	
Treat2		0.767			0.068	
	Day 14			Day 28		
Group	n	Mean	sd	n	Mean	sd
Control	3	32.81	7.918	3	50.63	12.361
10 x PEC	3	42.72	13.458	3	51.54	11.431
Sed		9.02			9.72	
Treat2		0.333			0.930	

n	Number of replicates
sd	Standard deviation
sed	Standard error of the difference between groups.
Treat1	Significance level of treatment variance ratio for comparison between groups.
Treat2	Significance level of treatment variance ratio for comparison between groups, which in this case this is equivalent to Student's *t*-test.
+++	Significance level of comparison with control group, using Student's *t*-test: $p<0.001$
**	Significance level of comparison with control group, using Dunnett's test : $p<0.01$

Findings:

A summary of the findings is presented in Table 89. Deviations in the ammonium and nitrate levels and respiration rate in control soil and soil treated with IN-T2921 were <25% at the end of the study.

Table 897 Summary of effects of IN-T2921 on ammonium and nitrate levels and respiration in soil amended with Lucerne meal

Application rate	Concentration (mg IN-T2921/kg soil)	% Deviation from non-treated control group after 28 days		
		Nitrogen transformation		Carbon transformation
		Ammonium	Nitrate	
1 × PEC	3.53	0	+18.6	-7.3 to +2.8
5 × PEC	17.65	0	+17.36	+8.4 to +14.4

10 × PEC	35.3	0	+1.8	-10.6 to -7.2

Effects on nitrate formation concentration and nitrate formation rate at the end of the study (56d).

Nominal IN-T2921 concentration	Mean NO_3^--N Levels (Day 28)		Mean Formation rate NO_3^--N (Day 28)	
	mg/100g dry soil	% Deviation from control[a]	mg /kg/d	% Deviation from control
Control 1	48.3	--	1.1	--
3.53 mg a.s/kg	57.38	18.6	1.4	27
17.65 mg a.s/kg	56.78	17.4	1.4	27
Reference toxicant	78.65	62.4	2.2	100
Control 2	50.63	--	1.2	--
35.3 mg a.s/kg	51.5499	1.8	1.2	0

Negative value = % inhibition, positive value = % stimulation

Conclusion:

IN-T2921, at soil concentrations equivalent to 1x, 5x and 10x the maximum conditions of Oxamyl field use had no significant effect on soil nitrogen transformation (maximum 18%) or carbon mineralisation (-10.6 to –7.2%). IN-T2921 therefore can be categorised as having low risk to soil microflora.

RMS comments and conclusion

The effects on soil nitrogen transformation study DuPont-4736, originally submitted under EU Rev8 Point IIA 8.5 and conducted with test material IN-T2921 technical metabolite, was conducted under guidelines OECD 216 (2000), OECD 217 (2000), and BBA VI 1-1 (1990).

A review of this study was made according to the current guideline (OECD 216, 2000) and the summary has been integrated with additional information and tables.

In the study the results were reported only as NO3- concentration. The RMS calculated the nitrate formation rate as requested in OECD 216 (see table included in the summary). After 28d, at 3.53, 17.65, and 35.3 mg/kg soil dw the effects on nitrate concentration were <25%, but the nitrate formation rate (mg /kg/d) was >25% different (actual 27%) after exposure to 3.53 and to 17.65 mg/kg soil dw (first subset of data), while it was <25% (actual 0%) at the 10x concentration of 35.3 mg/kg soil dw (second subset of data).. The contradicting results are possibly due to the fact that the statistical analysis were done on two subset of data, using two different controls, as indicated in the raw data reported above.

Validity criterion:

The guideline requires a CV of control replicates <= 15% to be valid. In this study, the CV for the nitrate formation rate at 28d was 5.7 % in the control reported in the first subset of data and 7.3% in the second control. Fulfilled.

Conclusion: results are contradictory and therefore the study is inconclusive. Clarification should be given about the different data reported for the controls in the two subsets of data used for analysis.

Summary of soil microflora endpoints

Table 908 Effects of Oxamyl 10GR, Oxamyl 10SL and metabolites on non-target soil microorganisms

Test item	Test	Test concentrations (mg a.s./kg soil d.w.)	Corresponding application rates relative to parent compound oxamyl (worst case Oxamyl 10GR application)	Parameter	% effect[b]	Reference
Oxamyl technical	28-day laboratory	12.0, 60.0	3x, 15x	Nitrate formation rate	<25% (-2,-2)	RF-0014.218.286.07[c]
Oxamyl 10GR	Not valid					DuPont-3793[d]
Oxamyl 10SL	28-day laboratory	1.5kg/ha 15 kg/ha 23 mg a.s./kg soil	< 1x <3x (solarization) 7x (drip irrigation, tomato) PEC not available	Nitrate formation rate	<25% (-10) <25% (+16) <25% (+23%)	DuPont-4114[e]
IN-A2213	56-day laboratory	4.9 49	3× 32×	Nitrate formation rate	<25% (+9.7) >25% (+35%)	DuPont-4131[c]
IN-D2708	Not valid					DuPont-4133[c]
IN-N0079	56-day laboratory	3.0, 15 30	16×, 82×, 163×	Nitrate formation rate	<25% (+6, +15) >25% (+28)	DuPont-4135[c]
IN-T2921	28-day laboratory Contradictory data Not reliable	3.53, 17.65 35.3	no PEC	Nitrate formation	>25% (+27, +27) <25% (0%)	DuPont-4736[c]

[a] Not corrected for purity.

[b] % deviation from the control.

[c] Studies cited or summarised in this document.

[d] Study cited or summarised in the Oxamyl EU Renewal Dossier, Document M-CP, Section 10 for Oxamyl 10GR, DuPont-40954 EU.

[e] Study cited or summarised in the Oxamyl EU Renewal Dossier, Document M-CP, Section 10 for Oxamyl 10SL, DuPont-42130 EU.

B.9.6 Effects on terrestrial non-target higher plants

B.9.6.1 Summary of screening data

Oxamyl was a new compound discovered in the 1970s. Early screening data may have been collected at the time, but are no longer available. The current document summarises available data to support this renewal dossier.

B.9.6.2 Testing on non-target plants

Seedling emergence

Study submitted in the EU Dossier in 2001 and included in the first EU approval review.

B.9.6.2/01

Reference: --	**Report:**	Porch, J. (2001); Oxamyl (DPX-D1410) 24L: A greenhouse study to investigate the effects on seedling emergence and early growth of ten terrestrial plant species **DuPont Report No.:** DuPont-5817 **Guidelines:** U.S. EPA 850.4100 (1996)

1. Test material: Oxamyl 24SL
   Lot/Batch #: D1410-421
   Purity: 240 g a.s./L

Materials and methods:

Non-target terrestrial plant response to Oxamyl 24L (Oxamyl 24 SC, 24% w/v Oxamyl) was evaluated on 10 common plant species. Effects on seedling emergence and early growth following soil surface application to planted seeds prior to emergence were assessed. Limit testing in the greenhouse (Tier 1) conducted at the maximum U.S. rate of 2.24 kg a.s./ha on all 10 species was conducted since preliminary testing indicated that none of the species would demonstrate 25% or greater effect. Tests were conducted in a low organic fine textured artificial sandy loam soil (pH 7.7, O.M. 3.1%, CEC, 9.8 meq/100 g). The test system was comprised of 8 replicates of 10 seeds/test rate and control. Standard plastic pots (approximately 16 cm diameter, 11 cm deep, with bottom drain holes) were used for all test species. The test item was applied to the soil surface immediately after sowing. Applications were made with a travelling belt laboratory sprayer calibrated to apply approximately 375 L/ha. Samples collected following test solution preparation were analyzed to confirm the test substance

concentration. Test duration was 21 days. The following conditions were maintained in the greenhouse for the duration of the tests: Temperature between 17.8°C and 38.8°C, Relative humidity ranging from 23.4 to 85.3%, Photoperiod of 16 hours with natural light augmented with high pressure sodium lamps. Total daily accumulated light intensity between 12.5 and 22.0 E/m2 (217µ and 382 µE/m2, respectively).

The number of emerged plants and living seedlings was recorded on days 7, 14, and 21. The number of surviving plants, mean shoot height, total plant dry weight, and a numeric visual rating of plant condition were recorded on day 21.

Means and standard deviations were calculated and the data were analyzed using Dunnett's t-test (= 0.05). Treatment group means were compared to the control means to determine if a 25% or greater adverse effect on seedling emergence, shoot height, or shoot weight was observed. Percent visual response was recorded as 100% - % visual response (as it is expressed in the raw data), such that 0% indicates no visual response and 100% indicates plant death or near death.

Findings:

The following table gives a summary of the mean % inhibition relative to the untreated control at 21 days for each species.

