Document Title

Summary of the ecotoxicological studies for fluopicolide

Data Requirement(s)

Regulation (EC) No 1107/2009 & Regulation (EU) No 283/2013

Document MCA

Section 8: Ecotoxicological studies

According to the Guidance Document SANCO/10181/2013 for applicants on preparing dossiers for the approval of a chemical active substance

Date

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Version history

Date [yyyy-mm-dd]	Data points containing amendments or additions[1] and brief description	Document identifier and version number

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4, 'How to revise an Assessment Report'.

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CA 8 ECOTOXICOLOGICAL STUDIES ON THE ACTIVE SUBSTANCE

Fluopicolide (AE C638206) was included in Annex I to Council Directive 91/414/EEC in 2010 (Commission Directive 2010/15/EU, Entry into Force on June 1, 2010). The expiration of approval of fluopicolide is May 31, 2023 (Commission Implementing Regulation (EU) 2017/1527). The Supplementary Dossier contains only data which were not submitted at the time of the Annex I inclusion of fluopicolide under Council Directive 91/414/EEC and which were therefore not evaluated during the first EU review. All data which were already submitted by Bayer AG (former Bayer CropScience) for the Annex I inclusion under Council Directive 91/414/EEC are contained in the Draft Assessment Report (DAR) and its Addenda, and are included in the Baseline Dossier provided by Bayer AG.

Fluopicolide is a fungicidal active substance developed by Bayer. It is the only active substance in Europe representing a class of chemistry (pyridinylmethyl-benzamides) with a unique mode of action via delocalization of a spectrin-like protein in the Oomycetes fungi.

Fluopicolide has a long track record of safe use in a large number of targeted crops within horticulture, e.g. cucumbers, lettuce and on arable crops (e.g. potato).

Fluopicolide is active against a wide range of Oomycete fungi, the causal agents of devastating plant diseases of economic importance in EU-27 such as potato late blight (*Phytophthora infestans*) or downy mildew diseases in a broad range of crops.

It provides effective, long lasting protection at low application rates against Oomycetes diseases at different stage of development of the fungi, giving flexibility of use to the farmer.

Fluopicolide can be formulated with other active ingredients in different types of formulations to optimise and complete its activity.

The development of resistances of Oomycetes against existing, well-established fungicide groups represent a threat for European farmers by increasing the complexity of their plant protection programs leading to severe economic impacts. With Fluopicolide, farmers in EU-27 have access to a modern tool for their integrated crop protection programs, contributing to effective and sustainable management of resistance development and preserving high level of protection against Oomycete diseases.

By reducing the Oomycete damages, applications of Fluopicolide on target crops contribute to the achievement of optimum yield and quality, thus securing sufficient supply of high-quality potatoes and horticultural produces for European consumer destinations and markets abroad, being it fresh or for the processing industry.

Relevant information for classification as detailed in the "*Combined Draft (Renewal) Assessment Report prepared according to Regulation (EC) N° 1107/2009 and Proposal for Harmonised Classification and Labelling (CLH Report) according to Regulation (EC) N° 1272/2008 – Volume 1, Level 2"* is provided in Document N1, Section 9.2, and highlighted in light grey.

CA 8.1 Effects on birds and other terrestrial vertebrates

CA 8.1.1 Effects on Birds

Studies on quail species, mallard ducks and finch species have been conducted with the active substance fluopicolide. Detailed information on acute, short-term and long-term effects of fluopicolide on birds is presented in the following chapters.

CA 8.1.1.1 Acute oral toxicity to birds

Table 8.1.1.1- 1: Acute oral toxicity to birds

Test substance	Test design	Test species	Endpoint	Reference
Fluopicolide	Acute toxicity	Mallard duck	LD_{50} > 2250 mg a.s./kg bw	[redacted] 2001; M-240576-01-1 KCA 8.1.1.1/01
			LD_{50} = 4248 mg a.s./kg bw a)	Extrapolated acc. to EFSA GD 2009
		Bobwhite quail	LD_{50} > 2250 mg a.s./kg bw	[redacted] 2001; M-240577-01-1 KCA 8.1.1.1/02
			LD_{50} = 4248 mg a.s./kg bw a)	Extrapolated acc. to EFSA GD 2009
		Zebra finch	LD_{50} = 1105 mg a.s./kg bw	[redacted] 2015; M-544294-01-1 KCA 8.1.1.1/03
		Bird acute geomean	**LD_{50} = 2711 mg a.s./kg bw** b)	Geometric mean acc. EFSA GD 2009

a) The study endpoint was extrapolated according to EFSA GD 2009. The extrapolation factor of 1.888 was derived from EFSA GD 2009, section 2.1.2, table 1 for studies in which 10 animals were dosed and no mortality occurred.

b) In accordance with EFSA GD 2009, the geometric mean LD_{50} of the three species mallard duck (LD_{50} = 4248 mg a.s./kg bw), bobwhite quail (LD_{50} = 4248 mg a.s./kg bw) and zebra finch (LD_{50} = 1105 mg a.s./kg bw) was used.

Data Point:	KCA 8.1.1.1/01
Report Author:	[redacted]
Report Year:	2001
Report Title:	AE C638206 Technical: An acute oral toxicity study with the mallard
Report No:	B003550
Document No:	M-240576-01-1
Guideline(s) followed in study:	USEPA (=EPA): FIFRA 71-1 (1982), OPPTS 850.2100 (1996)
Deviations from current test guideline:	Current Guideline: OECD 223 (2016) Regurgitation was not monitored. Birds were not housed individually but in pens containing five birds each. The space available for each bird in the pen was about 1350 cm2, and thus below the 2000 cm2 recommended in the guideline. These deviations are not expected to impact the study results.
Previous evaluation:	yes, evaluated and accepted in DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

Fluopicolide technical was administered orally to 10 adult mallard ducks (5 males and 5 females) at dose levels of 0, 292, 486, 810, 1350 and 2250 mg a.s./kg b.w. Birds were held at an average temperature of 22.7 °C with an average relative humidity of 60% and approximately 8 hours light per day. Birds were observed for 14 days for mortality and symptoms. Body weight and average feed consumption were measured for each dosage and control group.

No compound related mortalities occurred at any of the dose levels in the test. One male at the 1350 mg a.s./kg dosage was noted with a dropping left wing from day 1 through day 4 of the test, and one female at the 2250 mg a.s./kg dosage was noted with a leg injury from day 4 through day 13 of the test. Changes in body weight were comparable between the control and treatment groups and there were no apparent treatment-related effects upon body weight at any of the dosages tested. There were no treatment-related effects on feed consumption in any of the treatment groups. Postmortem examinations revealed no findings which were considered treatment related.

Based on this study the LD_{50} value for mallard duck exposed to fluopicolide was determined to be > 2250 mg a.s./kg b.w.

The study endpoint was extrapolated according to EFSA GD 2009. The extrapolation factor of 1.888 was derived from EFSA GD 2009, section 2.1.2, table 1 for studies in which 10 animals were dosed and no mortality occurred. The extrapolated LD_{50} was determined to be 4248 mg a.s./kg b.w.

I. Material and Methods:

Fluopicolide technical, purity: 97.1%, batch No.: 2050190/PP241024/2, single oral administration of the test substance in corn oil to 10 adult mallards (Anas platyrhynchos, 5 males and 5 females, 17 weeks old) per dose level: 0, 292, 486, 810, 1350 and 2250 mg a.s./kg b.w. (dosages were adjusted to 100% active substance).

Water and feed were provided ad libitum during acclimation and during the test, except during periods of fasting prior to testing. Birds were held at an average temperature of 22.7 ± 0.7°C (SD) with an average relative humidity of 60 ± 13% (SD). The photoperiod was approximately eight hours of light per day during acclimation and throughout the test. The birds were exposed to an average of approximately 194 lux. Each dosage group was assigned two pens. One pen contained five males and the other five females.

During the subsequent observation period of 14 days the birds were observed for mortality and symptoms. Body weight was measured at test initiation and on day 3, 7 and 14. Average feed consumption was determined by pen for each dosage group and control group for days 0-3, 4-7 and 8-14.

II. Results and Discussion:

Validity criteria (according to OECD 223, 2016)	Required	Obtained
Mortality in the control	≤ 10%	0%

Acute oral toxicity of fluopicolide techn. to birds

Test substance	Tech. as
Test object	Mallard ducks (male, female)
LD_{50} [mg a.s./kg b.w.]	> 2250
Lowest lethal effect dose (LLED) [mg a.s./kg b.w.]	> 2250
Lowest observed effect dose (LOED) [mg a.s./kg b.w.]	> 2250
No observed effect dose (NOED) [mg a.s./kg b.w.]	2250

Observations:

Mortality and clinical observations

No compound related mortalities occurred at any of the dose levels in the test. One male at the 1350 mg a.s./kg dosage was noted with a dropping left wing from day 1 through day 4 of the test, and one female at the 2250 mg a.s./kg dosage was noted with a leg injury from day 4 through day 13 of the test. With the above exceptions, all birds in the treatment groups were normal in appearance and behaviour for the duration of the test.

Body weight and feed consumption

Treatment (mg a.s./kg bw)	Mean bodyweight ± SD [g]							
	day 0	day 3	day 7	day 14	Δ d 0-3	Δ d 3-7	Δ d 7-14	Δ day 0-14
Females								
Control	986 ± 75	1025 ± 93	1020 ± 78	1018 ± 78	39 ± 23	-6 ± 30	-2 ± 18	32 ± 27
292	968 ± 55	1006 ± 75	989 ± 69	993 ± 45	37 ± 29	-16 ± 15	3 ± 32	24 ± 16
486	1016 ± 45	1060 ± 42	1074 ± 38	1067 ± 26	44 ± 13	13 ± 11	-7 ± 24	51 ± 29
810	991 ± 27	1024 ± 35	1027 ± 36	1036 ± 38	33 ± 34	2 ± 15	10 ± 21	43 ± 31
1350	991 ± 63	1022 ± 72	1020 ± 67	1016 ± 78	31 ± 19	-2 ± 18	-4 ± 20	26 ± 30
2250	982 ± 45	1048 ± 51	1019 ± 49	1025 ± 49	65 ± 24	-28 ± 39	6 ± 30	43 ± 21
Males								
Control	1151 ± 85	1192 ± 99	1199 ± 98	1202 ± 106	41 ± 21	7 ± 18	3 ± 19	51 ± 26
292	1130 ± 62	1183 ± 60	1200 ± 74	1227 ± 63	53 ± 39	18 ± 32	27 ± 14	97 ± 42
486	1124 ± 90	1182 ± 114	1151 ± 133	1122 ± 180	57 ± 31	-31 ± 85	-29 ± 70	-3 ± 138
810	1123 ± 95	1173 ± 91	1157 ± 96	1150 ± 93	50 ± 12	-16 ± 28	-7 ± 21	27 ± 35
1350	1156 ± 82	1190 ± 95	1214 ± 88	1202 ± 117	33 ± 37	24 ± 11	-12 ± 62	46 ± 100
2250	1079 ± 109	1154 ± 112	1164 ± 112	1150 ± 103	75 ± 31	10 ± 10	-15 ± 18	70 ± 24

Treatment (mg a.s./kg bw)	Mean food consumption [g/bird/d]		
	day 0-3	day 4-7	day 8-14
Females			
Control	141	122	157
292	124	112	124
486	141	120	129
810	145	135	139
1350	136	118	112
2250	170	97	112
Males			
Control	129	153	149
292	137	131	142
486	158	128	114
810	133	124	130
1350	161	140	170
2250	162	146	135

Gross pathology

Postmortem examinations revealed no findings which were considered treatment related.

III. CONCLUSIONS:

Based on this study the LD_{50} value for mallard duck exposed to fluopicolide was determined to be > 2250 mg as/kg b.w.

The study endpoint was extrapolated according to EFSA GD 2009. The extrapolation factor of 1.888 was derived from EFSA GD 2009, section 2.1.2, table 1 for studies in which 10 animals were dosed and no mortality occurred. The extrapolated LD_{50} was determined to be 4248 mg a.s./kg b.w.

Assessment and conclusion by applicant:

This study provides valid information on the acute toxicity of fluopicolide to Mallard ducks when administered via gavage and is considered reliable for use in risk assessments. No mortality or any other indication of toxicity was observed up to the top dose level of 2250 mg a.s./kg bw. The LD_{50} is > 2250 mg a.s./kg bw. The extrapolated LD_{50} is 4248 mg a.s./kg b.w.

Data Point:	KCA 8.1.1.1/02
Report Author:	[redacted]
Report Year:	2001
Report Title:	AE C638206 technical: An acute oral toxicity study with the nothern bobwhite
Report No:	B003551
Document No:	M-240577-01-1
Guideline(s) followed in study:	USEPA (=EPA): FIFRA 71-1 (1982), OPPTS 850.2100 (1996)
Deviations from current test guideline:	Current Guideline: OECD 223 (2016) Regurgitation was not monitored. Birds were not housed individually but in pens containing five birds each. The space available for each bird in the pen was about 795 cm2, and thus below the 1000 cm2 recommended in the guideline. These deviations are not expected to impact the study results.
Previous evaluation:	yes, evaluated and accepted in DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

Fluopicolide technical was administered orally to 10 adult bobwhite quails (5 males and 5 females) at dose levels of 0, 292, 486, 810, 1350 and 2250 mg a.s./kg b.w. Birds were held at an average temperature of 23.6 °C with an average relative humidity of 64% and approximately 8 hours light per day. Birds were observed for 14 days for mortality and sublethal symptoms. Body weight and average feed consumption were measured for each dosage and control group.

No compound related mortalities occurred at any of the dose levels in the test. All birds in the treatment groups were normal in appearance and behaviour for the duration of the test. There were no treatment related effects on body weight and feed consumption at any of the dosages tested. Postmortem examinations revealed no findings which were considered treatment related.

Based on this study the LD_{50} value for bobwhite quail exposed to fluopicolide was determined to be > 2250 mg as/kg b.w.

The study endpoint was extrapolated according to EFSA GD 2009. The extrapolation factor of 1.888 was derived from EFSA GD 2009, section 2.1.2, table 1 for studies in which 10 animals were dosed and no mortality occurred. The extrapolated LD_{50} was determined to be 4248 mg a.s./kg b.w.

1. Material and Methods:

Fluopicolide technical, purity: 97.1% w, batch No.: 2050190/PP241024/2, single oral administration of the test substance dispersed in corn oil to 10 adult bobwhite quail (Colinus virginianus, 5 males and 5 females, 25 weeks old) per dose level: 0, 292, 486, 810, 1350 and 2250 mg a.s./kg b.w. (dosages were adjusted to 100% active substance).

Water and feed were provided ad libitum during acclimation and during the test, except during periods of fasting prior to testing. Birds were held at an average temperature of 23.6 ± 0.56°C (SD) with an average relative humidity of 64 ± 11% (SD). The photoperiod was approximately eight hours of light per day during acclimation and throughout the test. The birds were exposed to an average of approximately 171 lux of illumination. Each dosage group was assigned two pens. One pen contained five males and the other five females.

During the subsequent observation period of 14 days the birds were observed for mortality and symptoms. Body weight was measured at test initiation and on day 3, 7 and 14. Average feed consumption was determined by pen for each dosage group and control group for days 0-3, 4-7 and 8-14. The light source was fluorescent light with eight hours with eight hours light and 16 hours dark at an average illumination of approximately 171 lux. Average temperature was 23.6°C ± 0.56°C (SD) and average relative humidity was 64% ± 11% (SD).

II. RESULTS AND DISCUSSION:

Validity criteria (according to OECD 223, 2016)	Required	Obtained
Mortality in the control	≤ 10%	0%

Acute oral toxicity of fluopicolide techn. to birds

Test substance	Tech. as
Test object	Bobwhite quail (male, female)
LD_{50} [mg a.s./kg b.w.]	> 2250
Lowest lethal effect dose (LLED) [mg a.s./kg b.w.]	> 2250
Lowest observed effect dose (LOED) [mg a.s./kg b.w.]	> 2250
No observed effect dose (NOED) [mg a.s./kg b.w.]	2250

Observations:

Mortality and clinical observations

No compound related mortalities occurred at any of the dose levels in the test. All birds in the treatment groups were normal in appearance and behaviour for the duration of the test.

Body weight and feed consumption

Treatment (mg a.s./kg bw)	Mean bodyweight [g]							
	day 0	day 3	day	day	Δ day 0-3	Δ day 3-7	Δ day 7-14	Δ day 0-14
Females								
Control	189 ± 6	194 ± 5	197 ± 6	200 ± 8	5 ± 2	3 ± 3	3 ± 3	11 ± 4
292	194 ± 6	200 ± 8	202 ± 9	208 ± 9	6 ± 3	2 ± 4	6 ± 3	13 ± 7
486	188 ± 5	194 ± 3	196 ± 4	199 ± 5	6 ± 3	2 ± 1	3 ± 2	11 ± 3
810	202 ± 14	206 ± 13	208 ± 12	213 ± 14	3 ± 3	3 ± 3	4 ± 10	10 ± 10
1350	184 ± 10	191 ± 10	193 ± 9	197 ± 10	7 ± 2	2 ± 1	3 ± 2	12 ± 3
2250	193 ± 9	199 ± 6	202 ± 8	205 ± 7	6 ± 3	3 ± 1	4 ± 2	12 ± 3
Males								
Control	190 ± 8	196 ± 7	200 ± 9	203 ± 7	6 ± 2	4 ± 2	3 ± 2	13 ± 3
292	193 ± 10	198 ± 10	199 ± 10	204 ± 11	5 ± 2	2 ± 3	5 ± 3	11 ± 2
486	202 ± 18	207 ± 19	208 ± 17	211 ± 19	6 ± 2	1 ± 2	3 ± 2	9 ± 2
810	194 ± 9	197 ± 11	199 ± 12	202 ± 10	3 ± 2	2 ± 1	3 ± 3	8 ± 4
1350	193 ± 10	199 ± 10	203 ± 10	206 ± 10	5 ± 2	4 ± 0	3 ± 1	12 ± 1
2250	192 ± 13	197 ± 12	200 ± 11	203 ± 13	5 ± 2	3 ± 3	3 ± 2	12 ± 2

Treatment (mg a.s./kg bw)	Mean food consumption [g/bird/d]		
	day 0-3	day 4-7	day 8-14
Females			
Control	21	24	20
292	26	29	24
486	23	25	21
810	16	18	18
1350	19	25	23
2250	28	32	25
Males			
Control	24	24	23
292	31	30	27
486	27	28	23
810	23	26	23
1350	16	22	20
2250	22	28	25

Gross pathology

Post-mortem examinations revealed no findings which were considered treatment related.

III. Conclusions:

Based on this study the LD_{50} value for bobwhite quail exposed to fluopicolide was determined to be > 2250 mg a.s./kg b.w.

The study endpoint was extrapolated according to EFSA GD 2009. The extrapolation factor of 1.888 was derived from EFSA GD 2009, section 2.1.2, table 1 for studies in which 10 animals were dosed and no mortality occurred. The extrapolated LD_{50} was determined to be 4248 mg a.s./kg b.w.

Assessment and conclusion by applicant:

This study provides valid information on the acute toxicity of fluopicolide to Bobwhite quails when administered via gavage and can be used for risk assessment. No mortality or any other indication of toxicity was observed up to the top dose level of 2250 mg a.s./kg bw. The LD_{50} is > 2250 mg a.s./kg bw. The extrapolated LD_{50} is 4248 mg a.s./kg b.w.

Data Point:	KCA 8.1.1.1/03
Report Author:	[redacted]
Report Year:	2015
Report Title:	Fluopicolide: An acute oral toxicity study with the zebra finch
Report No:	263-177
Document No:	M-544294-01-1
Guideline(s) followed in study:	U.S. Environmental Protection Agency Series 850 - Ecological Effects Test Guidelines OCSPP Number 850.2100 (2012)
Deviations from current test guideline:	Current Guideline: OECD 223 (2016) No deviations
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

Fluopicolide technical was administered orally to 10 three to six months old zebra finches (5 males and 5 females) at dose levels of 0, 125, 250, 500, 1000 and 2000 mg a.s./kg b.w. Birds were held at an average temperature of 22.4°C with an average relative humidity of 70% and approximately 8 hours light per day. Birds were observed for 14 days for mortality and sublethal symptoms. Body weight and average feed consumption were measured for each dosage and control group. Gross necropsies were performed on all mortalities and on three birds from the control group and from each treatment group at test termination, if available.

There were no mortalities in the control group or in the 125 and 250 mg a.s./kg treatment groups. There was 30% mortality at the 500 mg a.s./kg dosage level and 60% mortality at the 1000 and 2000 mg a.s./kg dosage levels. No apparent treatment-related effects on body weight among the surviving males and females in the 125, 250 and 500 mg a.s./kg treatment groups were observed. For the surviving males (1000 mg a.s./kg dosage level) and females (2000 mg a.s./kg dosage level) body weight gain was statistically lower from Day -1 to Day 3 and statistically higher from Day 7-14 than in the control. From day 3 to day 7 for the surviving males in the 1000 mg a.s./kg dosage level body weight gain was statistically higher than in the control. Based on this study the LD_{50} value for zebra finch exposed to fluopicolide was determined to be 1105 mg a.s./kg b.w.

I. Material and Methods:

The acute oral toxicity of fluopicolide technical (AE C638206-01-25, purity 100.5%) was investigated in a test with nominal dosages of 0, 125, 250, 500, 1000 and 2000 mg a.s./kg bw. Zebra finches (*Taeniopygia guttata*) approximately three to six months of age and weighing 12.8-17.5 g with a mean of 14.6 ± 0.9 grams at test initiation were used as test organisms. Test birds were housed indoors by dosage groups in batteries of pens. Each pen contained one male and female, randomly assigned to pens. Each dosage group was assigned five pens. Each pen had floor space that measured approximately 58 × 26 cm with a ceiling of 31 cm. Birds were maintained at 22.4 ± 0.2°C (SD) and an average relative humidity of 70 ± 3% (SD). The photoperiod was approximately eight hours of light per day during acclimation and throughout the test. The birds were exposed to an average of approximately 388 of lux.

The test substance was dosed using capsules. A single dose of the test substance in a capsule was orally intubated into the crop of each bird using a capsule dosing syringe. The capsule was coated with corn oil that had been dyed blue with a food-grade colorant to aid in the determination of regurgitation. Following dosing, birds were observed for at least a one-hour period for signs of regurgitation. Following the initial observation period, multiple cage side observations were performed on day 0 of the test. From test initiation until termination, all birds were observed at least twice daily. A record was maintained of all mortality, signs of toxicity, and abnormal behaviour.

Gross necropsies were performed on all mortalities and on three birds from the control group and from each treatment group at test termination, if available. The gross necropsies included, but were not limited to, a general examination of the exterior of the bird and an examination of the thoracic and abdominal cavities, including cardiovascular and respiratory systems, liver, spleen, gastro-intestinal tract, and urogenital system. Following test termination, all birds were disposed of by incineration.

II. Results and Discussion:

Validity criteria (according to OECD 223, 2016)	Required	Obtained
Mortality in the control	≤10%	0%

There were no mortalities in the control group, or in the 125 and 250 mg a.s./kg treatment groups. There was 30% mortality at the 500 mg a.s./kg dosage level and 60% mortality at the 1000 and 2000 mg a.s./kg dosage levels. Signs of toxicity noted at 250 mg a.s./kg bw and above included depression, reduced reaction to external stimuli, prostrate posture, ruffled appearance and lethargy. No indications of regurgitation were noted for any of the birds in the control group or for the birds from the 125, 250 and 500 mg a.s./kg treatment groups. There was 10% regurgitation seen at the 1000 mg a.s./kg dosage level and 30% regurgitation at the 2000 mg a.i./kg dosage level. All the birds that were noted as regurgitating resulted in mortality so regurgitation did not affect the calculation of an LD_{50} value.

When compared to the control group, there were no apparent treatment-related effects on body weight among the surviving males and females in the 125, 250 and 500 mg a.s./kg treatment groups. The slight loss in body weight from Day -1 to Day 3 was statistically significant at 1000 and 2000 mg a.s./kg. At the end of the observation period latest, all surviving birds had recovered from the initial body weight loss.

III. Conclusions:

The acute oral LD_{50} value for zebra finch exposed to fluopicolide was calculated to be 1105 mg a.s./kg. The no-mortality level was 250 mg a.s./kg. The no-observed- effect level was 125 mg a.s./kg, based on signs of toxicity observed at the 250 mg a.s./kg dosage level.

Assessment and conclusion by applicant:

This study provides valid information on the acute toxicity of fluopicolide to Zebra finches when administered via gavage and can be used for risk assessment. Toxicity was observed at 250 mg a.s./kg bw and above. The LD_{50} is 1105 mg a.s./kg bw.

CA 8.1.1.2 Short-term dietary toxicity to birds

Table 8.1.1.2- 1: Short-term dietary toxicity to birds

Test substance	Test design	Test species	Endpoint	Reference
Fluopicolide	dietary toxicity (short-term)	Bobwhite quail	LC_{50} > 5620 ppm LDD_{50} > 1744 mg a.s./kg bw/day	[redacted] 2002; M-240713-01-1 KCA 8.1.1.2/01
	dietary toxicity (short-term)	Mallard duck	LC_{50} > 5620 ppm LDD_{50} > 2343 mg a.s./kg bw/day	[redacted] 2002; M-240714-01-1 KCA 8.1.1.2/02
M-01 (2,6-dichloro-benzamide)	dietary toxicity (short-term)	Bobwhite quail	LC_{50} = 3867 ppm LDD_{50} = 1171 mg a.s./kg bw/day	[redacted] 2003; M-225551-01-2 KCA 8.1.1.2/03

Data Point:	KCA 8.1.1.2/01
Report Author:	[redacted]
Report Year:	2002
Report Title:	AE C638206 Technical: A dietary LC50 study with the Northern Bobwhite
Report No:	B003708
Document No:	M-240713-01-1
Guideline(s) followed in study:	U.S. Environmental Protection Agency: Series 850-Ecological Effects Test Guidelines OPPTS Number 850.2200 (1996) FIFRA Subdivision E, Section 71-2 (1982) OECD Guideline 205 (1984)
Deviations from current test guideline:	Method: Deviations from current guideline SANCO/3029/99 rev.4: Precision data could not be obtained directly as recoveries were determined at three different concentrations without replicates. However, the overall recovery data across different concentration demonstrate very good recoveries and the precision calculated form these data accounts for 3.1 and 0.6%, respectively, for both time points. The method can therefore be regarded as fit for purpose. Study: Current Guideline: OECD 205 (1984) The temperature of the brooding compartment was 40 ± 2°C, slightly greater than the 35 – 38°C recommended by the guideline. The relative humidity was 24 ± 5%, below the range of 50 – 75% recommended by the guideline. These deviations are not expected to impact the study results.
Previous evaluation:	yes, evaluated and accepted in DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

Bobwhite quail chicks (10 days old) were exposed for 5 days to nominal dietary concentrations of 562, 1000, 1780, 3160 and 5620 mg fluopicolide technical/kg feed (ppm); 30 chicks per control; 10 chicks

per test concentration; unknown sex. Birds were held at an average temperature of 27.3°C with an average relative humidity of 24% and approximately 16 hours light per day.
Birds were observed at least once daily for mortality, signs of toxicity and abnormal behaviour. Individual body weights were measured on day 0 at test initiation, on day 5 and at termination of the test on day 8. Samples were collected to verify the test concentrations administered and to confirm the stability and homogeneity of the test substance in the diets.
There was a single incidental mortality in the control group. There were no mortalities or overt signs of toxicity in the 562, 1000, 1780, 3160 and 5620 ppm groups. A single bird at 1000 ppm was noted with a swollen leg from the afternoon of day 4 until test termination. Additionally, one bird at 5620 ppm was euthanized on day 4 of the test due to a severe leg injury. All other birds at all test concentrations were normal in appearance and behaviour throughout the test. There were no effects on feed consumption at any of the concentrations tested. Compared to the control group, there were no treatment related effects on body weight at 562, 1000 and 1780 ppm. Reductions in body weight gain at 3160 and 5620 ppm were statistically significant during the exposure period and for the entire test period. Analysis of diet samples verified the test concentrations administered and confirmed the stability and homogeneity of the test substance in the diets.
The subacute dietary LC_{50} of fluopicolide to 10-day old Bobwhite quail chicks is determined to be > 5620 ppm, corresponding with an LDD_{50} of > 2064.2 mg a.s./kg bw/d.

I. Material and Methods:

Fluopicolide techn., purity: 97.1 %, Specification (Batch No. 2030190/PP241024/2, *Bobwhite quail* (*Colinus virginianus*) chicks (10-days-old) were exposed for 5 days to nominal dietary concentrations (techn. a.s.) of 562, 1000, 1780, 3160 and 5620 mg as/kg feed (ppm); 30 chicks per control; 10 chicks per test concentration; unknown sex. Dietary test concentrations were corrected for purity of the test substance. Water and feed were provided ad libitum during acclimation and during the test. During the test average temperature in the brooding compartment of the pens was 40 ± 2°C (SD). Average ambient room temperature for this study was 27.3 ± 0.6°C (SD) with an average relative humidity of 24 ± 5% (SD). The photoperiod was sixteen hours of light per day during acclimation and throughout the test. The birds were exposed to an average of approximately 134 lux from test initiation until termination, all birds were observed at least once daily; mortality, signs of toxicity and abnormal behaviour were assessed. Individual body weights were measured on day 0 at test initiation, on day 5 and at termination of the test on day 8. Following the exposure period, birds were maintained on untreated diet for a post observation period of 2 days.
Samples of the test diets were collected to verify the test concentrations administered and to confirm the stability and homogeneity of the test substance in the diets. Homogeneity of the test substance in the diet was evaluated by collecting six samples from the top, middle and bottom from the 562 and 5620 ppm a.s. test diets at preparation on Day 0. The homogeneity samples also served as verification samples for those concentrations. One verification sample was collected from the control diet and two verifications samples were collected from each remaining treatment group at preparation on Day 0. At the end of the exposure period (Day 5), one sample was collected from the control diet and two samples were collected from all treatment groups to determine stability of the test substance in the diet. The samples were collected from feed remaining in the feeders.

II. Results and Discussion:

Validity criteria (according to OECD 205, 1984)	Required	Obtained
Mortality in the control	≤ 10%	3.3% *
Test concentration maintained	≥ 80% of nominal over the 5 day exposure	Fulfilled
Effects in the lowest treatment level	No effects should occur	Fulfilled

* Incidental mortality (one bird)

Short-term dietary toxicity of fluopicolide to Bobwhite quails.

Test substance	Techn. a.s.	
Test object	Chicks (10 days)	
Exposure	dietary	
	[mg a.s./kg feed]	**[mg/kg bw/d]**
LC_{50} [mg a.s./kg feed]	> 5620	> 2064.2
Lowest lethal effect concentration (LLEC)	> 5620	> 2064.2
Lowest observed effect concentration (LOEC)	3160 *	1418.8*
No observed effect concentration (NOEC)	1780	837.7

* based on body weight

Observations:

Mortality and clinical observations

There was a single incidental mortality in the control group. There were no treatment related mortalities or overt signs of toxicity in the 562, 1000, 1780, 3160 and 5620 ppm treatment groups. A single bird at 1000 ppm was noted with a swollen leg from the afternoon of day 4 until test termination. Additionally, one bird in the 5620ppm group was euthanized on day 4 of the test due to a severe leg injury. All other birds at all test concentrations were normal in appearance and behaviour throughout the test.

Body weight and feed consumption

Treatment (ppm)	**Mean bodyweight ± SD [g]**					
	day 0	**day 5**	**day 8**	**Δ day 0-5[A]**	**Δ day 5-8[A]**	**Δ day 0-8[A]**
Chicks						
Control	20 ± 2	31 ± 3	40 ± 4	11 ± 2	8 ± 2	20 ± 3
562	19 ± 1	30 ± 2	38 ± 2	10 ± 1	8 ± 2	19 ± 2
1000	20 ± 2	29 ± 2	37 ± 3	10 ± 2	8 ± 2	18 ± 3
1780	20 ± 2	31 ± 4	39 ± 4	11 ± 2	8 ± 2	19 ± 3
3160	20 ± 2	29 ± 3	37 ± 4	9* ± 2	8 ± 2	17* ± 3
5620	20 ± 2	29 ± 4	36 ± 5	8* ± 2	8 ± 2	16* ± 4

* Statistically different from the control group at $p = 0.05$
[A] Mean change in weight were calculated from individual changes in bodyweight

Treatment (ppm)	**Mean food consumption [g]**	
	Exposure Period day 0-5	**Post-Exposure Period day 6-8**
Chicks		
Control	7	11
562	7	11
1000	11	12
1780	12	18
3160	11	13
5620	9	13

Treatment (ppm)	bw day 0 [g]	bw day 5 [g]	mean bw day 0-5 [g]	mean FC day 0-5 [g/bird/day]	FC/bw* [g/kg bw/day]	Dose* [mg/kg bw/d]
Chicks						
Control	20	31	25.5	7	274.5	0.0
562	19	30	24.5	7	285.7	160.6
1000	20	29	24.5	11	449.0	449.0
1780	20	31	25.5	12	470.6	835.7
3160	20	29	24.5	11	449.0	1418.8
5620	20	29	24.5	9	367.3	2064.2

bw = body weight, FC = Food consumption
* Values not presented in study report. Calculated on the basis of results for FC, bw and treatment rate given in study report.

Analytical results
None of the control samples showed any indication of the presence of the test substance fluopicolide. Diet samples were collected from the 562 and 5620 ppm a.s. test concentrations and were analysed to evaluate the homogeneity of the test substance in the diet. Means and standard deviations for the two test concentrations were 558 ± 14.4 ppm a.s. and 5790 ± 101 ppm a.s., respectively. Samples collected during the test to verify test substance concentrations for the 1000, 1780 and 3160 ppm a.s. diets had means of 1020 ppm a.s., 1780 ppm and 3300 ppm a.s., respectively. These values represented 102, 100 and 104% of nominal concentrations. Analysis of diet samples collected from feeders after being held at ambient temperature for 5 days averaged 103, 102, 103, 105 and 102% of the day 0 values for the 562, 1000, 1780, 3160 and 5620 ppm a.s. test concentrations.

Full details and acceptable validation data to support this method are presented within document M-CA-4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

III. CONCLUSIONS:

The subacute dietary LC_{50} of fluopicolide to 10-day old Bobwhite quail chicks is determined to be > 5620 ppm, corresponding with an LDD_{50} of > 2064.2 mg a.s./kg bw/d.

Assessment and conclusion by applicant:
This study provides valid information on the short-term dietary toxicity of fluopicolide to Bobwhite quail chicks when administered over 5 days in the diet and can be used for risk assessment. Toxicity was observed at 3160 ppm (1418.8 mg a.s./kg bw) and above. The LC_{50} is > 5620 ppm (LDD_{50} > 2064.2 mg a.s./kg bw).

Data Point:	KCA 8.1.1.2/02
Report Author:	[redacted]
Report Year:	2002
Report Title:	AE C638206 Technical: A dietary LC50 study with the mallard
Report No:	B003709
Document No:	M-240714-01-1
Guideline(s) followed in study:	U.S. Environmental Protection Agency: Series 850-Ecological Effects Test Guidelines OPPTS Number 850.2200 (1996) FIFRA Subdivision E, Section 71-2 (1982) OECD Guideline 205 (1984)
Deviations from current test guideline:	Method: Deviations from current guideline SANCO/3029/99 rev.4: Precision data could not be obtained directly as recoveries were determined at three different concentrations without replicates. However, the overall recovery data across different concentration demonstrate very good recoveries and the precision calculated form these data accounts for 3.1 and 0.6%, respectively, for both time points. The method can therefore be regarded as fit for purpose. Study: Current Guideline: OECD 205 (1984) The temperature of the brooding compartment was 30 ± 1°C, slightly below the 32 – 35°C recommended by the guideline. This deviation is not expected to impact the study results.
Previous evaluation:	yes, evaluated and accepted in DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

Mallard duck chicks (10 days old) were exposed for 5 days to nominal dietary concentrations of 562, 1000, 1780, 3160 and 5620 mg fluopicolide technical/kg feed (ppm); 30 chicks per control; 10 chicks per test concentration; unknown sex. Birds were held at an average temperature of 22.7°C with an average relative humidity of 50% and approximately 16 hours light per day. Birds were observed at least once daily for mortality, signs of toxicity and abnormal behaviour. Individual body weights were measured on day 0 at test initiation, on day 5 and at termination of the test on day 8. Samples were collected to verify the test concentrations administered and to confirm the stability and homogeneity of the test substance in the diets.

There were no mortalities in the control and test item groups. All birds in the control and in the treatment, groups were normal in appearance and behaviour throughout the test. No overt signs of toxicity were observed in all treatment groups. There were no treatment related effects on feed consumption at any of the concentrations tested. When compared to the control group, there were no apparent treatment related effects on body weight among birds at 562, 1000, 1780 and 3160 ppm during exposure period and for the entire test period. A statistically significant reduction of weight gain compared to the control was observed at 5620 ppm during the exposure and post-exposure period. None of the control sample showed any indication of the presence of the test substance. Analysis of diet samples verified the test concentrations administered and confirmed the stability and homogeneity of the test substance in the diets.

The subacute dietary LC_{50} of fluopicolide to 10-day old mallard duck chicks is determined to be > 5620 ppm, corresponding with an LDD_{50} of >2946.4 mg a.s./kg bw/d.

I. MATERIAL AND METHODS:

Fluopicolide techn., purity: 97.1 %, Batch No: 2050190/PP241024/2, Mallard duck (*Anas platyrhynchos*) (10-days-old) were exposed for 5 days to nominal dietary concentrations (techn. a.s.) of 562, 1000, 1780, 3160 and 5620 mg a.s./kg feed (ppm); 30 chicks per control; 10 chicks per test concentration; unknown sex. Dietary test concentrations were corrected for purity of the test substance. During the test the average temperature in the brooding compartment of the pens was 30 ± 1°C (SD). Average ambient room temperature for this study was 22.7 ± 0.5°C (SD) with an average relative humidity of 50 ± 6% (SD). The photoperiod was sixteen hours of light per day during acclimation and throughout the test. From test initiation until termination, all birds were observed at least once daily; mortality, signs of toxicity and abnormal behaviour were assessed. Individual body weights were measured on day 0 at test initiation, on day 5 and at termination of the test on day 8. Following the exposure period, birds were maintained on untreated diet for a post observation period of 3 days.
Samples of the test diets were collected to verify the test concentrations administered and to confirm the stability and homogeneity of the test substance in the diets. Homogeneity of the test substance in the diet was evaluated by collecting six samples from the top, middle and bottom from the 562 and 5620 ppm a.s. test diets at preparation on Day 0. The homogeneity samples also served as verification samples for those concentrations. One verification sample was collected from the control diet and two verifications samples were collected from each remaining treatment group at preparation on Day 0. At the end of the exposure period (Day 5), one sample was collected from the control diet and two samples were collected from all treatment groups to determine stability of the test substance in the diet. The samples were collected from feed remaining in the feeders.

II. RESULTS AND DISCUSSION:

Validity criteria (according to OECD 205, 1984)	Required	Obtained
Mortality in the control	≤ 10%	0%
Test concentration maintained	≥ 80% of nominal over the 5 day exposure	Fulfilled
Effects in the lowest treatment level	No effects should occur	Fulfilled

Short-term dietary toxicity of fluopicolide to mallard ducks

Test substance	Techn. a.s.	
Test object	Chicks (10 days)	
Exposure	dietary	
	[mg a.s./kg feed]	[mg/kg bw/d]
LDD_{50}	> 5620	> 2946.4
Lowest lethal effect concentration (LLEC)	> 5620	> 2946.4
Lowest observed effect concentration (LOEC)	5620*	2946.4
No observed effect concentration (NOEC)	3160	1394.8

* based on body weight

Observations:

Mortality and clinical observations

There were no mortalities in the control group and all control birds were normal in appearance and behaviour throughout the test. In addition, there were no mortalities or overt signs of toxicity in the 562, 1000, 1780, 2160 and 5620 ppm treatment groups. All birds in all treatment groups were normal in appearance and behaviour throughout the test.

Body weight and feed consumption

Treatment (ppm)	Mean bodyweight ± SD [g]					
	day 0	day 5	day 8	Δ day 0-5	Δ day 5-8	Δ day 0-8
Chicks						
Control	156 ± 17	287 ± 34	383 ± 43	132 ± 20	96 ± 13	228 ± 30
562	157 ± 19	288 ± 36	384 ± 50	131 ± 22	96 ± 22	227 ± 41
1000	157 ± 19	291 ± 41	390 ± 47	134 ± 26	100 ± 9	234 ± 30
1780	156 ± 20	275 ± 43	367 ± 57	119 ± 24	92 ± 18	212 ± 39
3160	156 ± 20	279 ± 36	365 ± 53	122 ± 20	86 ± 20	208 ± 36
5620	153 ± 16	259 ± 32	345 ± 43	106* ± 23	86 ± 15	192* ± 34

* Statistically different from the control group at p < 0.05

Treatment (ppm)	Mean food consumption [g]	
	Exposure Period day 0-5	Post-Exposure Period day 6-8
Chicks		
Control	96	152
562	110	168
1000	106	165
1780	90	144
3160	96	142
5620	108	153

Treatment (ppm)	bw day 0 [g]	bw day 5 [g]	mean bw [g]	mean FC day 0-5 [g/bird/day]	FC/bw* [g/kg bw/day]	Dose* [mg/kg bw/d]
Chicks						
Control	156	287	221.5	96	433.4	0.0
562	157	288	222.5	110	494.4	277.8
1000	157	291	224.0	106	473.2	473.2
1780	156	275	215.5	90	417.6	743.4
3160	156	279	217.5	96	441.3	1394.8
5620	153	259	206.0	108	524.7	2946.4

* Values not presented in study report. Calculated on the basis of results for FC, bw and treatment rate given in study report
bw = body weight, FC = Food consumption

Analytical results
None of the control samples showed any indication of the presence of the test substance fluopicolide. Diet samples were collected from the 562 and 5620 ppm a.s. test concentrations and were analysed to evaluate the homogeneity of the test substance in the diet. Means and standard deviations for the two test concentrations were 558 ± 14.4 ppm and 5790 ± 101 ppm, respectively. Samples collected during the test to verify test substance concentrations for the 1000, 1780 and 3160 ppm diets had means of 1020 ppm a.s., 1780 ppm and 3300 ppm, respectively. These values represented 102, 100 and 104% of nominal concentrations. Analysis of diet samples collected from feeders after being held at ambient temperature for 5 days averaged 100, 96, 101, 100 and 104% of the day 0 values for the 562, 1000, 1780, 3160 and 5620 ppm a.s. test concentrations.

Full details and acceptable validation data to support this method are presented within document M-CA-4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

III. CONCLUSIONS:

The subacute dietary LC_{50} of fluopicolide to 10-day old mallard duck chicks is determined to be > 5620 ppm, corresponding with an LDD_{50} of > 2946.4 mg a.s./kg bw/d.

Assessment and conclusion by applicant:
This study provides valid information on the short-term dietary toxicity of fluopicolide to Mallard duck chicks when administered over 5 days in the diet and can be used for risk assessment. Toxicity was observed at 5620 ppm (2946.4 mg a.s./kg bw) and above. The LC_{50} is > 5620 ppm (LDD_{50} > 2946.4 mg a.s./kg bw).

Data Point:	KCA 8.1.1.2/03
Report Author:	[redacted]
Report Year:	2003
Report Title:	A dietary LC50 study with the northern bobwhite AE C653711
Report No:	M-225551-01-2
Document No:	M-225551-01-2
Guideline(s) followed in study:	U.S. Environmental Protection Agency: Series 850-Ecological Effects Test Guidelines OPPTS Number 850.2200 (1996) FIFRA Subdivision E, Section 71-2 (1982) OECD Guideline 205 (1984)
Deviations from current test guideline:	Method: Deviations from current guideline SANCO/3029/99 rev.4: Recoveries were determined at three different concentrations in duplicate. However, the obtained data demonstrate very good recoveries and the precision. The method can therefore be regarded as fit for purpose. Study: Current Guideline: OECD 205 (1984) The mean concentration in the diet of day 0 and day 5 in the feeder was 78% in the lowest test concentration, slightly below the 80% recommended in the guideline. Since the mean concentration in the diet was always > 80% at higher concentrations relevant for endpoint derivation, this deviation is not expected to have impacted the study results. Body weight gain was affected at all test levels.
Previous evaluation:	yes, evaluated and accepted in DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

Bobwhite quail chicks (10 days old) were exposed for 5 days to nominal dietary concentrations of 562, 1000, 1780, 3160 and 5620 mg M-01 (2,6-dichlorobenzamide (code AE C653711))/kg feed (ppm); 30 chicks per control; 10 chicks per test concentration; unknown sex. Birds were held at an average temperature of 29.0°C with an average relative humidity of 59% and approximately 16 hours light per day.

Birds were observed twice daily, except on day 0 (4 observations) and day 8 (1 observation) for mortality, signs of toxicity and abnormal behaviour. Individual body weights were measured on day 0 at test initiation, on day 5 and at termination of the test on day 8. Samples were collected to verify the test concentrations administered and to confirm the stability and homogeneity of the test substance in the diets. There was no mortality in the control group and no treatment related mortalities in the 562, 1000 and 1780 ppm groups. In the 3160 and 5620 ppm groups 5 and 7 mortalities were observed. Clinical signs of toxicity were observed at 1000 ppm and above. Treatment related reductions in feed consumptions were observed at 3160 and 5620 ppm. Dose responsive reductions of body weight gain were observed at all treatment levels. Analysis of diet samples verified the test concentrations administered and confirmed the stability and homogeneity of the test substance in the diets, except for the lowest treatment level where only 78% of nominal was measured.

The subacute dietary LC_{50} of M-01 (2,6-dichlorobenzamide) to 10-day old Bobwhite quail chicks is determined to be 3867 ppm, corresponding with an LDD_{50} of 1171 mg p.m./kg bw/d.

I. Material and Methods:

M-01 (2,6-dichlorobenzamide (AE C653711)); purity: 98%, Lot number: I8499A, CAS Number: 2008-58-4. Bobwhite quail (*Colinus virginianus*) chicks (10-days-old) were exposed for 5 days to nominal dietary concentrations of 562, 1000, 1780, 3160 and 5620 mg p.m./kg feed (ppm); 30 chicks per control; 10 chicks per test concentration; unknown sex. Dietary test concentrations were corrected for purity of the test substance. Water and feed were provided *ad libitum* during acclimation and during the test. During the test average temperature in the brooding compartment of the pens was 38.4 ± 1.4°C (SD). Average ambient room temperature for this study was 29 ± 1.1°C (SD) with an average relative humidity of 59 ± 4% (SD). The photoperiod was sixteen hours of light per day during acclimation and throughout the test. The birds were exposed to an average of approximately 172 lux. With the exception of day 0 and day 8 of the test, all birds were observed twice daily. Birds were observed on four occasions on day 0 and once prior to test termination on day 8. Following the 5-day exposure period, birds were maintained on untreated diet for a post observation period of 3 days.
Samples of the test diets were collected to verify the test concentrations administered and to confirm the stability and homogeneity of the test substance in the diets. Homogeneity of the test substance in the diet was evaluated by collecting six samples from the top, middle and bottom from the 562 and 5620 ppm test diets at preparation on day 0. The homogeneity samples also served as verification samples for those concentrations. One verification sample was collected from the control diet and two verifications samples were collected from each remaining treatment group at preparation on day 0. At the end of the exposure period (day 5), one sample was collected from the control diet and two samples were collected from all treatment groups to determine stability of the test substance in the diet. The samples were collected from feed remaining in the feeders.

II. Results and Discussion:

Validity criteria (according to OECD 205, 1984)	Required	Obtained
Mortality in the control	≤ 10%	0%
Test concentration maintained	≥ 80% of nominal over the 5 day exposure	Fulfilled (except at 562 ppm)
Effects in the lowest treatment level	No effects should occur	reduced bw gain over 5-d treatment phase in all test levels

Short-term dietary toxicity of M-01 (2,6-dichlorobenzamide) to Bobwhite quails.

Test substance	M-01 (2,6-dichlorobenzamide)
Test object	Chicks (10 days)
Exposure	dietary
LC_{50} [mg p.m./kg feed]	3867
Lowest lethal effect concentration (LLEC) [mg p.m./kg feed]	3160
Lowest observed effect concentration (LOEC) [mg p.m./kg feed]	562 *
No observed effect concentration (NOEC) [mg p.m./kg feed]	Not determined

* based on body weight

Observations:

Mortality and clinical observations

There were no mortalities in the control group. There were no treatment-related mortalities in the 562, 1000 and 1780 ppm groups. A single incidental mortality occurred at 562 ppm after a chick had escaped from the brooding compartment; this was not considered treatment-related.

Cumulative mortalities

Treatment (ppm)	Exposure period						Post exposure period		
	day 0	day 1	day 2	day 3	day 4	day 5	day 6	day7	day 8
Chicks									
Control	0/30	0/30	0/30	0/30	0/30	0/30	0/30	0/30	0/30
562	0/10	0/10	0/10	1 [A]/10	1/10	1/10	1/10	1/10	1/10
1000	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
1780	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
3160	0/10	0/10	0/10	0/10	2/10	5/10	5/10	5/10	5/10
5620	0/10	0/10	0/10	1/10	6/10	7/10	7/10	7/10	7/10

[A] Mortality determined to be incidental, not included in the calculation of the LC50 value

Clinical signs of toxicity (including ruffled appearance, wing droop, lower limb weakness, loss of coordination, and lethargy) were noted at 1000 ppm and above.

Body weight and feed consumption

Treatment (ppm)	Mean bodyweight ± SD [g]					
	day 0	day 5	day 8	Δ day 0-5[A]	Δ day 5-8[A]	Δ day 0-8[A]
Chicks						
Control	30 ± 2	30 ± 3	40 ± 4	11 ± 2	10 ± 2	21 ± 3
562	20 ± 2	23 ± 3	33 ± 6	4 ± 2	10 ± 3	14 ± 4
1000	19 ± 2	20 ± 4	30 ± 7	1 ± 3	10 ± 4	11 ± 6
1780	20 ± 2	19 ± 3	27 ± 5	-1 ± 2	8 ± 3	7 ± 4
3160	19 ± 2	15 ± 2	22 ± 2	-4 ± 3	7 ± 1	3 ± 3
5620	20 ± 2	16 ± 3	23 ± 6	-6 ± 2	7 ± 3	1 ± 5

[A] Mean changes in weight were calculated from individual changes in bodyweight

Treatment (ppm)	Mean food consumption [g/bird/day]	
	Exposure Period day 0-5	Post-Exposure Period day 6-8
Chicks		
Control	8 ± 2	10 ± 2
562	14	11
1000	10	10
1780	9	9
3160	4	11
5620	5	10

Dose calculation was reported as presented below, note that the calculated dose at 1780 ppm is higher than at 3160 ppm, suggesting that the reduced feed consumption and body weight effects impair a proper assessment of the achieved doses. The study director proposed a dose conversion of the LC_{50} into a dietary LD_{50} of 1171 mg a.i./kg body weight/day as reasonable.

Treatment	Mean FC day 0-5 [g/bird/day]	Mean BW day 0-5 [g]	Dose [mg/kg bw/d]
Control	8	25	0
562	14	21	376
1000	10	20	528
1780	9	19	876
3160	4	17	715
5620	5	18	1468

BW = body weight, FC = Food consumption

Analytical results

None of the control samples showed any indication of the presence of the test item M-01 (2,6-dichlorobenzamide).

Diet samples were collected from the 562 and 5620 ppm M-01 (2,6-dichlorobenzamide) test concentrations and were analysed to evaluate the homogeneity of the test substance in the diet. Means and standard deviations for the two test concentrations were 569 ± 8.38 ppm and 5880 ± 150 ppm, respectively. Samples collected during the test to verify test substance concentrations for the 1000, 1780 and 3160 ppm diets had means of 1000 ppm, 1850 ppm and 3260 ppm, respectively. These values represented 100, 104 and 103% of nominal concentrations. Analysis of diet samples collected from feeders after being held at ambient temperature for 5 days averaged 78, 85, 84, 90 and 90% of the day 0 values for the 562, 1000, 1780, 3160 and 5620 ppm M-01 (2,6-dichlorobenzamide) test concentrations.

Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

III. Conclusions:

The subacute dietary LC_{50} of M-01 (2,6-dichlorobenzamide) to 10-day old Bobwhite quail chicks is determined to be 3867 ppm, corresponding with an LDD_{50} of 1171 mg p.m./kg bw/d.

Assessment and conclusion by applicant:

The assessment of the lethal toxicity endpoints is hampered by reduced feed consumption and body weight effects, so that calculation of the LDD_{50} is uncertain. No treatment-related mortality occurred up to 528 mg/kg bw/d.

CA 8.1.1.3 Sub-chronic and reproductive toxicity to birds

Table 8.1.1.3- 1: Reproductive toxicity to birds

Test substance	Test design	Test species	Endpoint	Reference
Fluopicolide	21 weeks feeding chronic, reproduction	Bobwhite quail	NOAEC ≥ 1000 ppm NOAEL ≥ 88.9 mg a.s./kg bw/d EC_{10} 46.7 (29.7 – 89.7) mg a.s./kg bw/d	[redacted] 2003; M-225403-01-2 KCA 8.1.1.3/01 EC_{10} calculation [redacted] 2019; M-660212-01-1 KCA 8.1.1.3/03
	21 weeks feeding chronic, reproduction	Mallard duck	NOEC ≥ 1000 ppm NOEL ≥ 140.8 mg a.s./kg bw/d EC_{10} 32.2 (31.1 – 33.4) mg a.s./kg bw/d	[redacted] 2003; M-225404-01-2 KCA 8.1.1.3/02 EC_{10} calculation [redacted] 2019; M-663971-01-1 KCA 8.1.1.3/04

Data Point:	KCA 8.1.1.3/01
Report Author:	[redacted]
Report Year:	2003
Report Title:	AE C638206 technical: A reproduction study with the Northern Bobwhite
Report No:	M-225403-01-2
Document No:	M-225403-01-2
Guideline(s) followed in study:	U.S. Environmental Protection Agency: Series 850-Ecological Effects Test Guidelines OPPTS Number 850.2300 (1996) FIFRA Subdivision E, Section 71-4 (1982) OECD Guideline 206 (1984)
Deviations from current test guideline:	Method: Deviations from current guideline SANCO/3029/99 rev.4: Recoveries were determined at five different concentrations in triplicate. However, the obtained data demonstrate very good recoveries and the precision. The method can therefore be regarded as fit for purpose. Study: Current Guideline: OECD 206 (1984) The birds were 28 weeks of age at the beginning of the study, slightly older than the 20 - 24 weeks recommended by the guideline. The floor area per pair was 0.1377 m2, below the 0.25 m2 recommended. The hatchlings were kept at a temperature of 38°C during their second week, higher than the 28 - 32 °C recommended. The humidity during egg storage was 87%, higher than the recommended 55 - 75%. These deviations are not expected to have impacted the study results.
Previous evaluation:	yes, evaluated and accepted in DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The aim of the study was to determine effects on reproduction of fluopicolide (AE C638206) to Bobwhite quail (*Colinus virginianus*). Fluopicolide technical was administered in the diet to three groups of sexually mature Bobwhite quails. Each group consisted of 16 breeding pairs and received nominal dietary concentrations of 0, 160, 400 or 1000 mg a.s./kg feed (ppm) over a period of 21 weeks. Birds were held at an average temperature of 21.3°C with an average relative humidity of 30.7% and approximately 8 hours light per day in the first 7 weeks. The photoperiod was increased to 17 hours of light per day during week 8. From onset of egg production eggs were collected daily. Eggs were selected randomly for egg shell thickness measurement and remaining eggs were candled to detect egg shell cracks. Cracked and abnormal eggs were discarded. All other eggs were placed in an incubator, candled again to determine embryo viability on day 11 and embryo survival on day 21. On day 21 eggs were placed into a hatcher. Hatchlings were weighed and kept on untreated diet until 14 days of age when they were weighed again and sacrificed. Adults were observed daily for mortality, abnormal behaviour and signs of toxicity. Adult body weight was measured at test initiation, on weeks 2, 4, 6, 8 and at adult sacrifice. Feed consumption was measured weekly for each pen for a 7-day period. Necropsies were performed on all adults surviving until adult sacrifice and on all adults that died during the test. In addition, effects upon egg production and quality, embryo development, hatchlings and 14-d chicks were examined. Endpoints were statistically evaluated for possible treatment related effects. According to OECD 206 guideline, the test results can be considered as valid. Samples were collected to verify the test concentrations administered and to confirm the stability and homogeneity of the test substance in the diets.

There were no treatment-related mortalities, overt signs of toxicity or treatment-related effects upon adult body weight or feed consumption at any of the concentrations tested. Additionally, there were no treatment-related effects upon any of the reproductive parameters measured at the 160-test concentration. At the 400 and 1000 ppm a.s. test concentrations there were significant reductions in hatchling body weights that were considered treatment related. Analysis of diet samples verified the test concentrations administered and confirmed the stability and homogeneity of the test substance in the diets.
Based upon the statistically significant effects on hatchling weight observed at 400 and 1000 ppm the no-observed-effect concentration (NOEC) for northern bobwhite quail exposed to fluopicolide technical in the diet during this study was 160 ppm a.s., which corresponds to a NOEL of 13.9 mg a.s./kg bw/d. However, the effects were considered not biologically relevant, so that the NOAEC from this study is considered to be 1000 ppm, corresponding to a NOAEL of 88.9 mg a.s./kg bw/d.

I. Material and Methods:

Fluopicolide (AE C638206) techn., purity: 96.1%, batch OP 2050046. Fluopicolide was administered in the diet to three groups of sexually mature Bobwhite quail (18 weeks old at test initiation) which approached their first breeding season. Each group consisted of 16 breeding pairs and received nominal dietary concentrations of either 0, 160, 400 or 1000 ppm a.s. over a period of 21 weeks (mean measured concentrations during the test: 162, 404 and 992 ppm a.s.).
Each pen was equipped with feed and water troughs. Weekly, sufficient feed for the feeding period was presented to the birds. Additional feed was weighed and added to the troughs as needed. The average temperature in the adult bobwhite quail study room during the course of the test was 21.3 ± 3°C with an average relative humidity of 30.7 ± 10.6 % (SD).
For the first 7 weeks of the test the birds were held under a photoperiod of 8 hours of light per day. The photoperiod was increased to 17 hours of light per day during week 8 to induce egg laying until adult sacrifice. Eggs were collected daily from the onset of egg production for 11 weeks and stored in a cold room. All eggs laid in a weekly interval were considered as one lot. At the end of the weekly interval, eggs were selected by indiscriminate draw for egg shell thickness measurement. The remaining eggs were candled to detect egg shell cracks. Cracked eggs and abnormal eggs were discarded. All eggs that were not discarded or used for egg shell thickness measurements were placed in an incubator at 37.4°C. Eggs were candled again on day 11 of incubation to determine embryo viability and on day 21 for determination of embryo survival. On day 21 of incubation, eggs were placed into a hatcher and allowed to hatch. Hatchlings were weighed and kept on untreated diet until 14 days of age when they were weighed again and sacrificed.
The adults were observed daily for mortality, abnormal behaviour, and signs of toxicity. Adult body weight was measured at test initiation, on weeks 2, 4, 6, 8 and at adult sacrifice. Feed consumption was measured weekly for each pen for a 7-day period. Necropsies were performed on all adults surviving until adult sacrifice and on all adults that died during the test.
Statistical evaluation for possible treatment related effects was conducted for the following endpoints: adult body weight, adult feed consumption, eggs cracked of eggs laid, eggshell thickness, viable 11-d embryos eggs per eggs set, live 3-week embryos per viable 11-d embryos, hatchlings per eggs set or per live 3-week embryos, 14-d survivor per eggs set or per hatchling, hatchling bodyweight and 14-d survivor bodyweight. The parameters "eggs laid per maximum laid", "hatchlings of maximum set" and "14-d survivor of maximum set" are not included in this summary since they lack any biological meaning.
Statistical evaluation: ANOVA, followed by Dunnett's multiple comparison procedure was used to determine statistically significant differences between the control group and each of the treatment groups. Except for adult bodyweight, the sample units were the individual pens within each experimental group. Percentage data were examined using Dunnett's method following an arcsine transformation. The pens in which adult mortality occurred were not used in statistical comparisons of the reproductive data.

Verification and homogeneity of the test substance in the diet was evaluated by collecting six samples from each of the 160, 400 and 1000 ppm a.s. treated diets on day 0 of week 1. Samples were collected from the top, middle and bottom of the left and right sections of the mixing vessel. Also collected on day 0 of week 1 was one sample from the control diet. Control and treatment group diet samples were also collected from the feed troughs on day 7 of week 1 to assess stability of the test substances under actual test conditions. Additionally, samples were collected from the control and treatment group diets during weeks 2, 3, 4, 8, 12, 16 and 20 of the test to measure/verify test concentrations.

II. RESULTS AND DISCUSSION:

Validity Criteria (according to OECD 206, 1984)	Obtained in this study
Adult mortality in control: ≤ 10 %	0%
Mean number of 14-day old survivors in the controls: ≥12 per hen	27 per hen
Eggshell thickness in control: ≥ 0.19 mm	0.234 mm
Concentration of the test item in the feed: ≥ 80 % of the nominal concentrations.	101- 108 % of the nominal concentrations

Findings:

Subchronic and reproduction toxicity to Bobwhite quail	
Test substance	Fluopicolide a.s.
Test object	Bobwhite quail
NOAEC for parental toxicity [ppm]	≥ 1000
NOAEL for parental toxicity [mg a.s./kg bw/d]	≥ 88.9
NOEC for reproduction [ppm]	160
NOEL for reproduction [mg a.s./kg bw/d]	13.9
NOAEC for reproduction [ppm]	≥ 1000
NOAEL for reproduction [mg a.s./kg bw/d]	≥ 88.9

Parental Toxicity

No mortalities occurred in the control, or in the 160 or 1000 ppm treatment groups. No overt signs of toxicity were observed at any of the concentrations tested. Four incidental mortalities occurred at 400 ppm. Due to the nature of the lesions observed at necropsy, all mortalities were considered to be incidental to treatment.

Incidental clinical observations noted during the test included those that normally are associated with injuries and pen wear. Such signs included head, neck, breast and foot lesions, bruising and abscesses on the head and neck, and feather loss. Clinical observations noted were typically associated with the incidental injuries, included lameness, lethargy and a thin general body condition. Except for incidental findings, all birds appeared normal throughout the study. The gross pathological examination of adult birds at termination of the study did not reveal findings that were considered treatment related.

There were no apparent treatment-related effects upon adult body weight at any of the concentrations tested. A very slight (4%) but statistically significant ($p < 0.05$), difference in body weight between females in the control group and those at 1000 ppm a.s. was not considered as treatment related since it was only observed in week 4 of the test.

Despite some occasions of statistically significant differences in individual weeks without any dose-response pattern, the overall food consumption in the treatments was comparable to the control.

The achieved dose was calculated in the report for the pre-egg laying phase (weeks 1-10), for the egg-laying phase (weeks 11-21), and for the total test duration (weeks 1-21). For use in this summary the overall dose over the total test duration is considered relevant.

Test Interval	Test Concentration [ppm a.s.]	Mean Body Weight [g]	Mean Feed Consumption [g/bird/day]	Daily Dietary Dose [mg a.s /kg bw/day]
Weeks 1-21	0	203	17	0
	160	201	17	13.9
	400	202	18	35.6
	1000	200	18	88.9

Reproduction Toxicity

There were no statistically significant treatment related adverse effects upon any of the reproductive parameters, except for hatchling weight that was statistically significantly lower at 400 and 1000 ppm.

Reproductive Performance per hen (absolute data)				
Parameter	**Control**	**160 ppm**	**400 ppm**	**1000 ppm**
Number of replicates	16	16	12	16
# of eggs laid	592	683	599	707
# of eggs laid / hen	37	43	50	44
# of eggs laid / hen / day	0.38	0.44	0.51	0.46
# of eggs cracked	15	2	4	12
# of eggs set	507	604	533	615
# of viable 11-d embryos	483	584	508	591
# of live 3-week embryos	479	584	504	585
# of normal hatchlings	458	563	468	559
# of 14-day survivors	424	514	418	495
# of 14-day survivors / hen	27	32	35	31
Eggshell thickness [mm]	0.234	0.233	0.236	0.233
Hatchling weight [g]	6.1	5.9	5.7*	5.5**
14-d survivor weight [g]	25	25	25	24

* $p < 0.05$ ** $p < 0.01$

Reproductive Performance (relative data)				
Parameter	**Control**	**160 ppm**	**400 ppm**	**1000 ppm**
% cracked eggs of eggs laid	2	0	1	2
% of viable 11-d embryos of eggs set	95	97	95	95
% of live 3-week embryos of viable embryos	99	100	99	99
% of hatchlings of live 3-week embryos	95	96	93	95
% of hatchlings of eggs set	90	93	88	90
% of 14-d survivors of eggs set	84	86	78	80
% of 14-d survivors of hatchlings	93	92	89	89

Reproductive Performance in % of control			
Parameter	**160 ppm**	**400 ppm**	**1000 ppm**
# of eggs laid	115%	101%	119%
# of eggs laid / hen	116%	135%	119%
# of eggs laid / hen/day	116%	134%	121%
# of eggs cracked	13%	27%	80%
# of eggs set	119%	105%	121%
# of viable 11-d embryos	121%	105%	122%
# of live 3-week embryos	122%	105%	122%
# of normal hatchlings	123%	102%	122%
# of 14-day survivors	121%	99%	117%
# of 14 -ay survivors / hen	119%	130%	115%
Eggshell thickness [mm]	100%	101%	100%
Hatchling weight [g]	97%	93%	90%
14-d survivor weight [g]	100%	100%	100%

Analytical results

None of the control samples showed any indication of the presence of the test substance fluopicolide. Diet samples were collected from the 160, 400 and 1000 ppm a.s. test concentrations, and were analysed to evaluate the homogeneity of the test substance in the diet. Means and standard deviations for the three test concentrations were 156 ± 0.753, 392 ± 18.0 and 992 ± 37.7 ppm a.s., respectively. Samples collected during the test to verify test substance concentrations for the 160, 400 and 1000 ppm a.s. diets had means and standard deviations of 162 ± 5.49, 404 ± 13.9 and 1020 ± 44.3 ppm a.s., respectively. These values represented 101, 101 and 102% of nominal concentrations, respectively. Analysis of diet samples collected from feeders after being held at ambient temperature for seven days averaged 108% of the day 0 values for each of the 160, 400 and 1000 ppm a.s. test concentrations, respectively.
Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

III. CONCLUSIONS:

Based on these findings the NOEC of parental toxicity is 1000 ppm a.s. (88.9 mg/kg bw/d). Based on slightly but statistically significantly reduced hatchling weight at 400 and 1000 ppm a.s, the NOEC for reproductive toxicity was set in the original study report at 160 ppm a.s., which corresponds to a NOEL of 13.9 mg a.s./kg body weight.
A statistical difference from control is observed in the hatchling weight at the two highest doses when estimated to one decimal place. However, reduction in hatchling weight is small (<10% compared to control) and could also reflect increased egg and hatchling numbers at the higher doses rather than being of adverse biological significance in terms of reproductive performance. There was essentially no treatment-related impact on 14d hatchling survivor numbers/female and 14d body weight and other reproductive parameters were unaffected.
However, the effects on hatchling weight were small, transient and considered not biologically relevant in the original DAR. Therefore, the NOAEC was assigned at 1000 ppm (NOAEL = 88.9 mg a.s./kg bw/d).

Assessment and conclusion by applicant:

The study is reliable, and the NOAEL of 88.9 mg a.s./kg bw/d can be used for risk assessment.

Data Point:	KCA 8.1.1.3/02
Report Author:	[redacted]
Report Year:	2003
Report Title:	AE C638206 technical: A reproduction study with the Mallard
Report No:	M-225404-01-2
Document No:	M-225404-01-2
Guideline(s) followed in study:	U.S. Environmental Protection Agency: Series 850-Ecological Effects Test Guidelines OPPTS Number 850.2300 (1996) FIFRA Subdivision E, Section 71-4 (1982) OECD Guideline 206 (1984)
Deviations from current test guideline:	Method: Deviations from current guideline SANCO/3029/99 rev.4: Recoveries were determined at five different concentrations in triplicate. However, the obtained data demonstrate very good recoveries and the precision. The method can therefore be regarded as fit for purpose. Study: Current Guideline: OECD 206 (1984) The birds were 25 weeks of age at the beginning of the study, younger than the 9 – 12 months recommended by the guideline. The floor area per pair was 0.675 m2, below the 1 m2 recommended. The hatchlings were kept at a temperature of 38°C during their first week, higher than the 32 - 35°C recommended. The humidity during egg incubation, hatching, and the first and second week of the hatchlings was lower than recommended by the guideline. These deviations are not expected to have impacted the study results.
Previous evaluation:	yes, evaluated and accepted in DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The aim of the study was to determine effects on reproduction of fluopicolide (AE C638206) to Mallard duck (*Anas platyrhynchos*). Fluopicolide technical was administered in the diet to three groups of sexually mature mallard ducks. Each group consisted of 16 breeding pairs and received nominal dietary concentrations of 0, 160, 400 or 1000 mg a.s./kg feed (ppm) over a period of 20 weeks. Birds were held at an average temperature of 18.4°C with an average relative humidity of 47.0% and approximately 8 hours light per day in the first 8 weeks. The photoperiod was increased to 17 hours of light per day during week 8. From onset of egg production eggs were collected daily for 12 weeks. Eggs were selected randomly for egg shell thickness measurement and remaining eggs were candled to detect egg shell cracks. Cracked and abnormal eggs were discarded. All other eggs were placed in an incubator, candled again to determine embryo viability on day 14 and embryo survival on day 21. On day 24 eggs were placed into a hatcher. Hatchlings were weighed and kept on untreated diet until 14 days of age when they were weighed again and sacrificed. Adults were observed daily for mortality, abnormal behaviour and signs of toxicity. Adult body weight was measured at test initiation, on weeks 2, 4, 6, 8 and at adult sacrifice. Feed consumption was measured weekly for each pen for a 7-day period. Necropsies were performed on all adults surviving until adult sacrifice and on all adults that died during the test. In addition, effects upon egg production and quality, embryo development and chick weight and survivability were examined. Endpoints were statistically evaluated for possible treatment related effects. According to OECD 206 guideline, the test results can be considered as valid. Samples were collected to verify the test concentrations administered and to confirm the stability and homogeneity of the test substance in the diets.

There were no treatment-related mortalities, overt signs of toxicity or treatment-related effects upon parental body weight or feed consumption at any of the concentrations tested. Additionally, there were no treatment-related effects upon any of the reproductive parameters measured at the 160, 400 or 1000 ppm test concentrations. Analysis of diet samples verified the test concentrations administered and confirmed the stability and homogeneity of the test substance in the diets. The no-observed effect

concentration (NOEC) for mallard duck exposed to Fluopicolide technical in the diet during the study was 1000 ppm (NOEL = 140.8 mg a.s./kg bw/d), the highest concentration tested.

I. MATERIAL AND METHODS:

Fluopicolide (AE C638206) technical, purity: 95.9%, batch OP 2050046. Fluopicolide was administered in the diet to three groups of sexually mature Mallard duck (25 weeks old at test initiation) which approached their first breeding season. Each group consisted of 16 breeding pairs and received nominal dietary concentrations of either 0, 160, 400 or 1000 mg a.s./kg feed (ppm) over a period of 20 weeks (mean measured concentrations during the test: 162, 404 and 1020 ppm a.s.).
Each pen was equipped with a bin feeder. Weekly, sufficient feed for the feeding period was presented to the birds. Additional feed was weighed and added to the troughs as needed. The average temperature in the adult mallard duck study room during the course of the test was 18.4 ± 2.7°C with an average relative humidity of 47 ± 15 % (SD).
For the first 8 weeks of the test the birds were held under a photoperiod of 8 hours of light per day. The photoperiod was increased to 17 hours of light per day during week 8 to induce egg laying until adult sacrifice. Eggs were collected daily from the onset of egg production for 12 weeks and stored in a cold room. All eggs laid in a weekly interval were considered as one lot. At the end of the weekly interval, eggs were selected by indiscriminate draw for egg shell thickness measurement. The remaining eggs were candled to detect egg shell cracks. Cracked eggs and abnormal eggs were discarded. All eggs that were not discarded or used for egg shell thickness measurements were placed in an incubator at 37.4°C. Eggs were candled again on day 14 of incubation to determine embryo viability and on day 21 for determination of embryo survival. On day 24 of incubation, eggs were placed into a hatcher and allowed to hatch. Hatchlings were weighed and kept on untreated diet until 14 days of age when they were weighed again and sacrificed.
The adults were observed daily for mortality, abnormal behaviour, and signs of toxicity. Adult body weight was measured at test initiation, on weeks 2, 4, 6, 8 and at adult sacrifice. Feed consumption was measured weekly for each pen for a 7-day period. Necropsies were performed on all adults surviving until adult sacrifice and on all adults that died during the test.
Statistical evaluation for possible treatment related effects was conducted for the following endpoints: adult body weight, adult feed consumption, eggs laid, eggs cracked of eggs laid, eggshell thickness, viable 14-d embryos eggs per eggs set, live 3-week embryos per viable 14-d embryos, hatchlings per eggs set or per live 3-week embryos, 14-d survivor per eggs set or per hatchling, hatchling bodyweight and 14-d survivor bodyweight. The parameters “eggs laid per maximum laid”, “hatchlings of maximum set” and “14-d survivor of maximum set” are not included in this summary since they lack any biological meaning.
Statistical evaluation: ANOVA, followed by Dunnett's multiple comparison procedure was used to determine statistically significant differences between the control group and each of the treatment groups. Except for adult bodyweight, the sample units were the individual pens within each experimental group. Percentage data were examined using Dunnett's method following an arcsine transformation.
Verification and homogeneity of the test substance in the diet was evaluated by collecting six samples from each of the 160, 400 and 1000 ppm a.s. treated diets on day 0 of week 1. Samples were collected from the top, middle and bottom of the left and right sections of the mixing vessel. Also collected on day 0 of week 1 was one sample from the control diet. Control and treatment group diet samples were also collected from the feed troughs on day 7 of week 1 to assess stability of the test substance under actual test conditions. Additionally, samples were collected from the control and treatment group diets during weeks 2, 3, 4, 8, 12, 16 and 20 of the test to measure/verify test concentrations.

II. RESULTS AND DISCUSSION:

Validity Criteria (according to OECD 206, 1984)	Obtained in this study
Adult mortality in control: ≤ 10 %	0%
Mean number of 14-day old survivors in the controls: ≥14 per hen	40 per hen
Eggshell thickness in control: ≥ 0.34 mm	0.395 mm
Concentration of the test item in the feed: ≥ 80 % of the nominal concentrations.	99 - 104 % of the nominal concentrations

Findings:

Subchronic and reproduction toxicity to Mallard duck	
Test substance	Fluopicolide a.s.
Test object	Mallard duck
NOEC for parental toxicity [ppm]	≥ 1000
NOEL for parental toxicity [mg a.s./kg bw/d]	≥ 140.8
NOEC for reproduction [ppm]	1000
NOEL for reproduction [mg a.s./kg bw/d]	≥ 140.8

Parental Toxicity

No mortalities occurred in the control, or in the 400 or 1000 ppm treatment groups. No overt signs of toxicity were observed at any of the concentrations tested. One incidental mortality occurred at 160 ppm (week 19). Due to the nature of the lesions observed at necropsy, this mortality was considered incidental to treatment.

Incidental clinical observations noted during the test included those that normally are associated with injuries and pen wear such as foot lesions. Except for incidental findings, all birds appeared normal throughout the study. The gross pathological examination of adult birds at termination of the study did not reveal findings that were considered treatment related.

There were no apparent treatment-related effects upon adult body weight or food consumption at any of the concentrations tested.

The achieved dose was calculated in the report for the pre-egg laying phase (weeks 1-10), for the egg-laying phase (weeks 11-21), and for the total test duration (weeks 1-21). For use in this summary the overall dose over the total study duration is considered relevant.

Test Interval	Test Concentration [ppm a.s.]	Mean Body Weight [g]	Mean Feed Consumption [g/bird/day]	Daily Dietary Dose [mg a.s /kg bw/day]
Weeks 1-20	0	1064	158	0
	160	1066	155	23.3
	400	1057	154	58.3
	1000	1072	151	140.8

Reproduction Toxicity

There were no statistically significant treatment related adverse effects upon any of the reproductive parameters, except for eggshell thickness that was statistically significantly lower at 400 ppm. Since the difference was slight and not concentration responsive, it was not considered to be treatment related.

Reproductive Performance per hen (absolute data)				
Parameter	**Control**	**160 ppm**	**400 ppm**	**1000 ppm**
Number of replicates	16	16	16	16
# of eggs laid	808	818	774	773
# of eggs laid / hen	51	51	48	48
# of eggs laid / hen/day	0.60	0.61	0.58	0.58
# of eggs cracked	3	7	14	6
# of eggs set	733	729	682	689
# of viable 14-d embryos	706	694	597	609
# of live 3-week embryos	705	683	594	600
# of normal hatchlings	646	595	548	505
# of 14-day survivors	635	588	535	499
# of 14-day survivors / hen	40	37	33	31
Eggshell thickness [mm]	0.395	0.389	0.397 *	0.385
Hatchling weight [g]	32	32	31	32
14-d survivor weight [g]	292	302	296	299

* $p < 0.05$

Reproductive Performance (relative data)				
Parameter	**Control**	**160 ppm**	**400 ppm**	**1000 ppm**
% cracked eggs of eggs laid	0	1	3	1
% of viable 14-d embryos of eggs set	97	95	85	90
% of live 3-week embryos of viable embryos	100	98	99	98
% of hatchlings of live 3-week embryos	92	86	92	82
% of hatchlings of eggs set	88	81	78	72
% of 14-d survivors of eggs set	87	80	77	71
% of 14-d survivors of hatchlings	99	99	97	99

Reproductive Performance in % of control			
Parameter	**160 ppm**	**400 ppm**	**1000 ppm**
# of eggs laid	101%	96%	96%
# of eggs laid / hen	100%	94%	94%
# of eggs laid / hen/day	102%	97%	97%
# of eggs cracked	233%	467%	200%
# of eggs set	99%	93%	94%
# of viable 11-d embryos	98%	85%	86%
# of live 3-week embryos	97%	84%	85%
# of normal hatchlings	92%	85%	78%
# of 14-day survivors	93%	84%	79%
# of 14-day survivors / hen	93%	83%	78%
Eggshell thickness [mm]	98%	95%	97%
Hatchling weight [g]	97%	93%	90%
14-d survivor weight [g]	100%	100%	100%

Analytical results

None of the control samples showed any indication of the presence of the test substance fluopicolide. Diet samples were collected from the 160, 400 and 1000 ppm a.s. test concentrations, and were analysed to evaluate the homogeneity of the test substance in the diet. Means and standard deviations for the three test concentrations were 156 ± 0.753, 392 ± 18.0 and 992 ± 37.7 ppm a.s., respectively. Samples collected during the test to verify test substance concentrations for the 160, 400 and 1000 ppm a.s. diets had means and standard deviations of 162 ± 5.49, 404 ± 13.9 and 1020 ± 44.3 ppm a.s., respectively. These values represented 101, 101 and 102% of nominal concentrations, respectively. Analysis of diet samples collected from feeders after being held at ambient temperature for seven days averaged 99, 104 and 104% of the day 0 values for each of the 160, 400 and 1000 ppm a.s. test concentrations, respectively.

Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

III. CONCLUSIONS:

Based on these findings the NOEC of parental toxicity and for reproductive effects is ≥ 1000 ppm a.s. (NOEL = 140.8 mg/kg bw/d). A small, but statistically significant reduction in shell thickness observed in the 400-ppm test group, was regarded as slight and inconsistent with respect to dose and not treatment-related. No statistical differences were observed in all other reproductive parameters and hence mallard duck reproductive capacity was considered to be unaffected by treatment up to 1000 ppm (140.8 mg/kg bw/d), the highest dose tested.

Assessment and conclusion by applicant:

The study is reliable and the NOEL of 140.8 mg/kg bw/d can be used for risk assessment.

ECx calculation for the bird reproduction studies

Data Point:	KCA 8.1.1.3/03
Report Author:	Kleinmann, J.; Wang, M.
Report Year:	2019
Report Title:	Calculation of EC10 and EC20 for fluopicolide for reproduction endpoints in northern Bobwhite
Report No:	19016-BAY-1
Document No:	M-660212-01-1
Guideline(s) followed in study:	None
Deviations from current test guideline:	Not applicable
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive summary

In the present study effect concentrations (EC_{10} and EC_{20}) were calculated from data of a reproduction study in northern bobwhite with exposure to fluopicolide (Temple et al., 2003). Calculations of EC_{10} and EC_{20} were conducted using ToxRat version 3.3.0.

Corresponding to the endpoints in the reproductive risk assessment for birds, the calculations have been performed based on achieved dietary doses, and the results are expressed accordingly in terms of mg/kg bw/d.

Effect concentrations are reported for those reproduction endpoints, for which a significant dose response was calculated. The resulting EC_{10} and EC_{20} values are summarised in the table below.

To provide additional information on the reliability of EC_x values, the 'normalised width' or *NW*, which is the ratio of the confidence interval of the EC_x and the median EC_x, was also calculated. The use of NW was recently proposed by EFSA (2015). The smaller the NW, the better the reliability.

EC_{10} and EC_{20} of reproduction endpoints from Temple et al. (2003) in northern bobwhite quail exposed to fluopicolide.

Endpoint	EC_{10} [mg/kg bw/d] (95% confidence interval)		EC_{20} [mg/kg bw/d] (95% confidence interval)	
Eggs laid per hen	No statistically significant concentration/response was found, i.e. the slope of the relationship is not significantly different from zero.			
Eggs cracked per eggs laid	No adverse effects detected [1]			
Eggshell thickness	No statistically significant concentration/response was found, i.e. the slope of the relationship is not significantly different from zero.			
Viable embryos per eggs set	No adverse effects detected [1]			
Live 3-week embryos per viable embryos	120.2 (n.d. – n.d.)	NW: -	127.6 (n.d. – n.d.)	NW: -
Hatchlings per hen	No statistically significant concentration/response was found, i.e. the slope of the relationship is not significantly different from zero.			
Hatchlings per eggs set	No statistically significant concentration/response was found, i.e. the slope of the relationship is not significantly different from zero.			
Hatchlings per viable embryos	No statistically significant concentration/response was found, i.e. the slope of the relationship is not significantly different from zero.			
Hatchlings per live 3-week embryos	No statistically significant concentration/response was found, i.e. the slope of the relationship is not significantly different from zero.			
14-day survivors per hen	No statistically significant concentration/response was found, i.e. the slope of the relationship is not significantly different from zero.			
14-day survivors per eggs set	No statistically significant concentration/response was found, i.e. the slope of the relationship is not significantly different from zero.			
14-day survivors per hatchling	No statistically significant concentration/response was found, i.e. the slope of the relationship is not significantly different from zero.			
Initial hatchling bodyweight	46.7 (29.7 – 89.7)	NW: 1.3	n.d.	
14-day survivor bodyweight	No statistically significant concentration/response was found, i.e. the slope of the relationship is not significantly different from zero.			

n.d.: not determined due to mathematical reasons (inappropriate data) or value is beyond the tested concentrations

[1] No ECx-values could be calculated since there was a positive response with increasing concentration, e.g. the number of eggs was slightly higher for higher concentrations.

ECx calculations were only possible for 2 endpoints (live 3-week embryos per viable eggs, initial hatchling bodyweight). The lowest EC_{10} was 46.7 mg/kg bw/d, albeit with a moderate fit (NW 1.3).

Assessment and conclusion by applicant:

The lowest EC_{10} was 46.7 mg/kg bw/d, albeit with a moderate fit (NW 1.3). This value is proposed for use in the avian reproductive risk assessment.

Data Point:	KCA 8.1.1.3/04
Report Author:	[redacted]
Report Year:	2019
Report Title:	Calculation of EC10 and EC20 for fluopicolide for reproduction endpoints in mallard
Report No:	19016-BAY-2
Document No:	M-663971-01-1
Guideline(s) followed in study:	None
Deviations from current test guideline:	Not applicable
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive summary

In the present study effect concentrations (EC_{10} and EC_{20}) were calculated from data of a reproduction studies in mallard with exposure to fluopicolide (Temple et al. 2003). Calculations of EC_{10} and EC_{20} were conducted using ToxRat version 3.3.0.

Corresponding to the endpoints in the reproductive risk assessment for birds, the calculations have been performed based on achieved dietary doses, and the results are expressed accordingly in terms of mg/kg bw/d.

Effect concentrations are reported for those reproduction endpoints, for which a significant dose response was calculated. The resulting EC_{10} and EC_{20} values are summarised in the table below.

To provide additional information on the reliability of EC_x values, the 'normalised width' or *NW*, which is the ratio of the confidence interval of the EC_x and the median EC_x, was also calculated. The use of NW was recently proposed by EFSA (2015). The smaller the NW, the better the reliability.

EC_{10} and EC_{20} of reproduction endpoints from Temple et al. (2003) in mallard exposed to fluopicolide.

Endpoint	EC_{10} [mg/kg bw/d] (95% confidence interval)		EC_{20} [mg/kg bw/d] (95% confidence interval)	
Eggs laid per hen	No statistically significant concentration/response was found, i.e. the slope of the relationship is not significantly different from zero.			
Eggs cracked per eggs laid	No statistically significant concentration/response was found, i.e. the slope of the relationship is not significantly different from zero.			
Eggshell thickness	No statistically significant concentration/response was found, i.e. the slope of the relationship is not significantly different from zero.			
Viable embryos per eggs set	105.0 (n.d. – n.d.)	-	n.d.	
Live 3-week embryos per viable embryos	No statistically significant concentration/response was found, i.e. the slope of the relationship is not significantly different from zero.			
Hatchlings per hen	32.2 (n.d. – n.d.)	NW: 0.07	88.2 (n.d. – n.d.)	NW: 0.03
Hatchlings per viable embryos	No statistically significant concentration/response was found, i.e. the slope of the relationship is not significantly different from zero.			
Hatchlings per live 3-week embryos	No statistically significant concentration/response was found, i.e. the slope of the relationship is not significantly different from zero.			
14-day survivors per hen	32.3 (n.d. – n.d.)	NW: 1.23	87.2 (n.d. – n.d.)	NW: 0.76
14-day survivors per eggs set	No statistically significant concentration/response was found, i.e. the slope of the relationship is not significantly different from zero.			
14-day survivors per hatchling	No statistically significant concentration/response was found, i.e. the slope of the relationship is not significantly different from zero.			
Initial hatchling bodyweight	No statistically significant concentration/response was found, i.e. the slope of the relationship is not significantly different from zero.			
14-day survivor bodyweight	No statistically significant concentration/response was found, i.e. the slope of the relationship is not significantly different from zero.			

n.d.: not determined due to mathematical reasons (inappropriate data) or value is beyond the tested concentrations

ECx calculations were only possible for 3 endpoints (viable embryos per eggs set, hatchlings per hen, 14-day survivors per hen). The lowest EC_{10} was 32.2 mg/kg bw/d, with an excellent fit (NW 0.07).

Assessment and conclusion by applicant:

The lowest EC_{10} was 32.2 mg/kg bw/d, with an excellent fit (NW 0.07). This value is proposed for use in the avian reproductive risk assessment.

CA 8.1.2 Effects on terrestrial vertebrates other than birds

Studies with mammals that have been conducted with the active substance fluopicolide are reported in the toxicology section.

CA 8.1.2.1 Acute oral toxicity to mammals

Table 8.1.2.1- 1: Acute oral toxicity data for mammals exposed to fluopicolide

Test species	Test design	Ecotoxicological Endpoint		Reference
Rat	Acute, oral	LD_{50}	> 5000 mg a.s./kg bw	[redacted] 2000; M-197224-01-1 KCA 5.2.1/01

CA 8.1.2.2 Long-term and reproduction toxicity to mammals

The wild mammal long-term risk assessment endpoint has been previously set at 20 mg/kg bw/d, being the NOAEL from the rabbit developmental toxicity study (Table 8.1.2.2-1). Thus, the position paper by [redacted]. 2006, (M-268483-01-1) is no longer relevant.

Table 8.1.2.2- 1: Long-term toxicity data for mammals exposed to fluopicolide

Test species	Test design	Ecotoxicological Endpoint		Reference
Rabbit	Long-term	NOAEL	20 mg a.s./kg bw/d	[redacted] 2004; M-202513-02-1 KCA 5.6.2/04

Current EFSA guidance (EFSA 2015 Technical report on the outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology) recommends formalising the presentation of the toxicity profile of relevance for selecting the wild mammal reproductive risk assessment endpoint in form of a tabled overview.

According to Appendix A of EFSA (2015), the results of 28-d oral toxicity studies, the sub-chronic oral toxicity studies, the multi-generation toxicity study and the developmental toxicity studies shall be compiled and evaluated.

Therefore, this overview is presented in Table 8.12.2-2, followed by more detailed information on the potentially relevant effects observed in each of these studies.

Overall, no specific toxicity on reproduction were observed. In rodents, moderate and often transient effects on body weight were typically observed at high dose levels. Effects of clear relevance for the population level occurred only in the rabbit developmental toxicity study (maternal death, premature deliveries).

Table 8.1.2.2- 2: Information from the mammalian toxicology section, relevant to identify the ecotoxicologically relevant reproductive endpoint of mammals for fluopicolide

Endpoint (phase)	Studies to check	Dose: observations	NOAEL proposal	Comment
		[mg/kg bw/d]		
1) Body weight change[1], behavioural effects and systemic toxicity[2]	28-day oral toxicity study (OECD 407)	Rat: M-199377-01-1 17.7: hepatocytic hypertrophy 179: BWG ↓, FC ↓, WC, clinical chemistry	Rat: 17.7	Large spacing between LOAEL and NOAEL
		Mouse: M-197343-01-1 115 (hepatocytic hypertrophy, liver weight ↑, clinical chemistry)	Mouse: ≥ 1111	No relevant effects up to top dose
	90-day oral toxicity study (OECD 408)	Rat: M-197622-01-1 114: urinalysis, clinical chemistry, ↑ liver weight, ↑ kidney weights, ↓ spleen weight, hepatocellular hypertrophy, histopathology in bone joints, histopathology in kidneys 1671: ↓ BW, ↓ BWG, ↓ FC, ↑ WC, haematology, clinical chemistry	Rat: 114	Effects at 114 mg/kg bw/d not relevant for wild mammal populations
		Mouse: M-205579-02-1 37.8: clinical chemistry 161: ↑ liver weight, hepatocellular hypertrophy 770: ↓ BWG, ↓ BW	Mouse: 161	Food consumption not measured
		Mouse: M-197623-01-1 46: hepatocellular hypertrophy 461: ↓ BWG (f), clinical chemistry, ↑ liver weight	Mouse: 60	Second mouse study conducted
	Multi-generation study (OECD 416)	Rat: M-232532-01-1 25.5: (hepatocyte hypertrophy) 103.4: ↓ BW, ↓ BWG, ↓ FC, ↑ liver weight, ↑ kidney weights, ↓ spleen weight	Rat: 25.5	Effects at 25.5 mg/kg bw/d not relevant for wild mammal populations
	Developmental studies (OECD 414)	Rat: M-202155-02-1 700: ↓ BW, ↓ BWG, ↓ FC	Rat: 60	Very large dose spacing
		Rabbit: M-202513-02-1 60: mortality, ↓ BW, ↓ BWG, ↓ FC	Rabbit: 20	
2) Indices of gestation, litter size, pup and litter weight[3]	Multi-generation study (OECD 416)	Rat: M-232532-01-1 2000 ppm: ↓ pup BW, ↓ pup BW G	Rat: 35.8	Dose from F0 females during gestation and lactation
	Developmental studies (OECD 414)	Rat: M-202155-02-1 700: ↓ mean foetal body weights, ↓ crown rump lengths & ↓ placental weights)	Rat: 60	Effects secondary to maternal toxicity
		Rabbit: M-202513-02-1 60: ↓ mean foetal body weights, ↓ crown rump lengths	Rabbit: 20	Effects secondary to maternal toxicity

Endpoint (phase)	Studies to check	Dose: observations	NOAEL proposal	Comment
		[mg/kg bw/d]		
3) Indices of viability, pre- and post-implantation loss	Multi-generation study (OECD 416)	Rat: M-232532-01-1 no effects up to highest dose	Rat: 127.3	Dose from F0 M prior to pairing
	Developmental studies (OECD 414)	Rat: M-202155-02-1 no effects up to highest dose	Rat: 700	
		Rabbit: M-202513-02-1 60: ↑ premature delivery	Rabbit: 20	15/23 dams aborted
4) Embryo/ foetal toxicity including teratological effects	Multi-generation study (OECD 416)	Rat: M-232532-01-1 no effects up to highest dose	Rat: 127.3	Dose from F0 M prior to pairing
	Developmental studies (OECD 414)	Rat: M-202155-02-1 700: increase in minor skeletal defects	Rat: 60	Effects of questionable relevance for wild mammals, large dose spacing
		Rabbit: M-202513-02-1 60: no effects 20: no effects	Rabbit: 20	No effects were seen up to the highest dose; however, high mortality in the high dose (60 mg/kg bw/d) precludes an adequate assessment of this group
5) Number aborting and number delivering early	Multi-generation study (OECD 416)	Rat: M-232532-01-1 no effects up to highest dose	Rat: 127.3	Dose from F0 M prior to pairing
	Developmental studies (OECD 414)	Rat: M-202155-02-1 no effects up to highest dose	Rat: 700	
		Rabbit: M-202513-02-1 60: ↑ premature delivery	Rabbit: 20	15/23 dams aborted
6) Systemic toxicity and effects on adult body weight	Multi-generation study (OECD 416)	Rat: M-232532-01-1 25.5: hepatocyte hypertrophy 103.4: ↓ BW, ↓ BWG, ↓ FC, ↑ liver weight, ↑ kidney weights, ↓ spleen weight	Rat: 25.5	Effects at 25.5 mg/kg bw/d not relevant for wild mammal populations
	Developmental studies (OECD 414)	Rat: M-202155-02-1 700: ↓ BW, ↓ BWG, ↓ FC	Rat: 60	Large dose spacing
		Rabbit: M-202513-02-1 60: mortality, ↓ BW, ↓ BWG, ↓ FC	Rabbit: 20	

Endpoint (phase)	Studies to check	Dose: observations	NOAEL proposal	Comment
		[mg/kg bw/d]		
7) Indices of post-natal growth[4], indices of lactation and data on physical landmarks	Multi-generation study (OECD 416)	Rat: M-232532-01-1 no effects up to highest dose	Rat: 127.3	Dose from F0 M prior to pairing
	Developmental studies (OECD 414)	Not relevant (no post-natal data)		
8) Survival and general toxicity up to sexual maturity	Multi-generation study (OECD 416)	Rat: M-232532-01-1 127.3: ↓ BW, ↓ BWG	Rat: 32.9	
	Developmental studies (OECD 414)	Not relevant (no post-natal data)		

More information on the most sensitive and relevant effects (% difference = in comparison with controls unless stated otherwise) in the studies tabled above:

Phase 1: Body weight changes, behavioural effects and systemic toxicity

M-199377-01-1: Rat 28-day study

0, 20, 200, 2000 & 20000 ppm corresponding with 0, 1.78, 17.8, 179 & 1770 mg/kg bw/d

Body weight gain (days 1-29) and absolute body weight (day 29) were reduced at 20000 ppm by 32/37% and 14/13% respectively in M/F. At 2000 ppm body weight gain and absolute body weight were reduced in females only, by 30% and 11% respectively (body weight changes were most marked at the start of the study for both doses). Food consumption at 20000 ppm was reduced by 41/28% in M/F during week 1. Water consumption was increased at 20000 ppm by 27/32% in M & F and at 2000 ppm by 18% in M. At 20000 ppm and 2000 ppm cholesterol was increased M & F whilst ALT was increased in M only. At 2000 ppm cholesterol was increased in M & F. Liver weights were increased at 20000 ppm by 25/23% (absolute) and 47/35% (relative) in M/F. Centrilobular hypertrophy was noted in both sexes from 200 ppm and changes associated with the accumulation of α2µ-globulin were noted in the kidneys of male rats (male rat specific finding). A NOAEL of 200 ppm (equivalent to 17.8 mg/kg bw/d) was proposed for toxicological purposes, however body weight effects observed in females at 2000 ppm may also be considered as ecotoxicologically relevant.

Therefore, the NOAEL for ecotox purposes may also be set at 200 ppm = 17.8 mg/kg bw/d, with however a large spacing factor of 10 to the lowest effect level.

M-197343-01-1: Mouse 28-day study

0, 6, 64, 640 & 6400 ppm corresponding with 0, 1.07, 11.6, 115 & 1111 mg/kg bw/d

No effects on body weight, food consumption or haematology. ALT was increased at 640 ppm and 6400 ppm in M and F (ALP was slightly, non-statistically significantly increased at 6400 ppm). Liver weights were increased at 6400 ppm by 33/50% (absolute) and 42/58% (relative) in M/F respectively. At 640 ppm absolute and relative liver weights were increased in females only by 19%. Hepatocellular hypertrophy was noted from 640 ppm.

A NOAEL of 64 ppm (11.6 mg/kg bw/d) was proposed for toxicological purposes, however no findings of ecotoxicological relevance was observed up to the top dose level (6400 ppm, equivalent to 1111 mg/kg bw/d).

M-197622-01-1: Rat 90-day study

0, 100, 1400 & 20000 ppm corresponding with 0, 7.9, 114 & 1671 mg/kg bw/d

Body weight gain (days 1-92) and absolute body weight (day 92) were reduced at 20000 ppm 30/18% and 41/29% respectively in M/F (most marked at week 1). Food consumption at 20000 ppm was reduced by 54/48% in M/F. Water consumption at this dose was increased by 44% in F (week 4). At 20000 ppm haematology parameters (Haematocrit, MCH, MCHC) were reduced in M & F and activated partial prothrombin time was slightly increased in M. At 20000 ppm cholesterol, total protein and GGT were increased in M & F and at 1400 ppm cholesterol was increased in M. Some changes to urinalysis parameters were noted at 1400 ppm & 20000 ppm (increased epithelial cells in M & increased volume and specific gravity in F). Liver weights were increased at 20000 ppm by 22% in F (absolute) and 51/49& (relative) in M/F). At 1400 ppm absolute liver weights were increased by 15% in M. Spleen weights were decreased at 20000 ppm by 45/40% in M/F and relative spleen weights were decreased by 24/29% in M/F. At 1400 ppm absolute spleen weights were reduced 10/16% in M/F and relative weights by 19% in F. Relative kidney weights were also increased by 11% in M at 1400 ppm. Histopathological findings comprised hypertrophy in the adrenal cortex at 20000 ppm, hepatocellular hypertrophy from 1400 ppm, trabecular hyperostosis in the bone joint from 1400 ppm and α2µ-globulin accumulation in the kidney of M (male rat specific effect).

A NOAEL of 100 ppm (7.4/8.4 mg/kg bw/d in M/F) was proposed for toxicological purposes, however findings of potential ecotoxicological relevance (body weight) were observed only at 20000 ppm, so that the NOAEL for ecotox purposes is 1400 ppm (114 mg/kg bw/d).