Table 99 Effects of Oxamyl 24 SC on emergence and early seedling growth (soil surface application prior to emergence)

Plant	Genus Species	% Inhibition on Day 21a			
		Emergence	Visual response	Total shoot dry wt.	Mean shoot ht.
Corn m	*Zea mays*	-1	-3	-12	-6
Oat m	*Avena sativa*	7	3	-14	-7
Onion m	*Allium cepa*	3	1	-1	0
Sorghum m	*Sorghum bicolor*	1	-1	12	7
Cucumber d	*Cucumis sativus*	1	-1	-14	-6
Oilseed rape d	*Brassica napus*	7	1	0	-8
Pea d	*Pisum sativum*	1	12	0	6
Soybean d	*Glycine max*	1	0	2	5
Sugar beet d	*Beta vulgaris*	-14	-4	9	-3
Tomato d	*Lycopersicon esculentum*	12*	0	16*	13*

[a] based on means of 8 replicates each comprised of 10 seeds and are reported for the most sensitive parameter.
* treatment group and control means are significantly different (t-test, $p<0.05$).

m- monocot; d =dicot.

Conclusion:

Response for 9 of the 10 species tested was not statistically different than the untreated control plants. Tomato emergence, total shoot dry weight and mean shoot height were statistically different than the control but the reduction was only 16%. Oxamyl 24 SL applied prior to emergence at the maximum U.S. labelled rate of 2.24 kg a.s./ha did not have an adverse impact on the 10 terrestrial plant species tested.

RMS comments and conclusion

The testing on non-target plants study DuPont-5817, originally submitted under EU Rev8 Point IIA 8.6 and conducted with test material Oxamyl 24SL, was conducted under guideline U.S. EPA 850.4100 (1996). The Applicant claims that this study fully meets the current guideline (OECD 208 [2006]) and that the study was reviewed by EFSA (2005) and used to select the nontarget plant endpoints. Therefore this study is relied upon.

A review of this study was done by the RMS based on the current guideline OECD 208 (2006).

The following deviations were noted: Lower relative humidity and higher temperature than recommended.

Each pot contained a total of ten seeds, while OECD recommends that big seeds (corn, soybean, tomato , cucumber, sugarbeet,) should be planted as one to two in a 15cm pot and rape or pea as three/pot.

In order for the test to be considered valid, the following performance criteria must be met in the controls:

• the seedling emergence is at least 70%: Fulfilled.

• the seedlings do not exhibit visible phytotoxic effects (e.g. chlorosis, necrosis, wilting, leaf and stem deformations) and the plants exhibit only normal variation in growth and morphology for that particular species: The conditions of control seedlings at 21d rated less than 100% normal in corn (97.5%), oat (97.5%), sugarbeet (96.3%), tomato (97.5%). Not completely fulfilled for all the species.

• the mean survival of emerged control seedlings is at least 90% for the duration of the study: a lower survival was recorded for oat (88.8%), sugarbeet (76.2), rape (87.5%), pea (85%). Not fulfilled for the listed species.

• environmental conditions for a particular species are identical and growing media contain the same amount of soil matrix, support media, or substrate from the same source. Fulfilled.

Conclusion: for four species, the validity criterion for seedling survival is not fulfilled. For several species the number of plants/pot were was high. Taking into account that the study was a limit test, the results can be used as **additional information.**

Vegetative vigour

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

B.9.6.2/02

Reference: CA 8.6.2/01	**Report:**	Bergfield, A. (2012); Oxamyl (DPX-D1410) 24SL: A greenhouse study to investigate the effects on vegetative vigor of ten terrestrial plant species following foliar exposure **DuPont Report No.:** DuPont-34275 **Guidelines:** U.S. EPA 123-1 (1992), OPPTS 850.4150 (1996), OPPTS 850.4250 (1996) **Deviations:** None **Testing Facility:** ABC Laboratories, Inc. (Missouri), Columbia, Missouri, USA **Testing Facility Report No.:** 68034 **GLP:** Yes **Certifying Authority:** Laboratories in the USA are not certified by any government agency, but are subject to regular inspections.

Executive summary:

Non-target terrestrial plant response to Oxamyl 24SL was evaluated on ten common plant species. Non-target terrestrial plant response to Oxamyl 24SL was evaluated on ten common plant species. The formulation contains solvents and is not approved in the EU. It is considered a worst case exposure scenario and would elicit greater responses compared to Oxamyl 10SL. Effects on vegetative vigour following a foliar application to seedlings were assessed. Testing was conducted at eight rates up to 2.24 kg a.s./hectare. Tests were conducted in non-sterile loamy sand soil (O.M. 2.1%, pH 5.6) or sandy loam soil (O.M. 2.0%, pH 5.9) under greenhouse conditions. For each species, the test system was comprised of six replicates of five plants each (30 plants) per test rate and control. Applications were made with a track sprayer calibrated to apply approximately 300 L water/ha. Test duration was 21 days. The number of plants surviving and numeric visual rating of plant conditions were recorded on Days 7, 14, and 21. Shoot height of all surviving plants was measured on Day 21. Dry weight was determined from plant shoots collected at test termination on Day 21. The rates producing the NOEC, ER_{50} and ER_{25} (rate resulting in 50% and 25% inhibitory response, respectively) were determined, when possible. The ER_{50} is >2.24 kg a.s./ha for all monocot and dicot species tested.

I. MATERIALS AND METHODS

A. MATERIALS

1.	Test material	Oxamyl 24SL
	Lot/Batch #:	D1410-524
	Purity:	24.5% (w/w) oxamyl by analysis
	Description:	Soluble concentrate
	CAS#	23135-22-0 for active ingredient
	Stability of test compound:	Not determined in the test system
2.	Control:	Domestic well water demineralized by reverse osmosis and deionized
	Test vehicle:	Domestic well water demineralized by reverse osmosis and deionized
	Toxic reference:	None
3.	Test organisms	Terrestrial Plants
	Species:	*Zea mays* (corn) *Avena sativa* (oat) *Allium cepa* (onion) *Lolium perenne* (perennial ryegrass) *Cucumis sativus* (cucumber) *Pisum sativum* (pea) *Brassica napus* (oilseed rape) *Glycine max* (soybean) *Beta vulgaris* (sugar beet) *Lycopersicon esculentum* (tomato)
	Age at dosing:	1 to 4 leaf stage depending on species
	Initial population:	6 replicates of 5 plants (30 plants total) each per treatment and control. Each plant was potted separately.
	Test chamber:	Plastic pots with drain holes, (10 cm diameter × 7 cm depth for onion and ryegrass, 15 cm diameter × 10 cm depth for all other species)
	Growth medium:	Natural sandy loam soil (O.M. 2.0%, pH 5.9, Organic Carbon 1.2%, CEC 10.7 meq/100 g) or loamy sand soil (O.M. 2.1%, pH 5.6, Organic Carbon 1.2%, CEC 10.3 meq/100 g)
	Watering:	Laboratory well water; initial top watering (foliage avoided) followed by sub-irrigation
4.	Environmental conditions (in-life period)	
	Temperature:	15.4 to 39.5°C
	Relative Humidity:	26 to 97%
	Photoperiod:	Average of 14 to 15 E/m^2 with 15-16 hours of daily light

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed

05 July to 26 July 2012 for corn, oat, cucumber, pea, oilseed rape, soybean, sugar beet, and tomato
22 August to 12 September 2012 for onion and perennial ryegrass

2. Experimental treatments

The effects of a surface (foliar) application of Oxamyl 24SL on the vegetative vigour of non-target plants were evaluated in a 21-day study. Seeds of corn, oat, onion, perennial ryegrass, cucumber, oilseed rape, pea, soybean, sugar beet, and tomato were planted at uniform depth (depth depending on species) in pots maintained in a greenhouse under controlled conditions. Natural soil loamy sand soil (O.M. 2.1%, pH 5.6) or sandy loam soil (O.M. 2.0%, pH 5.9) was used as a growth matrix. Nominal application rates of 0 (control), 0.0175, 0.0350, 0.0700, 0.140, 0.280, 0.560, 1.12, and 2.24 kg a.s./ha were used for all species. Applications were made using an automated laboratory track sprayer calibrated to apply approximately 300 L water/ha. Samples collected following test solution preparation were analyzed to confirm the test substance concentration. The average mean measured concentration (by HPLC) for the application on 05 July 2012 was 110% of nominal and on 22 August 2012 was 123% of nominal. The nominal test concentrations were used to calculate study endpoints. For each

species the test was comprised of 6 replicates of 5 individual plants per replicate equalling 30 plants per rate of test item and control. Plants were arranged in a randomized complete block by species.