M-205579-02-1: Mouse 90-day oral toxicity study 1

0, 50, 200, 800 & 3200 ppm corresponding with 0, 10.4, 37.8, 161 & 770 mg/kg bw/d in males & 0, 12.6, 52.8, 207 & 965 mg/kg bw/d in females

Body weight gain (days 1-90) was reduced by 7/14% in M/F (most marked during weeks 1 and 2 being reduced in males by 88% during days 1-8 and in females by 87% and 85% during days 1-8 and 1-15 respectively). Absolute body weight (day 8) at 3200 ppm was reduced by 10/7% in M/F (only 3% lower in both sexes on day 90). No food consumption was measured. At 3200 ppm, ALP was increased in males by 29%, cholesterol was reduced by 50/16% in M/F and albumin was reduced by 13% (both sexes). At 800 ppm cholesterol was reduced by 48.23% in M/F and albumin by 13/10% in M/F. At 200 ppm cholesterol was reduced by 25/21% in M/F. Absolute and relative liver weights were increased at 3200 ppm (20/25% and 30/34% respectively in M/F) and 800 ppm (10/13% and 14/16% respectively in M/F). Hepatocellular hypertrophy was noted from 800 ppm.

A NOAEL of 50 ppm (10.4/12.8 mg/kg bw/d in M/F) was proposed for toxicological purposes, however findings of potential ecotoxicological relevance (body weight) were observed only at 3200 ppm, so that the NOAEL for ecotox purposes is 800 ppm (161 mg/kg bw/d).

M-197623-01-1: Mouse 90-day oral toxicity study 2

0, 32, 320, 3200 & 6400 corresponding with 0, 4.7, 46, 461 & 944 mg/kg bw/d in males & 0, 6.2, 60, 629 & 1239 mg/kg bw/d in females

Body weight gain was reduced at 6400 ppm by 20/32% in M/F (most severe at week 1; 74/64 in M/F on days 1-8) and at 3200 ppm by 22% females only. No effect on absolute body weight or food consumption. At 6400 ppm ALT and creatinine were increased in both sexes and AST and ALP were increased in males only. At 3200 ppm ALT was increased in both sexes, AST was increased males and creatinine was increased in females. At 6400 ppm absolute liver weights were increased by 42/60% in M/F and relative liver weights were increased by 50/78% in M/F. At 3200 ppm absolute liver weights were increased 33/44% in M/F and relative weights were increased by 36/59% in M/F. Hepatocellular hypertrophy was noted from 320 ppm. A NOAEL of 320 ppm (46/60 mg/kg bw/d in M/F) was proposed for toxicological purposes, however body weight effects observed in females at 3200 ppm may also be considered as ecotoxicologically relevant.

Therefore, the NOAEL for ecotox purposes may also be set at 320 ppm = 60 mg/kg bw/d (dose for the females as the sex with body weight affected at 3200 ppm).

M-232532-01-1: Multi-generation study (0, 100, 500 & 2000 ppm)

Body weight gain was reduced at 2000 ppm in males by a maximum of 10% (week 0-8), whilst absolute body weights were reduced by 6% (week 8). In females at 2,000 ppm body weight gain was reduced by 14% during premating (weeks 0-10) and by max. 17% during gestation (GD 0-6), whilst absolute body weights were reduced by max. 7% during premating (week 10) and max. 8% during gestation (GD 13). Food consumption was reduced at 2000 ppm during premating (week 1) in males by 8% and in females by 9%, and in females during gestation (8% days 0-5) and lactation (13% days 7-13). At 2000 ppm liver weights were increased by 20/14% in M/F (absolute) and by 36/21% in M/F (relative), Kidney weights were increased by 10% in M (absolute) and by 16/7% in M/F (relative) and spleen weights reduced by 12/15% in M/F (absolute) and by 8% in F (relative). Hepatocyte hypertrophy was noted from 500 ppm in males and at 2000 ppm in males and females and histopathology findings in the kidneys were noted in both sexes at 2000 ppm. A NOAEL of 500 ppm was proposed for toxicological purposes; however, body weight changes at the LOAEL of 2000 ppm (equivalent to lowest achieved doses of 103.4/127.3 mg/kg bw/d in M/F) are also considered as ecotoxicologically relevant.

Therefore, a NOAEL for ecotox purposes may also be set at 500 ppm: equivalent to 25.5 mg/kg bw/d in males and 32.9 mg/kg bw/d (minimum achieved doses).

M-202155-02-1: Rat developmental toxicity study

0, 5, 60 & 700 mg/kg bw/d

At 700 mg/kg bw/d body weight gain (days 1-21, corrected for gravid uterine weight) was reduced by 12% and by a maximum of 24% on days 7-10. Absolute body weights were slightly reduced by a maximum of 3% from day 14. Food consumption was slightly reduced on days 1-4 by 5%.

A NOAEL of 60 mg/kg bw/d was proposed for maternal toxicity. This is also relevant from an ecotoxicology perspective, with however a large spacing factor of >10 to the lowest effect level.

M-202513-02-1: rabbit developmental toxicity study

0, 5, 20 & 60 mg/kg bw/d

At 60 mg/kg bw/d 3 animals were found dead and 15 were killed following abortion. Clinical signs comprised decreased defecation, hypoactivity, bristling coat, pultaceous faeces and discoloured urine. At 60 mg/kg bw/d body weight gain was reduced by 86% (days 6-29), whilst absolute body weights were reduced (max. 7% on day 29). Food consumption was decreased at 60 mg/kg bw/d (max. 54% days 26-29).

A NOAEL of 20 mg/kg bw/d was proposed for maternal toxicity, which is also relevant for ecotoxicology.

Phase 2: Indices of gestation, litter size, pup and litter weight

M-232532-01-1: Multi-generation study

0, 100, 500 & 2000 ppm

There was no effect on gestation length, gestation index or litter size in either F1 or F2 pups. Body weights of F1 pups were reduced at 2000 ppm from day 14 by approximately 8% in males and females and body weight gain at 2000 ppm (days 1-28) was lower by 9.6/7.9% in M/F. Body weights of F2 pups were reduced at 2000 ppm from day 14 (max. 13% in males and 20% in females), whilst body weight gain (days 1-28) was reduced by 14/11% in M/F (began on weaning and possibly related to palatability of the test substance in the diet).

A NOAEL for developing offspring of 500 ppm equivalent to 35.8 mg/kg bw/d (in F0 females during gestation and lactation) was proposed based on reduced body weights from day 14; this is also relevant to ecotoxicology.

M-202155-02-1: Rat developmental toxicity study

0, 5, 60 & 700 mg/kg bw/d

There was no effect on litter size, or on the number of live and dead foetuses; mean foetal body weights (-8%), crown rump lengths (-4%) and placental weights (-9%) were slightly, statistically significantly reduced at 700 mg/kg bw/d (secondary to maternal toxicity).

A NOAEL of 60 mg/kg bw/d was proposed for developmental toxicity, this NOAEL may also be relevant for ecotoxicology with regard to these parameters, with however a large spacing factor of >10 to the lowest effect level.

M-202513-02-1: Rabbit developmental toxicity study

0, 5, 20 & 60 mg/kg bw/d

At 60 mg/kg bw/d 2/23 dams died and 15/23 dams were killed following premature delivery. Therefore, the total number of live foetuses was reduced in this group (32 compared with 157 in controls), however, the mean number of live foetuses (per dam) was not affected by treatment. Mean foetal body weight (-14%) and crown rump length (-5.6%) were statistically significantly reduced at 60 mg/kg bw/d.

A NOAEL of 20 mg/kg bw/d was proposed for developmental toxicity, this NOAEL is also relevant for ecotoxicology.

Phase 3: Indices of viability, pre- and post-implantation loss

M-232532-01-1: Multi-generation study

0, 100, 500 & 2000 ppm

There was no effect on the number of implantations, the live birth index, or the viability index up to the highest dose tested. **Therefore, the ecotoxicologically relevant NOAEL for viability is >2000 ppm (equivalent to 103.4/127.3 mg/kg bw/d for M/F prior to pairing).**

M-202155-02-1: Rat developmental toxicity study

0, 5, 60 & 700 mg/kg bw/d

The was no effect on the number of corpora lutea, number of implantation sites, pre- or post-implantation loss or the mean number of resorptions per dam. **Therefore, the ecotoxicologically relevant NOAEL for viability is > 700 mg/kg bw/d.**

M-202513-02-1: Rabbit developmental toxicity study

0, 5, 20 & 60 mg/kg bw/d

At 60 mg/kg bw/d the incidence of premature deliveries was increased (15/23 dams) secondary to maternal toxicity at this dose. **Therefore, a NOAEL of 20 mg/kg bw/d is relevant for ecotoxicology.**

Phase 4: Embryo/foetal toxicity including teratological effect

M-232532-01-1: Multi-generation study

0, 100, 500 & 2000 ppm

There was no evidence of an adverse effect of fluopicolide on necropsy of offspring. Some absolute organ weights of offspring at 2000 ppm were lower than controls: Spleen 11%/17% in M/F, thymus 11%/9% in M/F (related to lower body weights). The relevant NOAEL for ecotoxicology is therefore > 2000 ppm (equivalent to 103.4/127.3 mg/kg bw/d in M/F for F0 females prior to pairing).

M-202155-02-1: Rat developmental toxicity study

0, 5, 60 & 700 mg/kg bw/d

There was an increase in minor skeletal defects at 700 mg/kg bw/d, secondary to maternal toxicity (aplastic, dysplastic or fused thoracic vertebral arches (0/148, 0/150, 1/153, 4/142), aplastic, dysplastic, fragmented, fused or dislocated thoracic vertebral centres (0/148, 0/150, 1/153, 10/142), fragmented or longitudinally displaced sternebra (0/148, 0/150, 0/153, 3/142), aplastic, dysplastic, shortened, fused or primordium of only 9 ribs (0/148, 0/150, 1/153, 6/142) as well as wavy and/or thickened ribs (1/148, 1/150, 0/153, 5/142). There was no evidence of an increase in any major skeletal malformations, or in any major or minor external or visceral malformations.

Therefore, the NOEL is 60 mg/kg bw/d, but considering only major malformations, the relevant NOAEL for ecotoxicology may be 700 mg/kg bw/d.

M-202513-02-1: Rabbit developmental toxicity study

0, 5, 20 & 60 mg/kg bw/d

There was no evidence of a treatment related effect at any dose; however, as 15/23 dams at 60 mg/kg bw/d delivered early, an assessment of this dose group could not be made.

Therefore, a NOAEL of 20 mg/kg bw/d was proposed for developmental toxicity; this NOAEL is also relevant for ecotoxicology regarding this endpoint.

Phase 5: Number aborting and number delivering early

M-232532-01-1: Multi-generation study

No effects on abortions or early deliveries. NOAEL 127.3 mg/kg bw/d for this endpoint.

M-202155-02-1: Rat developmental toxicity study

No effects on abortions or early deliveries. NOAEL 700 mg/kg bw/d for this endpoint.

M-202513-02-1: Rabbit developmental toxicity study

0, 5, 20 & 60 mg/kg bw/d

At 60 mg/kg bw/d the incidence of premature deliveries was increased (15/23 dams) secondary to maternal toxicity at this dose. **Therefore, a NOAEL of 20 mg/kg bw/d is relevant for ecotoxicology.**

Phase 6: Systemic toxicity and effects on adult body weight

M-232532-01-1: Multi-generation study

M-202155-02-1: Rat developmental toxicity study

M-202513-02-1: Rabbit developmental toxicity study

See Phase 1 (Body weight changes, behavioural effects, and systemic toxicity) for details on systemic and body weight effects for those 3 studies.

Phase 7: Indices of post-natal growth, indices of lactation and data on physical landmarks (e.g. body weight gain, ear, and eye opening, tooth eruption, hair growth, vaginal opening, and preputial separation)

M-232532-01-1: Multi-generation study (0, 100, 500 & 2000 ppm)

There were no effects on physical landmarks at any dose. Body weight was reduced in comparison with controls but was only statistically significant from day 14 coinciding with the time the pups began to eat the diet. Therefore, this is a direct effect of the test substance (owing to palatability of the test substance in the diet) rather than a developmental effect or an effect on lactation).

The relevant NOAEL for ecotoxicology regarding this endpoint is therefore > 2000 pm (equivalent to 103.4/127.3 mg/kg bw/d in M/F for F0 females prior to pairing).

Phase 8: Survival and general toxicity up to sexual maturation

M-232532-01-1: Multi-generation study (0, 100, 500 & 2000 ppm)

There was no effect on survival up to sexual maturation. In F1 pups body weight was reduced from day 14 to day 28 (max -8.7%/7.7% in M/F on day 28). Body weight gain (days 1-28) was also lower than controls in males (-9.6%) and females (-7.9%). In F2 pups body weight gain was reduced from day 14 (max -13% in males on day 21 and day 25 and max. -12% in females on day 21); body weight gain was also reduced (days 1-28) by -14% in males and -11% in females).

Therefore, the relevant NOAEL (ecotoxicology) for this endpoint is 500 ppm (equivalent to 25.5/32.9 mg/kg bw/d in M/F in F0 parents).

Higher Tier endpoint for seed eating mammals

Based on the different feeding behaviour of rabbits and rodents (mice) and the different observed effects in the toxicity studies, further described in M-683114-01-1 (and summarized in MCP FLC+FXA FS 350), it is considered justified to employ distinct risk assessments for rabbit scenarios (herbivores) with the rabbit endpoint, and for granivore scenarios of seed eating mice with the corresponding rodent endpoint.

The treatment of rodents (rats, mice) with fluopicolide mainly results in moderate effects on body weight changes, in the rat typically associated with initially reduced food consumption which is overcome by week 3. The duration of the environmental exposure scenario of mice to treated seeds in a landscape with freshly drilled oil seed rape fields can also be conservatively estimated not to exceed 3 weeks. Therefore, 3-week body weight effects in rodents were considered as appropriate point of departure for the risk assessment on seed eating mice in freshly drilled oil seed rape fields.

For the use in the Toxicity Exposure Ratio (TER) calculation, 3-week body weight effects were derived with a benchmark dose (BMD) calculation. For this purpose, body weight data for the first 3 weeks were excerpted from all dietary studies with fluopicolide in rodents (28-d, 90-d, chronic, reproduction) which include a comparable exposure setting in the initial 21 days.

BMDs were calculated with the tools recommended by EFSA (2017), and the reliability of the fit was assessed based on the criterion of normalized width (EFSA 2015). As a point of departure for the refined risk assessment, the $BMDL_{10}$ is proposed, i.e. the left confidence limit of the BMD for 10% effect on body weight. **The lowest reliable $BMDL_{10}$ was 119 mg/kg bw/d**.

This endpoint of 119 mg/kg bw/d was used as a refinement step for the seed eating mammal scenario. The seedling eater scenario was conducted with the rabbit endpoint of 20 mg/kg bw/d.

Toxicity of plant metabolites of fluopicolide

The available information of the toxicity of fluopicolide metabolites in leafy substrates of potential relevance for the representative uses and wild mammal risk assessment is compiled in Table 8.1.2.3-3. The M-01 metabolite (AEC653711, BAM) is sufficiently characterized as to allow a wild mammal risk assessment with the specific toxicity endpoints compiled below (LD50 1470 mg/kg bw; NOAEL 7.5 mg/kg bw/d). The toxicity data for metabolites is M-02, M-04 and M-05 are less extensive but confirm that they are not 10x more toxic than their parent, as would normally be assumed in the absence of data. Only metabolites M-06 and M-09 cannot be considered as characterised with experimental data, so that assuming 10x higher toxicity may be appropriate as a surrogate endpoint in case a quantitative risk assessment were required.

Table 8.1.2.2- 3: Comparison of the toxicity of the metabolites in leafy substrates for birds and mammal's assessment (same study collection as for the parent).

Data available	Endpoint derived
M-01 (AE C653711, BAM)	
Acute oral toxicity rat M-225484-01-1	LD_{50}: 2000 mg/kg (males) & 500 mg/kg bw (females)
Acute oral toxicity/range finding study rat M-228905-01-1	**LD_{50}: 1470 mg/kg bw** (males) & 2330 mg/kg bw (females)
13-week study rat M-234461-01-1	NOAEL: 14 mg/kg bw/d (↓ BWG, ↓FC, ↓ muscle tone at the LOAEL of 49 mg/kg bw/d)
3-generation reproduction study rat , M-301025-01-1	**NOAEL 7.5 mg/kg bw/d** (slight ↓ BW in dams and offspring at LOAEL of 14 mg/kg bw/d)
Teratology study in rabbits, M-301030-01-1	NOAEL: 30 mg/kg bw/d mortality, abortion, clinical signs, body weight loss and reduced food consumption at the LOAEL of 90 mg/kg bw/d). No effects on development
M-02 (AE C657188, PCA)	
Acute oral toxicity rat M-197257-02-1	**LD50: >2000 mg/kg bw/d**
28-day study rat, M-204953-03-1	**NOAEL: 1580 mg/kg bw/d** (no effects at highest dose tested)
M-04 (AE C657378)	
Acute oral toxicity rat M-221558-01-1	**LD50: > 2000 mg/kg bw**
28-day study rat M-221960-01-2	**NOAEL: 159.2/230.6 mg/kg bw/d** in M/F (no effects at highest dose tested)
M-05 (AE 1344122)	
Acute oral toxicity rat M-235328-01-1	**LD50: > 2000 mg/kg bw**
28-day study rat M-222343-01-1	**NOAEL: 152/167 mg/kg bw/d** in M/F (↓ BWG, kidney histopathology at LOAEL of 20,000 ppm)
M-06 (AE C643890)	
No experimental data available	
M-09 (AE B102859)	
Acute oral toxicity M-685650-01-1	**LD_{50}: 1030 mg/kg bw**

CA 8.1.3 Effects of active substance bioconcentration in prey of birds and mammals

Substances with a high bioaccumulation potential could theoretically bear a risk of secondary poisoning for birds and mammals if feeding on contaminated prey like fish or earthworms. For organic chemicals, a log P_{OW} > 3 is used to trigger an in-depth evaluation of the potential for bioaccumulation. The log P_{OW} value of fluopicolide is 2.9. Since the log P_{OW} does not exceed the trigger value of 3, fluopicolide is deemed to have a negligible potential to bioaccumulate in animal tissues. Nevertheless, a bioconcentration study was conducted with fluopicolide, and the lipid normalised BCF resulted in 65 L/kg ([redacted]2003; M-241273-01-1, KCA 8.2.2.3/01).

CA 8.1.4 Effects on terrestrial vertebrate wildlife (birds, mammals, reptiles and amphibians)

Risk to birds and mammals is assessed in Document MCP, Section 10.1.

Amphibians - Aquatic data

A 48-hour LC_{50} test was performed with *Xenopus laevis* tadpoles with a LC_{50} -48 hours > 1.0 mg a.s./L. These data indicate a greater sensitivity to fluopicolide exposure of fish compared to tadpoles ($LC_{50\ fish}$: 0.36 mg a.s./L vs $LC_{50\ tadpole}$: > 1 mg a.s./L), consistent with published literature summarised in [redacted] (2017; M-645423-01-1, please refer to summary below, KCA 8.1.4/01).

Based on this information, it is considered that there are sufficient data to conclude that early life-stage amphibians exposed via the aquatic environment would be protected by a risk assessment based on the relevant fluopicolide fish data.

Test species	Endpoint	Reference
Xenopus laevis - tadpoles	LC_{50} -48 hours: > 1 mg a.s./L	[redacted] 2010; M-393869-01-1 KCA 8.2.8/01
Oncorhynchus mykiss - rainbow trout	LC_{50} - 96 hours: 0.36 mg a.s./L	[redacted] 2003; M-240806-01-1 KCA 8.2.1/01

Amphibians – Terrestrial exposure (adult life stage)

Terrestrial amphibians avoid open fields and thus the majority of the population will be found in field margins, wetlands or surrounding hedges, woods or copses. Dermal exposure is considered to be the most relevant route of exposure, as compared to other terrestrial vertebrates, amphibian skin is more permeable (and so assumed to be more susceptible to chemical uptake) with no protective (interceptive) barrier such as fur or feathers and also because the food intake rates of amphibians are low. A risk screening approach is proposed based on available data and scientific knowledge, including:

- internal dose: calculated $LD_{50\ amphibian}$
- exposure rate: assume 100 % dermal absorption of full over-spray (no spray interception by vegetation or hiding by burrowing)
- available aquatic toxicity data for the specific chemical
- standardised species-specific allometric information on relevant skin surface area (3.041 cm^2) and bodyweight (1.395 g), as suggested in Weltje et al. (2017)

Test species	Endpoint	Reference
Xenopus laevis - tadpoles	LC_{50} -48 hours: > 1 mg a.s./L	[redacted] 2010; M-393869-01-1 KCA 8.2.8/01
Lepomis macrochirus - bluegill fish	BCFss: 65 L/kg (whole fish, lipid normalised)	[redacted] 2003; M-241273-01-1 KCA 8.2.2.3/01

As $LC_{50\ tadpole}$ data are available, the following simplified equation is proposed to calculate the LD_{50} (internal lethal dose) as a toxicity indicator:

$$LD_{50\ amphibian} = LC_{50\ tadpole} \times BCF_{fish}$$
$$= 1\ mg/L \times 65\ L/kg$$
$$= 65\ mg/kg$$

Then rate and species-specific exposure information are combined to indicate the potential dermal dose:

$$\text{Dermal dose} = \text{Application rate (kg/hectare)} \times (\text{exposed skin } (cm^2)/\text{bodyweight(g)})$$
$$= 0.1 \times (3.041/1.395)$$
$$= 0.218\ mg/kg$$

A simple toxicity: exposure ratio (TER) calculation using these derived values indicates low concern for terrestrial stage amphibians, as the TER_{acute} trigger value of 10 and $TER_{chronic}$ trigger value of 5 are clearly exceeded:

$$TER = 65\ (mg/kg) / 0.218\ (mg/kg)$$
$$= 298$$

Reptiles – Terrestrial exposure

Like terrestrial amphibians, reptiles avoid open fields and thus the majority of the population will be found in field margins, wetlands or surrounding hedges, woods or copses. Unlike amphibians, reptiles have a poorly penetrable skin, therefore dermal exposure is considered less relevant in reptiles and dietary exposure is considered further. The food intake rate of reptiles and amphibians is low because in poikilothermic animals there is no energy expenditure to maintain body temperature and therefore field metabolic rates are lower than for a bird or mammal of similar size (for lizards field metabolic rates are ~17 times lower, see [redacted] (1987); M-152436-01-1 (please refer to summary below, KCA 8.1.4/02)). Consequently, food intake risk to reptiles is considered to be lower than for species with higher food intake rates, such as birds, and therefore reptiles can be considered as protected by the avian risk assessment.

Data Point:	KCA 8.1.4/01
Report Author:	[redacted]
Report Year:	2017
Report Title:	An interspecies correlation model to predict acute dermal toxicity of plant protection products to terrestrial life stages of amphibians using fish acute toxicity and bioconcentration data
Report No:	M-645423-01-1
Document No:	M-645423-01-1
Guideline(s) followed in study:	--
Deviations from current test guideline:	Not applicable
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	Yes

Executive Summary

A study was performed to predict acute dermal toxicity of plant protection products (PPPs) to terrestrial amphibian life stages from (regulatory) fish data. By combining existing concepts, including interspecies correlation estimation (ICE), allometric relations, lethal body burden (LBB) and bioconcentration modelling, an equation was derived that predicts the amphibian median lethal dermal dose (LD_{50}) from standard acute toxicity values (96-h LC_{50}) for fish and bioconcentration factors (BCF) in fish. Where possible, fish BCF values were corrected to 5 % lipid and to parent compound. Then, BCF values were adjusted to an exposure duration of 96 h, in case steady state took longer to be achieved. The derived correlation equation is based on 32 LD_{50} values from acute dermal toxicity experiments with 15 different species of anuran amphibians, comprising 15 different PPPs. The developed ICE model can be used in a screening approach to estimate the acute risk to amphibian terrestrial life stages from dermal exposures to PPPs with organic active substances. This has the potential to reduce unnecessary testing of vertebrates.

Assessment and conclusion by applicant:

This study is considered valid as supportive information only.

Data Point:	KCA 8.1.4/02
Report Author:	[redacted]
Report Year:	1987
Report Title:	Field metabolic rate and food requirement scaling in mammals and birds
Report No:	A74185
Document No:	M-152436-01-1
Guideline(s) followed in study:	none
Deviations from current test guideline:	Not applicable
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	Yes

Executive Summary

Field metabolic rates (FMRs or H_F), all measured using doubly labeled water, of 23 species of eutherian mammals, 13 species of marsupial mammals, and 25 species of birds were summarized and analyzed allometrically (log_{10}—log_{10} regressions). FMR is strongly correlated with body mass in each of these groups. FMR scales differently than does basal or standard metabolic rate in eutherians (FMR slope = 0.81) and marsupials (FMR slope = 0.58), but not in birds (FMR slope — 0.64 overall, but 0.75 in passerines and 0.75 in all other birds). Medium—sized (240—550 g) eutherians, marsupials, and birds have similar FMRs, and these are ≈17 times as high as FMRs of like—sized ectothermic vertebrates such as iguanid lizards. For endothermic vertebrates, the energy cost of surviving in nature is enormous compared with that for ectotherms. Within the eutherians, marsupials, or birds, FMR scales differently for the following subgroups: rodents, passerine birds, herbivorous eutherians, herbivorous marsupials, desert eutherians, desert birds, and seabirds. Equations are given for use in predicting daily and annual FMR and food requirement of a species of terrestrial vertebrate, given its body mass.

Assessment and conclusion by applicant:

This study is considered valid as supportive information only.

CA 8.1.5 Endocrine disrupting properties

Potential endocrine-disrupting properties of fluopicolide are being evaluated according to EU Regulation 2018/605 (“ED criteria”) following recommendations of the ECHA-EFSA Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009.

CA 8.2 Effects on aquatic organisms

Table 8.2- 1: Endpoints used in risk assessment and additional valid studies for fluopicolide and its metabolites

Test substance	Test species	Endpoint	Reference
Fluopicolide	Fish, acute *Oncorhynchus mykiss*	**96 h LC_{50} 0.36 mg a.s./L (mm)** NOEC 0.16 mg a.s./L (mm)	[redacted] 2003; M-240806-01-1 KCA 8.2.1/01
	Fish, acute *Lepomis macrochirus*	96 h LC_{50} 0.75 mg a.s./L (mm) NOEC 0.56 mg a.s./L (mm)	[redacted] 2003; M-240805-01-1 KCA 8.2.1/02
	Fish, acute *Cyprinus carpio*	96 h LC_{50} 1.3 mg a.s./L (mm) NOEC 0.25 mg a.s./L (mm)	[redacted] 2003; M-219743-01-1 KCA 8.2.1/03
	Fish, acute *Brachydanio rerio*	96 h LC_{50} 1.8 mg a.s./L (mm) NOEC 1.0 mg a.s./L (mm)	[redacted] 2003; M-234508-01-2 KCA 8.2.1/04
	Fish, acute *Oryzias latipes*	96 h LC_{50} 0.7 mg a.s./L (mm) NOEC 0.44 mg a.s./L (mm)	[redacted] 2003; M-234510-01-2 KCA 8.2.1/05
	Fish, acute *Cyprinodon variegatus*	96 h LC_{50} 0.41 mg a.s./L (mm) NOEC 0.20 mg a.s./L (mm)	[redacted] 2003; M-223359-01-2 KCA 8.2.1/06
	Fish, acute *Pimephales promelas*	96 h LC_{50} 1.34 mg a.s./L (nom) NOEC 0.313 mg a.s./L (nom)	[redacted] 2015; M-533292-01-1 KCA 8.2.1/10
	Fish, chronic (ELS) *Pimephales promelas*	33 d NOEC 0.155 mg a.s./L (mm) **EC_{10} 0.278 mg a.s./L (mm)**	[redacted] 2003; M-241190-01-1 KCA 8.2.2.1/01 [redacted] 2018; M-643769-01-1 Calculation of EC_{10} endpoint. KCA 8.2.2.1/02
	Fish, BCF flow through *Lepomis macrochirus*	BCFss, lipid normalised 65 L/kg (whole fish)	[redacted] 2003; M-241273-01-1 KCA 8.2.2.3/01
	Invertebrate, acute *Daphnia magna*	**48 h EC_{50} > 1.8 mg a.s./L (mm)**	[redacted] 2003; M-240807-01-1 KCA 8.2.4.1/01

Test substance	Test species	Endpoint	Reference
	Invertebrate, acute *Crassostrea virginica*	96 h > 2.6 mg a.s./L EC_{50} (mm)	[redacted] 2003; M-225445-01-1 KCA 8.2.4.2/01
	Invertebrate, acute *Americamysis bahia*	96 h 3.2 mg a.s./L LC_{50} (mm)	[redacted] 2003; M-220513-01-2 KCA 8.2.4.2/02
	Invertebrate, chronic *Daphnia magna*	21 d 0.19 mg a.s./L NOEC (mm) EC_{10} Cannot be calculated	[redacted] 2003; M-241191-01-1 KCA 8.2.5.1/01 [redacted] 2018; M-617357-01-1 Endpoint recalculation. KCA 8.2.5.1/02
	Invertebrate, chronic *Americamysis bahia*	28 d 0.34 mg a.s./L NOEC (mm) **EC_{10} 0.18 mg a.s./L (mm)**	[redacted] 2019; M-544290-02-1 KCA 8.2.5.2/01
	Sediment dweller, chronic *Lumbriculus variegatus*	**28 d 1.98 mg a.s./kg NOEC (nom)**	[redacted] 2020; M-671529-03-1 KCA 8.2.5.4/02
	Algae *Pseudokirchneriella subcapitata* Green algae	72 h 4.3 mg a.s./L E_rC_{50} (mm) 72 h 3.0 mg a.s./L E_bC_{50} (mm) 72 h 2.4 mg a.s./L NOE$_r$C (mm) 72 h 2.6 mg a.s./L E_rC_{10} (mm)	[redacted] 2003; M-219737-01-2 KCA 8.2.6.1/01 [redacted] 2018; M-643768-01-1 Endpoint recalculation. KCA 8.2.6.1/05
	Algae, *Skeletonema costatum* (Marine diatom)	**72 h 0.073 mg a.s./L** **E_rC_{50} (nom)** 96 h 0.0612 mg a.s./L E_yC_{50} (nom) 72 h 0.0160 mg a.s./L E_rC_{05} (nom) 72 h 0.0424 mg a.s./L E_rC_{10} (nom)	[redacted] 2015; M-533278-01-1 KCA 8.2.6.2/07
	Algae *Navicula pelliculosa* (Freshwater diatom)	72 h 0.121 mg a.s./L E_rC_{50} (mm) 72 h 0.068 mg a.s./L E_yC_{50} (mm) 72 h 0.043 mg a.s./L NOE$_r$C (mm) 72 h 0.064 mg a.s./L E_rC_{10} (mm)	[redacted] 2020; M-678011-01-1 KCA 8.2.6.2/08

Test substance	Test species	Endpoint	Reference
	Aquatic macrophytes, *Lemna gibba*	**7 d E_rC_{50} > 3.2 mg a.s./L (mm) frond number & dry weight** NOE_rC 3.2 mg a.s./L (mm)	[redacted] 2003; M-220891-01-2 KCA 8.2.7/01
	Amphibian larvae, acute *Xenopus laevis*	48 h LC_{50} >1 mg a.s./L (nom) NOE_rC 0.125 mg a.s./L (nom)	[redacted] 2010; M-639869-01-1 KCA 8.2.8/01
M-01 (2,6-dichloro-benzamide (BAM; BCS-AA65784))	Fish, acute *Oncorhynchus mykiss*	**96 h LC_{50} 240 mg p.m./L (nom)**	[redacted] 2001; M-234311-01-2 KCA 8.2.1/07
	Invertebrate, acute *Daphnia magna*	**48 h EC_{50} 180 mg p.m./L (nom)**	[redacted] 2001; M-234306-01-2 KCA 8.2.4.1/02
	Algae *Pseudokirchneriella subcapitata* Green algae	72 h E_rC_{50} 120 mg p.m./L (nom) 72 h E_bC_{50} 60 mg p.m./L (nom) 72 h NOE_rC 40 mg p.m./L (nom) 72 h E_rC_{10} 49 mg p.m./L (nom)	[redacted] 2001; M-234304-01-2 KCA 8.2.6.1/03
	Algae *Navicula pelliculosa* (Freshwater diatom)	**72 h E_rC_{50} 92 mg p.m./L (mm)** 72 h E_yC_{50} 46 mg p.m./L (mm) 72 h NOE_rC 30 mg p.m./L (mm) 72 h E_rC_{10} 42 mg p.m./L (mm)	[redacted] 2020; M-678377-01-1 KCA 8.2.6.2/10
	Aquatic macrophytes, *Lemna gibba*	**7 d E_rC_{50} 97.6 mg p.m./L (nom), frond number** 7 d E_yC_{50} 71.8 mg p.m./L (nom) NOE_rC 25.0 mg p.m./L (nom) E_rC_{10} 51.0 mg p.m./L (nom)	[redacted] 2003; M-219725-01-2 KCA 8.2.7/02 [redacted] 2018; M-664031-01-1 Endpoint recalculation. KCA 8.2.7/03

Test substance	Test species	Endpoint	Reference
M-02 (3-chloro-5-trifluoromethylpyridine-2-carboxylic acid (PCA, BCS-AB43478))	Fish, acute *Oncorhynchus mykiss*	**96 h LC_{50} > 102 mg p.m./L (mm)**	[redacted] 2008; M-218631-01-2 KCA 8.2.1/08
	Algae, *Navicula pelliculosa* (Freshwater diatom)	**72 h E_rC_{50} 74 mg p.m./L (mm)** 72 h E_yC_{50} 72 mg p.m./L (mm) 72 h NOE_rC 42 mg p.m./L (mm) 72 h E_rC_{10} 48 mg p.m./L (mm)	[redacted] 2020; M-678012-01-1 KCA 8.2.6.2/09

Bold: Endpoints used in risk assessment
a.s.: active substance; p.m.: pure metabolite
nom = nominal concentrations, mm = mean measured concentration

CA 8.2.1 Acute toxicity to fish

Data Point:	KCA 8.2.1/01
Report Author:	[redacted]
Report Year:	2003
Report Title:	The 96 hour acute toxicity to the rainbow trout, Oncorhynchus mykiss, in a static system; AE C638206 Technical 97.1 percent w/w
Report No:	B003802
Document No:	M-240806-01-1
Guideline(s) followed in study:	OECD: 203 (1992); USEPA (=EPA): 72-1 (1982)
Deviations from current test guideline:	Method: Deviations from current guideline SANCO/3029/99 rev.4: Recoveries were only determined at two different concentrations in duplicate. However, the obtained data demonstrate very good recoveries and the precision calculated form these data accounts for 7.9 (AE C638206) and 8.2 % (AE C653711), respectively. The method can therefore be regarded as fit for purpose Study: Current Guideline: OECD 203 (2019) The pH of all test solutions was greater than 8.5 on days 0 and 1 (8.6 to 8.8). This deviation had no impact on the study since the validity criteria are met.
Previous evaluation:	yes, evaluated and accepted in DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Data Point:	KCA 8.2.1/11
Report Author:	[redacted]
Report Year:	2018
Report Title:	Statement - Certificate of analysis for fluopicolide acute toxicity study on rainbow trout (Young & Abedi, 2003; M-240806-01-1)
Report No:	M-634700-01-1
Document No:	M-634700-01-1
Guideline(s) followed in study:	--
Deviations from current test guideline:	Not applicable
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	Yes

Executive summary

An acute toxicity test was performed with the rainbow trout (*Oncorhynchus mykiss*) in a static system. Junvenile rainbow trouts were exposed to fluopicolide (tech.) at nominal concentrations of 0.65, 1.1, 1.8 3.0 and 5.0 mg a.s./L in well water for a 96- hour period. Additionally, a negative control was included. All treatments had 10 fish per test vessel (10 fish per treatment level). Test solutions were not renewed. Mortality, toxicity values and behaviour were recorded at time points 3, 6, 24, 48, 72 and 96 hours
Recoveries at 0 and 96h were below 80% so the biological results are based on mean measured concentrations of fluopicolide. There was no fluopicolide residue found in the dilution water or control samples. Analytical data indicated that the fluopicolide metabolite M-01 (AE C653711) did not form in the study samples. All samples were analysed by Gas Chromatography with MS detection (GC/MS).

The arithmetic mean measured concentrations of fluopicolide were 0.16, 0.29, 0.44, 0.70, and 1.2 mg a.s./L.
The study fulfils all validity criteria of the current version of OECD 203 guideline.
Mortality occurred at 0.44 mg a.s./L and above, with 100% mortality observed at 3h at 1.2 mg a.s./L.
Sublethal effects were observed at 0.29 mg a.s./L and above all over the study in surviving fish. The 96-hour LC_{50} of fluopicolide technical to rainbow trout is calculated as 0.36 mg a.s./L (95%CL = 0.29 to 0.44 mg a.s./L). The lowest observed concentration with mortality is 0.44 mg a.s./L. The highest concentration without mortality is 0.29 mg a.s./L. The NOEC (highest concentration without sublethal effects) is 0.16 mg a.s./L.

I. MATERIAL AND METHODS:

Test material	Fluopicolide (tech.) batch: 2050190//PP24102/02 purity 97.1 % w/w
Guideline(s) adaptation	None specified
Test species	Rainbow trout (*Oncorhynchus mykiss*)
Acclimation	At least 14 days Health during acclimation: less than 1% mortality
Organism age/size at study initiation	In control fish at the end of the study: Mean length: 4.6 ± 0.41 cm Mean body weight: 1.321 ± 0.3545 g
Test solutions	Nominal concentrations: 0.65 - 1.1 - 1.8 - 3.0 - 5.0 mg a.s./L. Corresponding mean measured concentrations: 0.16 - 0.29 - 0.44 - 0.70 and 1.2 mg a.s. / L. Samples were taken from all test chambers on day 0 and day 4. Controls: water The stock solution was filtered through a 0.45 µm filter as it was dispensed into the test chambers to remove undissolved test substance. This is the reason why the measured concentrations at t0 are in the range of 22 to 25% of nominal concentrations. After the filtration of the primary stock solution, there were no additional problems with solubility throughout the study.
Replication	No. of vessels per concentration (replicates): 1 No. of vessels per control (replicates): 1
Organisms per replicate	No. of organisms per vessel: 10
Exposure	static Total exposure duration: 96 hours
Test Vessel Loading	0.377 g fish/L test medium
Feeding during test	None
Test conditions	Temperature: 12.7 – 13.9°C Photoperiod: 16 hours light / 8 hours dark Light intensity: 1600 lux pH: 8.1 – 8.8. Water hardness: 94 mg $CaCO_3$/L Dissolved oxygen: 67 -113% saturation Conductivity: 1000 to 1300 µmhos/cm

Parameters Measured / Observations	Observations for death, and for abnormal appearance and behavior were performed at 3, 6, 24, 48, 72, and 96 hours (± 1 hour) Discrete measurements of temperature, dissolved oxygen, pH, and conductivity were obtained at test initiation, 24, 48, 72, and 96 hours, or within one hour of the designated time.
Chemical analysis	Samples were analysed by Gas Chromatography with MS detection (GC/MS) for the actual concentration of fluopicolide and M-01 (AE C653711) present in the test medium on day 0 and on day 4.
Data analysis	LC_{50} values and the 95%-confidence intervals were calculated for 3-, 6- and 24-hour time points with Spearman-Karber method and for the time points 48, 72 and 96 hours with binominal test method using CT-Tox version 1.1. The LC_{50} was estimated, using one of four statistical techniques: moving average, binominal, Spearman-Karber analysis or probit analysis. The appropriate method was determined according to the data characteristics.

II. Results and Discussion:

Validity criteria of OECD 203 (2019)	Required	Obtained
Measured concentration of the test substance	Mandatory	Performed and results are based on mean measured concentrations
Mortality in control during test	≤10%	0 %
Dissolved oxygen saturation	≥ 60%	67-113%

Analytical results:
Recoveries at 0 and 96h were below 80% (see table below) so the biological results are based on mean measured concentrations of fluopicolide. The results are reported on the basis of arithmetic mean measured concentrations. According to the EFSA technical report (2015)[1], the results should be based on geometric mean measured concentrations. However, the concentrations are stable over the 96 h of the test and in these conditions, both arithmetic and geometric means are similar when rounded, so there is no need to recalculate the endpoints.
There was no fluopicolide residue found in the dilution water or control samples. Analytical data indicated that the fluopicolide metabolite M-01 (AE C653711) did not form in the study samples.

Nominal conc. (mg a.s./L)	Arithmetic mean (mg a.s./L)	Geometric mean (mg a.s./L)	% of nominal	
			0 hour	96 hour
0.65	0.16	0.16	23	26
1.1	0.29	0.29	24	29
1.8	0.44	0.44	23	25
3.0	0.70	0.70	23	23
5.0	1.2	1.21	25	24

[1] EFSA (European Food Safety Authority), 2015. Technical report on the outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology. EFSA supporting publication 2015:EN-924. 62 pp.

Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4 with minor acceptable exceptions only.

Biological results:

Observations

At 3-hours, sub-lethal effects such as loss of equilibrium, erratic swimming, and swimming ceased were observed in the 0.29, 0.44, and 0.70 mg/L treatments. At 24-hours, sub-lethal effects were still observed in the 0.29 and 0.44 mg/L treatments. At 48 hours, sub-lethal effects were observed in the 0.29 mg/L treatment. At 72 or 96 hours, 10% sub-lethal effects continued in the 0.29 mg/L treatment.

Mortality

Exposure time (hours)	**3**	**6**	**24**	**48**	**72**	**96**
Arithmetic mean measured conc. (mg a.s./L)	**No of dead (%)**	**No of dead (%)**	**No of dead (%)**	**No of dead (%)**	**No of dead (%)**	**No of dead (%)**
Control	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
0.16	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
0.29	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
0.44	0 (0)	0 (0)	9 (90)	10 (100)	10 (100)	10 (100)
0.70	3 (30)	6 (60)	10 (100)	10 (100)	10 (100)	10 (100)
1.2	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)

III. CONCLUSIONS:

The study meets the validity criteria and the endpoints based on arithmetic mean measured concentrations are:

LC_{50} 96 hours (95% C.I.):	**0.36 mg a.s. / L (0.29 – 0.44 mg a.s. / L)**
lowest concentration with mortality	0.44 mg a.s. / L
highest concentration without mortality	0.29 mg a.s. / L
NOEC: highest concentration without sublethal effects	0.16 mg a.s. / L

Assessment and conclusion by applicant:
The study is reliable and the LC_{50} of 0.36 mg a.s./L and NOEC of 0.16 mg a.s./L can be used in risk assessment.

Data Point:	KCA 8.2.1/02
Report Author:	Young, B. M.
Report Year:	2003
Report Title:	The 96 hour acute toxicity to the bluegill sunfish, Lepomis macrochirus, in a static system; AE C638206 Technical 97.1 percent w/w
Report No:	B003801
Document No:	M-240805-01-1
Guideline(s) followed in study:	OECD: 203 (1992); USEPA (=EPA): 72-1 (1982)
Deviations from current test guideline:	Method: Deviations from current guideline SANCO/3029/99 rev.4: Recoveries were only determined at two different concentrations in duplicate. However, the obtained data demonstrate very good recoveries and the precision calculated form these data accounts for 2.4 (AE C638206) and 7.2 % (AE C653711), respectively. The method can therefore be regarded as fit for purpose.; Study: Current Guideline: OECD 203 (2019) The pH is greater than 8.5 (up to 8.7) on day 0 and 1 in the 2 highest concentrations. Since the variation within the study was very limited (between 8.4 and 8.7) and validity criteria are met, it is unlikely that this deviation had a significant impact on the results
Previous evaluation:	yes, evaluated and accepted in DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive summary

An acute toxicity test was performed with the bluegill sunfish, (*Lepomis macrochirus*) in a static system. Juvenile bluegill sunfish were exposed to fluopicolide (tech.) in nominal concentrations of 0.65, 1.1, 1.8, 3.0, and 5.0 mg a.s./L, in well water for a 96-hour period. Additionally, a negative control was included. All treatments had 10 fish per test vessel (i.e., 10 fish per treatment level). Test solutions were not renewed. Mortality, toxicity values and behaviour were recorded at time points 3, 6, 24, 48, 72 and 96 hours. Each test chamber was sampled for analysis of fluopicolide (tech.) and the metabolite M-01 (AE C653711) at 0 (prior to the introduction of the test organisms to the test chambers) and 96 hours (study termination). Samples were taken at mid-depth and did not include any extraneous materials. All samples were analysed by Gas Chromatography with electron capture detection (GC/ECD). Recoveries were below 80%, thus the results were based on mean measured concentrations of fluopicolide (tech.) which were 0.24, 0.37, 0.56, 1.0, and 1.7 mg a.s./L. The study fulfils all validity criteria of the current version of OECD 203 guideline. Sub-lethal effects were observed from 3 h to 24h at 1.0 mg a.s/L and above. Mortality occured at the same concentrations. Analysis of the mortality data by the trimmed Spearman-Karber method gave the following result. The 96-hour LC_{50} of fluopicolide (tech.) technical to bluegill sunfish was calculated as 0.75 mg a.s./L (95%, CL = 0.56 to 1.0 mg a.s./L). The lowest observed effect concentration (LOEC), based on sublethal effects, is 1.0 mg a.s./L. The no observed effect concentration (NOEC) is 0.56 mg a.s./L.

I. MATERIAL AND METHODS:

Test material	Fluopicolide (tech.) batch: 2050190//PP241024/2 purity 97.1 % w/w
Guideline(s) adaptation	None specified
Test species	Bluegill sunfish (*Lepomis macrochirus*)
Acclimation	At least 14 days Health during acclimation: less than 2% mortality
Organism age/size at study initiation	Mean length: 2.4 cm (2.1 – 2.6 cm) at the end of the study Mean body weight: 0.294 g (0.177 – 0.397 g) at the end of the study
Test solutions	Nominal concentrations: 0.65 - 1.1 - 1.8 - 3.0 - 5.0 mg a.s./L. Corresponding mean measured concentrations: 0.24 - 0.37 - 0.56 - 1.0 and 1.7 mg a.s./L. Samples were taken from all test chambers on day 0 and day 4. Controls: water Evidence of undissolved material: The stock solution was filtered through a 0.45 urn filter as it was dispensed into the test chambers to remove undissolved test substance. After the filtration of the primary stock solution, there were no additional problems with solubility throughout the study.
Replication	No. of vessels per concentration (replicates): 1 No. of vessels per control (replicates): 1
Organisms per replicate	No. of organisms per vessel: 10
Exposure	static Total exposure duration: 96 hours
Test Vessel Loading	0.196 g fish/L test medium
Feeding during test	None
Test conditions	Temperature: 21.6 - 22.2°C Photoperiod: 16 hours light / 8 hours dark Light intensity: 1650 lux pH: 8.4 – 8.7 Water hardness: 120 mg $CaCO_3$/L Dissolved oxygen: 77 -98% saturation Conductivity: 900 to 1000 µS/cm
Parameters Measured / Observations	Observations for death; and for abnormal appearance and behaviour, were performed at 3, 6, 24, 48, 72, and 96 hours (± 1 hour) Discrete measurements of temperature, dissolved oxygen, pH, and conductivity were obtained at test initiation, 24, 48, 72, and 96 hours, or within one hour of the designated time.
Chemical analysis	Samples were analysed by Gas Chromatography with electron capture detection (GC/ ECD) for the actual concentration of fluopicolide and metabolite M-01 (AE C653711 BAM) in the test medium on day 0 and on day 4.
Data analysis	LC_{50} values and the 95%-confidence intervals were calculated for 24-hour time points with Spearman-Karber method and for the time points 48, 72 and 96 hours with binominal test method using CT-Tox version 1.1. The LC_{50} was estimated, using one of four statistical techniques: moving average, binominal, Spearman Karber analysis or Probit analysis. The appropriate method was determined according to the data characteristics.

II. RESULTS AND DISCUSSION:

Validity criteria according to OECD 203 (2019)	Required	Obtained
Measured concentration of the test substance measured concentrations	Mandatory	Performed and results are based on mean measured concentrations
Mortality in control during test	≤ 10%	0 %
Dissolved oxygen saturation	≥ 60%	77 - 98%

Analytical results:
Recoveries were below 80% (see table below). The results should be expressed on the basis of geometric mean measured concentrations, but they were reported on the basis of arithmetic mean concentrations. The table below shows that there is no significant difference between the 2 means and a recalculation of the EC_{50} would not provide a different value so the biological results are based on arithmetic mean measured concentrations of fluopicolide.
There was no fluopicolide residue found in the dilution water or control samples. Metabolite M-01 (BAM, AE C653711) was not quantified in the samples.

Nominal conc. (mg a.s./L)	Arithmetic mean (mg a.s./L)	Geometric mean (mg a.s./L)	% of nominal concentrations	
			0 hour	96 hour
0.65	0.24	0.24	38	36
1.1	0.37	0.37	34	32
1.8	0.56	0.56	32	30
3.0	1.0	1.1	34-35	35
5.0	1.7	1.7	35	33

Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4 with minor acceptable exceptions only.

Biological results:

Observations

At 3-hours, sub-lethal effects of loss of equilibrium, erratic swimming, and lethargy were observed in the 1.0 and 1.7 mg/L treatments. At 6-hours, sub-lethal effects of loss of equilibrium, erratic swimming, and swimming ceased were observed in the 1.0 and 1.7 mg/L treatments. At 24-hours, sub-lethal effects were still observed in the 1.0 mg/L treatment. There were no sub-lethal effects observed at 48, 72 or 96 hours in any of the treatments.

Mortality

Exposure time (hours)	3	6	24	48	72	96
mean measured conc. (mg a.s./L)	No of dead (%)	No of dead (%)	No of dead (%)	No of dead (%)	No of dead (%)	No of dead (%)
Control	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
0.24	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
0.37	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
0.56	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
1.0	0 (0)	0 (0)	2 (20)	10 (100)	10 (100)	10 (100)
1.7	0 (0)	0 (0)	10 (100)	10 (100)	10 (100)	10 (100)

III. CONCLUSIONS:

The study meets the validity criteria and the endpoints based on mean measured concentrations of fluopicolide are:

LC_{50} 96 hours (95% C.I.):	**0.75 mg a.s. / L (0.56 – 1.0 mg a.s. / L)**
LOEC: lowest concentration with an effect	1.0 mg a.s./ L
NOEC: highest concentration without adverse effects	0.56 mg a.s. / L

Assessment and conclusion by applicant:

The study is reliable and the LC_{50} of 0.75 mg a.s./L and NOEC of 0.56 mg a.s./L can be used in the fluopicolide risk assessment.

Data Point:	KCA 8.2.1/03
Report Author:	[redacted]
Report Year:	2003
Report Title:	AE C638206: A 96-hour static acute toxicity test with the common carp (Cyprinus carpio)
Report No:	C036019
Document No:	M-219743-01-1
Guideline(s) followed in study:	OECD: 203 (1992); USEPA (=EPA): OPPTS 850.1075 (1996)
Deviations from current test guideline:	Method: Deviations from current guideline SANCO/3029/99 rev.4. Recoveries were determined at three different concentrations in triplicate. However, the obtained data demonstrate very good recoveries and the precision. The method can therefore be regarded as fit for purpose. Study: Current Guideline: OECD 203 (2019) Dissolved oxygen concentrations in the test solutions were >96% of saturation (8.3 mg/L) at test initiation, but dropped to slightly less than 60% saturation in the second highest concentration by 24 hours. Since the mortality in this test concentration is within the accepted limit (10%) for control and aeration was provided afterwards, therefore the impact of this deviation is considered negligible. The size of the fish was bigger than recommended by OECD guideline: mean of 4.5 instead of 3+/-1. But the weight of the fish is within the range specified in US EPA guideline, below 3.0 g. The pH is greater than 8.5 (up to 8.7) in all test concentrations and controls at test start and, in some occasions, later on. The validity criteria of the test were met so this slight deviation is not considered significant.
Previous evaluation:	Yes, evaluated and accepted in DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive summary

An acute toxicity test was performed with the common carp (*Cyprinus carpio*) in a static system. Juvenile common carps were exposed to fluopicolide in nominal concentrations of 0.13, 0.25, 0.50, 1.0 and 2.0 mg a.s./L, in well water for a 96-hour period. Additionally, a negative control and a solvent control were included. All treatments had 10 fish per test vessel (i.e. 10 fish per treatment level). Mortality, toxicity values and behaviour were recorded at time points 3, 24, 48, 72 and 96 hours.
Samples were taken from all test chambers on day 0, day 2 and day 4. Samples were analysed by High Performance Liquid chromatography using an ultraviolet detector for the actual concentration of fluopicolide in the test medium. Recoveries in the media were between 85.3 and 103% and no residues above the limit of quantification were measured in the controls.
The arithmetic mean measured concentrations of fluopicolide were 0.12, 0.25, 0.50, 0.98, and 2.0 mg a.s./L. There was a slight deviation from the validity criterion on oxygen saturation which is not considered to have impacted the reliability of the results. Common carp in the negative and solvent control groups appeared healthy and normal throughout the test. Sub-lethal effects were observed at 3h in the 2 highest concentrations, they had disappeared at 24h for the fish exposed to 0.98 mg a.s./L but were still observed in the surviving fish exposed to 2.0 mg a.s./L. All these fish were dead at 48h. At 0.98 mg a.s./L, 1 fish died at 48 h. At 0.50 mg a.s./L, sublethal effects were observed in 1 fish at 96h. The 96-hour LC_{50} value is 1.3 mg a.s./L, with a 95% confidence interval of 0.98 to 2.0 mg a.s./L. The no mortality concentration is considered to be 0.50 mg a.s./L, and the NOEC is 0.25 mg a.s./L.

I. MATERIAL AND METHODS:

Test material	Fluopicolide batch: OP 2350005; purity 99.4 %
Guideline(s) adaptation	None specified
Test species	Carp (*Cyprinus carpio*)
Acclimation	At least 14 days Health during acclimation: a single mortality in the 7-day period prior to the test
Organism age/size at study initiation	In control fish at the end of the study Mean length: 4.5 cm (3.9 – 5.2 cm) Mean body weight: 1.1 g (0.64 – 1.7 g) The fish used in the test slightly exceeded the OECD guideline recommended length of 3.0 ± 1.0 cm but were well within weight recommendations for both OPPTS and ASTM guidelines (<3.0 g and <5.0 g, respectively).
Test solutions	Nominal concentrations: 0.13 - 0.25 - 0.50 - 1.0 - 2.0 mg a.s./L. Corresponding mean measured concentrations: 0.12 - 0.25 - 0.50 - 0.98 and 2.0 mg a.s. / L. Samples were taken from all test chambers on day 0, day 2, and day 4. Controls: water Solvent control: 0.1 mL/L dimethyl formamide At test initiation, the solutions appeared clear and colorless with some white precipitate evident on the surface of the 1.0 and 2.0 mg/L solutions. At test termination, the 0.13, 0.25 and 0.50 mg/L solutions were clear and colorless, while the 1.0 and 2.0 mg/L solutions were slightly cloudy white, increasing in intensity with increasing concentration
Replication	No. of vessels per concentration (replicates): 2 No. of vessels per control (replicates): 2 No. of vessels per solvent control (replicates): 2
Organisms per replicate	No. of organisms per vessel: 10
Exposure	static Total exposure duration: 96 hours
Test Vessel Loading	0.38 g fish/L test medium
Feeding during test	None
Test conditions	Temperature: 21.7 – 23.2°C Photoperiod: 16 hours light / 8 hours dark Light intensity: 379 lux pH: 8.3 – 8.7. Water hardness: 130 mg $CaCO_3$/L Dissolved oxygen: 4.5 mg/L – 8.5 mg/L Gentle aeration was added to each test chamber to achieve > 60% saturation throughout the test Conductivity: 320 µmhos/cm
Parameters Measured / Observations	Observations for death, and for abnormal appearance and behaviour, were performed at 3, 24, 48, 72, and 96 hours Discrete measurements of temperature, dissolved oxygen and pH were obtained at test initiation, 24, 48, 72, and 96 hours.
Chemical analysis	Samples were analysed by High Performance Liquid chromatography using an ultraviolet detector for the actual concentration of fluopicolide and in the test medium on day 0, day 2 and on day 4.

Data analysis	LC_{50} values and the 95%-confidence intervals were calculated for 24-, 48-, 72- and 96-hour time points with binominal test using nonlinear interpolation between 0.98 and 2.0 mg/L with the computer program of C. E. Stephan. The no mortality concentration and the no-observed-effect-concentration (NOEC) were determined by visual interpretation of the mortality and observation data

II. RESULTS AND DISCUSSION:

Validity criteria according to OECD 203 (2019)	Required	Obtained
Measured concentration of the test substance	Mandatory	Performed and results are based on mean measured concentrations
Mortality in control during test	≤ 10%	0 %
Dissolved oxygen saturation	≥ 60%	4.5 mg/L – 8.5 mg/L*

*A dissolved oxygen concentration of 5.2 mg/L represents 60% saturation at 22°C in freshwater.

At 24h, the oxygen saturation dropped below 60% in one concentration (0.98 mg/L) only, a gentle aeration was then applied, in order to achieve > 60% saturation throughout the remainder of the test. As all fish appeared normal at this concentration at 24 h, and sufficient aeration was provided thereafter, it is considered that the breach of the validity criteria did not impact the results of the study.

Analytical results:
Recoveries in the media were between 85.3 and 103% (see table below). The biological results are based on arithmetic mean measured concentrations of fluopicolide.
There was no fluopicolide residue found in the control samples.

Nominal conc. (mg a.s./L)	Mean measured concentration (mg a.s./L)	% of nominal concentrations	Range of individual measurements (% of nominal)
0.13	0.12	92	85.3 -92.5
0.25	0.25	100	94.1 - 103
0.50	0.50	100	97.1 - 103
1.0	0.98	98	93.1 - 102
2.0	2.0	100	96.5 – 98.9

Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Biological results:

Observations

Common carp in the negative and solvent control groups appeared healthy and normal throughout the test. After 96-hours of exposure, fish in the 0.12 and 0.25 mg a.s./L treatment groups also appeared healthy and normal, with the exception of one incidental mortality in the 0.12 mg a.s./L treatment group. Since there were no effects observed among fish in the next higher concentration, this mortality was not considered to be treatment-related. While no mortalities occurred in the 0.50 mg/L treatment group, one fish was observed to have a loss of equilibrium by test termination, which was considered to be treatment-related. Observed effects at the two highest test concentrations after 3 hours were: loss of equilibrium, surfacing on surface, or lethargic behaviour.

Mortality

Exposure time (hours)	3	24	48	72	96
Mean measured conc. (mg a.s./L)	No of dead (%)	No of dead (%)	No of dead (%)	No of dead (%)	No of dead (%)
Control	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Solvent control	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
0.12	0 (0)	0 (0)	0 (0)	0 (0)	1 (5)*
0.25	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
0.50	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
0.98	0 (0)	0 (00)	1 (5)	1 (5)	2 (10)
2.0	1 (5)	16 (80)	20 (100)	20 (100)	20 (100)

* not considered as treatment related

III. CONCLUSIONS:

The endpoints based on arithmetic mean measured concentrations fluopicolide are:

LC_{50} 96 hours (95% C.I.):	**1.3 mg a.s. / L (0.98 – 2.0 mg a.s. / L)**
NOEC: highest concentration without adverse effects	0.25 mg a.s. / L

Assessment and conclusion by applicant:

The study is reliable and the LC_{50} of 1.3 mg a.s./L and NOEC of 0.25 mg a.s./L can be used in the fluopicolide risk assessment.

Data Point:	KCA 8.2.1/04
Report Author:	[redacted]
Report Year:	2003
Report Title:	AE C638206: A 96-hour static acute toxicity test with the zebra fish (Brachydanio rerio)
Report No:	M-234508-01-2
Document No:	M-234508-01-2
Guideline(s) followed in study:	OECD: 203 (1992); USEPA (=EPA): OPPTS 850.1075 (1996)
Deviations from current test guideline:	Method: Deviations from current guideline SANCO/3029/99 rev.4. Limited sets of validation recoveries were analysed. However, the average recoveries were within the acceptable range of 70–110% and the RSD values were below 20%. The analytical method can be regarded as fit for purpose. Study: Current Guideline: OECD 203 (2019) Some fish were bigger than recommended by OECD guideline: range 2.6 to 3.4, instead of 2+/-1 cm. But the weight of the fish is within the range specified in US EPA guideline, below 3.0 g. The pH is greater than 8.5 (up to 8.7) in all test concentrations and controls at test start and, in some occasions, later on. The validity criteria of the test were met so this slight deviation is not considered significant
Previous evaluation:	yes, evaluated and accepted in DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive summary

An acute toxicity test was performed with the zebra fish (*Brachydanio rerio*) in a static system. Juvenile zebra fish were exposed to fluopicolide in nominal concentrations of 0.13, 0.25, 0.50, 1.0 and 2.0 mg a.s./L in well water for a 96-hour period. Additionally, a negative control and a solvent control were included. All treatments had 10 fish per test vessel (i.e., 10 fish per treatment level). Mortality, toxicity values and behaviour were recorded at time points 4, 24, 48, 72, and 96 hours. Samples were analysed by High Performance Liquid Chromatography (HPLC) with UV-detection for the actual concentration of fluopicolide in the test medium on day 0, day 2 and on day 4. Recoveries in media were between 93.9 % and 109% of nominal concentrations and no residues above the limit of quantification were measured in the controls. The mean measured concentrations of fluopicolide were 0.12, 0.25, 0.51, 1.0, and 2.1 mg a.s./L.

The study fulfils all validity criteria of the current version of the OECD 203 guideline. Zebra fish in the negative and solvent control groups appeared healthy and normal throughout the test. After 96-hours of exposure, fish in the 0.12, 0.25, 0.51 and 1.0 mg a.s./L treatment groups also appeared healthy and normal, with no mortality or overt signs of toxicity observed. Sublethal effects and mortality were observed at 2.1 mg a.s./L. The 96-hour LC_{50} value is above 1.0 mg a.s./L and is estimated to be 1.8 mg a.s./L, obtained by nonlinear interpolation. The NOEC is 1.0 mg/L.

I. MATERIAL AND METHODS:

Test material	Fluopicolide (tech.) batch OP 2050046; purity 96.1 % w/w
Guideline(s) adaptation	None specified
Test species	Zebra fish (*Brachydanio rerio*)
Acclimation	At least 14 days Health during acclimation: 0 % mortality
Organism age/size at study initiation	In control fish at the end of the study: Mean length: 3.0 cm (2.6 cm - 3.4 cm) Mean body weight: 0.25 g (0.14 g – 0.36 g)
Test solutions	Nominal concentrations: 0.13 - 0.25 - 0.50 - 1.0 - 2.0 mg a.s./L Corresponding mean measured concentrations: 0.12 - 0.25 - 0.51 - 1.0 and 2.1 mg a.s. / L. Samples were taken from all test chambers on day 0, day 2 and day 4. Controls: water Solvent control: 0.1 ml/L dimethyl formamide Solutions appeared clear and colourless at test initiation and termination.
Replication	No. of vessels per concentration (replicates): 2 No. of vessels per control (replicates): 2
Organisms per replicate	No. of organisms per vessel: 10
Exposure	static Total exposure duration: 96 hours
Test Vessel Loading	0.12 g fish/L test medium
Feeding during test	None
Test conditions	Temperature: 21.7 – 23.7°C Photoperiod: 16 hours light / 8 hours dark Light intensity: 141 lux pH: 8.4 – 8.7 Water hardness: 132 mg $CaCO_3$/L Dissolved oxygen: 5.7 mg/L (67% of saturation)– 8.9 mg/L Conductivity: 320 µmhos/cm
Parameters Measured / Observations	Observations for death, and for abnormal appearance and behaviour, were performed at 4, 24, 48, 72, and 96 hours Discrete measurements of temperature, dissolved oxygen and pH, were obtained at test initiation, 24, 48, 72, and 96 hours. Hardness, alkalinity and specific conductance were measured in the dilution water at the beginning of the test.
Chemical analysis	Samples were analysed by High Performance Liquid Chromatography (HPLC) with UV-detection for the actual concentration of AE C638206 in the test medium on day 0, day 2 and on day 4.
Data analysis	LC_{50} values and the 95%-confidence intervals were calculated for 48, 72- and 96-hour time points with binominal test using nonlinear interpolation between 1.0 and 2.1 mg/L with the computer program of C. E. Stephan. There was <50% mortality in any treatment group at 24 hours, which precluded the statistical calculation of the 24-hour LC_{50} value. Therefore, the 24-hour LC_{50}, the no mortality concentration and the no observed- effect-concentration (NOEC) were determined by visual interpretation of the mortality and observation data.

II. RESULTS AND DISCUSSION:

Validity criteria according to OECD 203 (2019)	Required	Obtained
Measured concentration of the test substance	Mandatory	Performed and results are based on mean measured concentrations
Mortality in control during test	≤ 10%	0 %
Dissolved oxygen saturation	≥ 60%	5.7 mg/L – 8.9 mg/L*

* A dissolved oxygen concentration of 5.1 mg/L represents 60% saturation at 23°C in freshwater.

Analytical results:
Recoveries in media were between 93.9 % and 109% of nominal concentrations (see table below). The biological results are based on mean measured concentrations of fluopicolide (AE C638206).
There was no fluopicolide residue found in the control samples.

Nominal conc. (mg a.s./L)	Mean measured concentration (mg a.s./L)	% of nominal concentrations	Range of individual measurements (% of nominal)
0.13	0.12	92	93.9 - 97.9
0.25	0.25	100	96.2 – 104
0.50	0.51	102	94.1 - 109
1.0	1.0	100	95.6 - 104
2.0	2.1	105	99.7 - 107

Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4 with minor acceptable exceptions only.

Biological results:

Observations

Zebra fish in the negative and solvent control groups appeared healthy and normal throughout the test. After 96-hours of exposure, fish in the 0.12, 0.25, 0.51 and 1.0 mg/L treatment groups also appeared healthy and normal, with no mortality or overt signs of toxicity observed. Observed effects at the highest concentration were: erratic swimming, loss of equilibrium, lethargy, fish lying on bottom, surfacing or floating on surface.

Mortality

Exposure time (hours)	4	24	48	72	96
mean measured conc. (mg a.s./L)	No of dead (%)	No of dead (%)	No of dead (%)	No of dead (%)	No of dead (%)
Control	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Solvent Control	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
0.12	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
0.25	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
0.51	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
1.0	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
2.1	0 (0)	9 (45)	13 (65)	14 (70)	14 (70)

III. CONCLUSIONS:

The study meets the validity criteria and the endpoints based on arithmetic mean measured fluopicolide concentrations are:

LC_{50} 96 hours (95% C.I.):	**1.8 mg a.s. / L (1.0 – 2.1 mg a.s. / L)**
NOEC: highest concentration without adverse effects	1.0 mg a.s. / L

Assessment and conclusion by applicant:

The study is reliable and the LC_{50} of 1.8 mg a.s./L and NOEC of 1.0 mg a.s./L can be used in the fluopicolide risk assessment.

Data Point:	KCA 8.2.1/05
Report Author:	[redacted]
Report Year:	2003
Report Title:	AE C638206: A 96-hour static toxicity test with the ricefish (Oryzias latipes)
Report No:	M-234510-01-2
Document No:	M-234510-01-2
Guideline(s) followed in study:	ASTM: E729-88a (1994); OECD: 203 (1992); USEPA (EPA): OPPTS 850.1075 (1996)
Deviations from current test guideline:	Method: Deviations from current guideline SANCO/3029/99 rev.4: Limited sets of validation recoveries were analysed. However, the average recoveries were within the acceptable range of 70–110% and the RSD values were below 20%. The analytical method can be regarded as fit for purpose. Study: Current Guideline: OECD 203 (2019) The temperature range and size of medaka recommended in OECD guideline 203 from 2019 have changed in comparison to previous version of 1992. The fish in the test measure 2-3 cm instead of 1-2 cm and the temperature is in the range 21.8 – 23.3 which is not in the current range of 23-27°C. The pH is greater than 8.5 (up to 8.8) in all test concentrations and controls in almost all samples. The validity criteria of the test were met so these slight deviations are not considered significant.
Previous evaluation:	yes, evaluated and accepted in DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive summary

An acute toxicity test was performed with the ricefish (*Oryzias latipes*) in a static system. Juvenile ricefish were exposed to fluopicolide in nominal concentrations of 0.30, 0.44, 0.67, 1.0 and 1.5 mg a.s./L, in well water for a 96-hour period. Additionally, a negative control and a solvent control were included. All treatments had 10 fish per test vessel (i.e., 10 fish per treatment level). Mortality, toxicity values and behaviour were recorded at time points 3.5, 24, 48, 72, and 96 hours.
Samples were analysed by High Performance Liquid Chromatography with UV detection for the actual concentration of fluopicolide present in the test medium on day 0, day 2 and on day 4. Recoveries in media were between 90.8% and 103 %. The results were based on the arithmetic mean measured concentrations of fluopicolide which were 0.28, 0.44, 0.65, 0.99, and 1.5 mg/L. The study fulfils all validity criteria of the current version of OECD 203 guideline. Ricefish in the negative and solvent control groups appeared healthy and normal throughout the test. After 96-hours of exposure, fish in the 0.28 and 0.44 mg a.s./L treatment groups also appeared healthy and normal, with no mortality or overt signs of toxicity observed. Sublethal signs of toxicity were observed among fish in the 0.65, 0.99 and 1.5 mg a.s./L treatment groups during the test. The 96-hour LC_{50} value is 0.70 mg a.s./L, with a 95% confidence interval of 0.44 to 0.99 mg a.s./L. The no mortality concentration and NOEC is 0.44 mg a.s./L.

I. MATERIAL AND METHODS:

Test material	Fluopicolide (tech.) batch: OP 2050046 purity 96.1 % w/w
Guideline(s) adaptation	None specified
Test species	Ricefish (*Oryzias latipes*)
Acclimation	At least 14 days Health during acclimation: no sign of disease or stress
Organism age/size at study initiation	In control fish at the end of the study: Mean length: 2.6 cm (2.0 – 3.0 cm) Mean body weight: 0.15 (0.05 g – 0.22 g)
Test solutions	Nominal concentrations: 0.30 - 0.44 - 0.67 - 1.0 - 1.5 mg a.s./L Corresponding mean measured concentrations: 0.28 - 0.44 - 0.65 - 0.99 and 1.5 mg a.s. / L. Samples were taken from all test chambers on day 0, day 2 and day 4. Controls: water Solvent control: 0.1 ml/L dimethyl formamide Solutions appeared clear and colorless at test initiation and termination.
Replication	No. of vessels per concentration (replicates): 2 No. of vessels per control (replicates): 2
Organisms per replicate	No. of organisms per vessel: 10
Exposure	static Total exposure duration: 96 hours
Test Vessel Loading	0.06 g fish/L test medium
Feeding during test	None
Test conditions	Temperature: 21.8 – 23.3°C Photoperiod: 16 hours light / 8 hours dark Light intensity: 138 lux pH: 8.5 – 8.8. Water hardness: 134 mg $CaCO_3$/L Dissolved oxygen: 7.8 mg/L – 8.4 mg/L Conductivity: 330 µmhos/cm
Parameters Measured/ Observations	Observations for death, signs of toxicity and for abnormal behavior were performed at 3, 5, 24, 48, 72, and 96 hours Discrete measurements of temperature, dissolved oxygen and pH were obtained at test initiation, 24, 48, 72, and 96 hours Hardness, alkalinity and specific conductance were measured in the dilution water at the beginning of the test.
Chemical analysis	Samples were analysed by High Performance Liquid Chromatography with UV detection for the actual concentration of AE C638206 present in the test medium on day 0, day 2 and on day 4.
Data analysis	LC_{50} values and the 95%-confidence intervals were calculated for 24, 72- and 96-hour time points with binomial test and for the time point 48 hours with probit analysis using the computer program of C. E. Stephan (EPA). The no mortality concentration and the no-observed-effect-concentration (NOEC) were determined by visual interpretation of the mortality and observation data.

II. RESULTS AND DISCUSSION:

Validity criteria according to OECD 203 (2019)	Required	Obtained
Measured concentration of the test substance	Mandatory	Performed
Mortality in control during test	≤ 10%	0 %
Dissolved oxygen saturation	≥ 60%	7.8 mg/L – 8.4 mg/L*

* A dissolved oxygen concentration of 5.1 mg/L represents 60% saturation at 23°C in freshwater.

Analytical results:
Recoveries in media were between 90.8 % and 103 % (see table below). The biological results are based on arithmetic mean measured concentrations of fluopicolide.
There was no fluopicolide residue found in the control samples.

Nominal conc. (mg a.s./L)	Mean measured concentration (mg a.s./L)	% of nominal concentrations	Range of individual measurements (% of nominal)
0.30	0.28	93	90.8 - 98.3
0.44	0.44	100	97.4 – 103
0.67	0.65	97	94.7 - 101
1.0	0.99	99	97.7 – 102
1.5	1.5	100	99.3 - 101

Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4 with minor acceptable exceptions only.

Biological results:

Observations
Ricefish in the negative and solvent control groups appeared healthy and normal throughout the test. After 96-hours of exposure, fish in the 0.28 and 0.44 mg a.s./L treatment groups also appeared healthy and normal, with no mortality or overt signs of toxicity observed.
Sublethal signs of toxicity were observed among fish in the 0.65, 0.99 and 1.5 mg/L treatment groups during the test, these signs were: erratic swimming, loss of equilibrium, fish surfacing or lying on bottom.

Mortality

Exposure time (hours)	3.5	24	48	72	96
Mean measured conc. (mg a.s./L)	No of dead (%)	No of dead (%)	No of dead (%)	No of dead (%)	No of dead (%)
Control	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Solvent control	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
0.28	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
0.44	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
0.65	0 (0)	0 (0)	1 (5)	4 (20)	7 (35)
0.99	0 (0)	5 (25)	13 (65)	20 (100)	20 (100)
1.5	0 (0)	20 (100)	20 (100)	20 (100)	20 (100)

III. CONCLUSIONS:

The study meets the validity criteria and the endpoints based on arithmetic mean measured fluopicolide concentrations are:

LC_{50} 96 hours (95% C.I.):	**0.70 mg a.s. / L (0.44 – 0.99 mg a.s. / L)**
NOEC: Highest concentration without effect	0.44 mg a.s. / L

Assessment and conclusion by applicant:

The study is reliable and the LC_{50} of 0.70 mg a.s./L and the NOEC of 0.44 mg a.s./L can be used in the fluopicolide risk assessment.

Data Point:	KCA 8.2.1/06
Report Author:	[redacted]
Report Year:	2003
Report Title:	AE C638206 - Acute toxicity to sheepshead minnow (Cyprinodon variegatus) under static conditions
Report No:	M-223359-01-2
Document No:	M-223359-01-2
Guideline(s) followed in study:	USEPA (=EPA): FIFRA 72-3 (1982), OPPTS Draft 850.1075 (1996)
Deviations from current test guideline:	Method: Deviations from current guideline SANCO/3029/99 rev.4: Limited sets of validation recoveries were analysed. However, the average recoveries were within the acceptable range of 70–110% and the RSD values were below 20%. The analytical method can be regarded as fit for purpose.; Study: Current Guideline: OECD 203 (2019) The temperature range in the test 22-23°C does not fulfil the recommendations of the OECD guideline version 2019 (23-27°C) but is compliant with the OCSPP 850.1075 guideline. Similarly, the size of the fish is bigger than recommended 2.0-3.5 cm instead of 1-2 cm but the fish meet the requirements of the OCSPP guideline: weight < 3.0 g and longest fish less than twice the size of the smallest. Since these deviations are due to the lack of consistency between the 2 guidelines and validity criteria of the study are met, the study is considered as acceptable.
Previous evaluation:	yes, evaluated and accepted in DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Data Point:	KCA 8.2.1/12
Report Author:	[redacted]
Report Year:	2018
Report Title:	Statement - Certificate of analysis for fluopicolide acute toxicity study on Sheepshead minnow (Cafarella, 2003; M-223359-01-1)
Report No:	M-634697-01-1
Document No:	M-634697-01-1
Guideline(s) followed in study:	--
Deviations from current test guideline:	Not applicable
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	Yes

Executive summary

An acute toxicity test was performed with the sheepshead minnow (*Cyprinodon variegatus*) in a static system. Sheepshead minnow were exposed to fluopicolide in nominal concentrations of 0.26, 0.43, 0.72, 1.2 and 2.0 mg a.s./L, in natural filtered seawater for a 96-hour period. Additionally, a negative control and a solvent control were included. All treatments had 10 fish per test vessel (i.e., 10 fish per treatment level). Mortality and biological observations were recorded at time points 0, 6, 24, 48, 72 and 96 hours. Samples were analysed using gas chromatography equipped with electron capture detection (GC/ECD) for the actual concentration of fluopicolide present in the test medium on day 0 and on day 4. Recoveries in media were between 69% and 88%. The biological results are based on arithmetic mean measured concentrations of fluopicolide. These mean measured concentrations of fluopicolide were 0.20, 0.35, 0.58, 1.0, and 1.6 mg a.s./L. The study fulfils all validity criteria. Mortalities occured at 0.35 mg a.s./L and above. Sub lethal effects were observed at 6 h at the 2 highest concentrations until the fish died. At 0.58 mg/L effects started at 72 h and all fish were dead at 96h. The lowest observed effect concentration (LOEC), is 0.41 mg a.s./L. The no observed effect concentration (NOEC) is 0.20 mg a.s./L.

I. Material and Methods:

Test material	Fluopicolide (tech.) Lot No.: 2050190/PP241024/2 purity 97.7 % w/w
Guideline(s) adaptation	None specified
Test species	Sheepshead minnow (*Cyprinodon variegatus*)
Acclimation	At least 14 days Health during acclimation: No mortality was observed during the 48-hour period prior to test initiation
Organism age/size at study initiation	Mean length: 2.8 cm (2.0 cm – 3.5 cm) Mean body weight: 0.57 g (0.21 g – 1.1 g)
Test solutions	Nominal concentrations: 0.26 - 0.43 - 0.72 - 1.2 - 2.0 mg a.s./L. Corresponding mean measured concentrations: 0.20 - 0.35 - 0.58 - 1.0 and 1.6 mg a.s. / L. Samples were taken from all test chambers on day 0 and day 4. Controls: natural filtered seawater Solvent control: 0.10 mL/L dimethylformamide in seawater All exposure solutions were observed to be clear and colorless with no visible undissolved test substance.
Replication	No. of vessels per concentration (replicates): 1 No. of vessels per control (replicates): 1
Organisms per replicate	No. of organisms per vessel: 10
Exposure	static Total exposure duration: 96 hours
Test Vessel Loading	< 0.5 g fish/L test medium
Feeding during test	None

Test conditions	Temperature: 22 - 23°C Photoperiod: 16 hours light / 8 hours dark Light intensity: 76 – 90 footcandles pH: 7.5 - 7.9 Salinity: 32 ‰ - 33 ‰ Dissolved oxygen: 4.3 mg/L (60% saturation) – 7.2 mg/L (gentle aeration was initiated at the 72-hour observation interval to maintain dissolved oxygen concentrations > 60% of saturation)
Parameters Measured / Observations	Observations for death and sublethal effects were performed at 0, 6, 24, 48, 72, and 96 hours. Discrete measurements of temperature, dissolved oxygen, pH, and salinity were measured daily in each test vessel.
Chemical analysis	Samples were analysed using gas chromatography equipped with electron capture detection (GC/ECD) for the actual concentration of fluopicolide present in the test medium on day 0 and on day 4.
Data analysis	The LC_{50} was estimated, using one of three statistical techniques: moving average, binominal or probit analysis. The appropriate method was determined according to the data characteristics. The 96-hour LC_{50} value was determined by non-linear interpolation with 95% confidence intervals determined by binomial probability. For the calculations a computer program (Stephan 1982, U.S. EPA) was used. The NOEC is empirically determined.

II. Results and Discussion:

Validity criteria according to OECD 203 (2019)	**Required**	**Obtained**
Measured concentration of the test substance	Mandatory	Performed
Mortality and sublethal effects in controls during test	≤ 10%	0 %
Dissolved oxygen saturation	≥ 60%	4.3 mg/L – 7.2 mg/L*

* A dissolved oxygen concentration of 4.3 mg/L represents 60% saturation at 23°C and 32 ‰ in freshwater.

Analytical results:
Recoveries in media were between 69 % and 88 % (see table below). The biological results are based on arithmetic mean measured concentrations of fluopicolide.
According to the EFSA technical report (2015)[2], the concentrations should be calculated as geometric mean measured concentrations because the test is static, and the measured concentrations are below 80% of nominal. The geometric mean measured concentrations are provided below, they are very similar to the arithmetic mean measured concentrations. The LC_{50} calculated by non-linear interpolation with arithmetic and with geometric mean measured concentrations is the same. No residues of fluopicolide were measured in the controls above the limit of quantification (0.014 mg a.s./L).

[2] EFSA (European Food Safety Authority), 2015. Technical report on the outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology. EFSA supporting publication 2015:EN-924. 62 pp.

Nominal conc. (mg a.s./L)	Arithmetic mean (mg a.s./L)	% of nominal concentrations	Range of individual measurements* (% of nominal)	Geometric mean measured concentrations* (mg a.s./L)
0.26	0.20	76	69 - 81	0.19
0.43	0.35	80	72 - 88	0.34
0.72	0.58	81	79 - 83	0.58
1.2	1.0	83	83-83	0.99
2.0	1.6	82	80-85	1.6

Full details and acceptable validation data to support this method are presented within document M-CA-4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4 with minor acceptable exceptions only.

Biological results:

Observations
At 72-hours, sub-lethal effects of loss of equilibrium where observed for two fish and one fish exhibited erratic swimming in the 0.58 mg/L treatment. At 24-hours, two fish exhibited partial loss of equilibrium and several fish showed a complete loss of equilibrium in the 1.0 mg/L treatment. In the 1.6 mg/L treatment several fish already showed partial of complete loss of equilibrium already after 6 hours.

Mortality

Exposure time (hours)	6	24	48	72	96
Arithmetic mean measured conc. (mg a.s./L)	No of dead (%)	No of dead (%)	No of dead (%)	No of dead (%)	No of dead (%)
Control	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Solvent control	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
0.20	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
0.35	0 (0)	0 (0)	0 (0)	1 (10)	2(20)
0.58	0 (0)	0 (0)	2 (20)	7 (70)	10 (100)
1.0	0 (0)	0 (50)	10 (100)	10 (100)	10 (100)
1.6	0 (0)	10 (100)	10 (100)	10 (100)	10 (100)

III. CONCLUSIONS:

The study meets the validity criteria and the endpoints based on mean measured fluopicolide concentrations are:

LC_{50} 96 hours (95% C.I.):	**0.41 mg a.s. / L (0.20 – 0.58 mg a.s. / L)**
NOEC: highest concentration without adverse effects	0.20 mg a.s. / L*

* 0.19 mg a.s./L as geometric mean measured concentrations

Assessment and conclusion by applicant:

The study is reliable and the LC_{50} of 0.41 mg a.s./L and the NOEC of 0.20 mg a.s./L can be used in the fluopicolide risk assessment.

Data Point:	KCA 8.2.1/10
Report Author:	[redacted]
Report Year:	2015
Report Title:	Acute toxicity of fluopicolide technical to the fathead minnow (Pimephales promelas) under static conditions
Report No:	007SRLS14C38
Document No:	M-533292-01-1
Guideline(s) followed in study:	EU Directive 91/414/EEC; Regulation (EC) No. 1107/2009; US EPA OCSPP 850.1075 (1996); OECD Guideline 203 (1992). The afore mentioned guidelines were harmonized for various test parameters (i.e. temperature, light, etc.) to achieve optimal environmental conditions for the test organisms. Scientific discretion was implemented where guideline parameters do not fully converge
Deviations from current test guideline:	Method: none Study: Current Guideline: OECD 203 (2019) The size of the fish was bigger than recommended (2 cm +/-1), however it is compliant with OCSPP 850.1075 guideline which specifies a fish weight less than 3.0 g. Therefore, this deviation is not considered to be relevant.
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Justification for new vertebrate test performed after 14 June 2011: the study was conducted to fulfil US EPA request as stated in September 2013 in Fluopicolide Final Work Plan, Registration review, Case number 7055.

Executive summary

An acute toxicity test was performed with the fathead minnow (*Pimephales promelas*) in a static system. Juvenile fathead minnows were exposed to fluopicolide in nominal concentrations of 0.156, 0.313, 0.625, 1.25 and 2.50 mg a.s./L in soft processed water for a 96-hour period. Additionally, a negative control and a solvent control were included. All treatments had 10 fish per test vessel. Mortality and sublethal effects were recorded at time points 4, 24, 48, 72 and 96 hours. Samples were analysed using Liquid Chromatography Mass Spectrometry/Mass Spectrometry (LC-MS/MS) to determine the actual concentration of fluopicolide present in the test medium on day 0 and on day 4. Recoveries in media were between 88% and 100% and no residues above the limit of quantification were measured in the

controls. Biological results are based on nominal concentrations of fluopicolide. The study fulfils all validity criteria of the current version of the OECD 203 guideline. Sublethal effects were observed in the test concentrations of 0.625, 1.25 and 2.50 mg a.s./L from 4 h to the end of the test. Mortalities occured in the 2 highest concentrations. Analysis of the mortality data based on the nominal test concentrations gave the following result: The 96-hour LC_{50} value is 1.34 mg a.s./L. The highest concentration without observed effect (NOEC), is 0.313 mg a.s./L. The highest concentration without lethal effect is 0.625 mg a.s./L.

I. MATERIAL AND METHODS:

Test material	Fluopicolide (tech.) Batch number: ETFP00273 Specification: 102000016444-01 purity 100.5 % w/w
Guideline(s) adaptation	None specified
Test species	Fathead minnow (*Pimephales promelas*)
Acclimation	12 days Health during acclimation: No mortalities during 48 hours prior to testing, no treatments for disease.
Organism age/size at study initiation	Length and weight were only measured at test end: Mean length: 34.4 mm ± 1.24 mm Mean body wet weight: 0.3937 ± 0.0498 g
Test solutions	Nominal concentrations: 0.156 - 0.313 - 0.625 - 1.25 - 2.50 mg a.s./L. Corresponding mean measured concentrations: 0.148 - 0.307 - 0.613 - 1.16 and 2.23 mg a.s./ L. Control: Soft processed water Solvent control: 0.10 mL/L dimethylformamide No precipitates were present in any of the test levels
Replication	No. of vessels per concentration (replicates): 1 No of vessels per control (replicates): 1 No. of vessels per solvent control (replicates): 1
Organisms per replicate	No. of organisms per vessel: 10
Exposure	Static Total exposure duration: 96 hours
Test Vessel Loading	0.12 g fish/L test medium
Feeding during test	None
Test conditions	Temperature: 21.8 – 22.8°C Photoperiod: 16 hours light / 8 hours dark (30 min transition period) Light intensity: 660 – 815 lux pH: 7.3 – 8.1 Water hardness: 46 -54 mg/L Conductivity: 177.7 – 196.3 µmhos/com Dissolved oxygen: 92 – 95% saturation
Parameters Measured Observations	Survival (mortality) and sublethal behavioural effects Observation interval: approx. after 4, 24, 48, 72 and 96 hours Daily for dissolved oxygen, pH and temperature. Day 0 and 4 for hardness, alkalinity and conductivity.

Chemical analysis	Samples were analysed using Liquid Chromatography Mass Spectrometry/Mass Spectrometry (LC-MS/MS) to determine the actual concentration of fluopicolide present in the test medium on day 0 and on day 4.
Data analysis	The LC_{50} values were calculated using CETIS (version 1.8.7.4) statistical software. The NOEC, NOLEC and LOEC were empirically determined based upon observations data including lethal and sublethal effects.

II. RESULTS AND DISCUSSION:

Validity criteria according to OECD 203 (2019)	Required	Obtained
Measured concentration of the test substance	Mandatory	Performed
Mortality in control during test	≤ 10%	0 %
Dissolved oxygen saturation	≥ 60%	92 - 95%

Analytical results:
Recoveries in media were between 88 % and 100 % (see table below). Biological results are based on nominal concentrations of fluopicolide.
No residues of fluopicolide were measured in the controls above the limit of quantification (0.01 mg a.s./L).

Nominal conc. (mg a.s./L)	Arithmetic (mg a.s./L)	% of nominal concentrations	
		0 hour	48 hour
0.156	0.148	94	93
0.313	0.307	98	99
0.625	0.613	96	100
1.25	1.16	89	96
2.50	2.23	88	91

Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Biological results:

Observations
Sublethal effects were observed in the test concentrations with 0.625, 1.25 and 2.50 mg a.s./L. In the test concentration with 0.625 mg a.s./L all fish showed a dark coloration after 4 hours which remained till end of the study (96 hours). In the test concentrations with 1.25 mg a.s./L and 2.5 mg a.s./L the same sublethal effect was observed in all living fish over the whole test period. Additionally, labored respiration, erratic behaviour, fish lying on bottom and loss of equilibrium were observed in the two highest test concentrations during the test. All living fish in these concentrations showed sublethal effects, which remained until study end.

Mortality

Exposure time (hours)	4	24	48	72	96
Nominal conc. (mg a.s./L)	No of dead (%)	No of dead (%)	No of dead (%)	No of dead (%)	No of dead (%)
Control	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Solvent control	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
0.156	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
0.313	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
0.625	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
1.25	0 (0)	1 (10)	3 (30)	3 (30)	4 (40)
2.50	3 (30)	8 (80)	10 (100)	10 (100)	10 (100)

III. CONCLUSIONS:

The study meets the validity criteria and the endpoints based on nominal fluopicolide concentrations are:

LC_{50} 96 hours (95% C.I.):	1.34 mg a.s./L (1.08 – 1.66 mg a.s./L)
NOEC: highest concentration without observed effects	0.313 mg a.s./L
Highest concentration without lethal effect	0.625 mg a.s./L

Assessment and conclusion by applicant:

The study is reliable and the LC_{50} of 1.34 mg a.s./L and the NOEC of 0.313 mg a.s./L can be used in the fluopicolide risk assessment.

Data Point:	KCA 8.2.1/07
Report Author:	[redacted]
Report Year:	2001
Report Title:	2,6-dichlorobenzamide (BAM): Acute toxicity to rainbow trout (Oncorhynchus mykiss)
Report No:	M-234311-01-2
Document No:	M-234311-01-2
Guideline(s) followed in study:	OECD: 203 (1992); USEPA (=EPA): E 72-4 (1982), OPPTS 850.1075 (1996)
Deviations from current test guideline:	Method: Deviations from current guideline SANCO/3029/99 rev.4: Limited sets of validation recoveries were analysed. However, the average recoveries were within the acceptable range of 70–110% and the RSD values were below 20%. The analytical method can be regarded as fit for purpose. Study: Current Guideline: OECD 203 (2019) None
Previous evaluation:	yes, evaluated and accepted in DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive summary

An acute toxicity test was performed with the rainbow trout (*Oncorhynchus mykiss*) under semi-static conditions. Juvenile rainbow trouts were exposed to M-01 (2,6-dichlorobenzamide (BAM)) for a period of 96 hours, in groups of twenty (2 replicates of ten fish), to an aqueous solution of the test material over a range of concentrations of 100, 180, 320, 560 and 1000 mg/L. The control group was maintained under identical conditions but not exposed to the test material. The number of mortalities and any sub-lethal effects of exposure in each test and control vessel were determined 3 and 6 hours after the start of exposure and then daily throughout the test until termination after 96 hours.
Water samples were taken from the control and all test groups with surviving fish at 0 (fresh media), 24, 48, 72 (old and fresh media) and 96 (old media) hours for quantitative analysis by high performance liquid chromatography (HPLC with UV-detector). Measured concentrations were in the 80-120% range of nominal concentrations and no residues above the limit of quantification were measured in the controls. Recoveries were greater than 80% so the biological results are based on nominal concentrations of M-01 (2,6-dichlorobenzamide (BAM)).
The study fulfils all validity criteria of the OECD 203 guideline.
Sub-lethal effects of exposure were observed at test concentrations of 180, 320 and 560 mg/L. Mortality was observed at 320, 560 and 1000 mg/L.
The 96-hour LC_{50} based on nominal test concentrations is 240 mg/L with 95% confidence limits of 180 - 320 mg/L. The No Observed Effect Concentration is 100 mg/L.

I. MATERIAL AND METHODS:

Test material	M-01 (2,6-dichlorobenzamide (BAM)) AE C653711 (M-01) Batch: FUX001000/FUN81G02C Purity 99.5 % w/w
Guideline(s) adaptation	None specified
Test species	Rainbow trout (*Oncorhynchus mykiss*)
Acclimation	At least 14 days to test conditions Health during acclimation: no mortalities in the 7 days prior to the start of the test
Organism age/size at the end of the definitive test	Mean length: 4.5 ± 0.2 cm Mean body weight: 1.07 ± 0.19 g
Test solutions	Nominal concentrations: 100 – 180 – 320 – 560 - 1000 mg/L Controls: water Evidence of undissolved material: The prepared test media were all observed to be clear colourless solutions throughout the duration of the test.
Replication	No. of vessels per concentration (replicates): 2 No. of vessels per control (replicates): 2
Organisms per replicate	No. of organisms per vessel: 10
Exposure	Semi-static (daily renewal) Total exposure duration: 96 hours
Test Vessel Loading	0.54 g fish/L test medium (at the end of the test)
Feeding during test	None
Test conditions	Temperature: 14.0°C (discrete measurements), 12.8 to 13.8 °C (continuous measurement in one control vessel) Photoperiod: 16 hours light / 8 hours dark, with 20 min transition periods Light intensity: not reported pH: 7.4 – 7.9. Water hardness: 144-160 mg $CaCO_3$/L Dissolved oxygen: 69-95% saturation Conductivity: 495 µS/cm
Parameters Measured / Observations	Observations for death, and for abnormal appearance and behavior, were performed at 3, 6, 24, 48, 72, and 96 hours Discrete measurements of temperature, dissolved oxygen and pH were obtained at test initiation and after 24, 48, 72, and 96 hours, in fresh and old media. Temperature was also measured continuously in one control replicate.
Chemical analysis	Water samples were taken from the control and all surviving test groups at 0 (fresh media); 24, 48, and 72 (old and fresh media); and 96 (old media) hours for quantitative analysis by high performance liquid chromatography (HPLC with UV detector)
Data analysis	The LC_{50} value and associated confidence limits at 24 hours were calculated by the trimmed Spearman-Karber method using the ToxCalc computer software package and at 3, 6, 48, 72 and 96 hours the LC_{50} value was calculated using the geometric mean method (geometric mean of the concentration showing 0% mortality and the concentration showing 100% mortality). The confidence limits are these 2 concentrations.

II. RESULTS AND DISCUSSION:

Validity criteria according to OECD 203 (2019)	Required	Obtained
Measured concentration of the test substance	Mandatory	Performed
Mortality in control during test	≤ 10%	0 %
Dissolved oxygen saturation	≥ 60%	69 - 95%

Analytical results:
Recoveries were greater than 80% (see table below) so the biological results are based on nominal concentrations of M-01 (2,6-dichlorobenzamide (BAM)).
There was no M-01 (2,6-dichlorobenzamide (BAM)) residue found in the control samples.

Nominal conc. (mg a.s./L)	% of nominal concentrations[1]							
	0 hour	24 hour-old	24 hour-fresh	48 hour-old	48 hour-fresh	72 hour-old	72 hour-fresh	96 hour
100	103.5	101.5	101.5	102	103.5	104	105.5	105.5
180	105	104	101.5	102	104.5	107	103.5	104
320	103.5	101	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
560	102.5	100.5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
1000	102.5	100	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

[1] mean of replicates (n = 2)
n.d. – not determined (all fish died)

Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4 with minor acceptable exceptions only.

Biological results:

Observations

Sub-lethal effects of exposure were observed at test concentrations of 180, 320 and 560 mg/L. Sublethal effects were swimming at the surface, swimming at the bottom, loss of equilibrium and the presence of moribund fish.

Mortality

Exposure time (hours)	3	6	24	48	72	96
Nominal conc. (mg/L)	No of dead (%)	No of dead (%)	No of dead (%)	No of dead (%)	No of dead (%)	No of dead (%)
Control	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
100	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
180	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
320	0 (0)	0 (0)	9 (45)	20 (100)	20 (100)	20 (100)
560	0 (0)	0 (0)	20 (100)	20 (100)	20 (100)	20 (100)
1000	20 (100)	20 (100)	20 (100)	20 (100)	20 (100)	20 (100)

III. CONCLUSIONS:

The study meets the validity criteria and the endpoints based on nominal M-01 concentrations are:

LC_{50} 96 hours (95% C.I.):	**240 mg/L (180 – 320 mg/L)**
NOEC: highest concentration without adverse effects	100 mg/L

Assessment and conclusion by applicant:

The study is reliable and the LC_{50} of 240 mg/L can be used in the M-01 risk assessment.

Data Point:	KCA 8.2.1/08
Report Author:	[redacted]
Report Year:	2003
Report Title:	AE C657188: A 96-hour static acute toxicity test with the rainbow trout (Oncorhynchus mykiss)
Report No:	M-218631-01-2
Document No:	M-218631-01-2
Guideline(s) followed in study:	OECD: 203 (1992); ASTM Standard E729-88a (1994)
Deviations from current test guideline:	Method: Deviations from current guideline SANCO/3029/99 rev 4: Limited sets of validation recoveries were analysed. However, the average recoveries were within the acceptable range of 70–110% and the RSD values were below 20%. The analytical method can be regarded as fit for purpose. Study: Current Guideline: OECD 203 (2019) The pH at t0 was 8.6 in the test concentrations of 13 mg/L and above, this is greater than 8.5. Since there were no mortalities or sign of stress in any of the fish included in the test, this slight deviation had no impact on the study, which fulfils all validity criteria.
Previous evaluation:	yes, evaluated and accepted in DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive summary

An acute toxicity test was performed with the rainbow trout (*Oncorhynchus mykiss*) in a static system. Juvenile rainbow trouts were exposed to M-02 (AE C657188) at nominal concentrations of 6.3, 13, 25, 50 and 100 mg /L for a 96-hour period. Additionally, a negative control (dilution water) was included. One test chamber was maintained in each treatment and control group, with 10 trout in each test chamber. Observations of mortality and other signs of toxicity were made approximately 2, 24, 48, 72 and 96 hours after test initiation. Samples were taken at test initiation, after 48 hours and at test termination from all test chambers. Samples were analysed by high performance liquid chromatography (HPLC) using UV detection. Measured concentrations were in the 80-120% range of nominal concentrations and no residues above the limit of quantification were measured in the controls. The mean measured concentrations for the study were 6.3, 13, 25, 51 and 102 mg/L, representing 100, 100, 100, and 102% of nominal concentrations, respectively.
The study fulfils all validity criteria of the current version of OECD 203 guideline.

Biological results are based on mean measured concentrations. After 96-hours of exposure, trout in all of the M-02 (AE C657188) treatment groups appeared healthy and normal, with no mortality or overt signs of toxicity observed.
There were no effects observed at any concentration tested. The 96-hour LC_{50} value is > 102 mg/L, the highest concentration tested. The NOEC is 102 mg/L.

I. MATERIAL AND METHODS:

Test material	M-02 (AE C657188) 3-chloro-5-trifluoromethylpyridine-2-carboxylic acid Batch: MOY 4383 Purity: 99.8 % w/w
Guideline(s) adaptation	None specified
Test species	Rainbow trout (*Oncorhynchus mykiss*)
Acclimation	At least 14 days to test conditions Health during acclimation: no mortalities in the acclimation period
Organism age/size at the end of the definitive test	Juveniles Mean length: 5.2 cm (range 4.5 – 5.8 cm) Mean body weight: 1.2 g (range 0.72 – 1.6 g)
Test solutions	Nominal concentrations: 6.3 – 13 – 25 – 50 - 100 mg p.m./L. Mean measured concentrations: 6.3 – 12 – 25 – 51 - 102 mg p.m./L Controls: water Evidence of undissolved material: All test concentrations appeared clear and colourless at test initiation and termination.
Replication	No. of vessels per concentration (replicates): 1 No. of vessels per control (replicates): 1
Organisms per replicate	No. of organisms per vessel: 10
Exposure	Static Total exposure duration: 96 hours
Test Vessel Loading	0.29 g fish/L test medium
Feeding during test	None
Test conditions	Temperature: 11.9 - 13°C Photoperiod: 16 hours light / 8 hours dark, with 30- min transition periods Light intensity: 274 lux (at test initiation) pH: 8.3 – 8.6 Water hardness: 122 mg $CaCO_3$/L (at test initiation) Dissolved oxygen: 7.4 mg/L – 9.2 mg/L Conductivity: 280 µmhos/cm (at test initiation)
Parameters Measured Observations	Observations for mortality, signs of toxicity or abnormal behavior were made approximately 2, 24, 48, 72 and 96 hours after test initiation. Measurements of temperature, pH and dissolved oxygen in test chambers were conducted every 24 hours. Hardness, alkalinity and specific conductance were measured in the dilution water at the beginning of the test.