The test units were filled with soil. Three seeds were planted in each test pot. The seeds were planted and held under greenhouse conditions until they reached the appropriate size for testing (typically 1-4 leaf stage, 5-12 cm tall). One to three weeks after planting, depending on the species, the plants were thinned to one plant per test unit to minimize competitive effects and allow individual plant measurements. At application the plants had two to four leaves.

3. Observations

The visual response of the seedlings and number of surviving plants were recorded on Days 7, 14, and 21 after application. Additionally, shoot height and shoot dry weight were determined on Day 21.

4. Statistics

Shapiro-Wilk's test and Levene's test: Used to test data for normality and homogeneity of variance.

The data were analysed to determine the NOEC using an analysis of variance (ANOVA) and the Jonckheere-Terpstra test.

If there was a dose-response in the data for a given parameter, non-linear regression analysis (4-parameter logistic model) was used to determine the ER_{25}, ER_{50}, and their 95% confidence levels.

II. RESULTS AND DISCUSSION

A. FINDINGS

The results expressed as the ER_{50} as determined for the most sensitive parameter (shoot weight, shoot height, or all parameters) following foliar application of Oxamyl 24SL are summarised in the following table. No significant effects were observed on any of the measured parameters for any of the species tested. The effects observed at all concentrations did not indicate a monotonic response and are therefore not considered treatment related. A significant response was not confirmed by the effects noted in the other measured parameters.

Table 100 Effects of Oxamyl 24SL on ten non-target plants following post-emergent application (foliar application)

Species	Family	Genus/species	Oxamyl 24SL ER_{50} (kg a.s./ha)[a]	Parameter
Monocots:				
Corn	Gramineae	*Zea mays*	>2.24	All parameters
Oat	Gramineae	*Avena sativa*	>2.24	All parameters
Onion	Liliaceae	*Allium cepa*	>2.24	All parameters
Perennial Ryegrass	Gramineae	*Lolium perenne*	>2.24	All parameters
Dicots:				
Cucumber	Cucurbitaceae	*Cucumis sativus*	>2.24	All parameters
Pea	Leguminosae	*Pisum sativum*	>2.24	All parameters
Oilseed Rape	Brassicaceae	*Brassica napus*	>2.24	All parameters
Soybean	Leguminosae	*Glycine max*	>2.24	All parameters
Sugar Beet	Chenopodiaceae	*Beta vulgaris*	>2.24	All parameters
Tomato	Solanaceae	*Lycopersicon esculentum*	>2.24	All parameters

[a] Oxamyl 24SL nominally contains 24% a.s.

III. CONCLUSIONS

The NOEC, ER_{25}, and ER_{50} for Oxamyl 24SL (based on all endpoints) is >2.24 kg a.s./ha for all monocot species tested.

The NOEC, ER_{25}, and ER_{50} for Oxamyl 24SL (based on all endpoints) is >2.24 kg a.s./ha for all dicot species tested**.**

(Bergfield, A., 2012)

RMS comments and conclusion

The study was conducted under guideline U.S. EPA 123-1 (1992), OPPTS 850.4150 (1996), OPPTS 850.4250 (1996). A review of this study was done taking into account the current guideline OECD 227 (2006). The following deviations were noted: Lower relative humidity and higher temperature than recommended.

Validity criteria:

- the seedling emergence is at least 70 %. Not given in the test report. The % germination of the seeds lots is listed as provided by the supplier or by the Missouri Crop Improvement Association.

In the controls:
- the plants do not exhibit visible phytotoxic effects (e.g. chlorosis, necrosis, wilting, leaf and stem deformations). Plants exhibit only normal variation in growth and morphology for that particular species. The score for all species at 21d was 100% (no effect), except for pea 97%) and oilseed rape (98%), Not always completly fulfilled.
- the mean plant survival is at least 90 % for the duration of the study (actual 100%). Fulfilled.
- environmental conditions for a particular species are identical and growing media contain the same amount of soil matrix, support media, or substrate from the same source. Fulfilled

Conclusion:

One validity criterion cannot be verified. Considering the complete lack of the effect up to the highest tested concentration, the study can be considered **supportive of the** low toxicity to plants for the vegetative vigor.

B.9.6.3 Summary of screening data

B.9.6.4 Testing on non-target plants

B.9.7 Effects on other terrestrial organisms (flora and fauna)

Study submitted in the EU Dossier in 2001 and included in the first EU approval review.

B.9.7/01

Reference: --	**Report:**	Daly, R., Leva, D., Brugger, K. (2000); Summary of insecticide screening data for major metabolites of oxamyl **DuPont Report No.:** DuPont-5194 **Guidelines:** Not given

1. Test material: IN-A2213 technical metabolite, IN-D2708 technical metabolite, IN-N0079 technical metabolite, IN-T2921 technical metabolite
   Lot/Batch #: Not given
   Purity: Not given

Materials and methods:

The effects of four metabolites of Oxamyl (IN-A2213, IN-D2708, IN-N0079, and IN-T2921) were tested in standard 6-day insecticide assays using five insect species (Corn Plant Hopper, *Peregrinus maidis* (CPH); Two-Spotted Spider Mite, *Teranichus urticae* (TSSM); Green Peach Aphid, *Myzus persicae* (GPA); Fall Armyworm, *Spodoptera frugiperda* (FAW), and Southern Corn Rootworm, *Diabrotica undecimpunctata* (SCRW)). Treatments consisted of three replicates each of the four metabolites tested at 10, 50 and 250 ppm. Water controls, solvent controls, and toxic references were also tested. Effects were recorded 6 days after spray for CPH, TSSM, GPA and FAW and 48 hours after spray for SCRW. In total approximately 30 to 60 CPH, 30 to 60 TSSM, 90 to 150 GPA, and 15 FAW were tested per treatment. The controls and standards met activity thresholds for each species tested, therefore the test is considered valid. Activity thresholds are defined as follows: compounds are considered insecticidally active if they exhibit >80% mortality for CPH and GPA, >95% mortality for TSSM, a foliar feeding effect <5 for FAW, and >67% plant emergence or >80% mortality for SCRW. Percent mortality was recorded for CPH, GPA, and TSSM. Plant feeding damage was recorded for FAW. Plant emergence and percent mortality were recorded for SCRW.

Findings:

Four metabolites of Oxamyl (IN-A2213, IN-D2708, IN-N0079, and IN-T2921) show no insecticidal activity on five species of insects used in standard insecticide screens. There are no apparent differences in results from metabolites and controls (untreated and solvent). All untreated and solvent checks and standard data were within historical control values for this laboratory. The Southern Corn Rootworm plant emergence assay seemed to show some plant protection.

Table 101Percent mortality, plant damage due to feeding, or plant emergence of five test species after spray application of Oxamyl metabolites

		Corn Plant Hoppe	Two-Spotted Spider Mite	Green Peach Aphid	Fall Armyworm	Southern Corn Rootworm	
Compound	Rate (ppm)	% Mortality			Plant Feeding Damage[1]	Plant Emergence	% Mortality
Untreated	---	12	2	13	10	0	0
Solvent	---	3	0	20	10	0	0
IN-A2213	10	0	10.7	<20	10	50	---
	50	3.3	6.6	<20	10	33	6.7[2]
	250	3.7	3.7	<20	10	66	6.7
IN-D2708	10	3.8	5.7	<20	10	33	---
	50	0	2.6	<20	10	66	0
	250	0	5.7	<20	10	50	0
IN-N0079	10	3.6	4.7	<20	10	66	---
	50	1.3	4.6	<20	10	66	0
	250	1.2	3.9	<20	10	100	0
IN-T2921	10	3	1	<20	10	33	---
	50	0	8.9	<20	10	0	6.7

	250	0	4.5	<20	10	0	6.7

1 Foliar feedings are based on a 0 to 10 scale; O = no feeding/10 = total feeding
2 Fifteen SCRW were used in each test; 1 dead SCRW = 6.7% effect.

Conclusion:

Major metabolites of Oxamyl (IN-A2213, IN-D2708, IN-N0079, and IN-T2921) show no insecticidal activity on five species of insects used in standard insecticide screens. Comparisons of results from metabolites with the controls (untreated and solvent) yield no apparent differences. The SCRW assay seemed to show some plant protection by reducing feeding, but when the compounds were sprayed directly on the SCRW larvae, there was no insecticidal activity.

RMS comments and conclusion

The effects on other terrestrial organisms (flora and fauna) study DuPont-5194, originally submitted under EU Rev8 Point IIA 8.6 was conducted with test materials IN-A2213 technical metabolite, IN-D2708 technical metabolite, IN-N0079 technical metabolite, and IN-T2921 technical metabolite. Guidelines were not given. The study has been accepted during the first first EU approval review. Since there is no current guideline for an insecticide activity screening study, the study is relied upon**.**

B.9.8 Effects on biological methods for sewage treatment

Study submitted in the EU Dossier in 2001 and included in the first EU approval review.