Chemical analysis	Samples were taken at test initiation, after 48 hours and at test termination from all test chambers Samples were analysed by high performance liquid chromatography (HPLC) using variable wavelength detection set at 220 nm.
Data analysis	The absence of mortality in this study precluded the statistical calculation of LC_{50} values at 24, 48, 72 and 96 hours. Therefore, the LC_{50} values were estimated to be greater than the highest concentration tested. The no mortality concentration and the no-observed-effect-concentration (NOEC) were determined by visual interpretation of the mortality and observation data.

II. RESULTS AND DISCUSSION:

Validity criteria according to OECD 203 (2019)	**Required**	**Obtained**
Measured concentration of the test substance	Mandatory	Performed
Mortality in control during test	≤10%	0%
Dissolved oxygen saturation	≥ 60%	7.4 mg/L – 9.1 mg/L*

*A dissolved oxygen concentration of 6.5 mg/L represents 60% saturation at 12°C in freshwater.

Analytical results:
Recoveries were between 80 and 120% (see table below). Nevertheless, biological results are based on mean measured concentrations.
No residues of M-02 (AE C657188) were measured in the control above the limit of quantification (3 mg/L).

Nominal conc. (mg p.m./L)	**Mean measured conc. (mg p.m./L)**	**% of nominal concentrations**		
		0 hour	**48 hour**	**96 hour**
6.3	6.3	101	99.9	101
13	13	99.8	99.3	101
25	25	99.1	98.6	99.5
50	51	101	100	102
100	102	102	101	103

Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4 with minor acceptable exceptions only.

Biological results:

Observations

Rainbow trout in the negative control group appeared healthy and normal throughout the test. After 96-hours of exposure, trout in all of the M-02 (AE C657188) treatment groups also appeared healthy and normal, with no mortality or overt signs of toxicity observed.

Mortality

Exposure time (hours)	2	24	48	72	96
Mean measured conc. (mg p.m./L)	No of dead (%)	No of dead (%)	No of dead (%)	No of dead (%)	No of dead (%)
Control	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
6.3	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
13	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
25	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
51	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
102	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

III. CONCLUSIONS:

The study meets the validity criteria and the endpoints based on mean measured concentrations are:

LC_{50} 96 hours (95% C.I.):	**> 102 mg p.m./L (not applicable)**
NOEC: highest concentration without adverse effects	102 mg p.m./L

Assessment and conclusion by applicant:

The study is reliable and the LC_{50} of > 102 mg p.m./L can be used in the M-02 risk assessment.

Data Point:	KCA 8.2.1/09
Report Author:	[redacted]
Report Year:	2003
Report Title:	AE 1344122: A 96-hour static acute toxicity test with the rainbow trout (Oncorhynchus mykiss)
Report No:	C035531
Document No:	M-218630-01-1
Guideline(s) followed in study:	OECD: 203 (1992); ASTM Standard E729-88a (1994)
Deviations from current test guideline:	Method: Deviations from current guideline SANCO/3029/99 rev.4. Limited sets of validation recoveries were analysed. However, the average recoveries were within the acceptable range of 70–110% and the RSD values were below 20%. The analytical method can be regarded as fit for purpose. Study: Current Guideline: OECD 203 (2019) The pH at t0 was 8.6 in all the test concentrations, this is greater than 8.5. Since there were no mortalities or sign of stress in any of the fish included in the test, this slight deviation had no impact on the study which fulfils all validity criteria.
Previous evaluation:	yes, evaluated and accepted in DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive summary

An acute toxicity test was performed with the rainbow trout (*Oncorhynchus mykiss*) under static conditions. Juvenile rainbow trouts were exposed to a geometric series of five test concentrations of M-05 (AE 1344122) and a negative control (dilution water) for a 96-hour period. One test chamber was maintained in each treatment and control group, with 10 trout in each test chamber. Nominal test concentrations were 6.3, 13, 25, 50 and 100 mg p.m./L. Observations of mortality and other signs of toxicity were made approximately 6, 24, 48, 72 and 96 hours after test initiation. Samples were analysed by High Performance Liquid Chromatograph (HPLC with UV detector). Samples were collected from each test chamber at test initiation, after 48 hours and at test termination. Recoveries in the aged media were between 99% and 107% and no residues above the limit of quantification were measured in the controls. The mean measured concentrations for the study were 6.4, 14, 26, 50 and 101 mg p.m./L, representing 102, 108, 104, 100 and 101% of nominal concentrations, respectively. The study fulfils all validity criteria of the current version of OECD 203 guideline.
The biological results are based on arithmetic mean measured concentrations of the test item. Rainbow trout in the negative control group appeared healthy and normal throughout the test. After 96-hours of exposure, trout in all of the M-05 (AE 1344122) treatment groups also appeared healthy and normal, with no mortality or overt signs of toxicity observed. There were no effects observed at any concentration tested. The 96-hour LC_{50} value is > 101 mg p.m./L, the highest concentration tested. The NOEC is 101 mg p.m./L.

I. MATERIAL AND METHODS:

Test material	M-05 (AE 1344122) 3-methylsulfinyl-5-trifluoromethylpyridine-2-carboxylic acid Batch: YG3228 Purity 98.8 % w/w
Guideline(s) adaptation	None specified
Test species	Rainbow trout (*Oncorhynchus mykiss*)
Acclimation	At least 14 days no mortalities occurred and fish showed no signs of disease or stress
Organism age/size at study initiation	Mean length: 5.7 cm (range 5.1 – 6.0 cm); at test termination Mean body weight: 1.6 g (range 1.1 – 1.9 g); at test termination
Test solutions	Nominal concentrations: 6.3 – 13 – 25 – 50 - 100 mg p.m./L. Corresponding mean measured concentrations: 6.4 – 14 – 26 - 50 and 101 mg p.m./L. Controls: water Evidence of undissolved material: All test solutions appeared clear and colorless at test initiation and termination.
Replication	No. of vessels per concentration (replicates): 1 No. of vessels per control (replicates): 1
Organisms per replicate	No. of organisms per vessel: 10
Exposure	static Total exposure duration: 96 hours
Test Vessel Loading	0.39 g fish/L test medium
Feeding during test	None
Test conditions	Temperature: 11.9 – 12.8°C Photoperiod: 16 hours light / 8 hours dark with 30- min transition periods Light intensity: 289 lux pH: 8.2 – 8.6 Water hardness: 128 mg $CaCO_3$/L Dissolved oxygen: 7.8 – 9.3 mg/L (≥ 72% saturation) Conductivity: 320 µmhos/cm
Parameters Measured / Observations	Observations for death, and signs of toxicity or abnormal behavior, were performed at 6, 24, 48, 72, and 96 hours after test initiation. Discrete measurements of temperature, dissolved oxygen, pH were obtained at test initiation, 24, 48, 72, and 96 hours. Hardness, alkalinity and conductance were measured at the beginning of the test.
Chemical analysis	Samples were analysed by High Performance Liquid Chromatograph (HPLC with UV detector). Samples were collected from each test chamber at test initiation, after 48 hours and at test termination.
Data analysis	The absence of mortality in this study precluded the statistical calculation of LC_{50} values at 24, 48, 72 and 96 hours. Therefore, the LC_{50} values were estimated to be greater than the highest concentration tested. The no mortality concentration and the no-observed-effect-concentration (NOEC) were determined by visual interpretation of the mortality and observation data.

II. RESULTS AND DISCUSSION:

Validity criteria according to OECD 203 (2019)	Required	Obtained
Measured concentration of the test substance	Mandatory	Performed and results are based on mean measured concentrations
Mortality in control during test	≤ 10%	0 %
Dissolved oxygen saturation	≥ 60%	≥ 72% saturation

Analytical results:
Recoveries in the aged media were between 99 % and 107 % (see table below). The biological results are based on arithmetic mean measured concentrations of the test item.
No residues of M-05 (AE 1344122) were measured in the control above the limit of quantification (3 mg/L).

Nominal conc. (mg p.m./L)	Mean (mg p.m./L)	% of nominal concentrations		
		0 hour	48 hours	48 hours
6.3	6.4	102	101	102
13	14	104	104	107
25	26	103	103	103
50	50	98.7	99.3	99.9
100	101	99.7	101	102

Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4 with minor acceptable exceptions only.

Biological results:

Observations
Rainbow trout in the negative control group appeared healthy and normal throughout the test. After 96-hours of exposure trout in all of the M-05 (AE 1344122) treatment groups also appeared healthy and normal, with no mortality or overt signs of toxicity observed.

Mortality

Exposure time (hours)	3	6	24	48	72	96
mean measured conc. (mg p.m./L)	No of dead (%)	No of dead (%)	No of dead (%)	No of dead (%)	No of dead (%)	No of dead (%)
Control	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
6.4	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
14	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
26	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
50	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
101	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

III. CONCLUSIONS:

The study meets the validity criteria and the endpoints based on mean measured concentrations are:

LC_{50} 96 hours (95% C.I.):	**> 101 mg p.m./L** (not applicable)
NOEC: highest concentration without adverse effects	101 mg p.m./L

Assessment and conclusion by applicant:

The study is reliable and the LC_{50} > 101 mg p.m./L can be used in the M-05 risk assessment.

CA 8.2.2 Long-term and chronic toxicity to fish

CA 8.2.2.1 Fish early life stage toxicity test

Data Point:	KCA 8.2.2.1/01
Report Author:	[redacted]
Report Year:	2003
Report Title:	AE C638206: An Early Life-Stage Toxicity Test with the Fathead Minnow (Pimephales promelas) Under Flow-Through Conditions
Report No:	B004235
Document No:	M-241190-01-1
Guideline(s) followed in study:	OECD: 210 (1992); USEPA (=EPA): 850.1400 (1996)
Deviations from current test guideline:	Method: none Study: (current Guideline: OECD 210 (2013) The temperature deviated from the range of 25°C ± 1.5°C during a three-day period in which temperatures ranged from approximately 23.0 to 27.0°C, due to a mechanical malfunction. Since there was no corresponding increase in effects noted among the organisms during this period (Days 4-6 post-hatch), the variation in temperature is not believed to have adversely affected the study.
Previous evaluation:	yes, evaluated and accepted in DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Data Point:	KCA 8.2.2.1/02
Report Author:	[redacted]
Report Year:	2018
Report Title:	Statement - Certificate of analysis for fluopicolide toxicity study on early life stages of fathead minnow (Palmer et al, 2003; M-241190-01-1)
Report No:	M-634701-01-1
Document No:	M-634701-01-1
Guideline(s) followed in study:	--
Deviations from current test guideline:	Not applicable
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	Yes

Executive summary

A fish early life stage study was performed with fathead minnow (*Pimephales promelas*) in flow-through conditions for 33 days. Fluopicolide was applied at concentrations of 0.038 – 0.075 – 0.150 – 0.300 – 0.600 mg/L. A water control and a solvent control were also included. The test comprised 4 replicates of 20 fish for each group.

Mortality, clinical signs of toxicity and abnormal behaviour were recorded daily and were used to derive hatching success, time to hatch, and post-hatch survival parameters. In addition, growth was evaluated by determining total length, wet and dry weight at the end of the test.

Concentrations of fluopicolide were verified by HPLC/UV on days 0, 7, 14, 21, 28 and 33 for each concentration and control. Measured concentrations were in the 80-120% range of nominal concentrations and no residues above the LOQ were measured in the controls. The mean measured concentrations were: 0.037 – 0.076 – 0.155 – 0.304 – 0.585 mg/L.

The study fulfils all validity criteria of the current version of OECD 210 guideline.

There was a slight, yet notable delay in time to hatch in the 0.585 mg/L treatment group in comparison to the control groups. Hatching success was not significantly reduced at the highest concentration tested but larval survival at the end of the test was significantly reduced at 0.585 mg/L. Statistically significant effects on total length and wet weight were observed at 0.304 mg/L. However, there were no statistically significant effects on dry weight.

Therefore, the most sensitive parameters are wet weight and total length, and the corresponding overall NOEC is 0.155 mg/L (mean measured concentration).

I. MATERIAL AND METHODS:

Test material:	Fluopicolide (tech.) Lot/batch: 2050190/PP241024/2 Specification not reported Purity 97.7% w/w
Guideline(s) adaptation	None specified
Test species:	Fathead minnow (*Pimephales promelas*)
Organism Age at Experimental Start:	Embryos less than 24 h old
Test solutions	Nominal concentrations: of 0.038 – 0.075 – 0.150 – 0.300 – 0.600 mg/L (not corrected for purity) Arithmetic mean measured concentrations: 0.037 – 0.076 – 0.155 – 0.304 – 0.585 mg/L Controls: water control and solvent control (dimethylformamide 0.05 mL/L) Evidence of undissolved material: all of the test solutions appeared clear and colorless in the test chambers at test initiation and termination. A slight brown precipitate was observed in the diluter mixing chamber for the 0.600 mg/L replicates at test termination.
Replication:	No. of vessels per concentration (replicates): 4 No. of vessels per control (replicates): 4 No. of vessels per solvent control (replicates): 4
Organisms per replicate:	No. of fertilized eggs/embryos per vessel: 20
Exposure:	Flow-through Total exposure duration: 33 days (5-day-hatch and 28 d post-hatch)
Test Vessel Loading:	At the end of the test: 0.19 g fish/L of water in the tank or 0.015 g/L per 24 h
Feeding during test	Newly hatched larvae were fed live brine shrimp nauplii (*Artemia sp.*) three times per day during the test. Rations were adjusted each week to account for losses due to mortality.
Test conditions:	Temperature: 24.0 to 24.6°C (test chambers), temperature measured continuously in one water control replicate remained within the desired range of 25 ± 1°C, with the exception of a three-day period in which temperatures ranged from approximately 23.0 to 27.0°C, due to a mechanical malfunction. Photoperiod: 16:8 light:dark with gradual intensity changes at dawn and dusk Light intensity: 80 lux pH: 8.0 to 8.3 Water hardness: 124 to 144 mg/L as $CaCO_3$ Dissolved oxygen % saturation: ≥ 89% (7.3 mg/L) Conductivity: 350 µS/cm Begin of post-hatch period: day 5

Parameters Measured / Observations	Temperature was measured in each test chamber at the beginning and end of the test and at weekly intervals during the test, Temperature also was measured continuously in one negative control replicate. Dissolved oxygen was measured in alternating replicates of each treatment and control group at the beginning and end of the test, daily during the first seven days of the test and at weekly intervals during the test. Measurements of pH were made in alternating replicates of each treatment and control group at the beginning and end of the test and at weekly intervals during the test. Hardness, alkalinity and specific conductance were measured in alternating replicates of the water control and the highest concentration treatment group at the beginning of the test, at weekly intervals during the test and at test termination. During the first day of exposure, embryos were observed twice for mortality and eggs with fungus. Thereafter, until hatching was complete, observations of embryo mortality and the removal of dead embryos were performed once daily. During the 28-day post-hatch exposure period, the larvae were observed daily to evaluate the number of mortalities and the number of individuals exhibiting clinical signs of toxicity or abnormal behavior. From these observations, hatching success, time to hatch, and post-hatch growth and survival were evaluated. Post-hatch growth of the fathead minnows (total length, wet and dry weights) was evaluated at the conclusion of the 28-day post-hatch exposure period.
Sampling for chemical analysis	On Days 0, 7, 14, 21, 28 and 33 (test termination), water samples were collected from one alternating replicate test chamber of each treatment and control group to measure concentrations of the test substance. Fluopicolide was measured by HPLC/UV.
Data analysis:	Test endpoints analysed statistically for the juvenile fish were hatching success, larval survival and growth (total length, wet weight and dry weight). Data from the negative and solvent control groups for each parameter were compared using a t-test. Since no differences were detected between the two control groups ($p > 0.05$) for any of the parameters, the control data were pooled for comparison among the treatment groups. Discrete-variable data were analysed using Chi-square and Fisher's Exact test to identify treatment groups that showed a statistically significant difference ($p < 0.05$) from the controls. All continuous-variable data were evaluated for normality using Shapiro-Wilk's test, and for homogeneity of variance using Bartlett's test ($p = 0.01$). Since the data passed the assumptions of normality and homogeneity, ANOVA and Bonferroni's t-test were used ($p < 0.05$). All statistical tests were performed with TOXSTAT v3.5 or SAS v 8.2 software.

II. RESULTS AND DISCUSSION:

Validity criteria	Required by OECD 210, 1992	Required by OECD 210, 2013	Obtained
Dissolved oxygen concentration throughout the test	Between 60% - 100% saturation	≥ 60% saturation	≥ 89% saturation
Temperature range for the species	25 ± 2°	25 ± 1.5°	Fulfilled with the exception of a three-day period in which temperatures ranged from approximately 23.0°to 27.0°C due to a mechanical malfunction. Since there was no corresponding increase in effects noted among the organisms during this period (Days 4-6 post-hatch), the variation in temperature is not believed to have adversely affected the study
Water temperature difference between test chambers or between successive days at any time during the test	± 1.5° max	± 1.5° max	
Analytical measure of the test concentrations	Compulsory	Compulsory	Done
Hatching success of controls	> 66%	70%	83 and 85%
Post-hatch survival of controls	> 70%	> 75%	89 and 90%
Solubilising agent when used	No significant effect on survival nor any other adverse effects	Not required	Fulfilled

Analytical results:
The recoveries are in the range 80-120% (see table below). The results of the study are based on the arithmetic mean measured test concentrations.
No residues of fluopicolide were measured in the controls above the limit of quantification (20.5 µg/L).

Nominal conc. (mg/L)	Arithmetic mean (mg/L)	% of nominal concentrations					
		Day 0	Day 7	Day 14	Day 21	Day 28	Day 33
0.038	0.037	98.2	96.1	96.0	94.2	97.5	95.9
0.075	0.076	99.6	103	104	102	101	102
0.150	0.155	103	101	104	102	103	103
0.300	0.304	103	103	103	98.7	102	98.9
0.609	0.585	101	97.1	95.1	99.6	90.8	101

Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4 with minor acceptable exceptions only.

Biological results:

Observations

With the exception of one fish that appeared weak from Day 0 to 2 post-hatch, all surviving control larvae appeared normal. In general, the majority of the fish in the 0.037, 0.076, 0.155 and 0.304 mg/L treatment groups appeared normal throughout the test. There were a few sporadic observations of organisms that appeared smaller, weak or lethargic, or were swimming erratically or exhibiting a loss of equilibrium. These observations were few in number and did not occur in a concentration-responsive pattern.

In the 0.304 mg/L treatment group, one fish was observed to have an abnormal growth on the abdomen and dorsal fin. No other observations of abnormal growths were reported in any of the treatment groups during the test. There was a marked increase in observations of sublethal effects among surviving larvae in the 0.585 mg/L treatment group, including organisms that appeared smaller, weak or lethargic, or were swimming erratically, exhibiting a loss of equilibrium, or lying on the bottom. The increase in observations of sublethal effects in the 0.585 mg/L treatment group was considered to be treatment related.

Growth

In the 0.304 mg/L treatment group, the fish with an abnormal growth was excluded from calculation of mean wet weights since the growth markedly increased the weight of the organism.
There were statistically significant differences in total length and wet weight in the 0.304 mg/L treatment group in comparison to the pooled control.

Arithmetic mean measured concentrations (mg/L)	Mean total length (mm) ± SD	Mean wet weight (mg) ± SD	Mean dry weight (mg) ± SD
Water control	21.3 ± 0.61	68.1 ± 4.6	12.7 ± 1.3
Solvent control	21.5 ± 0.28	72.4 ± 3.8	12.8 ± 0.9
Pooled control	21.4 ± 0.45	70.3 ± 4.3	12.8 ± 1.0
0.037	21.3 ± 0.79	73.9 ± 8.5	13.0 ± 1.6
0.076	21.3 ± 0.54	73.2 ± 3.4	13.9 ± 0.6
0.155	21.1 ± 0.39	70.9 ± 3.9	13.9 ± 0.8
0.304	19.9 ± 0.29*	59.8 ± 3.0*	11.6 ± 0.6
0.585#	13.8 ± 1.1	17.5 ± 1.4	3.3 ± 0.2

excluded from statistical analysis since there was a significant effect on survival
* Statistically significant ($p \leq 0.05$, Bonferroni's t-test)

Time to hatch and hatching success

The fathead minnow embryos began hatching on Day 4 of the test and larvae were released into the test chambers on Day 5, when >90% of the surviving control embryos had hatched. There were no apparent differences in time to hatch between the control group and the 0.037, 0.076, 0.155 and 0.304 mg/L treatment groups. With the exception of one embryo in the 0.304 mg/L treatment group that hatched on Day 6, all surviving embryos hatched by Day 5 of the test. However, there was a slight, yet notable delay in time to hatch in the 0.585 mg/L treatment group in comparison to the control groups. Only 42% of the surviving embryos in the 0.585 mg/L treatment group had hatched by Day 5 of the test, in comparison to 100% in the negative and solvent control groups. The majority of the surviving embryos (58%) in the 0.585 mg/L treatment group hatched on Day 6 of the test.

There was a significant difference in hatching success between the pooled controls and the 0.037 and 0.076 mg/L treatment groups. However, at least two replicates in each of the 0.037 and 0.076 mg/L treatment groups had several embryos that were removed from the incubation cups on Day 5 due to the presence of a fungus, which was not related to treatment with the test substance. When the fungused embryos were excluded from calculations, hatching success in the 0.037 and 0.076 mg/L treatment groups was 78 and 95%, respectively, and was not significantly different from the pooled controls.

Arithmetic mean measured concentrations (mg/L)	Mean % hatching success	Mean % hatching success of non-fungused embryos
Water control	85	85
Solvent control	83	83
0.037	56*	78
0.076	66*	95
0.155	83	85
0.304	83	87
0.585	81	81

* Statistically significant difference ($p \leq 0.05$) from the pooled control using Fisher's Exact test.

Larvae survival on day 28 post-hatch

The decrease in survival in the 0.585 mg/L treatment group was statistically significant in comparison to the pooled controls.

Arithmetic mean measured concentrations (mg/L)	Mean % survival
Water control	90
Solvent control	89
0.037	82
0.076	98
0.155	91
0.304	89
0.585	17*

* Statistically significant difference ($p \leq 0.05$) from the pooled control using Fisher's Exact test.

III. CONCLUSIONS:

The study is considered to be valid and the endpoints based on arithmetic mean concentrations are:

	% hatching success	% post hatch survival	Dry weight	Wet weight	Total length
LOEC (mg/L) lowest concentration with an effect	> 0.585	0.585	0.585	0.304	0.304
NOEC (mg/L) **highest concentration without adverse effects**	0.585	0.304	0.304	**0.155**	**0.155**

Assessment and conclusion by applicant:

The study is reliable, and the NOEC is 0.155 mg/L. See below for additional endpoints.

Data Point:	KCA 8.2.2.1/03
Report Author:	[redacted]
Report Year:	2018
Report Title:	ECx calculation for fluopicolide study on early life stages of Pimephales promelas (Palmer et al. (2003), M-241190-01-1)
Report No:	M-643769-01-1
Document No:	M-643769-01-1
Guideline(s) followed in study:	none
Deviations from current test guideline:	Not applicable
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

ECx calculations for the ELS study (Palmer et al, 2003).

Endpoints for the following parameters were statistically determined in the existing report:

- Hatching success
- Post-hatch survival
- Length
- Fresh weight
- Dry weight

The NOEC for hatching success is the highest tested concentration (0.585 mg/L) and only 4.7% of effects were observed at this level. Therefore, it is not necessary to calculate EC_{10} and EC_{20} values for this parameter, they are both > 0.585 mg/L.

Nevertheless, ECx values can be calculated for the other parameters.

All recalculations were performed with the software ToxRat Professional Vers. 3.2.1 with the mean measured concentrations provided in the report.

Three linear regression models (Logit, Probit and Weibull) and 3-D non-linear regression model were compared. Only the most suitable model is presented: Probit for all parameters. Water control and solvent control were pooled when there were no statistically significant differences ($\alpha = 0.05$) according to Student-t test.

Endpoints (mg/L)	% post hatch survival	Dry weight	Wet weight	Total length
EC_{10} (95% confidence interval)	No significant dose-response relationship so no ECx can be calculated	0.307 (n.d.)	**0.278** (0.144-0.350)	0.338 (0.194-0.408)
EC_{20} (95% confidence interval)		0.356 (n.d.)	0.328 (0.203-0.397)	0.440 (0.332-0.498)
NOEC from the report	0.304	0.304	**0.155**	**0.155**

n.d. not determined due to mathematical reasons

According to the AGD, EC_{10} are prefered endpoints for risk assessment. Both NOEC and EC_{10} endpoints are robust but the EC_{10} is considered as more biologically relevant than the NOEC for this study because the level of effects at the NOEC for wet weight is below the level of effects in the controls and for length there is a reduction of only 1.5%. This NOEC value is artificially low due to the spacing factor between the LOEC and the NOEC. Therefore, it is proposed to use in the risk assessment, the EC_{10} for wet weight, the most sensitive parameter of the study.

The overall NOEC for the fish ELS study on fathead minnow with fluopicolide is 0.155 mg/L based on effects on length and wet weight. The lowest EC_{10} is 0.278 mg/L based on effects on wet weight.

Assessment and conclusion by applicant:

The study is reliable and the relevant endpoints for fluopicolide are the NOEC of 0.155 mg/L and the EC_{10} of 0.278 mg/L based on wet weight.

According to the AGD, EC_{10} are prefered endpoints for risk assessment. Both NOEC and EC_{10} endpoints are robust but the EC_{10} is considered as more biologically relevant than the NOEC for this study because the level of effects at the NOEC for wet weight is below the level of effects in the controls and for length there is a reduction of only 1.5%. This NOEC value is artificially low due to the spacing factor between the LOEC and the NOEC. Therefore, it is proposed to use in the risk assessment, the EC_{10} for wet weight, the most sensitive parameter of the study.

CA 8.2.2.2 Fish full life cycle test

Based on the triggers stated in the EU regulation 283/2013 on data requirements for active substances and the EFSA Aquatic Guidance Document, a fish full life cycle (FFLC) study is required for bioaccumulative and persistent substances. Fluopicolide has a BCF < 1000, and therefore does not meet the trigger of bioaccumulation and persistent. A FFLC may also be required due to endocrine disruption criteria. These are discussed in CA 8.2.3.

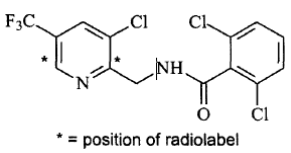
CA 8.2.2.3 Bioconcentration in fish

Data Point:	KCA 8.2.2.3/01
Report Author:	[redacted]
Report Year:	2003
Report Title:	Bioaccumulation and metabolism of [2,6-14C-pyridinyl]-AE C638206 in bluegill sunfish, Lepomis macrochirus, in a flow-through system
Report No:	B004340
Document No:	M-241273-01-1
Guideline(s) followed in study:	OECD: 305 (1981); USEPA (=EPA): Subdivision N 165-4 (1982)
Deviations from current test guideline:	Current Guideline: OECD 305 (2012) The concentration was not maintained within +/-20% of the mean for the high concentration (see details in the validity part below) because of the technical failure on day 16 only. This deviation is not considered to have affected the overall validity of the test since the worst case BCF were obtained from the low concentration where no deviations were observed.
Previous evaluation:	yes, evaluated and accepted in DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive summary

A bioconcentration study was performed with fluopicolide on bluegill sunfish (*Lepomis macrochirus*) using a 45-day flow-through system including a 24-day uptake and a 21-day depuration period. Three groups of 144 juvenile fish each were separately exposed to three treatments: (1) solvent control at 0.1 ml/L dimethylformamide; (2) treated at a nominal concentration of 0.8 µg/L [^{14}C]- fluopicolide in 0.1 mL/L dimethylformamide and (3) treated at a nominal concentration of 8.0 µg/L [^{14}C]- fluopicolide in 0.1 ml/L dimethylformamide. Water samples from each test chamber were analysed for total radioactive residues by liquid scintillation counting on day 0 and every Monday, Wednesday, and Friday during the uptake and depuration phases and by HPLC on days 0, 1, 7, 14 and 21. Fish were sampled at regular intervals throughout the uptake and depuration periods for determination of total radioactive residues and lipid content. Fish samples consisting of three tissue groups - whole body, edible tissue (muscle, skin and skeleton), and nonedible tissue (fins, head and viscera) - were taken throughout the uptake and depuration phases. Total radioactive residues in each tissue group were determined by combustion. Additional fish were collected, after achieving steady state, on days 21 and 24 of the exposure period for metabolite identification. The identity of single major residue component, unchanged fluopicolide, was confirmed by LC-MS. The study fulfils all validity criteria of the current version of the OECD 305 guideline. Mean measured concentration and standard deviation of test material in the water during the uptake period was 0.844 ± 0.028 µg/L [^{14}C]- fluopicolide equivalents at the low treatment (0.8 µg/L) and 7.265 ± 1.79 µg/L [^{14}C]- fluopicolide equivalents at the high treatment (8.0 µg/L). HPLC analysis demonstrated that the parent compound fluopicolide was stable in the low and high treatment water throughout the uptake phase. It was the only radioactive component detected in the water. There was one mortality in the solvent control treatment and two mortalities in the high treatment during the course of the study. These mortalities were not considered to be related to the test substance. The steady-state-BCF parent (based on whole fish) in the 0.8 µg/L test level is about 117 L/kg, the lipid normalized BCF is 65 L/kg.

I. MATERIAL AND METHODS:

Test material	Fluopicolide AE C638206 Batch code: R001737 Purity: 99.3% Radiochemical purity: 96.5%  * = position of radiolabel
Guideline(s) adaptation	None specified
Test species	Bluegill sunfish (*Lepomis macrochirus*)
Acclimation	Fish were acclimated to the test dilution water and the test temperature (i.e. 22 ± 2°C) for ≥ 14 days prior to initiation of testing. There were less than 3% of mortality in the population
Details on test organisms	- Weight at study initiation: 1.077 g (range 0.724-1.601 g) - Length at study initiation: 3.33 cm (range 3.0 – 3.9 cm) - Lipid content at test initiation: 17.3 % (ww) of whole fish
Test solutions	Nominal concentrations: 0.8 µg and 8 µg [^{14}C]-fluopicolide/L Measured concentrations: 0.844 and 7.265 µg [^{14}C]-fluopicolide/L Solvent control: 0.1 mL/L dimethylformamide Evidence of undissolved material: not reported
Replication	No. of vessels per concentration (replicates): 1 No. of vessels per solvent control (replicates): 1
Organisms per replicate	No. of organisms per vessel: 144
Exposure	Test type: Flow through Route of exposure: aqueous Total exposure duration: 24 days Total depuration duration: 21 days
Test Vessel Loading	Biomass loading rate: 2.2 g/L test tank at the start of the study and 0.43 g/L/day based on the flow of test solution through the tanks
Test conditions	Temperature: 21.2 - 24.0°C (continuous measurements), 21.6 – 22.7°C (discrete measurements) Photoperiod: 16:8 hours. Light intensity: 1800 lux (initial light intensity at the surface of the test system) pH: 8.2-8.5 Water hardness: 72 - 220 mg/L as $CaCO_3$ Oxygen saturation: 80-102%. The test aquaria were aerated during the study to maintain dissolved oxygen levels at or above 60% of saturation. TOC: 2.0 mg/L in dilution water Conductivity: 1000 µS/cm Alkalinity: 258 to 328 mg/L as $CaCO_3$
Feeding during test	During the uptake and depuration phase, the fish were fed twice daily with commercial fish food at a rate of approximately 2% of mean body weight per day. The amount of food was re-calculated (to adjust for loss due to mortality or sampling) each day based on the mean weight of fish sampled previously.

Parameters Measured / Observations	Discrete water quality measurements of temperature, dissolved oxygen, pH and conductivity were obtained in each test tank throughout the study. Continuous temperature monitoring in solvent control test tank. Fish were observed initially and daily during the exposure and depuration period for any mortality and/or abnormal appearance or behaviour. Total lipid content was determined in fish at study initiation and in treated fish sampled on days 7, 14, 21, 24 of uptake and days 7 and 18 of depuration.
Sampling for chemical analysis	Water analysis: On Day 0 and every Monday, Wednesday, and Friday thereafter during the uptake phase, the concentration of total radioactive residues in the test water were calculated by direct LSC as [^{14}C]-fluopicolide equivalents. On certain fish sampling days of the uptake phase (Study Days 0, 1, 7, 14, 21, 24) and on Day 1 of the depuration phase, duplicate water samples of 100 mL were removed from each aquarium using a glass pipette and stored in glass bottles. Samples were analysed by HPLC with UV detection. Fish analysis: Six fish were taken from each tank at each sampling and pooled into 2 groups of 3 fish. Three of these fish were taken for whole fish analysis and lipid analysis (lipid analysis on treated fish only). The remaining three fish from the control and treated tanks were weighed and dissected into edible and non-edible tissue. Six aliquots of each tissue sample were then combusted for analysis of total radioactive residue. Additional samples of fish were collected from each tank for metabolite characterization on Day 21 and Day 24. These fish were weighed in groups of six and dissected into edible and non-edible portions. Metabolites in extracts containing significant residues (>5%) were identified and quantified by comparison with authentic standards on HPLC. The identity of single major residue component, unchanged AE C6738206, was confirmed by LC-MS.

II. RESULTS AND DISCUSSION:

Validity criteria	Required (OECD 305, 1996)	Required (OECD 305, 2012)	Obtained
Water temperature variation over the whole test period	± 2°C		21.2 - 24°C*
Dissolved oxygen % saturation in all test vessels	60%		80 - 102%
Concentration of test substance in test chambers maintained within required range of the mean of the measured values during the uptake phase	±20%		low concentration: 93-105% of the mean high concentration: 23 – 116%**
The concentration of the test substance is below its limit of solubility in test water		Test concentration < water solubility of test item in test water	Yes***
Mortality or other adverse effects/disease in control and treated fish	10%		< 10%

* Temperature variation continuously monitored in solvent control aquarium

** On Day 16, a syringe pump malfunction in the high treatment reduced the flow of test solution for about 17 hours. The water concentrations by LSC on the following day dropped below the limit of ±20% of nominal concentration. After repair of the syringe pump, the water concentrations returned to normal values (102% of the mean measured uptake concentration). The concentration dropped below the required range for a short period of time in the highest concentration only, therefore the study should be considered as valid. Moreover, the worst case BCF values are obtained at the lowest concentration, where no issues of concentration stability was observed.

*** Water solubility of [2,6-^{14}C-pyridinyl]-fluopicolide = 3.02 mg/L

Analytical results:
Mean measured water concentrations as determined by LSC during the uptake period was 0.844 ± 0.0276 µg/L [^{14}C]-fluopicolide equivalents at the low treatment (0.8 µg/L) and 7.265 ± 1.79 µg/L [^{14}C]-fluopicolide equivalents at the high treatment (8.0 µg/L). This represented 106% of the low nominal concentrations and 91% of the high nominal concentration. Water concentrations ranged from 0.783 µg/L to 0.888 µg/L in the low treatment and 1.657 µg/L to 8.452 µg/L in the high treatment through the uptake phase. No radioactivity was detected in the solvent control tank.
Mean measured water concentration during day 1 and day 4 of depuration showed a decrease in [^{14}C]-fluopicolide equivalents in both treated tanks. After day 4 of depuration, no water samples were taken since concentrations of radioactive residue were practically not detectable. At several times during the uptake phase (days 0, 1, 7, 14 and 21) water samples from both the low and high treatment aquaria were analysed by HPLC to monitor any degradation of the test compound in aquarium water. No degradation of the test compound was observed.
Average daily concentrations of total radioactivity in water (mg/L, expressed as [^{14}C]-fluopicolide equivalents)

Study day	Nominal concentration: 0.8 µg/L	Nominal concentration: 8.0 µg/L
-3	0.898	8.405
-2	0.840	8.287
-1	0.867	8.435
0	0.783	7.597
2	0.819	7.864
4	0.859	7.916
7	0.888	8.200
9	0.867	7.829
11	0.863	7.992
14	0.853	8.002
16	0.830	1.657
16	0.829	5.014
16	0.815	6.345
17	0.859	7.426
18	0.836	7.933
21	0.876	8.319
23	0.862	8.433
24	0.825	8.452
25	0.035	0.203
28	0.006	0.006
Uptake Mean	0.844	7.265

Mean tissue residues expressed as µg/kg of [^{14}C]-fluopicolide equivalents.

Study day	Nominal concentration: 0.8 µg/L			Nominal concentration: 8.0 µg/L		
	Edible tissue	Whole fish	Non-edible tissue	Edible tissue	Whole fish	Non-edible tissue
0	n.d.	0.0 ± 0.0	n.d.	n.d.	0.0 ± 0.0	n.d.
1	26.3 ± 2.5	76.8 ± 4.2	124.1 ± 4.9	182.7 ± 26.3	594.1 ± 46.7	256.7 ± 89.6
3	23.9 ± 3.7	128.3 ± 15.2	127.3 ± 27.1	165.5 ± 22.4	680.4 ± 114.2	107.8 ± 39.4
7	29.1 ± 3.4	83.4 ± 10.5	156.6 ± 46.3	177.7 ± 13.6	847.0 ± 174.2	98.3 ± 47.7
10	30.4 ± 2.5	11.6 ± 10.7	151.9 ± 15.2	186.2 ± 10.7	720.3 ± 78.7	67.8 ± 9.4
14	27.9 ± 2.3	91.6 ±1.4	143.0 ± 6.7	193.3 ± 7.2	708.9 ± 74.4	37.1 ± 59.6
18	33.4 ± 2.9	101.2 ± 6.9	151.5 ± 9.5	210.8 ± 9.2	630.0 ± 35.0	1061.8 ± 59.3
21	43.3 ± 5.1	108.0 ± 7.7	186.6 ± 23.7	330.3 ± 16.6	766.6 ± 88.9	1313.8 ± 117.7
24	44.8 ± 3.6	87.4 ± 9.5	159.3 ± 8.9	301.8 ± 26.7	825.9 ± 143.2	1361.6 ± 74.8
25	15.0 ± 0.4	31.9 ± 4.5	51.9 ± 2.9	84.8 ± 2.6	174.3 ± 23.0	256.7 ± 29.6
28	9.2 ± 0.3	11.4 ± 0.4	17.2 ± 1.3	64.9 ± 1.3	95.0 ± 9.0	107.8 ± 4.7
31	7.5 ± 0.2	8.4 ± 0.3	13.1 ± 1.4	56.1 ± 0.5	78.5 ± 6.3	98.3 ± 4.0
35	5.9 ± 0.2	7.8 ± 0.4	9.1 ± 0.6	47.7 ± 0.8	47.5 ± 1.2	67.8 ± 6.4
42	4.5 ± 0.2	4.6 ± 0.4	6.0 ± 0.4	38.4 ± 0.9	44.4 ± 9.6	47.1 ± 6.6

n.d.: not determined

Due to a syringe pump malfunction in the high treatment on Day 16 of the uptake phase, the study was continued until Day 24 to re-establish steady state. Tissue residues remained stable in the low treatment throughout the uptake phase. In the high treatment, fish residues were stable by Day 17 and remained stable until the last day of uptake.

The determination of steady-state according to OECD guidelines requires three time points to be within ±20% of each other, no significant difference between time points, and a parallel curve with respect to the time axis. Due to the syringe pump malfunction in the high treatment on Day 16 of this study, these criteria were not met specifically for the high treatment, even though the overall plot reveals that steady-state was reached by Day 1. In the low treatment, the whole fish tissue residues for the final three time points of the uptake phase (Day 18, 21, and 24) were significantly different by a one-way ANOVA ($P<0.05$). However, the final three time points were within 20% of their average, the residues were not showing an upward trend, and a plot of whole fish tissue residues versus time for the low treatment revealed a parallel curve with respect to the time axis. In the high treatment, whole fish tissue residues from the final day of uptake (Day 24) showed a slight increase, but the final three time points of the uptake phase (Day 18, 21, and 24) met all of the requirements for steady-state according to OECD.

Depuration (% of maximum uptake concentration at day 24)

Depuration day	Nominal concentration: 0.8 µg/L			Nominal concentration: 8.0 µg/L		
	Edible tissue	Whole fish	Non-edible tissue	Edible tissue	Whole fish	Non-edible tissue
1	66	64	67	72	79	81
4	80	87	89	78	89	92
7	83	90	92	81	91	93
11	87	91	94	84	94	95
18	90	95	96	87	94	97

Depuration was fairly rapid in whole fish and non-edible tissue in both treatments, with greater than 90% of accumulated [^{14}C]-residues being eliminated from tissues after 7 days of the depuration period. Edible tissue residues in both treatments was slower to depurate with approximately 90% of [^{14}C]-residues eliminated from tissue after 18 days of depuration.
Fluopicolide was the only radioactive residue component in fillet tissues. Fluopicolide also represented from 67.7 to 75.9% of radioactive residue in viscera. Due to the low bioconcentration factors of AE C638206 determined in this study (<200 L/kg in non-edible tissue), identification of the minor metabolites was not pursued further.

Biological results (Bioconcentration part):
Mean lipid contents during the 45-day study period of whole fish were 12.9 ± 4.1% in the low treatment and 13.0 ± 3.8% in the high treatment. At the end of the uptake phase, the lipid contents were 9.0 and 9.8 % in the low and high concentrations respectively. These values are used to normalise the BCF to 5% lipid.
Substance uptake, depuration constants and bioconcentration factors are given in the table below.
The maximum observed bioconcentration factors for the low treatment were 53L/kg (Day 24), 156 L/kg (Day 3), and 221 L/kg (Day 21) for edible, whole fish, and non-edible tissues, respectively. The high treatment had maximum BCFs of 47 L/kg (Day 21), 114 L/kg (Day 24), and 187 L/kg (Day 24) for edible tissues, whole fish, and non-edible tissues respectively. Due to the variability in tissue concentrations and the rapid bioconcentration, the steady-state values were based on the average concentration of the Day 18, 21, and 24 time points.

	0.8 µg [2,6-^{14}C-pyridinyl]-fluopicolide/L			8 µg [2,6-^{14}C-pyridinyl]-fluopicolide/L		
	edible tissue	viscera tissue	whole fish	edible tissue	viscera tissue	whole fish
Time to reach 90% of steady state (days)	n.d.	n.d.	1.7	n.d.	n.d.	1.5
BCF_{SS} Steady-state BCF [tr] (L/kg)	48	197	117	40	175	104
Lipid content at day 24 (end of uptake)	n.d.	n.d.	9.0%	n.d.	n.d.	9.8%
Lipid normalized BCF_{ss}* (L/kg)	n.d.	n.d.	65	n.d.	n.d.	53
k_1 Overall uptake rate constant [L kg^{-1} day^{-1}]	n.d.	n.d.	164.4	n.d.	n.d.	152.1
k_2 Overall depuration rate constant [day^{-1}]	n.d.	n.d.	1.35	n.d.	n.d.	1.49
BCF_k* (L/kg) (= k_1/k_2)	n.d.	n.d.	122	n.d.	n.d.	102
Lipid normalised BCFk* (L/kg)	n.d.	n.d.	68	n.d.	n.d.	57
$t_{1/2}$ Depuration half-life [day]	n.d.	n.d.	0.51	n.d.	n.d.	0.47

n.d. = not determined
* not calculated in the report

III. CONCLUSIONS:

The steady-state-BCF parent (based on whole fish) in the 0.8 µg/L test level is about 117 L/kg, the lipid normalized BCF_{ss} is 65 L/kg.

Assessment and conclusion by applicant:

The study is reliable and the relevant endpoint for risk assessment is the lipid normalised BCF_{ss} of 65 L/kg.

CA 8.2.3 Endocrine disrupting properties

Potential endocrine-disrupting properties of fluopicolide are being evaluated according to EU Regulation 2018/605 ("ED criteria") following recommendations of the ECHA-EFSA Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009.

Four studies are currently ongoing;

Dossier node	Draft title	Study ID	Planned submission
KCA 8.2.3	*Xenopus* Eleutheroembryonic Thyroid Assay (XETA) analysis report	EBAC0095	November 2020 / 2nd Quarter 2021
KCA 8.2.3	Rapid Estrogen ACTivity In Vivo assay (REACTIV) assay analysis report	EBAC0100	November 2020 / 2nd Quarter 2021
KCA 8.2.3	Rapid Androgen Disruption Adverse-outcome Reporter (RADAR) assay analysis report	EBAC0101	November 2020 / 2nd Quarter 2021
KCA 8.2.3	Fish Short-Term Reproduction Assay (FSTRA) study report	EBAC0097	November 2020 / 2nd Quarter 2021

CA 8.2.4 Acute toxicity to aquatic invertebrates

CA 8.2.4.1 Acute toxicity to Daphnia magna

Data Point:	KCA 8.2.4.1/01
Report Author:	[redacted]
Report Year:	2003
Report Title:	The 48 hour acute toxicity to the water flea, Daphnia magna, in a static system; AE C638206 technical
Report No:	B003803
Document No:	M-240807-01-1
Guideline(s) followed in study:	OECD: 202 (1992); USEPA (=EPA): 72-2 (1982)
Deviations from current test guideline:	Method: Deviations from current guideline SANCO/3029/99 rev.4: Recoveries were only determined at two different concentrations in duplicate. However, the obtained data demonstrate very good recoveries and the precision calculated form these data accounts for 8.4 (AE C638206) and 10.5 % (AE C653711), respectively. The method can therefore be regarded as fit for purpose. Study: Current Guideline: OECD 202 (2004) The test was performed in soft water with a hardness of 40-48 mg CaCO3 /L instead of 140-250 mg/L. These are not optimal conditions for Daphnia magna however it has been demonstrated that long term culturing (125 days) of this species in soft water with 50 mg CaCO3 /L is possible without effect on survival. Only reproduction is affected (Lewis and Maki, 1981). Therefore, the results of the tests are considered to be reliable since the validity criteria are all met.
Previous evaluation:	yes, evaluated and accepted in DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive summary

An acute toxicity test was performed with water flea (*Daphnia magna*) in a static system. Juvenile daphnids (less than 24 hours old), were exposed to nominal concentrations of 0 (control) and 5.0 mg a.s./L of the test substance fluopicolide (tech) in well water (mean temperature of 19.5°C) for a 48-hour period. All treatments were in triplicate with 10 daphnids per test vessel. Test solutions were not renewed. Observations for immobility and for abnormal appearance and behaviour were performed at 3, 6, 24, and 48 hours. Samples of the freshly prepared test solutions from each treatment level were taken for analysis of fluopicolide (tech) and M-01 (BAM (AE C653711)) at 0 hour. At 48 hours (study termination) old test solutions from each treatment level (pooled replicates) were analysed. Samples were taken at mid depth and did not include any extraneous materials. All samples were analysed by Gas Chromatography with MS detection (GC/MS). The arithmetic mean measured concentration of fluopicolide (tech) was determined as 1.8 mg a.s./L (36% of nominal concentration) over the course of the study. The study fulfils all validity criteria of the current version of OECD 202 guideline. The test was performed in soft water with a hardness of 40-48 mg $CaCO_3$ /L instead of 140-250 mg/L. These are not optimal conditions for Daphnia magna however it has been demonstrated that long term culturing (125 days) of this species in soft water with 50 mg CaCO3 /L is possible without effect on survival. Only reproduction is affected [redacted], 1981 [M-669496-01-1]- see section CA 8.2.4.1/03 for a summary of this publication). Therefore, the results of the tests are considered to be reliable since the validity criteria are all met. The substance is stable over the course of the study. Consequently, all toxicity values were calculated based on the arithmetic mean measured concentrations. No M-01 (BAM (AE C653711)) residues were detected in any test sample at 0 and 48 hours. Test solutions at 0 hour ranged from 35 to 37% of nominal concentration, while at 48 hours were at 36% of nominal concentration. No mortality or sub-lethal effects were observed in the control or 1.8 mg/L treatments

during the study. The endpoints based on arithmetic mean measured concentrations are: EC_{50} 48 hours (95% C.I.) > 1.8 mg/L (not applicable), LOEC > 1.8 mg/L and NOEC = 1.8 mg/L.

I. MATERIAL AND METHODS:

Test material	Fluopicolide tech. Lot/batch 2050190//PP241024/2 Specification AE C638206 00 1C99 0005 Purity 97.1 % w/w
Guideline(s) adaptation	None specified
Test species	Water flea (*Daphnia magna*)
Organism age/size at study initiation	First instar neonates, less than 24 hours old
Test solutions	Limit test at 5 mg a.s./L (nominal concentration) corresponding to arithmetic mean measured concentrations of 1.8 mg a.s./L (maximum achievable concentration under test conditions). Controls: water control A stock solution was prepared in excess of solubility (5.0 mg/L) and stirred overnight to ensure maximum solubility. The stock solution was filtered and used directly as the treatment concentration. After the filtration of the primary stock solution, there were no additional problems with solubility throughout the study.
Replication	No. of vessels per concentration (replicates): 3 No. of vessels per control (replicates): 3
Organisms per replicate	No. of organisms per vessel: 10
Exposure	Static Total exposure duration: 48 hours
Feeding during test	None
Test conditions	Temperature: 18.9 - 20.1°C (continuous recording), 19.8 – 20.5°C (discrete recording) Photoperiod: 16 hours light / 8 hours dark with gradual intensity changes at dawn and dusk Light intensity: ca 710 lux pH: 7.3 – 7.9 Water hardness: 40 - 48 mg $CaCO_3$/L Dissolved oxygen: 86 - 105% saturation Conductivity: 270 - 280 µS/cm
Parameters Measured / Observations	Observations for immobility (i.e., lack of response to gentle prodding) and for abnormal appearance and behaviour were performed at 3, 6, 24, and 48 hours. Temperature was monitored continuously in a surrogate vessel filled with water. Discrete measurements of temperature, dissolved oxygen, pH, and conductivity were obtained at test initiation, 24, and 48 hours, or within one hour of the designated time.