B.9.8/01

Reference: --	**Report:**	Hertl, J. (2000); Oxamyl technical: Activated sludge, respiration inhibition test **DuPont Report No.:** DuPont-3348 **Guidelines:** OECD 209 (1984)

1. Test material: Pure Oxamyl (PAI)
   Lot/Batch #: D1410-196
   Purity: 96.9%

Materials and methods:

Effects of Oxamyl on biological methods for sewage treatment were determined in a 3-hours exposure laboratory study. Activated sludge with micro-organisms was obtained from a sewage plant and conditioned into an activated sludge suspension. The test flask for Control A was prepared at time "0" by adding 16 mL of synthetic sewage raised to a volume of 300 mL with tap water. The test was initiated by adding 200 mL of activated sludge inoculum. Approximately every 15 minutes, the above procedure was repeated, though the test substance and toxic reference treatments contained a known concentration of Oxamyl and 3,5-Dichlorophenol in the initial 300-mL solution, respectively. To initiate the study, 200 mL of activated sludge inoculum (containing 3 g dry weight/L) was first added to the Control A flask. At approximately 15-minute intervals, this procedure was repeated for the toxic reference and test substance treatments. The test was conducted between 19 – 20°C with a pH of 7. There were five test substance concentrations tested with one replicate per concentration, 100.0, 32.0, 10.0, 3.2 and 1.0 mg Oxamyl/L. Two controls (Control A and Control B – tap water and synthetic sewage inoculum) were tested in parallel. The toxic reference, 3,5-Dichlorophenol was tested at 32.0, 10.0, and 3.2

mg/L. The application order was as follows: Control A, reference substance concentrations, test substance concentrations, and Control B.

Findings:

The respiration rate in the control was determined to be 0.506 mg O2/liter-min. The respiration rates and percent inhibition values for the test substance groups of Oxamyl are summarised in the table below (Table 91). The sensitivity of the activated sludge to the toxic reference 3,5-dichlorophenol was within the range of 5-30 mg/L ($EC_{50}$for 3 hours incubation period was 5.4 mg/L); this is within accepted limits, indicating the validity of this test.

Table 912 Effects of Oxamyl on biological methods for sewage treatment

Test Substance (mg/L)	Mean respiration rate (mg O2/L*min)	Inhibition of respiration rate (%)
Control A	0.506	-
Control B	0.506	-
1.0	0.500	1.2
3.2	0.450	11.1
10.0	0.489	3.4
32.0	0.410	19.0
100.00	0.400	20.9

* negative values indicate an activation of the respiration rate

Conclusion:
Oxamyl exhibited a respiratory inhibition effect of 20.9% to activated sludge at 100 mg/L, the highest concentration tested. The EC_{50}value could not be determined due to the lack of any inhibitory effect of the test substance. However, it is clear that the EC_{50}value is greater than 100 mg/L because at this rate the percent inhibition was less than 50%. The EC20 value was 71.6 mg Oxamyl/L.

RMS comments and conclusion

The effects on biological methods for sewage treatment study DuPont-3348, originally submitted under EU Rev8 Point IIA 8.7 and conducted with test material pure oxamyl (PAI), was conducted under guideline OECD 209 (1984). The study has been re-evaluated according to the current OECD 209 (2010) and some differences in the test methology are expected.

To obtain both a NOEC and an ECx (e.g. EC50), the current guideline recommends six controls and five treatment concentrations in a geometric series with five replicates. In the study only one repliacate for test concentration was set (two in the control).

The present data set allows to conclude an EC50 > 100 mg/L. The EC20 is not reliable because of the very partial curve.

The RMS has recalculate the oxygen uptake in the control as 25 mg oxygen/g dw solid. The CV in control replicates was <30%. The validity criteria are fulfilled.

Conclusion: the test in acceptable. EC50 > 100 mg/L.

B.9.9 Monitoring data

No monitoring data is available for this active substance

B.9.10 Biological activity of metabolites potentially occurring in groundwater

Please refer to point B.9.8.

B.9.11 References relied on

List of information, tests and studies which are considered as relied upon by the RMS for the evaluation with a view to the approval of the active substance.

Studies marked in yellow are submitted for the first time.

Sorted by Annex Point

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner
B.9.3.2.1/02	Austin, H.	1999	Oxamyl 10L (10% w/w): A laboratory study to evaluate the effects on the aphid parasitoid *Aphidius rhopalosiphi* (Hymenoptera: Braconidae) Ecotox Ltd. DuPont-2609 Previously submitted at the EU level for Annex I inclusion Published: No	N	N		DuPont
B.9.6.2/02	Bergfield, A.	2012	Oxamyl (DPX-D1410) 24SL: A greenhouse study to investigate the effects on vegetative vigor of ten terrestrial plant species following foliar exposure ABC Laboratories, Inc. (Missouri) DuPont-34275 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 submitted	DuPont
B.9.2.1/06	[redacted]	2000	IN-T2921: Acute, 96-hour LC_{50} to rainbow trout, *Oncorhynchus mykiss* [redacted] DuPont-4439 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner
B.9.2.4.1/05	Boeri, R.L., Ward, T.J.	2000	IN-T2921: Acute, 48-hour, EC_{50} to *Daphnia magna* T.R. Wilbury Laboratories, Inc. DuPont-4441 Previously submitted at the EU level for Annex I inclusion Published: No	N	N		DuPont
B.9.2.5.1/01	Boeri, R.L., Ward, T.J.	2000a	Oxamyl technical: 21-day chronic, flow‑through toxicity to *Daphnia magna* T.R. Wilbury Laboratories, Inc. DuPont-2554 Previously submitted at the EU level for Annex I inclusion Published: No	N	N		DuPont
B.9.2.5.1/02	Boeri, R.L., Ward, T.J.	2000b	IN-D2708: Chronic, static-renewal toxicity to the daphnid, *Daphnia magna* T.R. Wilbury Laboratories, Inc. DuPont-3909 Previously submitted at the EU level for Annex I inclusion Published: No	N	N		DuPont
B.9.2.6.1/05	Boeri, R.L., Ward, T.J.	2001	IN-T2921: Influence on growth and growth rate of the alga, *Selenastrum capricornutum* T.R. Wilbury Laboratories, Inc. DuPont-4442 Previously submitted at the EU level for Annex I inclusion Published: No	N	N		DuPont

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner
B.9.2.1/04	[redacted]	1999	IN-D2708: Static, acute, 96-hour, (LC_{50}) test to rainbow trout, *Oncorhynchus mykiss* [redacted] DuPont-2507 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont
B.9.2.4.1/01	Boeri, R.L., Magazu, J.P., Ward, T.J.	1999b	Oxamyl technical: Acute, 48-hour EC_{50} to *Daphnia magna* T.R. Wilbury Laboratories, Inc. DuPont-2553 Previously submitted at the EU level for Annex I inclusion Published: No	N	N		DuPont
B.9.2.4.1/03	Boeri, R.L., Magazu, J.P., Ward, T.J.	1999a	IN-D2708: Static, acute, 48-hour limit test to *Daphnia magna* T.R. Wilbury Laboratories, Inc. DuPont-2510 Previously submitted at the EU level for Annex I inclusion Published: No	N	N		DuPont
B.9.2.6.1/01	Boeri, R.L., Magazu, J.P., Ward, T.J.	2000	Oxamyl technical: Influence on growth and growth rate of the alga, *Selenastrum capricornutum* T.R. Wilbury Laboratories, Inc. DuPont-2909 Previously submitted at the EU level for Annex I inclusion Published: No	N	N		DuPont

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner
B.9.2.6.1/03	Boeri, R.L., Magazu, J.P., Ward, T.J.	1999b	IN-D2708: Influence on growth and growth rate of the alga, *Selenastrum capricornutum* T.R. Wilbury Laboratories, Inc. DuPont-2511 Previously submitted at the EU level for Annex I inclusion Published: No	N	N		DuPont
B.9.2.6.1/04	Boeri, R.L., Magazu, J.P., Ward, T.J.	1999a	IN-N0079: Influence on growth and growth rate of the alga, *Selenastrum capricornutum* T.R. Wilbury Laboratories, Inc. DuPont-2514 Previously submitted at the EU level for Annex I inclusion Published: No	N	N		DuPont
B.9.1.1/02	[redacted]	1981	Single-dose oral toxicity study in mallard ducks [redacted] HLO 89-81 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont
B.9.2.4.2/03	Brougher, D.S., Martin, K.H., Gallagher, S.P., Bodle, E.S.	2013d	Oxamyl (DPX-D1410) technical (98% w/w): A 48-hour static acute toxicity test with the Cladoceran (*Ceriodaphnia dubia*) Wildlife International Ltd. (USA) DuPont-37399 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 submitted	DuPont

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner
B.9.2.4.2/04	Brougher, D.S., Martin, K.H., Gallagher, S.P., Bodle, E.S.	2013a	Oxamyl (DPX-D1410) technical (98% w/w): A 48-hour static acute toxicity test with *Hyalella azteca* Wildlife International Ltd. (USA) DuPont-37397 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 submitted	DuPont
B.9.2.4.2/05	Brougher, D.S., Martin, K.H., Gallagher, S.P., Bodle, E.S.	2013c	Oxamyl (DPX-D1410) technical (98% w/w): A 48-hour static acute toxicity test with the cladoceran (*Daphnia pulex*) Wildlife International Ltd. (USA) DuPont-37398 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 submitted	DuPont
B.9.2.4.2/07	Brougher, D.S., Martin, K.H., Gallagher, S.P., Bodle, E.S.	2013b	Oxamyl (DPX-D1410) technical (98% w/w): A 48-hour static acute toxicity test with *Chironomus tentans* Wildlife International Ltd. (USA) DuPont-37400 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 submitted	DuPont
B.9.2.4.2/02	Brougher, D.S., Martin, K.H., Gallagher, S.P., Krueger, H.O.	2013a	Oxamyl (DPX-D1410) technical (98% w/w): A 48-hour static acute toxicity test with the mayfly (*Centroptilum triangulifer*) Wildlife International Ltd. (USA) DuPont-37401 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 submitted	DuPont

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner
B.9.2.4.2/06	Brougher, D.S., Martin, K.H., Gallagher, S.P., Krueger, H.O.	2013b	Oxamyl (DPX-D1410) technical (98% w/w): A 48-hour static acute toxicity test with the caddisfly (*Chimarra atterima*) Wildlife International Ltd. (USA) DuPont-37402 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 submitted	DuPont
B.9.5/01	Cardinali, V.C.B.	2008	Effects of OXAMIL TÉCNICO on soil microorganisms: Nitrogen transformation test BIOAGRI Laboratorios Ltda. RF-0014.218.286.07 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 submitted	DuPont
B.9.5/04	Carter, J.	2001	IN-T2921: Assessment of the effects on soil microflora Huntingdon Life Sciences Ltd DuPont-4736 Previously submitted at the EU level for Annex I inclusion Published: No	N	N		DuPont
B.9.7/01	Daly, R., Leva, D., Brugger, K.	2000	Summary of insecticide screening data for major metabolites of oxamyl DuPont Stine-Haskell Research Center DuPont-5194 Previously submitted at the EU level for Annex I inclusion Published: No	N	N		DuPont

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner
B.9.2.2.1/02	[redacted]	1982	Early life stage toxicity of oxamyl to fathead minnow [redacted] HLR 877-81 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont
B.9.2.3/01	[redacted]	2015	Oxamyl (DPX-D1410) technical: 21-D amphibian metamorphosis assay (AMA) with south African clawed frog, *Xenopus laevis* [redacted]. DuPont-31032, Revision No. 1 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 submitted	DuPont
B.9.1.1/01	[redacted]	2000	Oxamyl technical: An acute oral toxicity study with the northern bobwhite [redacted] DuPont-2954 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont
B.9.1.1/03	[redacted]	1988a	A dietary LC_{50} study with the bobwhite [redacted] HLO 47-88 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner
B.9.1.1/04	[redacted]	1988b	A dietary LC_{50} study with the mallard [redacted] HLO 48-88 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont
B.9.3.1.1.1/02	Haupt, S.	2014	Oxamyl (DPX-D1410) technical (98% w/w): Acute oral and contact toxicity to the bumblebee, *Bombus terrestris* L. (Hymenoptera) Institut für Biologische Analytik und Consulting IBACON GmbH DuPont-39670 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 submitted	DuPont
B.9.3.1.1.2/02	Haupt, S.	2014	Oxamyl (DPX-D1410) technical (98% w/w): Acute oral and contact toxicity to the bumblebee, *Bombus terrestris* L. (Hymenoptera) Institut für Biologische Analytik und Consulting IBACON GmbH DuPont-39670 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 submitted	DuPont
B.9.8/01	Hertl, J.	2000	Oxamyl technical: Activated sludge, respiration inhibition test IBACON DuPont-3348 Previously submitted at the EU level for Annex I inclusion Published: No	N	N		DuPont

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner
B.9.2.2.1/03	[redacted]	2012	Oxamyl (DPX-D1410) technical (98% w/w): Early life-stage toxicity test with the sheepshead minnow, *Cyprinodon variegatus*, under flow-through conditions [redacted] DuPont-34270 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 submitted	DuPont
B.9.2.3/02	[redacted]	2012	Oxamyl technical (DPX-D1410): Short term reproduction assay with the fathead minnow, *Pimephales promelas*, determined under flow-through conditions [redacted] DuPont-31031 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 submitted	DuPont
B.9.2.4.2/08	Hicks, S.L.	2012	Oxamyl (DPX-D1410) technical (98% w/w): Effect on new shell growth of the eastern oyster (*Crassostrea virginica*) ABC Laboratories, Inc. (Missouri) DuPont-34273 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 submitted	DuPont

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner
B.9.2.5.2/01	Hicks, S.L.	2013	Oxamyl (DPX-D1410) technical (98% w/w): Life-cycle toxicity test of the saltwater mysid, *Americamysis bahia*, conducted under flow-through conditions ABC Laboratories, Inc. (Missouri) DuPont-34269 GLP: No Published: No	N	N		DuPont
B.9.2.2.1/01	[redacted]	1988	Early life stage toxicity of IN D1410-196 (oxamyl) to rainbow trout [redacted] HLR 468-88 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont
B.9.3.1.3/01	Klank, C.	2014	Oxamyl (DPX-D1410) technical (98% w/w): Honey bee (*Apis mellifera* L.) larval toxicity test (single feeding exposure) Eurofins Agroscience Services EcoChem GmbH DuPont-39678 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 submitted	DuPont

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner
B.9.4.1/01	Luhrs, U.	2001	Oxamyl 10G (10% w/w): Effects on reproduction and growth of the earthworm, *Eisenia fetida*, (Savigny 1826), in artificial soil IBACON DuPont-4296 Previously submitted at the EU level for Annex I inclusion Published: No	N	N		DuPont
B.9.4.1/05	Meinerling, M.	2014	Oxamyl (DPX-D1410) technical (98% w/w): Accumulation and elimination in earthworms (*Eisenia fetida*) in artificial soil Institut für Biologische Analytik und Consulting IBACON GmbH DuPont-38477 GLP: Yes Published: No	N	Y	Data protection on a MS by MS basis. The study provides additional data for the regulatory decision, conducted according to GLP and has not previously been protected or submitted in all MS.	DuPont
B.9.4.2.1/01	Meli, M.	2015	Oxamyl – Population level risk assessment for collembolans RIFCON GmbH DuPont-41996 EU GLP: No Published: No	N	N		DuPont

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner
B.9.4.1/02	Pavić, B.	2014	IN-A2213: Effects on reproduction and growth of the earthworm, *Eisenia fetida*, in artificial soil Institut für Biologische Analytik und Consulting IBACON GmbH DuPont-39672 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 submitted	DuPont
B.9.4.1/03	Pavić, B.	2015a	IN-D2708: Effects on reproduction and growth of the earthworm, *Eisenia fetida*, in artificial soil Institut für Biologische Analytik und Consulting IBACON GmbH DuPont-41042 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 submitted	DuPont
B.9.4.1/04	Pavić, B.	2015b	IN-N0079: Effects on reproduction and growth of the earthworm, *Eisenia fetida*, in artificial soil Institut für Biologische Analytik und Consulting IBACON GmbH DuPont-41045 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 submitted	DuPont
B.9.4.2.1/02	Pavić, B.	2014c	Oxamyl (DPX-D1410) technical (98% w/w): Effects on the collembola *Folsomia candida* in artificial soil with 5% peat Institut für Biologische Analytik und Consulting IBACON GmbH DuPont-39676 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 submitted	DuPont

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner
B.9.4.2.1/03	Pavić, B.	2014a	IN-A2213: Effects on the collembola *Folsomia candida* in artificial soil with 5% peat Institut für Biologische Analytik und Consulting IBACON GmbH DuPont-39673 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 submitted	DuPont
B.9.4.2.1/04	Pavić, B.	2015a	IN-D2708: Effects on the collembola *Folsomia candida* in artificial soil with 5% peat Institut für Biologische Analytik und Consulting IBACON GmbH DuPont-41043 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 submitted	DuPont
B.9.4.2.1/05	Pavić, B.	2015c	IN-N0079: Effects on the collembola *Folsomia candida* in artificial soil with 5% peat Institut für Biologische Analytik und Consulting IBACON GmbH DuPont-41046 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 submitted	DuPont