Chemical analysis	The parent test solutions were sampled at 0 hour (prior to the distribution of the test solutions to the test chambers). Composites of the replicates within treatment levels were sampled for analytical verification at 48 hours (study termination). At each sampling time point, water samples were taken at mid-depth and did not include any extraneous materials. Fluopicolide and metabolite M-01 (BAM) were determined by gas chromatography with MS detection.
Data analysis	Not applicable: no effects were observed during the study.

II RESULTS AND DISCUSSION:

Validity criteria (OECD 202, 2004)	Required	Obtained
Immobilisation and sub-lethal effects in control during test	≤ 10%	0 %
Dissolved oxygen concentration at the end of the test	≥ 3 mg/L	7.4 mg/L

Analytical results:

Since the concentration tested is in excess of maximum solubility of the test substance under the conditions of this study, low recoveries are observed (see table below). The substance is stable over the course of the study. Consequently, all toxicity values were calculated based on the arithmetic mean measured concentrations. No M-01 (BAM) residues were detected in any test sample at 0 and 48 hours. There was no M-01 (BAM) (AE C653711) residue found in the dilution water or control samples.

Nominal Concentration (mg a.s./L)	Day 0 Measured Concentration (mg a.s./L)	Day 0 % Nominal	Day 2 Measured Concentration (mg a.s./L)	Day 2 % Nominal	Arithmetic mean Measured Concentration (mg a.s./L)	% Mean Measured Concentration
5.0	1.77	35	1.80	36	1.80	36
	1.84	37	1.81	36		

Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4 with minor acceptable exceptions only.

Biological results:

Observations

No sub-lethal effects or abnormalities were observed in the control and in nominal concentration 5.0 mg/L.

Immobility

Exposure time (hours)	**0**	**3**	**6**	**24**	**48**
Arithmetic mean measured conc. (mg a.s./L)	**No of immobilized (%)**	**No of immobilized (%)**	**No of immobilized (%)**	**No of immobilized (%)**	**No of immobilized (%)**
Control	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
1.8	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

III. CONCLUSIONS:

The study meets the validity criteria and the endpoints based on arithmetic mean measured concentrations are:

EC_{50} 48 hours (95% C.I.):	**> 1.8 mg a.s. / L (not applicable)**
LOEC: lowest concentration with an effect	> 1.8 mg a.s. / L
NOEC: highest concentration without adverse effects	1.8 mg a.s. / L

Assessment and conclusion by applicant:

The study is reliable and the relevant endpoint for the fluopicolide risk assessment is the EC_{50} > 1.8 mg a.s./L.

Data Point:	KCA 8.2.4.1/02
Report Author:	[redacted]
Report Year:	2001
Report Title:	2,6-dichlorobenzamide (BAM): Acute toxicity to Daphnia magna
Report No:	1133/008
Document No:	M-234306-01-2
Guideline(s) followed in study:	OECD: 202 (1992); USEPA (=EPA): 72-2 (1982), OPPTS 850.1010 (1996)
Deviations from current test guideline:	Method: Deviations from current guideline SANCO/3029/99 rev.4: Limited sets of validation recoveries were analysed. However, the average recoveries were within the acceptable range of 70–110% and the RSD values were below 20%. The analytical method can be regarded as fit for purpose. Study: Current Guideline: OECD 202 (2004) No sublethal effects are mentioned in the study report so the validity criteria are based on immobilisation only.
Previous evaluation:	yes, evaluated and accepted in DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive summary

An acute toxicity test was performed with water flea (*Daphnia magna*) in a static system. Juvenile daphnids (less than 24 hours old), were exposed to nominal concentrations of 0 (negative control) and nominal concentrations of 10, 18, 32, 56, 100, 180, 320, 560 and 1000 mg/L of the test substance M-01 (2,6-dichlorobenzamide (BAM)) in dechlorinated tap water (room temperature of 21°C) for a 48 hour period. All treatments were in triplicate with 10 daphnids per test vessel. Test solutions were not renewed. Immobilization or adverse reactions to exposure were recorded at 24 and 48 hours after the start of exposure. Water samples were taken from the control and the test groups at 0 and 48 hours for quantitative analysis of the test item by High Performance Liquid Chromatography (HPLC) with UV detection. Analysis of the test preparations at 0 and 48 hours showed measured test concentrations to be near nominal and so it was considered justifiable to calculate the EC_{50} values in terms of the nominal test concentrations only. The study fulfils all validity criteria of the current version of OECD 202. No immobilisation was observed at the test concentrations up to 100 mg/Lmg/L. However, immobilisation was observed at 180 mg/L and above. The acute toxicity of the test material to the freshwater invertebrate *Daphnia magna* has been investigated and gives a 48-hour EC_{50} value of 180 mg/L with 95% confidence limits of 150 - 210 mg/L. The No Observed Effect Concentration at 48 hours is 100 mg/L.

I. MATERIAL AND METHODS:

Test material	M-01 (2,6-dichlorobenzamide (BAM)) Batch FUX001000/FUN81G02C Purity 99.5 % w/w
Guideline(s) adaptation	None specified
Test species	Water flea (*Daphnia magna*)
Organism age/size at study initiation	First instar neonates, less than 24 hours old
Test solutions	Nominal concentrations: 10 – 18 – 32 – 56 – 100 – 180 – 320 – 560 and 1000 mg/L Controls: Dechlorinated tap water Each jar contained approximately 200 mL of test solution
Replication	No. of vessels per concentration (replicates): 2 No. of vessels per control (replicates): 2
Organisms per replicate	No. of organisms per vessel: 10
Exposure	Static Total exposure duration: 48 hours
Feeding during test	None
Test conditions	Temperature: 21°C Photoperiod: 16 hours light / 8 hours dark with gradual intensity changes at dawn and dusk pH: 7.6 – 8.0 Water hardness: 155 mg as $CaCO_3$/L Dissolved oxygen: 8.0 mg/L (90%) – 8.3 mg/L (93%) Alkalinity: 128 mg/L as $CaCO_3$/L
Parameters Measured Observations	Immobilization or adverse reactions to exposure were recorded at 24 and 48 hours after the start of exposure Water temperature was recorded daily throughout the test. Dissolved oxygen concentrations and pH were recorded at the start and termination of the test. In addition, the temperature was recorded in one control vessel every hour.
Chemical analysis	Water samples were taken from the control and the test groups at 0 and 48 hours for quantitative analysis of the test item by High Performance Liquid Chromatography (HPLC) with UV detection
Data analysis	The EC_{50} values and associated confidence limits at 24 and 48 hours were calculated by the maximum-likelihood probit method using the ToxCalc computer software package (Version 5.0.23C, 1999).

II. RESULTS AND DISCUSSION:

Validity criteria (OECD 202, 2004)	Required	Obtained
Immobilisation and sub-lethal effects in control during test	≤ 10%	0 % immobilisation, no data on sublethal effects
Dissolved oxygen concentration at the end of the test	≥ 3 mg/L	8.0 mg/L – 8.1 mg/L

Analytical results:
Analysis of the test preparations at 0 and 48 hours (see table below) showed measured test concentrations near to nominal and so it was considered justifiable to calculate the EC values on the basis of nominal test concentrations.
There was no M-01 (2,6-dichlorobenzamide (BAM)) residue found in the control samples.

Nominal conc. (mg/L)	Arithmetic mean (mg/L)	% of nominal concentrations (mean of 2 measurements)	
		0 hour	48 hours
10	10.8	111	106
18	19.7	112	107
32	35.1	112	108
56	58.9	107	104
100	101	102	100
180	188	106	103
320	336	108	104
560	578	105	101
1000	1019	103	100

Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4 with minor acceptable exceptions only.

Biological results:

Immobility

Exposure time (hours)	24	48
Nominal conc. (mg /L)	No of immobilized (%)	No of immobilized (%)
Control	0 (0)	0 (0)
10	0 (0)	0 (0)
18	0 (0)	0 (0)
32	0 (0)	0 (0)
56	0 (0)	0 (0)
100	0 (0)	0 (0)
180	5 (25)	13 (65)
320	15 (75)	18 (90)
560	19 (95)	20 (100)
1000	20 (100)	20 (100)

III. CONCLUSIONS:

The study meets the validity criteria and the endpoints based on nominal concentrations are:

EC_{50} 48 hours (95% C.I.):	**180 mg / L (150 mg /L – 210 mg /L)**
NOEC: highest concentration without adverse effects	100 mg / L

Assessment and conclusion by applicant:

The study is reliable and the relevant endpoint for risk assessment is the EC_{50} of 180 mg/L.

Data Point:	KCA 8.2.4.1/03
Report Author:	[redacted]
Report Year:	1981
Report Title:	Effects of water hardness and diet on productivity of daphnia magna straus in laboratory culture
Report No:	M-669496-01-1
Document No:	M-669496-01-1
Guideline(s) followed in study:	not applicable
Deviations from current test guideline:	not applicable
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive summary:

This publication is included as it provides the additional information and justification that the study by [redacted] 2003 (M-240807-01-1) see above CA 8.2.4.1/01 is a valid study. The publication addresses the effects of different diet and water hardness, alone and in combination in long-term (multi-generation) studies. The summary below focuses only on the effects of water hardness.

The effects of diet and water hardness, alone and in combination, on life history characteristics of *Daphnia magna* were determined in two laboratory tests. Number of young on the first day of reproduction, total young and the number of generations were greater with increasing hardness. At the maximum test hardness of 350 mg/L (as $CaCO_3$), approximately 65 percent more young were produced than at the lowest hardness of 50 mg/L (as $CaCO_3$). Furthermore, time to sexual maturity was about one day shorter in the harder culture water. The only parameter not affected by water hardness is survival, with around 100% in all hardness conditions.

I. MATERIAL AND METHODS:

Two long-term static tests with periodic water renewal were conducted in a laboratory controlled for light (12 h illumination) and temperature (21 ± 1 °C). The unaerated tests were conducted under cool fluorescent lights that provided 300-400 ft-c of illumination. *Daphnia* were obtained from a stock culture that had been continuously maintained for over 2 years. Adults from this culture were isolated in separate aquaria 24 h before test initiation and young subsequently produced were then used the next day. The methodology for each test is described below.

Dissolved oxygen was monitored prior to test initiation but due to water renewal it was not thought necessary to determine it during testing. Water temperature and pH were also recorded at the beginning of each test.

Water hardness

The chronic effects of four water hardnesses, 50, 125, 225 and 350 mg/L as $CaCO_3$ on *D. magna* productivity were determined for over 125 days (multi-generations). Filtered well-water was blended with a deionized water to the desired test hardnesses. A supply of each hardness water was made prior to test initiation and used throughout the study to minimize variability in hardness during testing. Hardness of the well-water used in this study averaged 350 mg/l (± SD = 10 mg/L). Before use, total hardness of the test water was confirmed using a standard titration method. For each test hardness, three 1-L Pyrex beakers were utilized as test chambers. At test initiation, 800 mL of the test water and 5 daphnids (<24 h old) were added to the chamber. The test chambers were then covered with glass to prevent evaporation of the test waters. The test waters were renewed three times a week for the test duration. Daphnids were fed daily a combination of a green algae (*Selenastrum capricornutum* Printz) suspension and a trout chow-dehydrated alfalfa mixture. Time to sexual maturity, adult survival and number of newborns observed on the first day of reproduction were monitored daily during the study. On the first day of reproduction after enumeration, all adults and all but five juveniles were discarded. The five remaining juveniles were then added to a cleaned test chamber containing water of the same total hardness from which they were withdrawn to initiate the next generation. The data from this test as well as that of the second test were statistically analysed using t-tests.

II. RESULTS AND DISCUSSION:

Dissolved oxygen exceeded 7.0 mg/L (> 78% saturation) at the beginning of the tests. The pH ranged from 6.8 to 7.1 and the mean water temperature was 21°C (± SD = 2°C).

Water hardness

Laboratory productivity of *Daphnia* reared in waters at four different total hardnesses

Total hardness [1]	Total young	Mean number of generations	Mean days to sexual maturity	Mean brood size [2]	Adult survival %
50	5239	49	8.7 (0.5) *	7.2 (0.3) *	98
125	5629	48	8.3 (0.2) *	7.8 (0.1) *	100
225	7543	52	7.8 (0.04) *	9.7 (0.2) *	100
350	8660 *3	55	7.6 (0.05) *	10.9 (0.4) *	100

[1] As mg/L $CaCO_3$
[2] Young observed on first day of reproduction per adult
[3] Differs from 7543
* = significant difference at 0.05 level; ( ) = ± S.D.

Overall, significant increases in daphnid productivity were observed with increasing water hardness. Total young and average number of newborn observed on the first day of reproduction for daphnids reared in waters of 225 and 350 mg/L hardness were significantly greater ($P < 0.05$) than for the species cultured at the lower hardnesses. Mean number of generations also increased in the harder culture waters. In addition, mean times to sexual maturity were progressively shorter for *Daphnia* reared in waters of increasing hardness ($P < 0.05$). Approximately 65% more total young were produced in the highest test hardness water (350 mg/L) than were observed in waters of the lowest hardness (50 mg/L). This was reflected by the production of approximately 50% more young on the first day of reproduction by adults reared in the harder water. In addition, six more generations of daphnids were produced and the time to sexual maturity was reduced about one day for daphnids reared in the harder water. The only parameter not affected by hardness was adult survival which was nearly 100% in all cases.

The daphnids used in the hardness test were obtained from a culture unit where the hardness of the water averaged over 300 mg/L as $CaCO_3$. Therefore, the results observed in this study at the higher hardness may reflect adaptation by the daphnids to hard water. Other strains of *D. magna grown* and adapted to lower hardnesses may not react in the same way.

III. CONCLUSIONS:

High water hardness (225 or 350 mg $CaCO_3$/L) increases significantly the reproduction performance of *Daphnia magna*. The only parameter not affected by water hardness is survival, with around 100% in all hardness conditions (50 to 350 mg $CaCO_3$/L).

Assessment and conclusion by applicant:

The publication provides supporting information to evaluate the validity of the study by Young and Abedi 2003 (M-240807-01-1) see above CA 8.2.4.1.01. It demonstrates that Daphnia survival is not affected by low water hardness in long-term studies, therefore no impact is expected in an acute study.

CA 8.2.4.2 Acute toxicity to an additional aquatic invertebrate species

Data Point:	KCA 8.2.4.2/01
Report Author:	[redacted]
Report Year:	2003
Report Title:	AE C638206 - Acute toxicity to Eastern oysters (Crassostrea virginica) under flow-through conditions
Report No:	C038657
Document No:	M-225445-01-1
Guideline(s) followed in study:	USEPA (=EPA): FIFRA 72-3 (1989), OPPTS 850.1025 draft (1996)
Deviations from current test guideline:	Method: Deviations from current guideline SANCO/3029/99 rev.4: Limited sets of validation recoveries were analysed. However, the average recoveries were within the acceptable range of 70–110% and the RSD values were below 20%. The analytical method can be regarded as fit for purpose. Study: Current Guideline: OCSPP 850.1025 (1996) No deviations
Previous evaluation:	yes, evaluated and accepted in DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Data Point:	KCA 8.2.4.2/03
Report Author:	[redacted]
Report Year:	2018
Report Title:	Statement - Certificate of analysis for fluopicolide acute toxicity study on Eastern oysters (Dionne, 2003; M-225445-01-1)
Report No:	M-634698-01-1
Document No:	M-634698-01-1
Guideline(s) followed in study:	--
Deviations from current test guideline:	not applicable
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	Yes

Executive summary

An acute toxicity test was performed with eastern oyster (*Crassostrea virginica)* under flow-through conditions. The oysters (similar age), were exposed to nominal concentrations of 0.39, 0.65, 1.1, 1.8 and 3.0 mg a.s./L. of the test substance fluopicolide in unfiltered natural seawater (salinity of 32‰ - 33‰ and temperature of 20°C - 21°C.) for a 96-hour period. Negative control (natural filtered sea water) and a solvent control (0.100 mL/L dimethylformamide) were included. All treatments include 20 organisms per test vessel. Biological observations were made at test initiation and at each subsequent 24-hour interval until termination of the test. All samples were analysed for fluopicolide using gas chromatography with electron capture detection (GC/ECD) at test initiation and after 96 hours. Analysis of the test preparations at 0 and 96 hours were between 82% and 100% of the nominal concentrations. Results reported are based on mean measured concentrations of the test substance. The mean measured concentrations were: 0.33, 0.64, 1.0, 1.6 and 2.6 mg a.s./L. The study fulfils all validity criteria of FIFRA Guideline 72-3. No sublethal effects were observed among any of the exposed oysters throughout the test concentration range. A significant difference was detected between the dilution water control and the solvent control data. Therefore, treatment data was compared to solvent control data. At test termination, one dead oyster was observed in the 0.64 mg a.s./L treatment level. No mortality was observed among oysters at any of the remaining treatment levels tested or the controls. The EC_{50} at 96 hours for shell growth of eastern oysters (*Crassostrea virginica*) has been investigated and gives a value of > 2.6 mg a.s./L. The No Observed Effect Concentration is 2.6 mg a.s./L.

I. MATERIAL AND METHODS:

Test material	Fluopicolide Lot No: 2050190//PP241024/2 Purity 97.7 % w/w
Guideline(s) adaptation	None specified
Test species	Eastern oyster (*Crassostrea virginica*)
Acclimation	10 days prior testing. During this period the salinity was progressively increased from 15 to 32‰. No mortality during the 7 days before test initiation
Organism age/size at study initiation	The oysters were of similar age and had a mean valve height of 36 ± 4.6 mm (N = 30).
Test solutions	Nominal concentrations: 0.39 - 0.65 - 1.1 - 1.8 and 3.0 mg a.s./L Mean measured concentrations: 0.33 - 0.64 - 1.0 - 1.6 and 2.6 mg a.s./L Controls: natural unfiltered sea water Solvent control: 0.100 mL/L dimethylformamide
Replication	No. of vessels per concentration (replicates): 2 No. of vessels per control (replicates): 2
Organisms per replicate	No. of organisms per vessel: 20
Exposure	Flow through (flow rate of the recirculating test solution was 1.75 L/minute or about 5.25 L per oyster per hour) Total exposure duration: 96 hours
Feeding during test	Concentrated volumes of algae (*Tetraselmus maculate)* suspension (approximately 10^7 cells/mL) were added to each test aquarium 3 times daily

Test conditions	Temperature: 20 °C – 21 °C Photoperiod: 16 hours light / 8 hours dark with gradual intensity changes at dawn and dusk pH: 7.5 – 8.1. Dissolved oxygen: 4.5 mg/L (60% saturation)– 7.5 mg/L (aeration was initiated at the 72-hour observation interval to raise and maintain dissolved oxygen levels at ≥ 60% of air saturation) Salinity: 32 ‰ – 33 ‰
Parameters Measured / Observations	Biological observations (e.g., visible abnormalities, such as excessive mucous production or a failure to siphon and feed, as evidenced by a lack of fecal and pseudofecal production) and observations of the physical characteristics of the test solutions were made at test initiation and at each subsequent 24-hour interval until termination of the test. Sublethal effects were determined by a comparison of the performance and appearance of the exposed oysters to that of the control oysters. After 96 hours new shell growth was measured microscopically to the nearest 0.1 mm. Immobilization or adverse reactions to exposure were recorded at 24 and 48 hours after the start of exposure the pH, temperature, salinity, and dissolved oxygen concentration were measured daily in each replicate aquarium. In addition, temperature was monitored continuously in one replicate of the highest concentration.
Chemical analysis	All samples were analysed for fluopicolide using gas chromatography with electron capture detection (GC/ECD) at test initiation and after 96 hours
Data analysis	During the exposure of eastern oysters to fluopicolide, no concentration tested caused > 50% reduction; therefore, the EC_{50} value was empirically estimated to be greater than the highest test concentration. The No-Observed-Effect Concentration (NOEC) for the 96-hour exposure period was statistically determined by using Williams' Test

II. Results and Discussion:

Validity criteria (OCSPP 850.1025, 1996)	**Required**	**Obtained**
Mortality and sublethal effects in control during test	≤ 10%	0 %
New shell growth in control oysters	≥ 2mm	2.2 mm (0.6 mm, SD)

Analytical results:
Analysis of the test preparations at 0 and 96 hours (see table below) were between 82 % and 100 % of the nominal concentrations. Results reported are based mean measured concentrations of the test substance.

Nominal Concentration (mg a.s./L)	**Mean measured Concentration (mg a.s./L)**	**% Nominal**	**Day 0 Measured Concentration (mg a.s./L)**	**Day 4 Measured Concentration (mg a.s./L)**
Control	< 0.028	-	< 0.028	< 0.028
Solvent control	< 0.028	-	< 0.028	< 0.028
0.39	0.33	85 %	0.34	0.32
0.65	0.64	98 %	0.62	0.66
1.1	1.0	91 %	1.1	0.94
1.8	1.6	88 %	1.7	1.5
3.0	2.6	86 %	2.7	2.5

Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4 with minor acceptable exceptions only.

Biological results:

Observations
No sublethal effects were observed among any of the exposed oysters throughout the test concentration range. The table below presents the mean shell growth of oysters at each treatment level. A significant difference was detected between the dilution water control and the solvent control data; therefore, treatment data was compared to solvent control data.

Nominal conc. (mg a.s./L)	**Mean Shell deposition at 96h , mm (SD)**	**Mean reduction, %**
Control	2.2 (0.6)	NA
Solvent Control	2.8 (0.8)	NA
0.33	2.2 (0.6)	21
0.64	2.8 (1.0)	0
1.0	2.8 (1.1)	0
1.6	2.4 (1.1)	14
2.6	1.7 (0.6)	39

NA – not applicable
Immobility

At test termination, one dead oyster was observed in the 0.64 mg a.s./L treatment level. No mortality was observed among oysters at any of the remaining treatment levels tested or the controls.

III. Conclusions:

The study meets the validity criteria and the endpoints based on mean measured concentrations are:

EC_{50} 96 hours (95% C.I.):	**> 2.6 mg a.s. /L (not applicable)**
NOEC: highest concentration without adverse effects	2.6 mg a.s. /L

Assessment and conclusion by applicant:

The study is reliable and the relevant endpoint for risk assessment is the EC_{50} > 2.6 mg a.s./L.

Data Point:	KCA 8.2.4.2/02
Report Author:	[redacted]
Report Year:	2003
Report Title:	AE C638206 - acute toxicity to mysids (Americamysis bahia) under static conditions
Report No:	M-220513-01-2
Document No:	M-220513-01-2
Guideline(s) followed in study:	USEPA (=EPA): FIFRA 72-3 (1982)
Deviations from current test guideline:	Method: Deviations from current guideline SANCO/3029/99 rev.4: Limited sets of validation recoveries were analysed. However, the average recoveries were within the acceptable range of 70–110% and the RSD values were below 20%. The analytical method can be regarded as fit for purpose. Study: Current Guideline: OCSPP 850.1035 (1996) The oxygen saturation has to be between 60 and 100% during the whole test. The saturation during the test dropped below 60% (4.1 mg/L for 25° and 32‰ salinity) at 96h in all test vessels. The lowest saturation was around 44 %. The saturation in the highest test concentration was significantly lower than in the other groups at 72h. The recommended salinity should be 20‰ but it was 32‰ in the test. The maximum solvent concentration should be 0.1 mL/L but it was 0.25 mL/L during the test. The pH was slightly out of the recommended range 7.5-8.5, it was 7.4 in one replicate of 1 concentration at 96h. This slight deviation is not considered relevant. The validity criterion of the guideline is met but influence of the poor oxygenation on the test results cannot be ruled out as the saturation decreased with increasing concentrations. Consequently, the toxicity of fluopicolide may be over-estimated.
Previous evaluation:	yes, evaluated and accepted in DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive summary

An acute toxicity test was performed with mysids (*Americamysis bahia*) in a static system. The mysids (less than 24 hours old) were exposed to nominal concentrations of 0.65, 1.1, 1.8, 3.0 and 5.0 mg a.s./L of the test substance fluopicolide in filtered natural seawater (salinity of 32‰ and temperature of 25°C – 26°) for a 96-hour period. Negative controls with natural filtered sea water and a solvent control with 0.250 ml/L dimethylformamide were tested. All treatments include 10 organisms per test vessel. Biological observations were made at test initiation and at each subsequent 24-hour interval until termination of the test. All samples were analysed for fluopicolide using gas chromatography with electron capture detection (GC/ECD) at test initiation and after 96 hours. Analysis of the test preparations at 0 and 96 hours were between 82% and 91% of the nominal concentrations. Results reported are based on mean measured concentrations of the test substance. The mean measured concentrations were: 0.57, 0.96, 1.6, 2.7 and 4.2 mg a.s./L. The study fulfils all validity criteria of the U.S. EPA's Pesticide Assessment Guidelines (Subdivision E, Series 72-3; U.S. EPA, 1982). All surviving mysids exposed to the 2.7 and 4.2 mg a.s./L treatment levels were lethargic. The LC_{50} 96 hours (95% C.I.) of the test material to mysids *(Americamysis bahia)* has been investigated and gives a value of 3.2 mg a.s./L (2.7 - 4.2 mg a.s./L). The No Observed Effect Concentration is 1.6 mg a.s./L.

I. MATERIAL AND METHODS:

Test material	Fluopicolide Lot No 2050190//PP241024/2 Purity 97.7 % w/w
Guideline(s) adaptation	None specified
Test species	Mysid (*Americamysis bahia*)
Organism age/size at study initiation	The mysids were < 24 hours old.
Test solutions	Nominal concentrations: 0.65, 1.1, 1.8, 3.0 and 5.0 mg a.s./L Mean measured concentrations: 0.57, 0.96, 1.6, 2.7 and 4.2 mg a.s./L Controls: natural filtered sea water Solvent control: 0.250 mL/L dimethylformamide
Replication	No. of vessels per concentration (replicates): 2 No. of vessels per control (replicates): 2
Organisms per replicate	No. of organisms per vessel: 10
Exposure	Static Total exposure duration: 96 hours
Feeding during test	Live brine shrimp nauplii (*Artemia salina*) were added to each test vessel once daily
Test conditions	Temperature: 25 °C – 26 °C Photoperiod: 16 hours light / 8 hours dark with gradual intensity changes at dawn and dusk at 76 to 94 footcandles pH: 7.4 – 8.0 Dissolved oxygen: 3.0 mg/L – 6.7 mg/L (40% saturation corresponds to 2.7 mg/L) Salinity: 32 ‰. Recommended salinity in the guideline is 20‰.
Parameters Measured / Observations	Biological observations were made at test initiation and at each subsequent 24-hour interval until termination of the test. pH, temperature, salinity, and dissolved oxygen concentration were measured daily in each test vessel. In addition, temperature was monitored continuously in one replicate of the highest concentration.
Chemical analysis	All samples were analysed for fluopicolide using gas chromatography with electron capture detection (GC-ECD) at test initiation and after 96 hours
Data analysis	The LC_{50} was calculated by a computer program (Stephan, 1982) by comparison of the three statistical methods: moving average angle analysis, probit analysis, and nonlinear interpolation with 95% confidence limits calculated by binomial probability. The 96-hour LC_{50} value was estimated by non-linear interpolation, with corresponding 95% confidence intervals calculated by binomial probability. The No-Observed-Effect Concentration (NOEC) during the 96-hour exposure period was also determined but the method is not reported.

II. RESULTS AND DISCUSSION:

Validity criteria (OCSPP 850.1035, 1996)	Required	Obtained
Mortality and sublethal effects in control(s) during test	≤ 10%	0 % in control, 5 % in solvent control

Analytical results:

Analysis of the test preparations at 0 and 96 hours (see table below) were between 82 % and 91 % of the nominal concentrations. Results reported are based on mean measured concentrations of the test substance.

Nominal Concentration (mg a.s./L)	Day 0 Measured Concentration (mg a.s./L)	Day 4 Measured Concentration (mg a.s./L)	Mean measured Concentration (mg a.s./L)	% of Nominal
Control	< 0.046	< 0.045	-	-
Solvent control	< 0.046	< 0.045	-	-
0.65	0.55	0.58	0.57	87
1.1	0.93	0.99	0.96	87
1.8	1.6	1.6	1.6	91
3.0	2.7	2.7	2.7	90
5.0	4.2	4.1	4.2	84

Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4 with minor acceptable exceptions only.

Biological results:

Observations
All surviving mysids exposed to the 2.7 and 4.2 mg a.s./L treatment levels were lethargic.

Immobility

Exposure time (hours)	24	48	72	96
Arithmetic mean measured conc. (mg a.s./L)	Cumulative Mortality (%)	Cumulative Mortality (%)	Cumulative Mortality (%)	Cumulative Mortality (%)
Control	0	0	0	0
Solvent Control	0	0	0	5
0.57	0	0	0	0
0.96	0	0	0	5
1.6	0	0	0	0
2.7	0	0	0	15
4.2	0	35	45	95

III. CONCLUSIONS:

The study meets the validity criteria and the endpoints based on mean measured concentrations are:

LC_{50} 96 hours (95% C.I.):	**3.2 mg a.s. /L (2.7 – 4.2 mg a.s./L)**
NOEC: highest concentration without adverse effects	1.6 mg a.s. /L

Assessment and conclusion by applicant:

The study has deficiencies which may have resulted in toxicity over-estimation. The relevant endpoint for the fluopicolide risk assessment is the LC_{50} of 3.2 mg a.s./L.

CA 8.2.5 Long-term and chronic toxicity to aquatic invertebrates

CA 8.2.5.1 Reproductive and development toxicity to Daphnia magna

Data Point:	KCA 8.2.5.1/01
Report Author:	[redacted]
Report Year:	2003
Report Title:	Effects on the life-cycle of the water flea (Daphnia magna) in a static renewal system AE C638206 technical 97.7 percent w/w
Report No:	B004236
Document No:	M-241191-01-1
Guideline(s) followed in study:	OECD 211 (1998); SSEPA (=EPA) 72-4 (1986)
Deviations from current test guideline:	Method: Deviations from current guideline SANCO/3029/99 rev.4: Limited sets of validation recoveries were analysed. However, the average recoveries were within the acceptable range of 70-110% and the RSD values were below 20%. The analytical method can be regarded as fit for purpose with regard to this toxicity study. Study: Current Guideline: OECD 211 (2012) No deviations
Previous evaluation:	yes, evaluated and accepted in DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Data Point:	KCA 8.2.5.1/02
Report Author:	[redacted]
Report Year:	2018
Report Title:	Statistical re-evaluation of fluopicolide daphnia reproduction study (Young & Abedi, 2003; M-241191-01-1)
Report No:	M-617757-01-1
Document No:	M-617757-01-1
Guideline(s) followed in study:	none
Deviations from current test guideline:	Not applicable
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	Yes

Executive summary

A chronic toxicity of fluopicolide (tech.) test was performed with water flea (*Daphnia magna*) in a semi-static system. First-instar neonates daphnids (less than 24 hours old) were exposed to nominal concentrations of 0.028, 0.056, 0.11, 0.23, 0.45 and 0.90 mg/L fluopicolide in well water (mean temperature of 20.4°C) for 21 days. A water control was included. All treatments consisted of ten individual replicates with a single daphnid per test chamber and three group replicates with five daphnids per test chamber. Observations for death, immobility, and abnormal appearance or behaviour were performed daily on each replicate chamber (both individual and group). Starting on day 6, all individual daphnids were checked daily for initial neonate production. Samples of the freshly prepared test solutions from each treatment were taken at test initiation and each week on Friday. Samples from old test solutions from each treatment were taken at study termination and each week on Monday. Fluopicolide and the metabolite M-01 (BAM) were measured by Gas Chromatography with Electron Capture Detector (GC/ECD). No residues of fluopicolide were measured in the control above the limit of quantification (LOQ= 0.025 mg a.s./L). There were no M-01 (BAM) residues found in any test solution greater than the limit of quantification (0.025 mg a.s./L). The arithmetic mean measured concentrations are 0.024, 0.047, 0.096, 0.19, 0.37 and 0.74 mg a.s./L. The statistical analysis of the results presented in the report does not fulfil current standards and leads to inconclusive results for the most sensitive endpoint: number of off-spring per adult. Therefore, a new analysis of the results has been performed according to the OECD guideline 211 (2012). No sub-lethal effects were observed in any of the treatments during the study. Survival of adult daphnids was not significantly reduced at any of the treatment concentrations when compared to the control group. There were no immobile or dead neonates observed in any treatment level. Total living neonates per adult were significantly reduced at the 0.048, 0.37, and 0.74 mg/L mean measured concentration when compared to the control group. In a static renewal exposure for 21 days, fluopicolide has chronic effects on the reproduction of the water flea *D. magna* at a mean measured concentration of 0.74 mg/L. No effects are observed at a mean measured concentration of 0.37 mg a.s./L. However, the overall NOEC is 0.19 mg a.s./L on the basis of off-spring production.

I. MATERIAL AND METHODS:

Test material:	Fluopicolide (tech.) Lot/batch: 2050190//PP241024/2 Code No: AE C638206 00 1C99 0005 Purity 97.7% w/w
Guideline(s) adaptation	None specified
Test species:	Water flea (*Daphnia magna)*
Organism Age at Experimental Start:	1st instar neonates less than 24 h old
Test solutions:	Nominal concentrations: 0.028 – 0.056 – 0.11 – 0.23 – 0.45 – 0.90 mg a.s./L Arithmetic mean measured concentrations: 0.024 – 0.048 – 0.096 – 0.19 – 0.37 – 0.74 mg a.s./L Controls: water control Evidence of undissolved material: not reported
Replication:	No. of vessels per concentration (replicates): 10 individual replicates and 3 group replicates No. of vessels per control (replicates): 10 individual replicates and 3 group replicates
Organisms per replicate:	No. of organisms per vessel: 1 for the individual replicates and 5 for the group replicates
Exposure:	Semi-static test, renewal every Monday, Wednesday, Friday Total exposure duration: 21 days
Test Vessel Loading:	80 mL of test solution / daphnid
Feeding during test:	Green algae (*Pseudokirchneriella subcapitata*) daily: 2.0×10^7 cells/daphnid/day or 0.23 mg C/daphnid/day and fish food suspension at each medium renewal at 5.0 mg dry solids/mL or 0.22 mg C/daphnid/day
Test conditions:	Temperature: 19.8 to 20.8°C in fresh medium and 19.4 to 20.6°C in aged medium Photoperiod: 16:8 light:dark with gradual intensity changes at dawn and dusk Light intensity: 630-700 lux pH: 7.4 to 7.8 in fresh medium and 8.1 to 8.6 in aged medium Water hardness: 170 to 180 mg/L as $CaCO_3$ Dissolved oxygen % saturation: 66% (5.4 mg/L) to 94% in fresh medium and 75 to 120% in aged medium Conductivity: 800 to 1000 µS/cm
Parameters Measured / Observations	Discrete measurements of temperature, dissolved oxygen, pH, and specific conductivity were obtained at each renewal period. Water quality parameters were measured on the newly prepared parent stock solutions and on one replicate of each old test solution, alternating the replicate each time. Observations for death, immobility, and abnormal appearance or behavior were performed daily on each replicate chamber (both individual and group). Starting on Day 6, all individual daphnids were checked daily for initial neonate production. The time to first brood was recorded for each replicate. Following the onset of neonate production for the individual daphnids, all living, dead, or immobile neonates were counted and removed from test chambers each day. At termination of the study, surviving adult daphnids were measured from the apex of the helmet to the base of the spine using a dissecting microscope and ocular micrometer. Adult daphnids for each replicate were then dried and weighed.

Sampling for chemical analysis	Samples of the freshly prepared test solutions from each treatment were taken at test initiation and each week on Friday. Samples from old test solutions from each treatment were taken at study termination and each week on Monday. Fluopicolide and the metabolite BAM were measured by Gas Chromatography with Electron Capture Detector (GC/ECD).
Data analysis:	All statistical analyses were performed using TOXSTAT® (Version 3.4). Dichotomous data were analysed by 2 × 2 contingency tables and Fisher's Exact Test. All other continuous data were initially subjected to a Chi-Square Test to assess departures from normality and a Bartlett's Test to determine homogeneity of variance. To assess treatment effects, a one-way analysis of variance (ANOVA) was used with a Bonferroni t-Test. If data was not normally distributed or had heterogeneity of variance, then a non-parametric statistical procedure was used (i.e. Wilcoxon's Rank Sum).

II. RESULTS AND DISCUSSION:

Validity criteria (OECD 211, 2012)	Required	Obtained
Mortality of the parent animals in control at the end of the test	≤ 20%	8%
Mean number of living off-spring produced per parent animal surviving in control at the end of the test	≥ 60	236

Analytical results:
No residues of fluopicolide were measured in the control above the limit of quantification (0.025 mg/L). There were no M-01 (BAM) residues found in any test solution greater than the limit of quantification (0.025 mg/L).

Few recoveries were observed to be below 80% but the substance was proved to be stable over the renewal interval (variation between new and old medium less than 20%), therefore according to EFSA the technical report (2015), arithmetic means can be used to express the results. The geometric mean measured concentrations are 0.024, 0.047, 0.096, 0.19, 0.37 and 0.74 mg/L, they are not significantly different from the arithmetic mean measured concentrations used to express the results (see table below).

Nominal conc. (mg a.s./L)	Arithmetic mean (mg a.s./L)	% of nominal concentrations						
		Day 0 New	Day 3 Aged	Day 7 New	Day 10 Aged	Day 14 New	Day 17 Aged	Day 21 Aged
0.028	0.024	79	86	92	90	85	85	88
0.056	0.048	76	80	85	95	85	87	92
0.11	0.096	86	81	91	93	85	90	86
0.23	0.19	76	80	86	89	77	86	86
0.45	0.37	79	78	89	92	78	79	80
0.90	0.74	81	79	85	92	77	81	84

Geometric mean concentrations according to OECD formula can be calculated since few measurements were below 80%, however, the measurements at 21 d have to be ignored because the concentration in the corresponding fresh medium are unknown.

The geometric concentrations are: 0.024, 0.047, 0.096, 0.189 and 0.370-0.740 mg a.s./L. They are not significantly different from the arithmetic mean concentrations.

Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4 with minor acceptable exceptions only.

Biological results:

Observations
No sub-lethal effects were observed in any of the treatments during the study.

Growth
There were no significant differences (P > 0.05) from the control group by Wilcoxon's Rank Sum Test on dry weight or length.

Arithmetic mean measured concentrations (mg a.s./L)	Mean dry weight (mg) ± SD	Mean length (mm) ± SD
Control	0.90 ± 0.08	5.0 ± 0.10
0.024	0.87 ± 0.14	4.8 ± 0.19
0.048	0.96 ± 0.15	4.9 ± 0.21
0.096	0.78 ± 0.21	4.8 ± 0.40
0.19	0.87 ± 0.09	4.9 ± 0.23
0.37	0.87 ± 0.11	5.0 ± 0.11
0.74	0.99 ± 0.15	4.9 ± 0.12

Adult survival

Survival of adult daphnids was not significantly reduced at any of the treatment concentrations when compared to the control group.

Arithmetic mean measured concentrations (mg a.s./L)	% survival at day 21
Control	92
0.024	92
0.048	100
0.096	96
0.19	96
0.37	96
0.74	96

Reproduction data

There were no immobile or dead neonates observed in any treatment level. Total living neonates per adult were significantly reduced at the 0.048, 0.37 and 0.74 mg/L mean measured concentration when compared to the control group. The statistically significant difference at 0.048 and 0.37 were not considered biologically significant since there were no effects at the 0.096, and 0.19 mean measured concentrations.

Arithmetic mean measured concentrations (mg a.s./L)	Mean number of living neonates per adult[1]	Day of 1st brood (mean)[1]
Control	236	8.5
0.024	214	8.7
0.048	207#	8.5
0.096	197	8.6
0.19	212	8.8
0.37	210#	8.7
0.74	184*	9

Significant difference ($P < 0.05$) from the control group by Wilcoxon's Rank Sum Test, however, not considered biologically significant.
* Significant difference ($P < 0.05$) from the control group by Wilcoxon's Rank Sum Test.
[1] SD not reported

The statistical analysis of the results presented in the report and in the table above does not fulfil current standards and leads to inconclusive results for the most sensitive endpoint: number of off-spring per adult.
Therefore, a new analysis of the results has been performed according to the OECD guideline 211 (2012), i.e. determination of effects on the total number of living offspring per introduced parent, and on time to 1st brood, in addition to the parameters already analysed in the report: total number of living offspring per surviving parent, immobility at 21 days (called parent survival), dry weight and length. Moreover, new endpoints such as EC_{10} and EC_{20} were determined when mathematically possible. All calculations were performed with ToxRat 3.2.1.

III. Conclusions:

The study meets the validity criteria and the endpoints based on arithmetic mean concentrations are:

Endpoint (mg/L)	From the new analysis	From the report
Immobility at 21 d		
NOEC	0.74	0.74
EC_{10}	Cannot be calculated: less than 10% of effects at the highest concentration	Not available
EC_{20}		Not available
Number of off-spring per introduced parent		
NOEC	**0.19**	Not available
EC_{10}	Cannot be calculated: no dose response relationship	Not available
EC_{20}		Not available
Number of off-spring per surviving parent		
NOEC	**0.19**	0.37
EC_{10}	Cannot be calculated: no dose response relationship	Not available
EC_{20}		Not available
Time to 1st brood		
NOEC	0.37	Not available
EC_{10}	Cannot be calculated for mathematical reasons	Not available
EC_{20}		Not available

Endpoint (mg/L)	From the new analysis	From the report
Dry weight		
NOEC	0.74	0.74
EC_{10}	Cannot be calculated: less than 10% of effects at the highest concentration	Not available
EC_{20}		Not available
Length		
NOEC	See comment below	0.74
EC_{10}	Cannot be calculated: less than 10% of effects at the highest concentration	Not available
EC_{20}		Not available

The statistical analysis concludes that statistically significant effects on length are observed at the lowest (0.024 mg/L) and at the highest concentration (0.74 mg/L). The levels of effects at these 2 concentrations were 3.1 and 2.9% inhibition, respectively. However at 0.096 mg/L, the inhibition was higher (3.6%) despite no statistical significance. The inhibition on length ranged from 0.1% at 0.37 mg/L to 3.6% at 0.096 mg/L with no dose response relationship. Since no significant effects on dry weight was determined and since the inhibition on length is very low (less than 4%) and not concentration dependent, these statistical effects are not considered to be biologically relevant.

The overall NOEC is 0.19 mg/L on the basis of off-spring production.

Assessment and conclusion by applicant:

The study is reliable and the relevant endpoint for the fluopicolide risk assessment is the NOEC of 0.19 mg a.s./L.

CA 8.2.5.2 Reproductive and development toxicity to an additional aquatic invertebrate species

Data Point:	KCA 8.2.5.2/01
Report Author:	[redacted]
Report Year:	2019
Report Title:	Fluopicolide - Life-cycle toxicity test with mysids (Americamysis bahia)
Report No:	12709.6377
Document No:	M-544290-02-1
Guideline(s) followed in study:	OCSPP Draft Guideline 850.1350 U.S. Environmental Protection Agency's OCSPP 850.1350 (Draft, U.S. EPA, 1996) and the Standard Guide for Conducting Life-Cycle Toxicity Tests with Saltwater Mysids (ASTM, 2008)
Deviations from current test guideline:	Method: No deviation Study: Current Guidelines: OCSPP 850.1350 (1996) and ASTM 1191-03 (2008) The study was performed according to both OCSPP 850.1350 and ASTM E 1191-03 guidelines. The validity criteria of ASTM guidelines were applied to this study. The photoperiod was selected according to ASTM guideline: 16h light whereas OCSPP recommends 14h. The temperature for the test was selected according to OCSPP guidelines: 25-26°C whereas ASTM guideline recommends 27°C. These conditions were selected to be consistent with the culturing conditions.
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive summary

The objective of this study was to determine the chronic (full life-cycle) toxicity to the mysid *Americamysis bahia* exposed to fluopicolide technical under flow-through conditions. Two generations of saltwater mysids F0 juveniles (starting < 24 hours post release continued to sexual maturity) and F1 (mysids were collected during reproductive phase) were continuously exposed to the test substance in diluted, filtered natural seawater. The F1 groups were evaluated for 96 hours for observations of mortality. After 28 days, the F0 generation was terminated and the results of the exposure were evaluated for potential chronic effects. All treatments (4 replicates/each concentration and control) include 20 organisms per aquaria. For the definitive 28-day chronic toxicity test nominal fluopicolide concentrations of 0.08 8, 0.18, 0.35, 0.70, 1.4 and 2.8 mg a.s./L were selected. A negative control was included. All exposure solutions were analysed for fluopicolide by gas chromatography with micron-electron capture detection (GC/μECD). Recoveries were between 76 and 111%. Therefore, results were based on arithmetic mean measured concentrations: 0.076, 0.16, 0.34, 0.66, 1.2 and 2.3 mg a.s./L. No residues of fluopicolide were found in the control samples above the maximum detectable limit (MDL). The study fulfils all validity criteria of the ASTM E 1191-03 guideline. Based on mean measured concentrations of fluopicolide and F0 mean number of offspring per female (the most sensitive indicator of toxicity), the No-Observed-Effect Concentration (NOEC) is determined to be 0.34 mg a.s./L. The Lowest-Observed-Effect Concentration (LOEC) for mysids was determined to be 0.66 mg a.s./L. The EC_{10} is 0.18 mg a.s./L.

I. MATERIAL AND METHODS:

Test material	Fluopicolide technical Batch No.: ETFP000273 Purity 100.5% w/w
Guideline(s) adaptation	None specified
Test species	Saltwater mysid (*Americamysis bahia*) from in-house culture
Organism age/size at study initiation	Juvenile mysids, approximately 24 hours old
Test solutions	Nominal concentrations: 0.088, 0.18, 0.35, 0.70, 1.4 and 2.8 mg a.s./L Arithmetic mean measured concentrations: 0.076, 0.16, 0.34, 0.66, 1.2 and 2.3 mg a.s./L Controls: Dilute, filtered, natural seawater Evidence of undissolved material: Not reported
Replication	No. of aquaria per concentration (replicates): 4 No. of aquaria per control (replicates): 4
Organisms per replicate	No. of organisms per aquaria: 20 Immature mysids were held in 1 retention chamber per aquarium until sexual maturity. Then they were held in pairing chambers (max 5 per aquarium). During the reproductive phase, groups of up to 10 offspring per replicate, 40 per treatment were collected and evaluated. Offspring were removed from adult mysid chambers in each replicate vessel and placed in separate pairing chambers within that replicate. One F1 group was established and monitored for each replicate vessel.
Exposure	Flow-through: 7.7 aquarium volume addition per day Total exposure duration: 28 days
Loading rate	Maximum biomass loading did not exceed 0.0026 g/L flowing solution per day or 0.02 g/L of solution at any time, in any replicate exposure aquarium
Feeding during test	Live brine shrimp nauplii (*Artemia* sp.), twice daily. At least one of these feedings was with brine shrimp nauplii enriched with Selco®, a substance high in saturated fatty acids
Test conditions	Temperature: 25 – 27°C Photoperiod: 16 hours light, 8 hours dark with a 15- to 30-min transition period Light intensity: 230 to 360 lux pH: 7.4 – 8.2 Dissolved oxygen: 4.50 to 6.89 mg/L (62 to 94% saturation range) Salinity: 19 - 21 ‰

Parameters Measured/ Observations	In order to observe the mysids during the exposure period, the numbers of dead and living organisms were counted and any abnormal appearance or behavior was recorded. Survival of the test organisms was estimated for the first 12 days of the test, i.e. prior to pairing of the mysids. After males and females had been paired (day 13) definitive counts of survival were made and the number of dead males and females, the number of off-spring produced by each individual female and any abnormal appearance or behavior was recorded. Observations were daily throughout the study. At the time an F1 generation pairing chamber was established and daily thereafter for 96 hours, observations of stress, abnormal behavior (including discoloration, immobilization and inability to maintain position in the water column) and survival were made. Body length and dry weight were determined at the end of the test. Temperature, dissolved oxygen concentration, pH and salinity were measured in each replicate on day 0 and alternated between replicates daily thereafter throughout the exposure period for each treatment level and the control.
Chemical analysis	Prior to the start of the definitive exposure, samples were removed from one replicate of each treatment level and the control and analysed for fluopicolide concentration. In addition, a sample of effluent from each of the saturator columns was also analysed during the pretest period. During the in-life phase, samples were removed from alternating replicate solutions of each treatment level and the control on days 0, 7, 14, 21 and 28 for analysis of fluopicolide concentration. In addition, one sample of each saturator column's effluent was analysed at each sampling interval during the exposure period. All exposure solutions were analysed for fluopicolide by gas chromatography with micron-electron capture detection (GC/µECD).
Data analysis	Data for the survival endpoints (e.g., 28-day survival, male and female survival and F_1 survival) were analysed using Fisher's Exact Test with Bonferroni-Holm's Adjustment. For the other parameters, the Dunnett's Multiple Comparison Test was conducted. CETIS™ was used to perform the statistical computations. EC_x values were determined by non-linear regression. Calculations were possible only for the number of off-spring per female because of the lack of dose response relationship for the other parameters. The initial report has been amended to exclude 3 pairing chambers from the statistical analysis of reproduction data. These chambers were excluded because they either contained 2 males or 2 females and were therefore not capable of producing offspring.

II. RESULTS AND DISCUSSION:

Validity criteria (ASTM 1191-03 Guideline, 2008)	Required	Obtained
F_0 survival	≥ 70%	84
Reproductively active females	≥ 75%	100
Reproductive performance (offspring per female)	≥ 8	21.5

Analytical results:

Recoveries were between 76 and 111% (see table below). Therefore, results were based on arithmetic mean measured concentrations. No residues of fluopicolide were found in the control samples above the MDL (minimum detectable limit = 0.012 mg a.s./L).

Nominal Concentration (mg/L)	Arithmetic mean measured concentration (mg/L)	% of nominal concentrations	Range of individual measurements (% of nominal) *
0.088	0.076	87	76 - 100
0.18	0.16	91	78 - 111
0.35	0.34	98	91 - 106
0.70	0.66	94	80 - 111
1.4	1.2	86	79 - 93
2.8	2.3	82	79 - 89

* Values were calculated on the basis of measured concentrations given in the study report (Table 3)

Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Biological results:

At test termination, mean survival of 84% was observed among male mysids in the control. Mean survival of 85, 69, 87, 72, 80 and 78% was observed among male mysids exposed to the 0.076, 0.16, 0.34, 0.66, 1.2 and 2.3 mg/L treatment levels, respectively. Fisher's Exact Test with Bonferroni-Holm's Adjustment determined no significant difference in male survival among organisms exposed to any of the treatment levels tested compared to the control (84%).
At test termination, mean survival of 94% was observed among female mysids in the control.
Mean survival of 89, 100, 97, 86, 89 and 89% was observed among female mysids exposed to the 0.076, 0.16, 0.34, 0.66, 1.2 and 2.3 mg/L treatment levels, respectively. Fisher's Exact Test with Bonferroni-Holm's Adjustment determined no significant difference in female survival among organisms exposed to any of the treatment levels tested compared to the control (94%).
Following 28 days of exposure, mean survival of 84% was observed among organisms in the control. Mean survival of 85, 77, 90, 80, 83 and 79% was observed among mysids exposed to the 0.076, 0.16, 0.34, 0.66, 1.2 and 2.3 mg/L treatment levels, respectively. Fisher's Exact Test with Bonferroni-Holm's Adjustment determined no significant difference in survival among organisms exposed to any of the treatment levels tested compared to the control (84%).
Following the 96-hour observation period, mean percent survival of 95% was observed among F1 mysids in the control. Mean percent survival of 100, 98, 95, 90, 85 and 73% was observed among F1 mysids exposed to the 0.076, 0.16, 0.34, 0.66, 1.2 and 2.3 mg/L treatment levels, respectively. Fisher's Exact Test with Bonferroni-Holm's Adjustment determined no significant difference in F1 mysid survival among organisms exposed to any of the treatment levels compared to the control (95%).

At test termination, the mean number of off-spring per female for organisms in the control was 21.5. The mean number of off-spring per female was 18.8, 16.4, 16.4, 12.9, 9.4 and 6.0 among mysids exposed to the 0.076, 0.16, 0.34, 0.66, 1.2 and 2.3 mg/L treatment levels, respectively.
Dunnett's Multiple Comparison Test determined a significant difference in the mean number of off-spring per female among organisms exposed to the 0.66, 1.2 and 2.3 mg/L treatment levels compared to the control (21.5).
The average total body length of male mysids in the control was 7.14 mm. The average total body length of male mysids exposed to the 0.076, 0.16, 0.34, 0.66, 1.2 and 2.3 mg/L treatment levels was 7.31, 7.64, 7.41, 7.04, 7.00 and 6.89 mm, respectively. Dunnett's Multiple Comparison Test determined no significant difference in the total body length of male mysids exposed to any of the treatment levels compared to the control (7.14 mm).
The average total body length of female mysids in the control was 7.41 mm. The average total body length of female mysids exposed to the 0.076, 0.16, 0.34, 0.66, 1.2 and 2.3 mg/L treatment levels was 7.67, 7.81, 7.46, 7.19, 7.24 and 7.00 mm, respectively. Dunnett's Multiple Comparison Test determined a significant difference in the total body length of female mysids exposed to the 2.3 mg/L treatment level compared to the control (7.41 mm).
The average dry body weight of male mysids in the control was 0.78 mg. The average dry body weight of male mysids exposed to the 0.076, 0.16, 0.34, 0.66, 1.2 and 2.3 mg/L treatment levels was 0.81, 0.90, 0.83, 0.80, 0.77 and 0.76 mg, respectively. Dunnett's Multiple Comparison determined no significant difference in the dry body weight of male mysids exposed to any of the treatment levels compared to the control (0.78 mg).
The average dry body weight of female mysids in the control was 1.10 mg. The average dry body weight of female mysids exposed to the 0.076, 0.16, 0.34, 0.66, 1.2 and 2.3 mg/L treatment levels was 1.08, 1.39, 1.11, 1.01, 1.00 and 0.96 mg, respectively. Dunnett's Multiple Comparison Test determined no significant difference in the dry body weight of female mysids exposed to any of the treatment levels compared to the control (1.10 mg).

Survival (F_0)

Arithmetic mean measured conc. (mg/L)	Mean male survival[a)] + (SD) [%]	Mean female survival[a)] + (SD) [%]	Mean post-pairing survival[b)] + (SD) [%]	Mean 28-day survival[b)] + (SD) [%]
Control	84 (16)	94 (7)	88 (11)	84(12)
0.076	85 (18)	89 (10)	87 (13)	85 (15)
0.16	69 (11)	100 (0)	83 (6)	77 (8)
0.34	87 (11)	97 (6)	94 (5)	90 (4)
0.66	72 (20)	86 (8)	81 (8)	80 (8)
1.2	80 (7)	89 (10)	87 (6)	83 (7)
2.3	78 (16)	89 (21)	83 (16)	79 (16)

[a] Calculations of male and female survival began after pairing.
[b)] Calculations of survival are of both male and female mysid combined.

Survival (F_1)

Arithmetic mean measured conc. (mg/L)	Survival + (SD) [%]
Control	95 (6)
0.076	100 (0)
0.16	98 (5)
0.34	95 (10)
0.66	90 (8)
1.2	85 (19)
2.3	73 (26)

Reproduction

Arithmetic mean measured conc. (mg/L)	Percent of Females Producing Young + (SD)	Mean Number of Young Per Surviving Female + (SD)
Control	100 (0)	21.5 (2.9)
0.076	100 (0)	18.8 (2.7)
0.16	100 (0)	16.4 (4.8)
0.34	95 (10)	16.4 (0.4)
0.66	95 (10)	12.9[a] (4.5)
1.2	90 (12)	9.4[a] (1.3)
2.3	90 (12)	6.0[a] (3.1)

[a] Significant difference compared to the control, based on Dunnett's Multiple Comparison Test

Growth parameters after 28 days

Arithmetic mean measured conc. (mg/L)	Mean total length ± SD (mm)		Mean dry weight ± SD (mg)	
	Males + (SD)	Females + (SD)	Males + (SD)	Females + (SD)
Control	7.14 (0.13)	7.41 (0.13)	0.78 (0.02)	1.10 (0.08)
0.076	7.31 (0.09)	7.67 (0.09)	0.81 (0.02)	1.08 (0.14)
0.16	7.64 (0.12)	7.81 (0.16)	0.90 (0.02)	1.39 (0.06)
0.34	7.41 (0.11)	7.46 (0.15)	0.83 (0.05)	1.11 (0.13)
0.66	7.04 (0.21)	7.19 (0.20)	0.80 (0.04)	1.01 (0.07)
1.2	7.00 (0.29)	7.24 (0.19)	0.77 (0.01)	1.00 (0.10)
2.3	6.89 (0.13)	7.00[a] (0.20)	0.76 (0.07)	0.96 (0.09)

[a] Significant difference compared to the control, based on Dunnett's Multiple Comparison Test

III. CONCLUSIONS:

The study meets the validity criteria. The most sensitive endpoint was mean number of off-spring per female. EC_x values could be calculated for this parameter only.
The results, based on arithmetic mean measured concentrations, are:

Parameter	NOEC (mg a.s./L)	LOEC (mg a.s./L)	EC_{10} (mg a.s./L)	EC_{20} (mg a.s./L)
F_0 male survival at test end	2.3	> 2.3	Cannot be calculated, lack of dose-response	
F_0 female survival at test end	2.3	> 2.3	Cannot be calculated, lack of dose-response	
F_0 survival at test end (male and female)	2.3	> 2.3	Cannot be calculated, lack of dose-response	
F_1 survival at 96h post-release	2.3	> 2.3	Cannot be calculated, lack of dose-response	
Nb of offspring per female	0.34	0.66	0.18	0.38
Body length of males	2.3	> 2.3	Cannot be calculated, lack of dose-response	
Body length of females	1.2	2.3	Cannot be calculated, lack of dose-response	
Dry weight of males	2.3	> 2.3	Cannot be calculated, lack of dose-response	
Dry weight of females	2.3	> 2.3	Cannot be calculated, lack of dose-response	

Assessment and conclusion by applicant:

The study is reliable and the endpoint relevant for the fluopicolide risk assessment is the EC_{10} of 0.18 mg a.s./L.

CA 8.2.5.3 Development and emergence in Chironomus riparius

No study is avaiable and a study is not required.

CA 8.2.5.4 Sediment dwelling organisms

Data Point:	KCA 8.2.5.4/01
Report Author:	[redacted]
Report Year:	2003
Report Title:	AE C638206: A prolonged sediment toxicity test with Chironomus riparius using spiked sediment
Report No:	M-223565-01-2
Document No:	M-223565-01-2
Guideline(s) followed in study:	OECD: 218 (2001)
Deviations from current test guideline:	Method: Deviations from current guideline SANCO/3029/99 rev.4: Recoveries were determined at three different concentrations in duplicate. However, the obtained data demonstrate very good recoveries and the precision. The method can therefore be regarded as fit for purpose. Study: Current Guideline: OECD 218 (2004) The study does not meet the validity criteria
Previous evaluation:	yes, evaluated and accepted in DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Supportive only

Executive summary

A prolonged sediment toxicity test was performed with midges (*Chironomus riparius)* using sediment spiked with the test substance fluopicolide. Groups of midges were exposed to a geometric series of six test concentrations, a negative (dilution water) control and a solvent control (1.0 mL acetone/kg) for approximately 28 days. Four replicate test chambers were maintained in each treatment and control group, with 20 midges in each chamber for a total of 80 individuals per test concentration. Nominal test concentrations were 0.50, 1.3, 3.1, 7.8, 20 and 49 mg fluopicolide/kg. The NOEC and LOEC were determined by visual interpretation of the dose-response pattern and statistical analyses of the mean development times, emergence rates and development rates. Overlying water, pore water and sediment samples from the analytical sampling test chambers were collected from the negative control, solvent control, the lowest test concentration (0.50 mg a.s./kg) and the highest test concentration (49 mg a.s./kg) on day 0, day 7 and day 28. Samples were analysed by HPLC analysis. The endpoints were based on the nominal concentrations. The test does not meet the validity criteria of the OECD guideline 218. All emerged midges appeared normal during the test, with no observations of abnormal behaviour before or after emergence. The EC_{50} value based on percent emergence of midges *(Chironomus riparius)* exposed to sediment-incorporated fluopicolide is greater than 49 mg a.s./kg, the highest nominal concentration tested. There were no treatment related effects observed on mean development times, emergence rates and development rates. The LOEC is greater than 49 mg a.s./kg and the NOEC is 49 mg a.s./kg.

I. MATERIAL AND METHODS:

Test material:	Name of substance: Fluopicolide (AE C638206) Batch No.: OP 2050046 Purity 96.1% w/w
Guideline adaptation	alpha cellulose was used as carbon source for the sediment instead of peat moss
Test species:	Midge *(Chironomus riparius)*
Organism age:	First instar larvae, one to four days old at exposure initiation
Preparation of spiked sediment	Dry formulated sediment (1500 g) was weighed into seven 2000 mL plastic bottles to make batches of treated sediment for each test concentration. Each bottle received 1.5 mL of the appropriate acetone stock solution and then was placed in a rotary mixer. The sediment and test substance were mixed for approximately 39 hours. After mixing was complete, batches of sediment were distributed among replicate test chambers. Sediment was added to the appropriate replicates to a depth of 2 cm from the bottom of the jar and then 600 mL of ultraviolet sterilized well water was slowly added to the test chambers. Test chambers were then placed in an environmental chamber, covered, and allowed to settle for approximately 48 hours, prior to the addition of test organisms.
Test solutions	Nominal sediment concentrations: 0.50, 1.3, 3.1, 7.8, 20 and 49 mg a.s./kg sediment dry weight. Arithmetic mean measured concentration in lowest and highest test rate - in sediment: 0.131 and 55.6 mg a.s./kg - in overlying water: 0.104 and 3.932 mg a.s./L - in sediment pore water: 0.418 and 3.517 mg a.s./L Water control: approx. 150 mL sediment and 600 mL dilution water Solvent control and test concentrations: approx. 150 mL sediment treated with acetone or the stock solution in acetone, 600 mL dilution water Evidence of undissolved material: At test initiation the overlying water in all test chambers appeared clear and colourless. At test termination the overlying water in all test chambers appeared slightly cloudy tan.
Replication:	<u>Vessels to measure biological response:</u> No. of vessels per concentration (replicates): 4 No. of vessels per control (replicates): 4 No of vessels per solvent control (replicates): 4 <u>Additional vessels with organisms for analytical sampling on day 7 and 28:</u> No. of vessels in the lowest and highest concentration (replicates): 2 No. of vessels per control (replicates): 2 No. of vessels per solvent control (replicates): 2 <u>Additional vessels without organisms for analytical sampling on day 0:</u> No. of vessels in the lowest and highest concentration (replicates): 1 No. of vessels per control (replicate): 1 No. of vessels per solvent control (replicates): 1
Organisms per replicate:	No. of organisms per vessel: 20
Exposure:	Static conditions Total exposure duration: 28 days
Feeding during test	Larvae were fed 10 – 30 mg of ground rabbit pellets throughout the test.

Test conditions:	Water temperature: 20.4 to 20.6°C (daily measurements), 20 to 21°C (continuous measurements) Photoperiod: 16:8 light: dark with a 30-min transition period Light intensity: 366 lux (at test initiation) pH: 7.9 to 8.5 Water hardness: 128 to 132 mg/L as $CaCO_3$ Dissolved oxygen (mg/L): 6.1 to 8.6 Specific Conductance (µmhos/cm): 310-320 Alkalinity (mg/L as $CaCO_3$): 182-186
Sediment	Artificial sediment: 14% kaolin clay 80% industrial quartz sand 0.5% dolomite 5% alpha-cellulose 0.01% humic acid
Parameters Measured / Observations	Temperature and dissolved oxygen were measured in the overlying water of one alternating test chamber in each treatment and control group at the beginning of the test, three times per week and at the end of the test. Temperature was also measured continuously in a beaker of water placed adjacent to the test chamber. The pH of the water was measured on samples of overlying water collected from one alternating replicate test chamber at the beginning of the test, once per week and at the end of the test. Dissolved oxygen was measured on samples of overlying water collected from one alternating replicate test chamber at test initiation, three times per week during the test, and at test termination. The test chambers were observed three times per week during the first 13 days of the test to make visual assessments of any abnormal behavior (e.g., leaving sediment, unusual swimming). During the period of expected emergence following day 13, test chambers were observed on a daily basis and the sex and number of fully emerged midges were recorded. The total number of midges emerged in each replicate was determined at test termination.
Sampling for chemical analysis	Overlying water, pore water and sediment samples from the analytical sampling test chambers were collected from the negative control, solvent control, the lowest test concentration (0.50 mg a.s./kg) and the highest test concentration (49 mg a.s./kg) on Day 0, Day 7 and Day 28. Water samples were collected from mid-depth of the water column. Samples were analysed by HPLC analysis.
Data analysis:	The NOEC and LOEC were determined by visual interpretation of the dose-response pattern and statistical analyses of the mean development times, emergence rates and development rates. All comparisons were made between pooled control and treatment groups, as the Student's t-test showed no statistical difference between the negative and solvent control group. Data were analysed using a two-tailed Dunnett's test, as appropriate, to identify those treatment levels that were statistically different from the pooled control group. In addition to the Dunnett's test, the development time was analysed using the Kruskal-Wallis test. A Chi-square test was performed to check normality, and the homogeneity of variance was checked using the Bartlett's test. All statistical procedures were performed using SAS version 8.2 and Toxstat version 3.5.

II. RESULTS AND DISCUSSION:

Validity criteria (OECD 218, 2004)	Required	Obtained
Emergence in the controls	≥ 70%	94% and 93%
Emergence period in the controls	between day 12 and 23	between day 14 and day 28
Dissolved oxygen % saturation at test end in all test vessels	> 60%	> 6.1 mg/L (60% = 5.5 mg/L in test conditions)
Water pH in all test vessels	between 6 and 9	7.9 to 8.5
Water temperature variation over the whole exposure period	± 1°C	20 - 21°C

Analytical results:
No fluopicolide residues were measured in sediment the overlaying water and sediment pore water of the control and solvent control above the limits of quantification.

Nominal test concentration (mg a.s./kg sediment dry weight)	Arithmetic mean measured sediment concentrations (mg a.s./kg)	% of nominal concentration		
		Day 0	Day 7	Day 28
0.5	0.131	78.4	-*	-*
49	55.6	110	113	118

* Measured concentration was beneath the LOQ, Limit of quantification = 0.208 g a.s./kg

Nominal concentration (mg a.s./kg sediment dry weight)	Measured concentration (mg a.s./L)			
	Day 0	Day 7	Day 28	Arithmetic mean
Overlying water				
0.5	< LOQ	< LOQ	< LOQ	< LOQ
49	0.712	1.04	1.38	3.132
Sediment pore water				
0.5	0.780	0.345	0.129	0.418
49	4.670	3.060	2.820	3.517

LOQ: Limit of quantification = 0.104 mg a.s./L

Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Biological results:

All emerged midges appeared normal during the test, with no observations of abnormal behavior before or after emergence.

Mean percent emergence and mean emergence rate during the life-cycle exposure with fluopicolide (AE C683206) and midge (*Chironomus riparius*).

Emergence was first noted on day 14 of the test. There were no significant differences ($p > 0.05$) in comparison of mean emergence rates between the pooled control and any of the treatment groups. Therefore, there were no treatment related effects on emergence rates.

Nominal test concentration (mg a.s./kg sediment dry weight)	Cumulative emergence after 28 days		
	Mean Percent Emergence	Mean Male Emergence Rate	Mean Female Emergence Rate
Control	94	37	38
Solvent Control	93	34	40
Pooled control	93	-	-
0.5	91	39	34
1.3	90	32	40
3.1	95	40	36
7.8	89	38	38
20	88	45	25
49	91	31	42

Mean development time of midges during the life-cycle exposure with fluopicolide (AE C683206) and midge (*Chironomus riparius*)

There were significant differences ($p<0.05$) in comparisons of development times between the pooled control and the 3.1, 7.8, and 20 mg a.s./kg treatment groups using a two-tailed Dunnett's test. However, there were no significant differences ($p > 0.05$) between the pooled control and the 0.50, 1.3 or 49 mg a.s./kg treatment groups. The differences in development times were relatively small, were not concentration dependent and the range of values in the treatment groups overlapped with the pooled controls (with exception of the 20 mg a.s./kg group). Therefore, the differences observed in development time were not believed to be treatment related. There were no significant differences ($p > 0.05$) in comparison of development rates between the pooled control and any of the treatment groups. Therefore, there were no treatment related effects on development rates.

Nominal test concentration (mg a.s./kg sediment dry weight)	Mean development time in days	Mean development rate[#]
Control	20.3	0.0521
Solvent Control	20.4	0.0524
Pooled control	20.4	0.0522
0.5	19.6	0.0541
1.3	18.5	0.0570
3.1	17.6*	0.0600
7.8	18.5*	0.0571
20	17.5*	0.0600
49	18.7	0.0563

*Dunnett's test shows statistical differences from pooled controls ($p < 0.05$)
[#]represents portion of larval development per day.

III. CONCLUSIONS:

The test does not meet the validity criteria of the OECD guideline 218.
No adverse effects up to and including the highest concentration (49 mg a.s./kg) were observed.
Endpoints based on nominal sediment concentrations are:

Endpoint	Nominal test concentration (mg a.s./kg sediment dry weight)	
	NOEC	LOEC
Percent emergence	≥ 49	> 49
Mean development time	≥ 49	> 49
Mean development rate	≥ 49	> 49

EC_{10} and EC_{20} values cannot be calculated because of the lack of dose response relationship.

Assessment and conclusion by applicant:

The study is not considered valid.

Data Point:	RCA 8.2.5.4/02
Report Author:	[redacted]
Report Year:	2020
Report Title:	Amendment 2 to final report - Fluopicolide, technical: A study on the chronic toxicity to the sediment dweller Lumbriculus variegatus
Report No:	19P7LA
Document No:	M-671529-03-1
Guideline(s) followed in study:	OECD Guideline 225: "Sediment-water Lumbriculus toxicity test using spiked sediment", October 2007
Deviations from current test guideline:	Method: No deviation Study: Current Guideline: OECD 225 (2007) No deviations
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

A chronic study with sediment dwelling worms (*Lumbriculus variegatus*) in a 28-day spiked-sediment test was investigated. Fluopicolide was applied at nominal sediment concentrations of 0.126, 0.316, 0.79, 1.98 and 4.94 mg a.s./kg dry sediment. Additionally, a water control and a solvent control were included. The test comprised 4 replicates of 10 individual species for the control and the treatment and 6 replicates of 10 individual species for the solvent control. The total number of worms per replicate, survival and the total dry weight of the worms per replicate were assessed at test end (day 28).

Concentrations of fluopicolide in sediment, pore water and overlying water were verified by HPLC-MS/MS on day 0, day 14 and day 28 for each concentration and the controls. The measured concentrations of the pure substance in sediment, pore water and overlying water resulted in a mass

balance of 97 to 119% of the nominally applied pure substance throughout the test period. In the control and solvent control, the measured concentrations in sediment, pore water and overlying water were below the limit of detection (0.003 mg a.s./kg dry sediment and 0.15 µg/L).

The study fulfils all validity criteria of the OECD Guideline 225.

At test termination (test day 28), the total number of worms per replicate at the highest concentration was statistically different from the pooled control. The endpoint based on nominal sediment concentrations was: NOEC (28 d) = 1.98 mg a.s./kg dry sediment.

I. MATERIAL AND METHODS

Test material:	Fluopicolide, technical Batch code: AE C638206-01-35 Specification No.: Not reported Purity: 98.8 % w/w
Guideline(s) adaptation	None specified
Test species:	*Lumbriculus variegatus*
Culturing conditions	The culture conditions were 20 ± 2 °C, 16h light and 8 h dark Cultured in crystallising dishes containing quartz sand and reconstituted water In the culture, the worms are fed with fish food suspension, TetraMin®
Organism age/size at study initiation:	Synchronised adult worms of similar size
Preparation of spiked sediments	The test item (33 mg) was dissolved in 200 mL of acetone (stock solution of 165 mg/L) by stirring (10 min) and manual shaking. A series of solutions to prepare the different concentrations levels in the sediment was prepared by diluting the stock solution with the same solvent used to prepare the stock solution. Each of the application solutions of the different concentration levels was spiked onto a defined quantity of the sediment (10 g quartz sand per replicate). The spiked sand was left for evaporation of the solvent and was then mixed into the quantity of formulated sediment necessary for all replicates of one concentration level to achieve the desired nominal concentration levels in mg/kg dry sediment. For each concentration level the same volume of the corresponding application solution per quantity of sediment was used. To ensure that the test item added to the sediment was evenly distributed within the sediment, the bulk formulated sediments were thoroughly mixed using a power drill with stainless steel propeller.
Test concentrations	Nominal sediment concentrations: 0.126, 0.316, 0.79, 1.98 and 4.94 mg a.s./kg dry sediment Controls: water Solvent control: acetone (200 mL)
Replication:	No. of vessels per concentration (replicates): 4 No. of vessels per control (replicates): 4 No. of vessels per solvent control (replicates): 6
Organisms per replicate:	No. of organisms per vessel: 10

Exposure:	Static conditions (with periodic compensation of evaporated water) Spiked sediment test Total exposure duration: 28 days
Feeding during test	Feed in sediment (*Urtica* powder and cellulose)
Test conditions:	Water temperature: 19.6-21.0°C (manual measurements in test vessels) and 19.0-20.7°C (automatic measurement; 1h intervals in a separate vessel) Photoperiod: 16 h light, 8 h dark Light intensity: 175 - 268 lux pH: 7.2 - 8.3 (overlying water) Water hardness: 286 - 339 mg/L $CaCO_3$ (overlying water) Dissolved oxygen: 7.2 - 8.7 mg/L (> 81 % saturation) Ammonium content: max. 8.76 mg/L as NH_4^+ (overlying water) Sediment water ratio approx. 1:3.5
Sediment	Artificial sediment according to OECD guideline No. 225 (2007); addition of feed to sediment before application. Composition in % dry weight sediment: % peat: 4.8 % organic carbon: 2.5 % sand: 75 % clay: 20 % *Urtica* powder: 0.37 % Cellulose powder: 0.13 % Calcium carbonate: 0.23 % deionised water: 30-50
Parameters Measured / Observations	Temperature, dissolved oxygen content and pH were measured in one test vessel of each concentration level and one test vessel of the controls at the start and the end of the exposure period and once per week. Total water hardness was measured in one replicate of the control and one test vessel at the highest concentration at the start and the end of the exposure period and once per week. Ammonium content was measured in one replicate of the control and one test vessel at the highest concentration at the start and the end of the exposure period. Light intensity was measured once during the test over the testing area. Temperature was recorded additionally, at hourly intervals, throughout the test. The total number of worms per replicate, survival and the total dry weight of the worms per replicate were assessed at test end (day 28).
Sampling for chemical analysis	During the in-life phase of the definitive study, sediment, pore water and overlying water samples were taken from the control, solvent control and all concentration levels. Directly after spiking the sediment samples were taken and kept as a reserve. Further samples were taken at end of equilibration (day 0 of exposure), on day 14 and at the end of the exposure period; these samples were analysed. Chemical analysis of sediment, pore water and overlying water samples were performed by HPLC-MS/MS.

Data analysis:	The total number of worms per replicate and the total dry weight of the worms per replicate were assessed. In order to estimate mortalities, the numbers of worms that did not react to a gentle stimulus were considered to be dead. To determine significant differences between the controls (control and solvent control) the replicates of each control were tested for normal-distribution and for homogeneity of variances; thereafter a pair-wise comparison test was used. Since no statistically significant difference was observed between the control and solvent control for any of the parameters, the statistical evaluation of the effects was performed against the pooled controls. Normal distribution and variance homogeneity of the data were assessed by R-by-S test and Cochran's test, respectively. The Williams test was used where variance homogeneity was confirmed. The Multiple sequentially-rejective Welch-t-test after Bonferroni-Holm was used where variance homogeneity was not confirmed. Probit analysis was performed to calculate the EC_x-values. The statistical software package ToxRat Professional 3.3.0 was used for these calculations. All statistical calculations were done based on the nominal test item concentrations.

II. RESULTS AND DISCUSSION

Validity criteria (OECD 225, 2007)	Required	Obtained
Average increase factor of the number of living worms in the control replicates at the end of exposure	≥ 1.8	2.3
Average increase factor of the number of living worms in the solvent control replicates at the end of exposure	≥ 1.8	2.1
pH of the overlying water	6-9	7.2 - 8.3
Dissolved oxygen concentration measured in at least one replicate per concentration level and control at least once per week:	> 30%	≥ 81%

Analytical results:

The measured concentrations of the pure substance in sediment, pore water and overlying water resulted in a mass balance of 97 to 119% of the nominally applied pure substance throughout the test period. The biological results are therefore expressed based on nominal concentrations (pure a.s.).

Analytical results: Measured concentrations of fluopicolide in sediment

Nominal sediment concentration [mg a.s./kg]	Day 0		Day 14		Day 28	
	Measured concentration [mg a.s./kg]	% of nominal	Measured concentration [mg a.s./kg]	% of nominal	Measured concentration [mg a.s./kg]	% of nominal
Control	< LOD	-	< LOD	-	< LOD	-
Solvent control	< LOD	-	< LOD	-	< LOD	-
0.126	0.139	111	0.121	96	0.117	93
0.316	0.287	91	0.268	85	0.255	81
0.790	0.798	101	0.649	82	0.638	81
1.98	1.85	93	1.58	80	1.54	78
4.94	4.88	99	4.21	85	3.89	79

LOD = Limit of detection (0.003 mg a.s./kg dry sediment)

Analytical results: Measured concentrations of fluopicolide in pore water and overlying water

Nominal sediment concentration [mg a.s./kg]	Day 0	Day 14	Day 28
	Pore water - measured concentration [µg a.s./L]		
Control	< LOD	< LOD	< LOD
Solvent control	< LOD	< LOD	< LOD
0.126	12.1	8.74	7.18
0.316	29.5	23.6	21.2
0.790	104	57.3	48.8
1.98	257	164	150
4.94	673	520	448
	Overlying water - measured concentration [µg a.s./L]		
Control	< LOD	< LOD	< LOD
Solvent control	< LOD	< LOD	< LOD
0.126	2.15	3.51	5.73
0.316	5.70	13.6	14.0
0.790	15.7	30.6	34.0
1.98	38.4	91.7	106
4.94	122	260	307

LOD = limit of detection (0.15 µg/L)

Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Biological results:

Survival

After 28 days of exposure, no mortality was observed up to the highest concentration level. In all replicates 10 or more worms were found. Therefore, the parameter survival was not affected up to the highest test item concentration level.

Reproduction

The total number of worms (including adult and regenerated worms) found at the end of the test was evaluated as a surrogate measurement of the reproduction endpoint.

Total number of worms per replicate per treatment after 28 day of exposure

Nominal sediment concentration [mg a.s./kg]	Mean (± SD)	Reduction in % of pooled controls
Control	23.3 ± 4.65	-
Solvent control	21.3 ± 3.61	-
Pooled control	22.1 ± 3.93	-
0.126	22.3 ± 4.03	-0.7
0.316	21.3 ± 2.75	3.8
0.790	23.3 ± 3.59	-5.2
1.98	22.5 ± 5.97	-1.8
4.94	16.5 ± 1.73	25.3*

SD: Standard deviation, *Significant difference to the pooled control (Williams test)

Biomass

Total biomass of the animals per replicate (mean values and SD per treatment) in mg dry weight

Nominal sediment concentration [mg a.s./kg]	Mean (± SD)	Reduction in % of pooled controls
Control	41.8 ± 6.25	-
Solvent control	37.6 ± 2.75	-
Pooled control	39.3 ± 4.67	-
0.126	35.5 ± 1.44	9.6
0.316	35.0 ± 0.80	10.8
0.790	35.6 ± 1.64	9.4
1.98	36.4 ± 0.20	7.5
4.94	35.5 ± 2.3	9.6

SD: Standard deviation

Since the difference between the solvent control and the control biomass is around 10%, a reduction around 10% of biomass in the treatment groups is not considered as biologically relevant. Moreover, no dose response is observed for this parameter.

III. CONCLUSION

The study meets the validity criteria and the endpoints based on nominal sediment concentrations are:

Endpoint	Survival	Reproduction	Biomass (dry weight)
LOEC – 28 days: lowest concentration with an effect	> 4.94 mg a.s./kg	4.94 mg a.s./kg	> 4.94 mg a.s./kg
NOEC – 28 days: highest concentration without an effect	4.94 mg a.s./kg	1.98 mg a.s./kg	4.94 mg a.s./kg
EC_{10} / EC_{20} / EC_{50}	4.94 mg a.s./kg	-[A]	-[A]

[A] EC_x values could not be determined (No clear concentration-response relationship)

Assessment and conclusion by applicant:

This study is considered reliable for risk assessment and the endpoint (based on nominal concentrations) is:

NOEC (28 d) = 1.98 mg a.s./kg

CA 8.2.6 Effects on algal growth

CA 8.2.6.1 Effects on growth of green algae

The 72-hour toxicity test with the freshwater alga (*Scenedesmus subspicatus*) by [redacted] 2004; M-227278-01-1 was originally submitted for the Annex I inclusion – however since it was conducted with a straight SC 480 formulation is not considered relevant for this section.

Data Point:	KCA 8.2.6.1/02
Report Author:	[redacted]
Report Year:	2003
Report Title:	AE C638206: A 96-hour toxicity test with the freshwater alga (Selenastrum capricornotum)
Report No:	M-219737-01-2
Document No:	M-219737-01-2
Guideline(s) followed in study:	EU (=EEC): 92/69/EEC C.3 (1992); OECD: 201 (1984); USEPA (=EPA): OPPTS: 850.5400 (1996)
Deviations from current test guideline:	Method: Deviations from current guideline SANCO/3029/99 rev.4: Limited sets of validation recoveries were analysed. However, the average recoveries were within the acceptable range of 70–110% and the RSD values were below 20%. The analytical method can be regarded as fit for purpose. Study: Current Guideline: OECD 201 (2011) The light intensity was slightly lower than recommended in OECD guideline: it was 3900-4500 lux instead of 4440-8880 lux, but this is compliant with OCSPP guideline which recommends 4300 lux +/- 15%. Since the growth of the controls was satisfactory and met the validity criteria, this deviation is not considered relevant.
Previous evaluation:	yes, evaluated and accepted in DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive summary

The green alga, *Selenastrum capricornutum, (*syn *Pseudokirchneriella subcapitata)* was exposed to a geometric series of five test concentrations of fluopicolide, a negative (culture medium) control and a solvent (dimethylformamide) control under static conditions for 96 hours. Three replicate test chambers were maintained in each treatment group and six replicates for each control group. The selected nominal test concentrations of fluopicolide were 0.31, 0.63, 1.2, 2.5 and 5 mg a.s./L. Fluopicolide was analysed by high performance liquid chromatography (HPLC) using an ultraviolet (UV) detector. Samples of the test solutions were collected at approximately 0 and 96 hours to measure concentrations of the test substance. Measured concentrations were in the 80-120% range of nominal concentrations and no residues above the limit of quantification (LOQ) were measured in the controls. The mean measured concentrations were: 0.30, 0.59, 1.2, 2.4 and 4.3 mg a.s./L. The study meets the validity criteria of the guideline OECD 201. Samples collected on day 0 had recoveries of 96, 94, 93, 94 and 87% of nominal concentrations respectively. Samples collected on day 4 had recoveries of 96, 93, 91, 94 and 84% of nominal concentrations, respectively. Mean measured test concentrations were 0.30, 0.59, 1.2, 2.4 and 4.3 mg a.s./L, respectively. Mean measured test concentrations were used to calculate the EC_{50} values. The 72-hour E_bC_{50} and E_rC_{50} values are 3.0 and > 4.3 mg a.s./L, respectively. The 96-hour E_bC_{50} and E_rC_{50} values are 2.7 and > 4.3 mg a.s./L, respectively. The 72-hour NOAErC, is 2.4 mg a.s./L.

I. MATERIAL AND METHODS:

Test material	Fluopicolide Batch No. OP2350005 Specification: AE C638206 00 1C99 0006 Purity 99.4 %
Guideline(s) adaptation	None specified
Test species	Algae *Pseudokirchneriella subcapitata* (UTCC 37) formerly *Selenastrum capricornutum*
Culturing conditions	Algal cells used in this test were obtained from cultures that had been actively growing in culture medium for at least two weeks prior to test initiation. The culture was last transferred to fresh medium three days prior to test initiation The algal cells were cultured and tested in freshwater algal medium as defined in ASTM Standard guide 1218-90E
Test solutions	Nominal concentrations: 0.31, 0.63, 1.3, 2.5, 5.0 mg a.s./L Corresponding mean measured concentration 0.30, 0.59, 1.2, 2.4, 4.3 mg a.s./L Control: culture medium Solvent control: 0.1 ml/L dimethylformamide All solutions were clear and colourless at test initiation. At test termination the 5.0 mg/L solution had visible precipitation.
Replication	No. of vessels per concentration (replicates): 3 No. of vessels per control (replicates): 6 No. of vessels per solvent control (replicates): 6
Exposure	Static Total exposure duration: 96 hours
Initial cells density	1×10^4 cells/mL in each test group
Test conditions	Temperature: 23.7 – 24.2°C Photoperiod: continuous light Light intensity: 3900 to 4500 lux pH: 7.7 – 9.0 Growth medium same as culture medium: Yes Type of light: Cool white fluorescent tubes.
Parameters Measured / Observations	Test medium samples were collected from each replicate of the treatment and control group for the determination of algal cell densities. Samples were collected at approximately 24-hour intervals during the 96-hour exposure and were held for a maximum of one day under refrigerated conditions sufficient to inhibit growth until cell counts could be performed. Temperature was measured twice daily. Light intensity was measured at test initiation, pH was measured at test initiation and termination.
Sampling for chemical analysis	Samples of the test solutions were collected at approximately 0 and 96 hours to measure concentrations of the test substance. Samples at test initiation were collected from the individual batches of test solution prepared for each treatment and control group prior to addition of the algae. At test termination, samples were collected from the pooled replicates from each treatment and control group. Fluopicolide was analysed by high performance liquid chromatography (HPLC) using an ultraviolet (UV) detector.

Data analysis	The calculations, as well as all statistical analyses, were conducted using "The SAS System for Windows", Version 8.02 or TOXSTAT version 3.5. Nonlinear regression or linear interpolation was used to calculate EC50 values and their corresponding 95% confidence intervals. The data were evaluated for normality and homogeneity of variance (p=0.05) using the Shapiro-Wilk's and Levene's tests, respectively. The treatment groups then were compared to the pooled control using Dunnett's test (p=0.05). If the assumptions of normality and homogeneity of variances were not met an attempt was made to correct the condition by log transformation of the data. If the data failed the assumptions of normality and/or homogeneity of variances and transformations would not correct the problem Dunnett's test was still used to make the comparisons.

II. RESULTS AND DISCUSSION:

Validity criteria (OECD 201, 2011)	Required	Obtained
Increase of biomass in the control cultures	16	103 (72h), 365 (96 h)
Mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3) in the control cultures	≤ 35%	7.6% (72h), 12.0 (96h)
Coefficient of variation of average specific growth rates in replicate control cultures	≤ 7%	3.2% (72 h), 2.0 (96h)

Analytical results:
Nominal concentrations selected for use in this study were 0.31, 0.63, 1.3, 2.5 and 5.0 mg a.s./L. Mean measured test concentrations were 0.30, 0.59, 1.2, 2.4 and 4.3 mg a.s./L representing 97, 94, 92, 96 and 86% of nominal, respectively. Mean measured test concentrations were used to calculate the EC_{50} values.
No residues of fluopicolide were measured in the controls above the limit of quantification (201 µg/L).

Nominal Concentration (mg a.s./L)	Mean Measured Concentration (mg a.s. /L)	0-hour % Nominal	96-hour % Nominal
0.31	0.30	95.5	95.5
0.63	0.59	94.2	93.3
1.3	1.2	92.6	91.4
2.5	2.4	94.0	94.1
5.0	4.3	87.2	84.4

Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4 with minor acceptable exceptions only.

Biological results:

Mean measured concentrations (mg a.s./L)	Biomass inhibition (%)		Growth rate inhibition (%)	
	72 h	96 h	72 h	96 h
0.30	-3.2	0.17	-0.51	0.29
0.59	5.2	6.9	0.97	1.6
1.2	9.4	17*	1.8	4.4#
2.4	29*	39*	6.9#	11*
4.3	81*	86*	39*	36*

* Statistically significant difference ($p<0.05$) from the pooled control replicates using Dunnett's test
# Although statistically significant a 6.9 and 4.4% inhibition from the control was not considered to be treatment related since this amount of inhibition was considered to be within an acceptable limit for *Selenastrum*

Exponential growth in the control: yes

III. CONCLUSIONS:

The study meets the validity criteria of the guideline. The endpoints based on mean measured concentrations are:

E_rC_{50} 72 hours, 96 hours (95% CI)	**>4.3 mg a.s./L (not calculable)**
E_bC_{50}, 72 h	3.0 mg a.s./L (2.8-3.2)
E_bC_{50}, 96 h	2.7 mg a.s./L (2.4-2.9)
NOAEC 72 hours (Growth rate) highest concentration without adverse effects	2.4 mg a.s. /L
NOAEC 72 hours (Biomass, Cell density) highest concentration without adverse effects	1.2 mg a.s./L

Additional endpoints were calculated to fulfill the data requirements, they are presented below.

Data Point:	KCA 8.2.6.1/05
Report Author:	[redacted]
Report Year:	2018
Report Title:	ECx calculation for fluopicolide study on the green algae Pseudokirchneriella subcapitata (Desjardins et al. (2003), M-219737-01-1)
Report No:	M-643768-01-1
Document No:	M-643768-01-1
Guideline(s) followed in study:	none
Deviations from current test guideline:	Not applicable
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	Yes

In the existing report, endpoints for the following parameters were statistically determined at 72 and 96 h:

- Growth rate
- Biomass (area under the curve)
- Cell density

Endpoints based on cell density are not requested by the current version of the OECD guideline 201, so the EC_{10} and EC_{20} were not calculated for this parameter.

Nevertheless, ECx values can be calculated for both biomass and growth rate parameters. All recalculations were performed with the software ToxRat Professional Vers. 3.2.1 with the mean measured concentrations provided in the report. Three linear regression models (Logit, Probit and Weibull) and 3-D non-linear regression model were compared. Only the most suitable model is presented (Probit for all parameters). Water control and solvent control were pooled when there were no statistically significant differences ($\alpha = 0.05$) according to Student-t test.

E_rC_{10} 72 hours (95% CI)	2.6 mg a.s./L (2.1-3.0)
E_rC_{20} 72 hours (95% CI)	3.3 mg a.s./L (2.9-3.5)
$E_bC_{10,}$ 72 h (95% CI)	2.2 mg a.s./L (1.5-2.7)
E_bC_{20} 72 hours (95% CI)	3.1 mg a.s./L (2.7-3.4)
E_rC_{10} 96 hours (95% CI)	1.7 mg a.s./L (1.3-2.0)
E_rC_{20} 96 hours (95% CI)	2.0 mg a.s./L (1.7-2.3)
$E_bC_{10,}$ 96 h (95% CI)	1.1 mg a.s./L (0.6-1.5)
E_bC_{20} 96 hours (95% CI)	1.5 mg a.s./L (1.0-1.8)

Assessment and conclusion by applicant:

The study is reliable and the endpoint relevant for the fluopicolide risk assessment is the 72h-E_rC_{50} > 4.3 mg a.s./L

Data Point:	KCA 8.2.6.1/03
Report Author:	[redacted]
Report Year:	2001
Report Title:	2,6-dichlorobenzamide (BAM): Algal inhibition test
Report No:	1133/007
Document No:	M-234304-01-2
Guideline(s) followed in study:	EU (=EEC): 67/548/EEC; EU (=EEC): 92/69/EEC C.3 (1992); OECD: 201 (1984); USEPA (=EPA): J 122-2, OPPTS 850.5400 (1996)
Deviations from current test guideline:	Method: Deviations from current guideline SANCO/3029/99 rev.4: Limited sets of validation recoveries were analysed. However, the average recoveries were within the acceptable range of 70–110% and the RSD values were below 20%. The analytical method can be regarded as fit for purpose. Study: Current Guideline: OECD 201 (2011) The pH in control increased by more than 1.5 unit at 96 h. No measurement was performed at 72h. Since this change is the consequence of the important growth (factor 185 over 96h) and since the validity criteria are met, this deviation is not considered relevant. The light conditions were not measured, but the test was performed with continuous illumination at approximatively 7000 lux, which is in the recommended range. However, the growth in control replicates was satisfactory so there is no impact of this deviation on the results of the test.
Previous evaluation:	yes, evaluated and accepted in DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive summary

The green alga, *Pseudokirchneriella subcapitata* was exposed to a geometric series of five test concentrations to the fluopicolide metabolite M-01 (2-6-dichlorobenzamide) and a negative (culture medium) control under static conditions for 96 hours. After 96 h exposure a re-growth experiment of a maximum duration of 144h was performed. The selected nominal test concentrations of fluopicolide were 10, 20, 40, 80 and 160 mg a.s./L. Fluopicolide was analysed by high performance liquid chromatography (HPLC) using an ultraviolet (UV) detector. Samples of the test solutions were collected at 0, 24, 48, 72 and 96 hours to measure concentrations of the test substance. Measured concentrations were in the 80-120% range of nominal concentrations and no residues above the limit of quantification (LOQ) were measured in the control. Therefore, results were based on nominal concentrations. The study meets the validity criteria of the guideline OECD 201. The 72-hour E_rC_{50} and E_bC_{50} values are 120 and 60 mg a.s./L, respectively. The 96-hour E_rC_{50} and E_bC_{50} values are 140 and 62 mg a.s./L, respectively. The determined NOEC is 40 mg a.s./L.

I. MATERIAL AND METHODS:

Test material	M-01 (2,6-dichlorobenzamide, BAM, AE C653711) Batch number: FUX001000/FUN81G02C Purity: 99.5%
Guideline(s) adaptation	None specified
Test species	Freshwater green algae *Pseudokirchneriella subcapitata* Strain CCAP 278/4
Culturing conditions	The culture was maintained in the laboratory at a temperature of 21 ± 1°C under continuous illumination (intensity approximately 7000 lux) and constant aeration. The culture medium used for both the range-finding and definite tests was the same as that used to maintain the stock culture.
Test solutions	Nominal concentrations: 10 – 20 – 40 – 80 – 160 mg /L Controls: culture medium Evidence of undissolved material: At 0 hours the control and all test concentrations were clear colourless solutions. No evidence of precipitate was reported at 96h.
Replication	No. of vessels per concentration (replicates): 3 No. of vessels per control (replicates): 3
Exposure	Static Total exposure duration: 96 hours + re-growth experiment of 144 h without exposure
Initial cells density	1×10^4 cells/mL in each test group
Test conditions	Temperature of controls and test solution: 24 – 1°C Photoperiod: continuous light Light intensity approximately 7000 lux pH at t0: 7.0 -7.1, pH at 96h: 7.7 - 9.8. In controls: 7.1-9.7 Water hardness: not specified Conductivity: not specified Growth medium same as culture medium: Yes Type of light: artificial (not specified)
Parameters Measured Observations	The pH of each control and test flask was determined at initiation of the test and after 96-hour exposure. The temperature within the incubator was measured every hour. Cell densities were determined at 0, 24, 48, 72 and 96 hours.
Sampling for chemical analysis	Water samples were taken from the control and each test group (individual replicate samples) at 0 and 96 hours for quantitative analysis by HPLC with UV detector.
Data analysis	One-way analysis of variance incorporating Bartlett's test for homogeneity of variance and Dunnett's multiple comparison procedure for comparing several treatments with a control was carried out on the area under the growth curve data. The area under the growth curve data was transformed to its square root prior to analysis as statistical analysis of the untransformed data indicated that significant differences were apparent at 20 mg/1 but not 40 mg/L. All statistical analyses were performed using the SAS computer software package. EC_x values were determined graphically. The 95% confidence limits were calculated using the method of Litchfield and Wilcoxon

II. RESULTS AND DISCUSSION:

Validity criteria (OECD 201, 2011)	Required	Obtained
Increase of biomass in the control cultures	≥16	185
Mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3) in the control cultures	≤ 35%	6.3%
Coefficient of variation of average specific growth rates in replicate control cultures	≤ 7%	1.79%

Analytical results:
All recoveries were within the range of 80 – 120% of nominal (see table below). Thus, the biological results are based on nominal concentrations. No residues of fluopicolide were measured in the control above the limit of quantification (0.96 mg/L).

Nominal Concentration (mg /L)	0-hour Measured Concentration (mg /L)	0-hour % Nominal	72-hour Measured Concentration (mg /L)	72-hour % Nominal
10	10.5	105	10.4	104
20	20.8	104	21.4	107
40	41.1	103	42.3	106
80	81.5	102	84.2	105
160	160	100	163	102

Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4 with minor acceptable exceptions only.

Biological results:

All test cultures were inspected microscopically at 96 hours. There were no abnormalities detected in any of the control or test cultures.

Culture densities (cells/mL, 10^4)

Nominal concentrations (mg /L)	Biomass inhibition at 72-h (%)	Biomass inhibition at 96 h (%)	Growth rate inhibition at 72 h (%)	Growth rate inhibition at 96 h (%)
10	5	6	1	0
20	17	16	3	0
40	13	11	0	-2*
80	67	65	23	15
160	96	98	79	74

* growth increase compared to controls

Exponential growth in the control: yes

Re-growth experiment:
Re-growth was observed to have occurred in the control and 10, 20, 40 and 80 mg/L test cultures after 72 hours of recovery, and in the 160 mg/L test culture after 144 hours of recovery. These results indicate that the test material was algistatic in effect.

III. CONCLUSIONS:

The study meets the validity criteria and the endpoints based on nominal concentrations are:

E_rC_{50} 72 hours (95% C.I.):	**120 mg/L (C.I. not reported)**
E_rC_{50} 96 hours (95% C.I.):	140 mg/L (C.I. not possible to calculate)
E_rC_{10} 72 hours (95% C.I.):	49 mg/L
E_bC_{50} 72 hours (95% C.I.):	60 mg/L (51-71 mg/L)
E_bC_{50} 96 hours (95% C.I.):	62 mg/L (52-74 mg/L)
E_bC_{10} 72 hours (95% C.I.):	13 mg/L
NOEC: highest concentration without adverse effects	40 mg /L

Assessment and conclusion by applicant:

The study is reliable and the endpoint relevant for the M-01 (AE C653711) risk assessment is the 72h-E_rC_{50} of 120 mg/L.