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner
B.9.4.2.1/06	Pavić, B.	2014d	Oxamyl (DPX-D1410) technical (98% w/w): Effects on reproduction of the predatory mite *Hypoaspis aculeifer* in artificial soil with 5% peat Institut für Biologische Analytik und Consulting IBACON GmbH DuPont-39677 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 submitted	DuPont
B.9.4.2.1/07	Pavić, B.	2014b	IN-A2213: Effects on reproduction of the predatory mite *Hypoaspis aculeifer* in artificial soil with 5% peat Institut für Biologische Analytik und Consulting IBACON GmbH DuPont-39674 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 submitted	DuPont
B.9.4.2.1/08	Pavić, B.	2015b	IN-D2708: Effects on reproduction of the predatory mite *Hypoaspis aculeifer* in artificial soil with 5% peat Institut für Biologische Analytik und Consulting IBACON GmbH DuPont-41044 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 submitted	DuPont

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner
B.9.4.2.1/09	Pavić, B.	2015d	IN-N0079: Effects on reproduction of the predatory mite *Hypoaspis aculeifer* in artificial soil with 5% peat Institut für Biologische Analytik und Consulting IBACON GmbH DuPont-41047 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 submitted	DuPont
B.9.6.2/01	Porch, J.	2001	Oxamyl (DPX-D1410) 24L: A greenhouse study to investigate the effects on seedling emergence and early growth of ten terrestrial plant species Wildlife International Ltd. (USA) DuPont-5817 Previously submitted at the EU level for Annex I inclusion Published: No	N	N		DuPont
B.9.2.4.2/01	Rebstock, M.	2012	Oxamyl (DPX-D1410) technical (98% w/w): Acute toxicity with the mysid shrimp, *Americamysis bahia*, determined under flow-through test conditions ABC Laboratories, Inc. (Missouri) DuPont-34271 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 submitted	DuPont

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner
B.9.2.7/01	Rebstock, M.	2012	Oxamyl (DPX-D1410) technical (98% w/w): 7-day growth inhibition test with the freshwater aquatic plant, duckweed, *Lemna gibba* ABC Laboratories, Inc. (Missouri) DuPont-34272 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 submitted	DuPont
B.9.1.1/05	[redacted]	1982b	The effects of dietary inclusion of oxamyl on reproduction in the bobwhite quail [redacted] HLO 453-82 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont
B.9.1.1/06	[redacted]	1982a	The effects of dietary inclusion of oxamyl on reproduction in the mallard duck [redacted] HLO 337-82 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont
B.9.3.1.2/01	Schmitt, H.	2014	Oxamyl (DPX-D1410) technical (98% w/w): Assessment of effects to the honeybee, *Apis mellifera* L., in a 10 days chronic feeding test under laboratory conditions Eurofins Agroscience Services, Gmbh DuPont-39665 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 submitted	DuPont

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner
B.9.3.1.1.1/01	Schur, A.	1999	Oxamyl technical: Acute oral and contact toxicity to the honey bee, *Apis mellifera* L. GAB Biotechnologie, Gmbh DuPont-2740 Previously submitted at the EU level for Annex I inclusion Published: No	N	N		DuPont
B.9.3.1.1.2/01	Schur, A.	1999	Oxamyl technical: Acute oral and contact toxicity to the honey bee, *Apis mellifera* L. GAB Biotechnologie, Gmbh DuPont-2740 Previously submitted at the EU level for Annex I inclusion Published: No	N	N		DuPont
B.9.5/02	Wachter, S.	2000b	IN-A2213: Assessment of the effects on soil microflora GAB Biotechnologie, Gmbh DuPont-4131 Previously submitted at the EU level for Annex I inclusion Published: No	N	N		DuPont
B.9.5/03	Wachter, S.	2000a	IN-D2708: Assessment of the effects on soil microflora GAB Biotechnologie, Gmbh DuPont-4133 Previously submitted at the EU level for Annex I inclusion Published: No	N	N		DuPont

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner
B.9.3.2.2/01	Walker, H.	2000	Oxamyl (DPX-D1410) 10L: A laboratory study to determine the LC_{50} and evaluate the sublethal effects on the predatory mite *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae) Ecotox Ltd. DuPont-4037 Previously submitted at the EU level for Annex I inclusion Published: No	N	N		DuPont
B.9.2.1/01	[redacted].	2000a	Oxamyl Technical: Static, acute, 96-hour, (LC_{50}) test to rainbow trout, *Oncorhynchus mykiss* [redacted] DuPont-2907 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont
B.9.2.1/02	[redacted]	2000b	Oxamyl Technical: Static-renewal, acute, 96-hour, (LC_{50}) test to bluegill sunfish, *Lepomis macrochirus* [redacted] DuPont-2908 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner
B.9.2.1/03	[redacted]	1999a	IN-A2213: Static, acute, 96-hour limit test to rainbow trout, *Oncorhynchus mykiss* [redacted] DuPont-2500 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont
B.9.2.1/05	[redacted]	1999b	IN-N0079: Static, acute, 96-hour, (LC_{50}) test to rainbow trout, *Oncorhynchus mykiss* [redacted] DuPont-2512 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont
B.9.2.4.1/02	Ward, T.J., Magazu, J.P., Boeri, R.L.	1999a	IN-A2213: Static, acute, 48-hour limit test to *Daphnia magna* T.R. Wilbury Laboratories, Inc. DuPont-2502 Previously submitted at the EU level for Annex I inclusion Published: No	N	N		DuPont
B.9.2.4.1/04	Ward, T.J., Magazu, J.P., Boeri, R.L.	1999b	IN-N0079: Acute, static, 48-hour toxicity (EC_{50}) test to *Daphnia magna* T.R. Wilbury Laboratories, Inc. DuPont-2513 Previously submitted at the EU level for Annex I inclusion Published: No	N	N		DuPont

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner
B.9.2.6.1/02	Ward, T.J., Magazu, J.P., Boeri, R.L.	1999	IN-A2213: Influence on growth and growth rate of the alga, *Selenastrum capricornutum* T.R. Wilbury Laboratories, Inc. DuPont-2505 Previously submitted at the EU level for Annex I inclusion Published: No	N	N		DuPont

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Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner
B.9.1.1/01	[redacted]	2000	Oxamyl technical: An acute oral toxicity study with the northern bobwhite [redacted] DuPont-2954 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont
B.9.1.1/02	[redacted]	1981	Single-dose oral toxicity study in mallard ducks [redacted] HLO 89-81 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont
B.9.1.1/03	[redacted]	1988a	A dietary LC_{50} study with the bobwhite [redacted] HLO 47-88 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont
B.9.1.1/04	[redacted]	1988b	A dietary LC_{50} study with the mallard [redacted] HLO 48-88 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner
B.9.1.1/05	[redacted]	1982b	The effects of dietary inclusion of oxamyl on reproduction in the bobwhite quail [redacted] HLO 453-82 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont
B.9.1.1/06	[redacted]	1982a	The effects of dietary inclusion of oxamyl on reproduction in the mallard duck [redacted] HLO 337-82 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont
B.9.2.1/01	[redacted]	2000a	Oxamyl Technical: Static, acute, 96-hour, (LC_{50}) test to rainbow trout, *Oncorhynchus mykiss* [redacted] DuPont-2907 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner
B.9.2.1/02	[redacted]	2000b	Oxamyl Technical: Static-renewal, acute, 96-hour, (LC_{50}) test to bluegill sunfish, *Lepomis macrochirus* [redacted] DuPont-2908 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont
B.9.2.1/03	[redacted]	1999a	IN-A2213: Static, acute, 96-hour limit test to rainbow trout, *Oncorhynchus mykiss* [redacted] DuPont-2500 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont
B.9.2.1/04	[redacted]	1999	IN-D2708: Static, acute, 96-hour, (LC_{50}) test to rainbow trout, *Oncorhynchus mykiss* [redacted]. DuPont-2507 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont
B.9.2.1/05	[redacted]	1999b	IN-N0079: Static, acute, 96-hour, (LC_{50}) test to rainbow trout, *Oncorhynchus mykiss* [redacted] DuPont-2512 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner
B.9.2.1/06	[redacted]	2000	IN-T2921: Acute, 96-hour LC_{50} to rainbow trout, *Oncorhynchus mykiss* [redacted] DuPont-4439 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont
B.9.2.2.1/01	[redacted]	1988	Early life stage toxicity of IN D1410-196 (oxamyl) to rainbow trout [redacted] HLR 468-88 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont
B.9.2.2.1/02	[redacted]	1982	Early life stage toxicity of oxamyl to fathead minnow [redacted] HLR 877-81 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont
B.9.2.2.1/03	[redacted]	2012	Oxamyl (DPX-D1410) technical (98% w/w): Early life-stage toxicity test with the sheepshead minnow, *Cyprinodon variegatus*, under flow-through conditions [redacted] DuPont-34270 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 submitted	DuPont

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner
B.9.2.3/01	[redacted]	2015	Oxamyl (DPX-D1410) technical: 21-D amphibian metamorphosis assay (AMA) with south African clawed frog, *Xenopus laevis* [redacted] DuPont-31032, Revision No. 1 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 submitted	DuPont
B.9.2.3/02	[redacted]	2012	Oxamyl technical (DPX-D1410): Short term reproduction assay with the fathead minnow, *Pimephales promelas*, determined under flow-through conditions [redacted] DuPont-31031 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 submitted	DuPont
B.9.2.4.1/01	Boeri, R.L., Magazu, J.P., Ward, T.J.	1999b	Oxamyl technical: Acute, 48-hour EC_{50} to *Daphnia magna* T.R. Wilbury Laboratories, Inc. DuPont-2553 Previously submitted at the EU level for Annex I inclusion Published: No	N	N		DuPont
B.9.2.4.1/02	Ward, T.J., Magazu, J.P., Boeri, R.L.	1999a	IN-A2213: Static, acute, 48-hour limit test to *Daphnia magna* T.R. Wilbury Laboratories, Inc. DuPont-2502 Previously submitted at the EU level for Annex I inclusion Published: No	N	N		DuPont

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner
B.9.2.4.1/03	Boeri, R.L., Magazu, J.P., Ward, T.J.	1999a	IN-D2708: Static, acute, 48-hour limit test to *Daphnia magna* T.R. Wilbury Laboratories, Inc. DuPont-2510 Previously submitted at the EU level for Annex I inclusion Published: No	N	N		DuPont
B.9.2.4.1/04	Ward, T.J., Magazu, J.P., Boeri, R.L.	1999b	IN-N0079: Acute, static, 48-hour toxicity (EC_{50}) test to *Daphnia magna* T.R. Wilbury Laboratories, Inc. DuPont-2513 Previously submitted at the EU level for Annex I inclusion Published: No	N	N		DuPont
B.9.2.4.1/05	Boeri, R.L., Ward, T.J.	2000	IN-T2921: Acute, 48-hour, EC_{50} to *Daphnia magna* T.R. Wilbury Laboratories, Inc. DuPont-4441 Previously submitted at the EU level for Annex I inclusion Published: No	N	N		DuPont
B.9.2.4.2/01	Rebstock, M.	2012	Oxamyl (DPX-D1410) technical (98% w/w): Acute toxicity with the mysid shrimp, *Americamysis bahia*, determined under flow-through test conditions ABC Laboratories, Inc. (Missouri) DuPont-34271 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 submitted	DuPont

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner
B.9.2.4.2/02	Brougher, D.S., Martin, K.H., Gallagher, S.P., Krueger, H.O.	2013a	Oxamyl (DPX-D1410) technical (98% w/w): A 48-hour static acute toxicity test with the mayfly (*Centroptilum triangulifer*) Wildlife International Ltd. (USA) DuPont-37401 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 submitted	DuPont
B.9.2.4.2/03	Brougher, D.S., Martin, K.H., Gallagher, S.P., Bodle, E.S.	2013d	Oxamyl (DPX-D1410) technical (98% w/w): A 48-hour static acute toxicity test with the Cladoceran (*Ceriodaphnia dubia*) Wildlife International Ltd. (USA) DuPont-37399 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 submitted	DuPont
B.9.2.4.2/04	Brougher, D.S., Martin, K.H., Gallagher, S.P., Bodle, E.S.	2013a	Oxamyl (DPX-D1410) technical (98% w/w): A 48-hour static acute toxicity test with *Hyalella azteca* Wildlife International Ltd. (USA) DuPont-37397 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 submitted	DuPont
B.9.2.4.2/05	Brougher, D.S., Martin, K.H., Gallagher, S.P., Bodle, E.S.	2013c	Oxamyl (DPX-D1410) technical (98% w/w): A 48-hour static acute toxicity test with the cladoceran (*Daphnia pulex*) Wildlife International Ltd. (USA) DuPont-37398 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 submitted	DuPont

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner
B.9.2.4.2/06	Brougher, D.S., Martin, K.H., Gallagher, S.P., Krueger, H.O.	2013b	Oxamyl (DPX-D1410) technical (98% w/w): A 48-hour static acute toxicity test with the caddisfly (*Chimarra atterima*) Wildlife International Ltd. (USA) DuPont-37402 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 submitted	DuPont
B.9.2.4.2/07	Brougher, D.S., Martin, K.H., Gallagher, S.P., Bodle, E.S.	2013b	Oxamyl (DPX-D1410) technical (98% w/w): A 48-hour static acute toxicity test with *Chironomus tentans* Wildlife International Ltd. (USA) DuPont-37400 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 submitted	DuPont
B.9.2.4.2/08	Hicks, S.L.	2012	Oxamyl (DPX-D1410) technical (98% w/w): Effect on new shell growth of the eastern oyster (*Crassostrea virginica*) ABC Laboratories, Inc. (Missouri) DuPont-34273 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 submitted	DuPont
B.9.2.5.1/01	Boeri, R.L., Ward, T.J.	2000a	Oxamyl technical: 21-day chronic, flow‑through toxicity to *Daphnia magna* T.R. Wilbury Laboratories, Inc. DuPont-2554 Previously submitted at the EU level for Annex I inclusion Published: No	N	N		DuPont

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner
B.9.2.5.1/02	Boeri, R.L., Ward, T.J.	2000b	IN-D2708: Chronic, static-renewal toxicity to the daphnid, *Daphnia magna* T.R. Wilbury Laboratories, Inc. DuPont-3909 Previously submitted at the EU level for Annex I inclusion Published: No	N	N		DuPont
B.9.2.5.2/01	Hicks, S.L.	2013	Oxamyl (DPX-D1410) technical (98% w/w): Life-cycle toxicity test of the saltwater mysid, *Americamysis bahia*, conducted under flow-through conditions ABC Laboratories, Inc. (Missouri) DuPont-34269 GLP: No Published: No	N	N		DuPont
B.9.2.6.1/01	Boeri, R.L., Magazu, J.P., Ward, T.J.	2000	Oxamyl technical: Influence on growth and growth rate of the alga, *Selenastrum capricornutum* T.R. Wilbury Laboratories, Inc. DuPont-2909 Previously submitted at the EU level for Annex I inclusion Published: No	N	N		DuPont

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner
B.9.2.6.1/02	Ward, T.J., Magazu, J.P., Boeri, R.L.	1999	IN-A2213: Influence on growth and growth rate of the alga, *Selenastrum capricornutum* T.R. Wilbury Laboratories, Inc. DuPont-2505 Previously submitted at the EU level for Annex I inclusion Published: No	N	N		DuPont
B.9.2.6.1/03	Boeri, R.L., Magazu, J.P., Ward, T.J.	1999b	IN-D2708: Influence on growth and growth rate of the alga, *Selenastrum capricornutum* T.R. Wilbury Laboratories, Inc. DuPont-2511 Previously submitted at the EU level for Annex I inclusion Published: No	N	N		DuPont
B.9.2.6.1/04	Boeri, R.L., Magazu, J.P., Ward, T.J.	1999a	IN-N0079: Influence on growth and growth rate of the alga, *Selenastrum capricornutum* T.R. Wilbury Laboratories, Inc. DuPont-2514 Previously submitted at the EU level for Annex I inclusion Published: No	N	N		DuPont
B.9.2.6.1/05	Boeri, R.L., Ward, T.J.	2001	IN-T2921: Influence on growth and growth rate of the alga, *Selenastrum capricornutum* T.R. Wilbury Laboratories, Inc. DuPont-4442 Previously submitted at the EU level for Annex I inclusion Published: No	N	N		DuPont

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner
B.9.2.7/01	Rebstock, M.	2012	Oxamyl (DPX-D1410) technical (98% w/w): 7-day growth inhibition test with the freshwater aquatic plant, duckweed, *Lemna gibba* ABC Laboratories, Inc. (Missouri) DuPont-34272 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 submitted	DuPont
B.9.3.1.1.1/01	Schur, A.	1999	Oxamyl technical: Acute oral and contact toxicity to the honey bee, *Apis mellifera* L. GAB Biotechnologie, Gmbh DuPont-2740 Previously submitted at the EU level for Annex I inclusion Published: No	N	N		DuPont
B.9.3.1.1.1/02	Haupt, S.	2014	Oxamyl (DPX-D1410) technical (98% w/w): Acute oral and contact toxicity to the bumblebee, *Bombus terrestris* L. (Hymenoptera) Institut für Biologische Analytik und Consulting IBACON GmbH DuPont-39670 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 submitted	DuPont
B.9.3.1.1.2/01	Schur, A.	1999	Oxamyl technical: Acute oral and contact toxicity to the honey bee, *Apis mellifera* L. GAB Biotechnologie, Gmbh DuPont-2740 Previously submitted at the EU level for Annex I inclusion Published: No	N	N		DuPont

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner
B.9.3.1.1.2/02	Haupt, S.	2014	Oxamyl (DPX-D1410) technical (98% w/w): Acute oral and contact toxicity to the bumblebee, *Bombus terrestris* L. (Hymenoptera) Institut für Biologische Analytik und Consulting IBACON GmbH DuPont-39670 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 submitted	DuPont
B.9.3.1.2/01	Schmitt, H.	2014	Oxamyl (DPX-D1410) technical (98% w/w): Assessment of effects to the honeybee, *Apis mellifera* L., in a 10 days chronic feeding test under laboratory conditions Eurofins Agroscience Services, Gmbh DuPont-39665 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 submitted	DuPont
B.9.3.1.3/01	Klank, C.	2014	Oxamyl (DPX-D1410) technical (98% w/w): Honey bee (*Apis mellifera* L.) larval toxicity test (single feeding exposure) Eurofins Agroscience Services EcoChem GmbH DuPont-39678 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 submitted	DuPont

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner
B.9.3.2.1/02	Austin, H.	1999	Oxamyl 10L (10% w/w): A laboratory study to evaluate the effects on the aphid parasitoid *Aphidius rhopalosiphi* (Hymenoptera: Braconidae) Ecotox Ltd. DuPont-2609 Previously submitted at the EU level for Annex I inclusion Published: No	N	N		DuPont
B.9.3.2.2/01	Walker, H.	2000	Oxamyl (DPX-D1410) 10L: A laboratory study to determine the LC_{50} and evaluate the sublethal effects on the predatory mite *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae) Ecotox Ltd. DuPont-4037 Previously submitted at the EU level for Annex I inclusion Published: No	N	N		DuPont
B.9.4.1/01	Luhrs, U.	2001	Oxamyl 10G (10% w/w): Effects on reproduction and growth of the earthworm, *Eisenia fetida*, (Savigny 1826), in artificial soil IBACON DuPont-4296 Previously submitted at the EU level for Annex I inclusion Published: No	N	N		DuPont

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner
B.9.4.1/02	Pavić, B.	2014	IN-A2213: Effects on reproduction and growth of the earthworm, *Eisenia fetida*, in artificial soil Institut für Biologische Analytik und Consulting IBACON GmbH DuPont-39672 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 submitted	DuPont
B.9.4.1/03	Pavić, B.	2015a	IN-D2708: Effects on reproduction and growth of the earthworm, *Eisenia fetida*, in artificial soil Institut für Biologische Analytik und Consulting IBACON GmbH DuPont-41042 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 submitted	DuPont
B.9.4.1/04	Pavić, B.	2015b	IN-N0079: Effects on reproduction and growth of the earthworm, *Eisenia fetida*, in artificial soil Institut für Biologische Analytik und Consulting IBACON GmbH DuPont-41045 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 submitted	DuPont

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner
B.9.4.1/05	Meinerling, M.	2014	Oxamyl (DPX-D1410) technical (98% w/w): Accumulation and elimination in earthworms (*Eisenia fetida*) in artificial soil Institut für Biologische Analytik und Consulting IBACON GmbH DuPont-38477 GLP: Yes Published: No	N	Y	Data protection on a MS by MS basis. The study provides additional data for the regulatory decision, conducted according to GLP and has not previously been protected or submitted in all MS.	DuPont
B.9.4.2.1/01	Meli, M.	2015	Oxamyl – Population level risk assessment for collembolans RIFCON GmbH DuPont-41996 EU GLP: No Published: No	N	N		DuPont
B.9.4.2.1/02	Pavić, B.	2014c	Oxamyl (DPX-D1410) technical (98% w/w): Effects on the collembola *Folsomia candida* in artificial soil with 5% peat Institut für Biologische Analytik und Consulting IBACON GmbH DuPont-39676 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 submitted	DuPont

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner
B.9.4.2.1/03	Pavić, B.	2014a	IN-A2213: Effects on the collembola *Folsomia candida* in artificial soil with 5% peat Institut für Biologische Analytik und Consulting IBACON GmbH DuPont-39673 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 submitted	DuPont
B.9.4.2.1/04	Pavić, B.	2015a	IN-D2708: Effects on the collembola *Folsomia candida* in artificial soil with 5% peat Institut für Biologische Analytik und Consulting IBACON GmbH DuPont-41043 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 submitted	DuPont
B.9.4.2.1/05	Pavić, B.	2015c	IN-N0079: Effects on the collembola *Folsomia candida* in artificial soil with 5% peat Institut für Biologische Analytik und Consulting IBACON GmbH DuPont-41046 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 submitted	DuPont

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner
B.9.4.2.1/06	Pavić, B.	2014d	Oxamyl (DPX-D1410) technical (98% w/w): Effects on reproduction of the predatory mite *Hypoaspis aculeifer* in artificial soil with 5% peat Institut für Biologische Analytik und Consulting IBACON GmbH DuPont-39677 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 submitted	DuPont
B.9.4.2.1/07	Pavić, B.	2014b	IN-A2213: Effects on reproduction of the predatory mite *Hypoaspis aculeifer* in artificial soil with 5% peat Institut für Biologische Analytik und Consulting IBACON GmbH DuPont-39674 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 submitted	DuPont
B.9.4.2.1/08	Pavić, B.	2015b	IN-D2708: Effects on reproduction of the predatory mite *Hypoaspis aculeifer* in artificial soil with 5% peat Institut für Biologische Analytik und Consulting IBACON GmbH DuPont-41044 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 submitted	DuPont

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner
B.9.4.2.1/09	Pavić, B.	2015d	IN-N0079: Effects on reproduction of the predatory mite *Hypoaspis aculeifer* in artificial soil with 5% peat Institut für Biologische Analytik und Consulting IBACON GmbH DuPont-41047 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 submitted	DuPont
B.9.5/01	Cardinali, V.C.B.	2008	Effects of OXAMIL TÉCNICO on soil microorganisms: Nitrogen transformation test BIOAGRI Laboratorios Ltda. RF-0014.218.286.07 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 submitted	DuPont
B.9.5/02	Wachter, S.	2000b	IN-A2213: Assessment of the effects on soil microflora GAB Biotechnologie, Gmbh DuPont-4131 Previously submitted at the EU level for Annex I inclusion Published: No	N	N		DuPont
B.9.5/03	Wachter, S.	2000a	IN-D2708: Assessment of the effects on soil microflora GAB Biotechnologie, Gmbh DuPont-4133 Previously submitted at the EU level for Annex I inclusion Published: No	N	N		DuPont

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner
B.9.5/04	Carter, J.	2001	IN-T2921: Assessment of the effects on soil microflora Huntingdon Life Sciences Ltd DuPont-4736 Previously submitted at the EU level for Annex I inclusion Published: No	N	N		DuPont
B.9.6.2/01	Porch, J.	2001	Oxamyl (DPX-D1410) 24L: A greenhouse study to investigate the effects on seedling emergence and early growth of ten terrestrial plant species Wildlife International Ltd. (USA) DuPont-5817 Previously submitted at the EU level for Annex I inclusion Published: No	N	N		DuPont
B.9.6.2/02	Bergfield, A.	2012	Oxamyl (DPX-D1410) 24SL: A greenhouse study to investigate the effects on vegetative vigor of ten terrestrial plant species following foliar exposure ABC Laboratories, Inc. (Missouri) DuPont-34275 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 submitted	DuPont

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner
B.9.7/01	Daly, R., Leva, D., Brugger, K.	2000	Summary of insecticide screening data for major metabolites of oxamyl DuPont Stine-Haskell Research Center DuPont-5194 Previously submitted at the EU level for Annex I inclusion Published: No	N	N		DuPont
B.9.8/01	Hertl, J.	2000	Oxamyl technical: Activated sludge, respiration inhibition test IBACON DuPont-3348 Previously submitted at the EU level for Annex I inclusion Published: No	N	N		DuPont