Data Point:	KCA 8.2.6.1/04
Report Author:	[redacted]
Report Year:	2002
Report Title:	Effect to Pseudokirchneriella subcapitata (green alga) in a growth inhibition test; AE C638206 technical 97.1 percent w/w
Report No:	B003804
Document No:	M-240808-01-1
Guideline(s) followed in study:	OECD: 201 (1992); USEPA (=EPA): 123-2 (1982)
Deviations from current test guideline:	Current Guideline: OECD 201 (2011) The study does not meet the validity criteria (see below)
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Supportive only

Executive summary

Algal cultures of *Pseudokirchneriella subcapitata* with an initial nominal cell count of approximately 1.0×10^4 cells/mL were exposed to a nominal concentration of 5.0 mg a.s./L or maximum achievable concentration of fluopicolide in AAP media for a 96-hour period. The study design included six replicate algal cultures each without test substance as the control treatment and six replicate algal cultures each with test substance. At 24-hour intervals, the cell density (cells/mL) of each culture was counted under a microscope using a haemocytometer. Samples were analysed by Gas Chromatography with MS detection (GC/MS) for determination of fluopicolide and the metabolite M-01 (AE C653711). The recoveries were not in the range of 80-120% but the concentrations are stable over the study duration. Thus, biological results after 72 and 96 hours are based on arithmetic mean measured concentrations of fluopicolide. The arithmetic mean measured concentrations was 1.8 mg a.s./L. The study does not meet the validity criteria of the current version of OECD 210 guideline. 1.8 mg a.s./L (arithmetic mean) represents 36% of the nominal concentrations. Test solutions at 0 hour ranged from 29 to 33% of nominal, while at 96 hours they were 41% of nominal concentration indicating stability of the test substance. Since the highest concentration tested was in excess of the maximum solubility of the test substance, the low percent of nominal was expected. In order to assure that fluopicolide (AE C638206) would not hydrolyse to the major metabolite M-01 (AE C653711) under study conditions, the concentration of M-01 (AE C653711) in the samples are measured as well. The 72- and 96-hour endpoints are based on arithmetic mean. The 72 and 96-hour E_bC_{50} (biomass) and E_rC_{50} (growth rate) values of fluopicolide to *Pseudokirchneriella subcapitata* are greater than the mean measured concentration of 1.8 mg a.s./L. The no observed effect concentration (NOEC) is less than 1.8 mg a.s./L. The lowest observed effect concentration (LOEC) was 1.8 mg/L.

I. MATERIAL AND METHODS:

Test material	Fluopicolide (AE C638206) Batch number: 2050190//PP241024/2 Purity: 97.1%
Guideline(s) adaptation	None specified
Test species	Freshwater green algae *Pseudokirchneriella subcapitata* Strain #1648
Culturing conditions	The culture was maintained in the laboratory at a temperature of 24.7 to 25.4°C under continuous illumination (intensity approximately 4800 to 5100 lux). The culture medium (AAP) used for the test was the same as that used to maintain the stock culture.
Test solutions	Nominal concentrations: 5.0 mg a.s./L. Limit test at solubility limit Mean measured concentration: 1.8 mg a.s./L Controls: AAP medium Evidence of undissolved material: Test solution was prepared in excess of solubility and filtered prior to study initiation. No evidence of undissolved material after filtering reported in the test solution.
Replication	No. of vessels per concentration (replicates): 6 No. of vessels per control (replicates): 6
Exposure	Static Total exposure duration: 96 hours
Initial cells density	1×10^4 cells/mL in each test group
Test conditions	Temperature of controls and test solution: 24.0 – 25.8°C Photoperiod: continuous light Light intensity 4200-4500 lux pH in the control: 7.6 – 8.8 Conductivity: 150 µmhos/cm Type of light: artificial (cool white fluorescent bulbs)
Parameters Measured / Observations	Discrete measurements of temperature, pH, dissolved oxygen and salinity were obtained at test initiation and at test termination (i.e. 96 hours). Cell densities were determined at 24, 48, 72 and 96 hours.
Sampling for chemical analysis	Water samples were taken on day 0 and on day 4 (test termination). Samples taken at initiation were taken directly from parent test solutions after adequate mixing, and prior to the addition of algae. Samples taken at termination were composite from all replicates within a treatment. Samples were analysed by Gas Chromatography with MS detection (GC/MS) for determination of fluopicolide and metabolite BAM.
Data analysis	To test the assumption of normality and equal variances, algal biomass and growth rate for 24, 48, 72 and 96 hours were subjected to a Shapiro-Wilk's Test and F-test to determine homogeneity of variance. To assess treatment effect for homoscedastic and normally distributed data, a t-Test was used to determine if the exposure concentration exhibited a response significantly different ($p \leq 0.05$) than the control group. All statistical analyses were performed using TOXSTAT (Version 3.4) statistical software for personal computers with conclusions of statistical significance at the $\alpha = 0.05$ (95% confidence level).

II. RESULTS AND DISCUSSION:

Validity criteria (OECD 201, 2011)	Required	Obtained 72 h	Obtained 96 h
The increase of biomass in the control cultures.	≥ 16	50.8	187
Mean coefficient of variation for section-by-section specific growth rates in the control cultures	≤ 35%	47.1%	38.9
Coefficient of variation of average specific growth rates during the whole test period in replicate control cultures	≤ 7%	3.59%	1.19

Analytical results:
The recoveries were not in the range of 80-120% of nominal (see table below), but the concentrations are stable over the study duration. Thus, biological results after 72 and 96 hours are based on arithmetic mean measured concentrations of fluopicolide. No residues of fluopicolide were found in the control and solvent control samples above the limit of detection (0.0075 mg a.s./L). M-01 (BAM) metabolite did not form during the test.

Nominal concentration (mg a.s./L)	Arithmetic mean measured concentration (mg a.s./L)	0-hour Measured Concentration (mg a.s./L)	0-hour % Nominal	96-hour Measured Concentration (mg a.s./L)	96-hour % Nominal
5	1.8036	1.5625	31.25	2.0446	40.9

Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Biological results:

72 hours

Arithmetic mean measured concentration (mg a.s./L)	Mean cell number (cells/mL × 10^4)	Specific growth rate (h^{-1})	Inhibition of average specific growth rate (%)
Control	50.8	0.0544	-
1.80	39.5	0.0510	6*

*Significantly different ($p \leq 0.05$) from control group by t-test.

Arithmetic mean measured concentration (mg a.s./L)	Area under the growth curve (biomass integral)	Inhibition of Biomass integral (%)
Control	9504000	-
1.80	7484000	21*

*Significantly different ($p \leq 0.05$) from control group by t-test.

96 hours

Arithmetic mean measured concentration (mg a.s./L)	Mean cell number (cells/mL × 10^4)	Specific growth rate (h^{-1})	Inhibition of average specific growth rate (%)
Control	187	0.0545	-
1.80	124	0.0501	8*

*Significantly different ($p \leq 0.05$) from control group by t-test.

Arithmetic mean measured concentration (mg a.s./L)	Area under the growth curve (biomass integral)	Inhibition of Biomass integral (%)
Control	37804000	-
1.8036	26804000	29*

*Significantly different ($p \leq 0.05$) from control group by t-test.

III. CONCLUSIONS:

The study does not meet the validity criteria. The 72- and 96-hour endpoints based on arithmetic mean concentrations are:

E_rC_{50} 72 hours (95% C.I.):	**> 1.80 mg a.s./L (n.d.)**
LOE_rC 72 hours: lowest concentration with a significant effect compared to the control	= 1.80 mg a.s./L
NOE_rC 72 hours: highest concentration without a significant effect compared to the control	< 1.80 mg a.s./L
E_rC_{50} 96 hours (95% C.I.):	**> 1.80 mg a.s./L (n.d.)**
LOE_rC 96 hours: lowest concentration with a significant effect	= 1.80 mg a.s./L
NOE_rC 96 hours: highest concentration without a significant	< 1.80 mg a.s./L
E_bC_{50} 72 hours (95% C.I.)	**> 1.80 mg a.s./L (n.d.)**
LOE_bC 96 hours: lowest concentration with a significant effect	= 1.80 mg a.s./L
NOE_bC 96 hours: highest concentration without a significant	< 1.80 mg a.s./L
E_bC_{50} 96 hours (95% C.I.)	**> 1.80 mg a.s./L (n.d.)**
LOE_bC 96 hours: lowest concentration with a significant effect compared to the control	= 1.80 mg a.s./L
NOE_bC 96 hours: highest concentration without a significant effect compared to the control	< 1.80 mg a.s./L

n.d.: not determined due to mathematical reasons or inappropriate data

Assessment and conclusion by applicant:

The study is not valid, a valid study is submitted in this dossier

CA 8.2.6.2 Effects on growth of an additional algal species

Data Point:	KCA 8.2.6.2/01
Report Author:	[redacted]
Report Year:	2003
Report Title:	Effect to Anabaena flos-aquae (blue-green alga) in a growth inhibition test AE C638206 technical 97.1 percent w/w
Report No:	B004237
Document No:	M-241192-01-1
Guideline(s) followed in study:	OECD: 201 (1992); USEPA (=EPA): 123-2 (1982)
Deviations from current test guideline:	Method: Deviations from current guideline SANCO/3029/99 rev.4: Limited sets of validation recoveries were analysed. However, the average recoveries were within the acceptable range of 70–110% and the RSD values were below 20%. The analytical method can be regarded as fit for purpose with regard to this toxicity study. Study: Current Guideline: OECD 201 (2011) The study does not meet the validity criteria (see below)
Previous evaluation:	yes, evaluated and accepted in DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive summary

Algal cultures of *Anabaena flos-aquae* with an initial nominal cell count of approximately 1.0×10^4 cells/mL were exposed to the nominal concentration of 5.0 mg a.s./L or maximum achievable concentration of fluopicolide in Algal Assay Procedure (AAP) media for a 96-hour period. The study design included six replicate algal cultures each without test substance (control treatment) and six replicate algal cultures each with test substance. At 24-hour intervals, the cell density (cells/mL) of each culture was counted under a microscope using a haemocytometer. Water samples were taken on day 0 and on day 4. At test initiation they were taken directly from parent test solutions after adequate mixing, and prior to the addition of algae. Samples taken at termination were composite from all replicates within a treatment. The extracted fluopicolide and fluopicolide metabolite M-01 (AE C653711) were measured by injection to gas chromatography with electron capture detector (GC/ECD). The recoveries were not in the range of 80-120% but the concentrations are stable over the study duration. Thus, biological results after 72 and 96 hours are based on arithmetic mean measured concentrations of fluopicolide. The arithmetic mean measured concentrations was: 2.2 mg a.s./L. The study does not meet the validity criteria of the current version of OECD 201 guideline. All toxicity values were calculated based on the mean measured fluopicolide concentration of 2.2 mg a.s./L. Since the test solution was prepared in excess of solubility and filtered prior to study initiation, the mean measured fluopicolide value represents the maximum achievable concentration under study conditions. The 72- and 96-hour endpoints are based on arithmetic mean. The 72- and 96-hour E_bC_{50} (biomass) and E_rC_{50} (growth rate) values of fluopicolide technical to *Anabaena flos-aquae* are greater than 2.2 mg a.s./L. The no observed effect concentration (NOEC) is 2.2 mg a.s./L. The lowest observed effect concentration (LOEC) could not be determined under the conditions of this study.

I. MATERIAL AND METHODS:

Test material	Fluopicolide (AE C638206 (tech)) Batch number: 2050190//PP241024/2 Purity: 97.1% w/w
Guideline(s) adaptation	None specified
Test species	Freshwater blue-green algae *(Anabaena flos-aquae* strain 1444*)*
Culturing conditions	The algal cells were cultured and tested in freshwater AAP medium. Cultures were maintained at test temperature (24.6 to 25.3°C) and under continuous light (approx. 1900 lux).
Test solutions	Nominal concentrations: 5.0 mg a.s./L. Corresponding mean measured concentrations (0-96 h): 2.2 mg a.s./L. Controls: filtered medium control Evidence of undissolved material: Precipitate noted in stock solution of the preliminary test. Stock solution was filtered afterwards through a 0.45 µm filter to remove undissolved material. No information on precipitations given for main test, but stock solution for main test also filtered through a 0.45 µm filter.
Replication	No. of vessels per concentration (replicates): 6 No. of vessels per control (replicates): 6
Exposure	Static Total exposure duration: 96 hours
Initial cell density	1×10^4 cells/mL at test initiation
Test conditions	Temperature: 25.0 – 25.4°C Photoperiod: continuous light Light intensity: 2300 to 2400 lux pH of controls (0 – 96 hours): 7.6 – 8.6 Conductivity: 500 µS/cm Type of light: artificial (Cool white fluorescent tubes)
Parameters Measured / Observations	Test temperature was monitored continuously in a surrogate filled vessel within the environmental chamber. Discrete measurements of temperature, pH, dissolved oxygen were obtained at test initiation, and at test termination (i.e. 96 hours). Cell density was determined daily on a sub-sample from each flask by counting cells with a hemacytometer and compound microscope.
Sampling for chemical analysis	Water samples were taken on day 0 and on day 4. At test initiation they were taken directly from parent test solutions after adequate mixing, and prior to the addition of algae. Samples taken at termination were composite from all replicates within a treatment. The extracted fluopicolide and fluopicolide metabolite (AE C653711) were measured by injection to gas chromatography with electron capture detector (GC/ECD).
Data analysis	All statistical analyses were performed using TOXSTAT® (Version 3.4) with conclusions of the statistical significance at the $\alpha = 0.05$ (95% confidence interval).

II. RESULTS AND DISCUSSION:

Validity criteria (OECD 201, 2011)	**Required**	**Obtained 72h**	**Obtained 96h**
Increase of biomass in the control cultures	≥ 16	15.4*	53*
Mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3) in the control cultures	≤ 35%	62.9%*	52.7%*
Coefficient of variation of average specific growth rates in replicate control cultures	≤ 10%	4.28%*	4.46%*

* Values not given in study report; calculated on the basis of cell density data for controls given in study report

Analytical results:
Recoveries were not within the range of 80 – 120% of nominal (see table below). Since the highest concentration tested is in excess of the maximum solubility of the test substance under the conditions of this study, the low percent of nominal is understandable. Thus, biological results after 96 hours are based on arithmetic mean measured concentrations of fluopicolide. No residues of fluopicolide were found in the control above the limit of quantification (LOQ = 0.025 mg/L). The fluopicolide metabolite AE C653711 was not detected in any test sample on 0 and 96 hours above the limit of quantification (LOQ = 0.025 mg/L).

Nominal Concentration (mg a.s./L)	Arithmetic mean measured concentrations after 96 hours	% of nominal concentrations	
		0-hour	96-hour
5.0	2.2	44*	44

*Mean of two measurements (injections to GC/ECD)

Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev. 4 with minor acceptable exceptions only.

Biological results:

72 hours

Arithmetic mean measured concentrations (mg a.s./L)	Mean cell number after 72 h (cells/mL × 10^4)	Specific growth rate (h^{-1})	Inhibition of average specific growth rate (%)
Control	154000	0.03788	-
2.2	147000	0.03713	2

Arithmetic mean measured concentrations (mg a.s./L)	Area under the growth curve (biomass integral)	Inhibition of Biomass integral (%)
Control	2860200	-
2.2	2460000#	14

Significantly different (t-test, $p \leq 0.05$) from control group

96 hours

Arithmetic mean measured concentrations (mg a.s./L)	Mean cell number after 96 h (cells/mL × 10^4)*	Specific growth rate (h^{-1})	Inhibition of average specific growth rate (%)
Control	529000	0.04120	-
2.2	596000	0.04253	-3

Arithmetic mean measured concentrations (mg a.s./L)	Area under the growth curve (biomass integral)	Inhibition of Biomass integral (%)
Control	10812000	-
2.2	11128000	-3

III. CONCLUSIONS:

The study does not meet the validity criteria. The 72- and 96-hour endpoints based on arithmetic mean are:

E_rC_{50} 72 hours:	**> 2.2 mg a.s./L**
LOE_rC 72 hours: lowest concentration with a significant effect compared to the control	> 2.2 mg a.s./L
NOE_rC 72 hours: highest concentration without a significant effect compared to the control	2.2 mg a.s./L
E_bC_{50} 72 hours (95% C.I.):	**> 2.2 mg a.s./L**
LOE_bC 72 hours: lowest concentration with a significant effect compared to the control	2.2 mg a.s./L
NOE_bC 72 hours: highest concentration without a significant effect compared to the control	< 2.2 mg a.s./L
E_rC_{50} 96 hours (95% C.I.):	**> 2.2 mg a.s./L**
LOE_rC 96 hours: lowest concentration with a significant effect compared to the control	> 2.2 mg a.s./L
NOE_rC 96 hours: highest concentration without a significant effect compared to the control	2.2 mg a.s./L
E_bC_{50} 96 hours (95% C.I.):	**> 2.2 mg a.s./L**
LOE_bC 96 hours: lowest concentration with a significant effect compared to the control	> 2.2 mg a.s./L
NOE_bC 96 hours: highest concentration without a significant effect compared to the control	2.2 mg a.s./L

Assessment and conclusion by applicant:

The study is not valid for risk assessment, but a qualitative conclusion can be drawn from the study about the lack of significant effects on *Anabaena flos-aquae* at the fluopicolide solubility limit.

Data Point:	KCA 8.2.6.2/02
Report Author:	[redacted]
Report Year:	2003
Report Title:	AE C638206 - Acute toxicity to the marine diatom, Skeletonema costatum, under static conditions
Report No:	M-223563-01-2
Document No:	M-223563-01-2
Guideline(s) followed in study:	EU (=EEC): L383A - C.3 (1992); OECD: 201 (1984); USEPA (=EPA): OPPTS Draft 850.5400 (1996)
Deviations from current test guideline:	Method: Deviations from current guideline SANCO/3029/99 rev.4: Recoveries were determined at three different concentrations in triplicate. However, the obtained data demonstrate very good recoveries and the precision. The method can therefore be regarded as fit for purpose. Study: Current Guideline: OECD 201 (2011) The study does not meet the validity criteria
Previous evaluation:	yes, evaluated and accepted in DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Supportive only

Executive summary

The objective of this study was to determine the effect of fluopicolide on the growth of the marine diatom *Skeletonema costatum* over 96h. For the culturing and the test, the same medium was used: AES (artificially enriched seawater) prepared with sterile, filtered natural seawater. Nominal concentrations were: 0.0012, 0.0041, 0.014, 0.045, 0.15 and 0.50 mg a.s./L. Additionally, water and solvent controls (dimethylformamide at 0.1 µL/mL) were included. The test comprised 3 replicates for each concentration/control. Samples analysed at 96 hours of exposure were removed from individually composited replicate solutions of the treatment levels and controls. Samples were analysed by gas chromatographic analysis with electron capture detection (GC/ECD). Some recoveries were not in the range of 80 – 120% of nominal but the substance is stable over the test duration. Thus, biological results after 72 hours and 96 hours are based on arithmetic mean concentrations. The corresponding arithmetic mean measured concentrations (0 – 96 h): 0.0014, 0.0044, 0.016, 0.046, 0.14 and 0.47 mg a.s./L. The study is not considered valid according to the OECD 201 guideline. Cells exposed to the 0.14 and 0.47 mg a.s./L treatment levels were observed to be bloated. Cells exposed to the remaining treatment levels and the controls were observed to be normal. The 72- and 96-hour endpoints based on arithmetic mean measured concentrations are: E_rC_{50} 72 hours: 0.066 mg a.s./L, E_bC_{50} 72 hours: 0.051 mg a.s./L, The recovery experiment performed with cultures exposed to 0.14 and 0.47 mg a.s./L showed that fluopicolide has an algistatic, rather than algicidal effect on the growth of *Skeletonema costatum*.

I. MATERIAL AND METHODS:

Test material	Fluopicolide (AE C638206) Lot No: 2050190/PP241024/2 Purity: 97.7% w/w
Guideline(s) adaptation	Evaluation of recovery was included in the study
Test species	Marine diatom (*Skeletonema costatum*, strain CCMP 1332)
Culturing conditions	Stock cultures were maintained at a shaking rate of 60 ± 10 rpm, a temperature of 20 ± 2°C, a photoperiod of 14 hours light: 10 hours darkness and a light intensity of of 4300 to 5400 lux. For the culturing and the test, the same medium was used: AES (artificially enriched seawater) prepared with sterile, filtered natural seawater. Inoculum used to initiate the toxicity test with AE C638206 was taken from a stock culture that had been transferred to fresh medium six days before testing.
Test solutions	Nominal concentrations: 0.0012, 0.0041, 0.014, 0.045, 0.15 and 0.50 mg a.s./L Corresponding arithmetic mean measured concentrations (0 – 96 h): 0.0014, 0.0044, 0.016, 0.046, 0.14 and 0.47 mg a.s./L Controls: water and solvent controls (dimethylformamide at 0.1 µL/mL) Evidence of undissolved material: All test solutions were observed to be clear, colorless and did not contain any visible undissolved test substance.
Replication	No. of vessels per concentration (replicates): 3 No. of vessels per control (replicates): 3 No. of vessels per solvent control (replicates): 3
Exposure	Static Total exposure duration: 96 hours Recovery period: up to 9 days for concentrations 0.15 and 0.50 mg a.s./L
Initial cell density	7.7×10^4 cells/mL at test initiation
Test conditions	Temperature: 18–21°C Photoperiod: continuous light Light intensity: 4000-46000 lux pH of test and controls (0 – 96 h): 7.9 – 8.7 Salinity: 30 +/- 2 g/L Conductivity: 39 000 – 41 000 µmhos/cm Type of light: artificial
Parameters Measured / Observations	Temperature was measured continuously in a flask of water adjacent to the test flasks in the environmental chamber. Minimum and maximum temperatures were recorded daily. Light intensity was measured at hour 0 and at each 24-hour interval during the exposure period. Water quality parameters (pH and conductivity) were measured at test initiation and at the termination of the 96-hour exposure period. At each subsequent 24-hour interval, cell counts were conducted on the three replicate vessels of the treatment and the control vessels using a hemacytometer and compound microscope. Recovery: the subcultures were microscopically examined every other day to determine whether or not cell growth had resumed. The subculture was discontinued after a substantial increase in cell density (i.e. > 10X) was observed.

Sampling for chemical analysis	At test initiation and test termination one sample was removed from each test solution and the controls for analysis of AE C638206 concentration. Samples analysed at 0-hour were removed from the test and control solutions prior to division into the replicate test vessels. Samples analysed at 96 hours of exposure were removed from individually composited replicate solutions of the treatment levels and controls. Samples were analysed by gas chromatographic analysis with electron capture detection. (GC/ECD).
Data analysis	EC_x values (e.g. x = 50) and confidence intervals were calculated for the standard exposure period, using a commercial program (TOXSTAT®). NOEC were determined with Williams' test after checking normality and variance homogeneity.

II. RESULTS AND DISCUSSION:

Validity criteria (OECD 201, 2011)	Required	Obtained 72h	Obtained 96 h
Increase of biomass in the control cultures	≥ 16	84	87
Mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3) in the control cultures	≤ 35%	80.8%	110.5%
Coefficient of variation of average specific growth rates in replicate control cultures	≤ 10%	1.18%	3.04%

The study is not considered valid according to the OECD 201 guideline (2011).

Analytical results:
Some recoveries were not in the range of 80 – 120% of nominal (see table below) but the substance is stable over the test duration. Thus, biological results after 72 hours and 96 hours are based on arithmetic mean concentrations.

Nominal Concentration (mg a.s./L)	Arithmetic mean measured concentrations after 96 hours (mg a.s./L)	% of nominal concentrations*	
		0-hour	96-hour
Control	-	< 0.000054	0.00037**
Solvent control	-	< 0.000054	0.00015**
0.0012	0.0014	100.0	141.7
0.0041	0.0044	92.7	122.0
0.0140	0.0160	100.0	128.6
0.0450	0.0460	95.6	111.1
0.1500	0.1400	86.7	100.0
0.5000	0.4700	92.0	98.0

* Values not given in study report; calculated on the basis of nominal concentrations and concentrations measured after 0 and 96 hours.

** A compound present in the matrix is believed to have co-eluted at the same retention time as the analyte and separation was not achieved.

The 96-hour sample from the 0.045 mg a.s./L (nominal) treatment level, without algae present, yielded a measured concentration of 0.051 mg a.s./L. The equivalent replicate 96-hour test solution with algae present was 0.050 mg a.s./L, indicating that the presence of algae in the test solution had no impact on the concentration of fluopicolide.

Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Biological results:

Cells exposed to the 0.14 and 0.47 mg a.s./L treatment levels were observed to be bloated. Cells exposed to the remaining treatment levels tested and the controls were observed to be normal.

72 hours

Arithmetic mean measured concentrations (mg a.s./L)	0-72 h average specific growth rates [days]	Inhibition of average specific growth rate [%]
Water control	0.73	
Solvent control	0.65	-
0.0014	0.62	5
0.0044	0.66	-2
0.0160	0.65	0
0.0460	0.51	22
0.1400	-0.35[#]	154
0.4700	-0.56[#]	186

Significantly reduced compared to solvent control based on William's test

Arithmetic mean measured concentrations (mg a.s./L)	Area under the growth curve (biomass integral)	Inhibition of Biomass integral (%)
Water control	495600	
Solvent control	263700	0
0.0014	289700	-10
0.0044	306000	-16
0.0160	331900	-26
0.0460	164000	38
0.1400	133200	51[#]
0.4700	-152800	158[#]

Significantly reduced compared to solvent control based on William's test

96 hours

Arithmetic mean measured concentrations (mg a.s./L)	Mean cell number after 96 h per mL	Inhibition of cell densities [%]
Water control	667500	-
Solvent control	768300	-
Pooled control	717900	-
0.0014	818300	-14
0.0044	709200	1
0.0160	684200	5
0.0460	839200	-17
0.1400	12500	98[#]
0.4700	1700	100[#]

Significantly reduced compared to pooled control based on William's test

III. CONCLUSIONS:

The study does not meet the validity criteria and the 72- and 96-hour endpoints based on arithmetic mean measured concentrations are given in the table below. In general, fluopicolide has an algistatic, rather than algicidal effect on the growth of *Skeletonema costatum* at 0.14 and 0.47 mg a.s./L.

E_rC_{50} 72 hours (95% C.I.):	0.066 mg a.s./L (0.046 – 0.075 mg a.s./L)
LOE_rC 72 hours: lowest concentration with a significant effect compared to the control	0.140 mg a.s./L
NOE_rC 72 hours: highest concentration without a significant effect compared to the control	0.046 mg a.s./L
E_bC_{50} 72 hours (95% C.I.):	0.051 mg a.s./L (0.035 – 0.068 mg a.s./L)
LOE_bC 72 hours: lowest concentration with a significant effect	0.140 mg a.s./L
NOE_bC 72 hours: highest concentration without a significant	0.046 mg a.s./L
Cell density EC_{50} 96 hours (95% C.I.):	0.093 mg a.s./L (0.090 – 0.094 mg a.s./L)
Cell density LOEC 96 hours: lowest concentration with a significant effect compared to the control	0.140 mg a.s./L
Cell density NOEC 96 hours: highest concentration without a significant effect compared to the control	0.046 mg a.s./L

Assessment and conclusion by applicant:

The study is not valid. A valid study is submitted in the dossier.

Data Point:	KCA 8.2.6.2/03
Report Author:	[redacted]
Report Year:	2003
Report Title:	AE C638206 - Acute toxicity to the freshwater diatom, Navicula pelliculosa
Report No:	C037812
Document No:	M-223560-01-1
Guideline(s) followed in study:	EU (=EEC): L383A - C.3 (1992); OECD: 201 (1984); USEPA (=EPA): OPPTS Draft 850.5400 (1996)
Deviations from current test guideline:	Method: Deviations from current guideline SANCO/3029/99 rev.4: Limited sets of validation recoveries were analysed. However, the average recoveries were within the acceptable range of 70–110% and the RSD values were below 20%. The analytical method can be regarded as fit for purpose. Study: Current Guideline: OECD 201 (2011) The study does not meet the validity criteria
Previous evaluation:	yes, evaluated and accepted in DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities.
Acceptability/Reliability:	Yes

Executive summary

The objective of this study was to determine the effect of fluopicolide on the growth of the freshwater diatom *Navicula pelliculosa* over 96 h. Nominal concentrations were 0.0010, 0.0026, 0.0064, 0.016, 0.040 and 0.10 mg a.s./L. Additionally, water and solvent controls (dimethylformamide at 0.1 µL/mL) were included. The test comprised 3 replicates for each concentration control.
Samples analysed at 0-hour were removed from the test and control solutions prior to division into the replicate test vessels. Samples analysed at 96 hours of exposure were removed from individually composited replicate solutions of the treatment levels and controls. Samples were analysed by gas chromatographic analysis with electron capture detection (GC/ECD). Some recoveries were not in the range of 80 – 120% of nominal but the substance is stable over the test duration. Thus, biological results after 72 hours and 96 hours are based on arithmetic mean concentrations. The corresponding arithmetic mean measured concentrations (0 – 96 h): 0.0013, 0.0030, 0.0071, 0.018, 0.044 and 0.11 mg a.s./L. The study is not considered valid according to the OECD 201 guideline. Cells exposed to all treatment levels and the controls were observed to be normal. The 72 and 96 hours endpoints are: E_rC_{50} 72 hours: 0.069 mg a.s./L , E_bC_{50} 72 hours: 0.029 mg a.s./L. The recovery experiment performed with cultures exposed to 0.10 mg a.s./L showed that the effects of fluopicolide were algistatic and not algicidal.

I. MATERIAL AND METHODS:

Test material	AE C638206 (Fluopicolide) Lot No: 2050190/PP241024/2 Purity: 97.7% w/w
Guideline(s) adaptation	Evaluation of recovery was included in the study
Test species	Freshwater diatom (*Navicula pelliculosa* strain 667)
Culturing conditions	The AAP medium used to prepare the exposure solutions was formulated in the same manner as the culture medium. Culture conditions similar to testing conditions (e.g. 24 ± 2°C, continuous lighting at 3900 – 4700 lux)
Test solutions	Nominal concentrations: 0.0010, 0.0026, 0.0064, 0.016, 0.040 and 0.10 mg a.s./L Corresponding arithmetic mean measured concentrations (0 – 96 h): 0.0013, 0.0030, 0.0071, 0.018, 0.044 and 0.11 mg a.s./L Controls: water and solvent controls (dimethylformamide at 0.1 µL/mL) Evidence of undissolved material: No visible undissolved test substance observed in stock solution
Replication	No. of vessels per concentration (replicates): 3 No. of vessels per control (replicates): 3 No. of vessels per solvent control (replicates): 3
Exposure	Static Total exposure duration: 96 hours Recovery period: up to 9 days for concentration 0.10 mg a.s./L
Initial cell density	approx. 1×10^4 cells/mL at test initiation
Test conditions	Temperature: 23-24°C Photoperiod: continuous light Light intensity: 4000 to 4600 lux pH of controls and test solutions (0 – 96 h): 7.1 – 9.4 Conductivity: 90 to 100 µmhos/cm Type of light: artificial
Parameters Measured / Observations	Temperature was measured continuously in a flask of water adjacent to the test flasks in the environmental chamber. Minimum and maximum temperatures were recorded daily. Light intensity was measured at hour 0 and at each 24-hour interval during the exposure period. Water quality parameters (pH and conductivity) were measured at test initiation and at the termination of the 96-hour exposure period. At each subsequent 24-hour interval, cell counts were conducted on the three replicate vessels of the treatment and the control vessels using a hemacytometer and compound microscope. Recovery: the subcultures were microscopically examined every other day to determine whether or not cell growth had resumed. The subculture was discontinued after a substantial increase in cell density (i.e. > 10X) was observed.

Sampling for chemical analysis	At test initiation and test termination one sample was removed from each test solution and the controls for analysis of AE C638206 concentration. Samples analysed at 0-hour were removed from the test and control solutions prior to division into the replicate test vessels. Samples analysed at 96 hours of exposure were removed from individually composited replicate solutions of the treatment levels and controls. Samples were analysed by gas chromatographic analysis with electron capture detection. (GC/ECD).
Data analysis	EC_x values (e.g. x = 50) and confidence intervals were calculated for the standard exposure period, using a commercial program (TOXSTAT®).

II. RESULTS AND DISCUSSION:

Validity criteria (OECD 201, 2011)	Required	Obtained 72 h	Obtained 96 h
Increase of biomass in the control cultures	16	24.6	104
Mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3) in the control cultures	≤ 35%	64.3%	51.1%
The coefficient of variation of average specific growth rates in replicate control cultures	≤ 10%	6.08%	3.91%

The study is not considered valid according to the OECD 201 guideline (2011).

Analytical results:
Some recoveries were not in the range of 80 – 120% of nominal (see table below) but the substance is stable over the test duration. Thus, biological results after 72 hours and 96 hours are based on arithmetic mean concentrations.

Nominal Concentration (mg a.s./L)	Arithmetic mean measured concentrations after 96 hours (mg a.s./L)	% of nominal concentrations*	
		0-hour	96-hour
Control	-	0.000070**	<0.000054
Solvent control	-	<0.000053	<0.000054
0.0010	0.0013	130	130
0.0026	0.0030	112	119
0.0064	0.0071	108	114
0.0160	0.0180	113	113
0.0400	0.0440	115	108
0.1000	0.1100	120	99

* Values not given in study report; calculated on the basis of nominal concentrations and concentrations measured after 0 and 96 hours.
** A compound present in the matrix is believed to have co-eluted at the same retention time as the analyte and separation was not achieved.

The 96-hour sample from the 0.016 mg a.s./L (nominal) treatment level, without algae present, yielded a measured concentration of 0.017 mg a.s./L. The equivalent replicate 96-hour test solution with algae present was 0.018 mg a.s./L, indicating that the presence of algae in the test solution had no impact on the concentration of fluopicolide.

Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4 with minor acceptable exceptions only.

Biological results:

Cells exposed to all treatment levels tested and the controls were observed to be normal.

72 hours

Arithmetic mean measured concentrations (mg a.s./L)	**0-72 h average specific growth rates [days]**	**Inhibition of average specific growth rate [%]**
Control	1.09	-
Solvent control	1.01	-
Pooled control	1.05	-
0.0013	0.99	6
0.0030	1.04	1
0.0071	1.01	4
0.0180	0.94#	10
0.0440	0.70#	33
0.1100	0.24#	77

Significantly reduced compared to pooled control based on William's test

Arithmetic mean measured concentrations (mg a.s./L)	**Area under the growth curve (biomass integral)**	**Inhibition of Biomass integral (%)**
Control	190000	-
Solvent control	138300	-
Pooled control	164200	-
0.0013	128600	22
0.0030	143100	13
0.0071	126200	23
0.0180	106600#	35
0.0440	49400#	70
0.1100	15200#	91

Significantly reduced compared to pooled control based on William's test

96 hours

Arithmetic mean measured concentrations (mg a.s./L)	**Mean cell number after 96 h per mL**	**Inhibition of cell density [%]**
Control	1038300	-
Solvent control	914200	-
Pooled control	976300	-
0.0013	1006700	-3
0.0030	1095000	-12
0.0071	877500	10
0.0180	806700#	17
0.0440	402500#	59
0.1100	50000#	95

Significantly reduced compared to pooled control based on William's test

Recovery for algistatic/algicidal properties:

A sample was removed from the composite of the three replicates vessels of the 0.10 mg a.s./L test concentrations at test termination. The samples were then diluted with fresh medium to get subcultures with a nominal concentration of 0.0010 mg a.s./L that were incubated for 9 days. The subculture from 0.20×10^4 cells/mL to 15.8×10^4 cells/mL in 4 days (multiplication by 79). These observations indicate that fluopicolide has an algistatic, rather than an algicidal effect on *Navicula costatum*.

III. CONCLUSIONS:

The study does not meet the validity criteria and the 72- and 96-hour endpoints based on arithmetic mean measured concentrations are given in the table below. The 4-day recovery phase conducted at termination of the definitive test indicated that the effects of fluopicolide (AE C638206) were algistatic and not algicidal.

E_rC_{50} 72 hours (95% C.I.):	0.069 mg a.s./L (0.063 – 0.075 mg a.s./L)
LOE_rC 72 hours: lowest concentration with a significant effect compared to the control	0.0180 mg a.s./L
NOE_rC 72 hours: highest concentration without a significant effect compared to the control	0.0071 mg a.s./L
E_bC_{50} 72 hours (95% C.I.):	0.029 mg a.s./L (0.020 – 0.036 mg a.s./L)
LOE_bC 72 hours: lowest concentration with a significant effect	0.0180 mg a.s./L
NOE_bC 72 hours: highest concentration without a significant	0.0071 mg a.s./L
Cell density EC_{50} 96 hours (95% C.I.):	0.037 mg a.s./L (0.033 – 0.041 mg a.s./L)
Cell density LOEC 96 hours: lowest concentration with a significant effect compared to the control	0.0180 mg a.s./L
Cell density NOEC 96 hours: highest concentration without a significant effect compared to the control	0.0071 mg a.s./L

Assessment and conclusion by applicant:

The study is not valid. A valid study is submitted in the dossier.

Data Point:	KCA 8.2.6.2/04
Report Author:	[redacted]
Report Year:	2003
Report Title:	72-hour toxicity screening tests with the freshwater diatom (Navicula pelliculosa) Codes: AE C638206, AE C653711, AE C657188, AE 1344122
Report No:	149A-181
Document No:	M-225549-01-2
Guideline(s) followed in study:	OECD: 201 (1984); USEPA (=EPA): OPPTS 850.5400 (1996)
Deviations from current test guideline:	Current Guideline: OECD 201 (2011) The study validity cannot be assessed due to the lack of information in the report (screening study).
Previous evaluation:	yes, evaluated and accepted in DAR (2005)
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive summary

The purpose of this study was to evaluate the toxic effect of the parent compound fluopicolide in comparison to the toxic effect of the metabolites M-01 (AE C653711), M-02 (AE C657188) and M-05 (AE 1344122) on the freshwater diatom, *Navicula pelliculosa* in a static system for 72 h. Algal medium supplemented with silica and selenium was used for culturing and the test. Algae were kept under continuous light. Nominal concentrations for the parent compound were: 0.03 and 0.3 mg a.s./L and for the metabolites 0.03, 0.3 and 3.0 mg/L. Additionally, water and solvent controls (dimethylformamide at 0.1 µL/mL) were included. The test comprised 3 replicates for each concentration and control. Number of cells/mL were counted at 72 hours using a haemocytometer and a microscope. pH was measured on day 0 and day 4. No chemical analysis of test concentrations was done. The validity criteria of biomass increase in cultures was met according to OECD 201 guideline. Further validity criteria could not be calculated on the basis of the data given in the report.

The 72-hour EC_{50} for cell density for the parent compound fluopicolide is reported as 0.072 mg a.s./L, with 95% confidence limits of 0.046 and 0.114 mg a.s./L. The 72-hour EC_{50} value for cell density for the metabolites M-01 (AE C653711), M-02 (AE C657188) and M-05 (AE 1344122) are > 3.0 mg a.s./L, the highest test concentration. The available endpoint is based on cell count not on growth rate, area under the curve or yield.

I. MATERIAL AND METHODS:

Test material	Fluopicolide (AE C638206) M-01 (AE C653711 (BAM, 2,6-dichlorobenzamide)) M-02 (AE C657188 (PCA, 3-chloro-5-trifluoromethyl-pyridine-2-carboxylic acid)) M-05 (AE 1344122 (3-methylsulfinyl-5-trifluoro-methylpyridine-2-carboxylic acid)) No further data on test materials
Guideline(s) adaptation	None specified
Test species	Freshwater diatom (*Navicula pelliculosa*)
Culturing conditions	Medium used for culture and test: algal medium supplemented with silica and selenium. Algae were lighted with artificial (cool white fluorescent light), continuous illumination
Test solutions	Nominal concentrations (parent compound): 0.03 and 0.3 mg/L Nominal concentrations (metabolites): 0.03, 0.3 and 3.0 mg/L Controls: water and solvent control (dimethylformamide at 0.1 µL/mL) Evidence of undissolved material: All test solutions were clear and colorless
Replication	No. of vessels per concentration (replicates): 3 No. of vessels per control (replicates): 3 No. of vessels per solvent control (replicates): 3
Exposure	Static Total exposure duration: 72 hours
Initial cell density	approx. 1×10^4 cells/mL at test initiation
Test conditions	Temperature: 23.6 to 24.6°C Continuous light: 3890 – 4700 lux pH of controls and solutions (0 – 96 h): 7.5 – 9.0 Type of light: artificial (cool white fluorescent light)
Parameters Measured / Observations	Number of cells/mL at 72 hours using a haemocytometer and a microscope. pH measured on day 0 and day 4. Timing of further physical measurements not specified in study report.
Sampling for chemical analysis	Screening study: No chemical analysis of test concentrations.
Data analysis	Calculation of EC_{50} values and confidence limits not specified in study report.

II. RESULTS AND DISCUSSION:

Validity criteria (OECD 201, 2011)	Required	Obtained
Increase of biomass in the control cultures within the 72-hour test period.	16	139
Mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3) in the control cultures	≤ 35%	Cannot be calculated from the data in the report
Coefficient of variation of average specific growth rates during the 72-hour test period in replicate control cultures	≤ 10%	Cannot be calculated from the data in the report

Biological results:

Nominal test concentration (mg a.s./L)	Fluopicolide	
	Mean cell density after 72 hours	Percent inhibition*
Control	1388333	-
Solvent control	1126667	-
Pooled control	1257500	-
0.030	1175000	6.6
0.30	9000	99

* % inhibition relative to the pooled control

Nominal test concentration (mg a.s./L)	M-01 (AE C653711)		M-02 (AE C657188)		M-05 (AE 1344122)	
	Mean cell density after 72 hours	Percent inhibition*	Mean cell density after 72 hours	Percent inhibition*	Mean cell density after 72 hours	Percent inhibition *
Control	1388333		1388333	-	1388333	-
Solvent control	1126667		1126667	-	1126667	-
Pooled control	1257500	-	1257500	-	1257500	-
0.030	1088333	13	1050000	17	1073333	15
0.30	1110000	12	1060000	16	1065000	15
3.0	1128333	10	1098333	13	1045000	17

* % inhibition relative to the pooled control

III. CONCLUSIONS:

The validity criteria of biomass increase in cultures was met according to OECD 201 guideline. Further validity criteria could not be reviewed on the basis of the data given in the report. The 72 h endpoints based on nominal concentrations are given in the table below. The available endpoint is based on cell count not on growth rate, area under the curve or yield.

Endpoint	Fluopicolide	M-01 (AE C653711)	M-02 (AE C657188)	M-05 (AE 1344122)
72-hour EC_{50} (mg/L)	0.072	> 3.0	> 3.0	> 3.0
95% Confidence Limits (mg/L)	0.046 – 0.114	-*	-*	-*

* Confidence limits cannot be calculated with data obtained.

Assessment and conclusion by applicant:

The study is not acceptable and should not be used in risk assessment.

Data Point:	KCA 8.2.6.2/05
Report Author:	[redacted]
Report Year:	2003
Report Title:	A 96-hour toxicity test with the freshwater diatom Navicula pelliculosa Final Report AE C653711
Report No:	M-225556-01-2
Document No:	M-225556-01-2
Guideline(s) followed in study:	EU (=EEC): Method C3 (1992); OECD: 201 (1984); USEPA (=EPA): OPPTS 850.5400 (1996)
Deviations from current test guideline:	Method: Deviations from current guideline SANCO/3029/99 rev.4: Recoveries were determined at two different concentrations in triplicate. However, the obtained data demonstrate very good recoveries and the precision. The method can therefore be regarded as fit for purpose. Study: Current Guideline: OECD 201 (2011) The study does not meet the validity criteria
Previous evaluation:	yes, evaluated and accepted in DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Supportive only

Executive summary

The objective of this study was to determine the toxicity of M-01 (2,6-dichlorobenzamide) to the freshwater diatom, *Navicula pelliculosa,* over a 96-hour exposure period under static test conditions for 96 hours. Eight replicates were maintained in the control group and four replicate test chambers were maintained in each treatment group. M-01 (2,6-dichlorobenzamide) was applied at nominal concentrations of 0.43, 0.94, 2.07, 4.55 and 10 mg/L. Measured test concentrations were determined from samples of test medium collected from each treatment and control group at the beginning and end of the test. Samples were analysed by high performance liquid chromatography (HPLC) using UV detection. Measured concentrations were in the 80-120% range of nominal concentrations and no residues above the limit of quantification (LOQ) were measured in the controls. The arithmetic mean measured concentrations were: 0.43, 0.96, 2.08, 4.59 and 10 mg /L. The study does not fulfil the validity criteria of the current version of OECD 201 guideline. There were no noticeable changes in cell morphology in any of the tested concentrations when compared to the control. There was evidence of cell aggregation in all treatment levels including the controls. Because aggregation was observed in all treatment levels it was not considered to be treatment-related. The 72 and 96-hour E_bC_{50} and E_rC_{50} values are > 10 mg/L, the highest measured concentration tested. The 72- and 96-hour NOAEC for each growth parameter is 10 mg/L.

I. MATERIAL AND METHODS:

Test material	M-01 (2,6-dichlorobenzamide, AE C653711) Lot No: I8499A Purity 98 %
Guideline(s) adaptation	None specified
Test species	*Navicula pelliculosa* UTEX 667
Culturing conditions	Algal cells used in this test were obtained cultures that had been actively growing in culture medium for at least two weeks prior to test initiation. The culture was last transferred to fresh medium four days prior to test initiation. The algal cells were cultured and tested in freshwater algal medium.
Test solutions	Nominal concentrations: 0.43 - 0.94 - 2.07 - 4.55 - 10 mg/L Corresponding mean measured concentration: 0.43 - 0.96 - 2.08 - 4.59 - 10 mg /L Controls: culture medium (freshwater algal medium) All test solutions appeared clear and colourless
Replication	No. of vessels per concentration (replicates): 8 No. of vessels per control (replicates): 4
Exposure	Static Total exposure duration: 96 hours
Initial cells density	1×10^4 cells/mL in each test group
Test conditions	Temperature: 24.1 – 24.4°C Photoperiod: continuous light Light intensity at surface of test vessels: 3890 to 4700 lux pH: 7.5 – 7.8 Growth medium same as culture medium: Yes Type of light: cool white fluorescent tubes
Parameters Measured / Observations	Samples were collected at approximately 24-hour intervals during the 96-hour exposure and were held for a maximum of three days under refrigerated conditions sufficient to inhibit growth until cell counts could be performed. pH was measured at study start and end. Temperature was measured continuously in the environmental chamber and twice daily in a vessel adjacent to the test vessels.
Sampling for chemical analysis	Samples of test solutions were taken at test initiation (0 hour) and at test termination (96 hours) for analysis of AE C653711. Samples were analysed by high performance liquid chromatography (HPLC) using UV detection.
Data analysis	The calculation of cell densities, areas under the growth curve (biomass), growth rates and percent inhibition values, as well as all statistical analyses, were conducted using "The SAS System for Windows, Version 8.02. Non-linear regression was used to calculate EC_{50} values and their corresponding 95% confidence intervals for cell density (EC_{50}), biomass (E_bC_{50}) and growth rate (ErC_{50}) for each 24-hour exposure period, when possible. The data were evaluated for normality and homogeneity of variance (p=0.05) using the Shapiro-Wilk's and Levene's tests, respectively. The treatment groups then were compared to the negative control using Dunnett's test (p = 0.05). The results of the statistical analyses, as well as an evaluation of the concentration-response pattern, were used to determine the NOAEC relative to each parameter at 72 and 96 hours.

II. RESULTS AND DISCUSSION:

Validity criteria (OECD 201, 2011)	Required	Obtained
Increase of biomass in the control cultures	16	119
Mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3) in the control cultures	≤ 35%	43 %
Coefficient of variation of average specific growth rates in replicate control cultures	≤ 10%	0.34%

Analytical results:

Recoveries were 98 - 103% of nominal concentrations (see table below). The biological results are based on mean measured concentrations of M-01 (AE C653711). No residues of fluopicolide were measured in the controls above the limit of quantification (0.0 mg a.s./L).

Nominal Concentration (mg /L)	Mean Measured Concentration (mg /L)	0-hour % Nominal	96-hour % Nominal
0.43	0.43	102	97.6
0.94	0.96	103	102
2.07	2.08	102	99.1
4.55	4.59	102	100
10	10	102	102

Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Biological results:

Mean area under the growth curve (biomass) and percent inhibition

Mean measured concentrations (mg /L)	Inhibition % at 24 h	Inhibition % at 48 h	Inhibition % at 72 h*	Inhibition % at 96 h
0.43	-13	-17	-10	-6.4
0.96	-7.3	1.7	0.66	-0.052
2.08	-7.7	6.7	2.6	0.92
4.59	-10	-3.0	-0.066	1.2
10	-4.1	3.5	5.8	5.0

*No statistically significant differences ($p > 0.05$) at 72-hours from the negative control replicates using Dunnet`s test

Algae growth rate

Mean measured Concentrations (mg /L)	Inhibition % at 24 h	Inhibition % at 48 h	Inhibition % at 72 h*	Inhibition % at 96 h
0.43	-5.3	-4.2	-0.69	-0.33
0.96	-3.2	0.82	-0.27	-0.041
2.08	-3.3	2.5	-0.59	0.50
4.59	-4.6	-0.39	0.42	0.57
10	-1.9	1.3	1.6*	0.27

*Although statistically significant a 1.6% inhibition from the control was not considered to be treatment related since this amount of inhibition was considered to be within an acceptable limit for *Navicula*.

III. CONCLUSIONS:

The study is not considered to be valid. The endpoints based on arithmetic mean measured concentrations are:

E_rC_{50} 72 hours, 96 hours (95 % CI)	**> 10 mg /L (not applicable)**
E_bC_{50} 72 hours, 96 hours (95 % CI)	**> 10 mg /L (not applicable)**
NOAEC: highest concentration without adverse effects (based on biomass and growth rate)	10 mg /L

Assessment and conclusion by applicant:

The study is not acceptable and should not be used in risk assessment.

Data Point:	KCA 8.2.6.2/06
Report Author:	[redacted]
Report Year:	2003
Report Title:	A 96-hour toxicity test with the freshwater diatom Navicula pelliculosa Final Report AE 1344122
Report No:	M-225547-01-2
Document No:	M-225547-01-2
Guideline(s) followed in study:	EU (=EEC): Method C3 (1992); OECD: 201 (1984); USEPA (=EPA): OPPTS 850.5400 (1996)
Deviations from current test guideline:	Method: Deviations from current guideline SANCO/3029/99 rev.4: Recoveries were determined at two different concentrations in triplicate. However, the obtained data demonstrate very good recoveries and the precision. The method can therefore be regarded as fit for purpose. Study: Current Guideline: OECD 201 (2011) The study does not meet the validity criteria (see below)
Previous evaluation:	yes, evaluated and accepted in DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Supportive only

Executive summary

The objective of this study was to determine the toxicity of M-05 (AE 1344122, 3-methylsulfinyl-5-trifluoro-methylpyridine-2-carboxylic acid) to the freshwater diatom *Navicula pelliculosa,* over a 96-hour exposure period under static test conditions. Eight replicates were maintained in the control group and four replicate test chambers were maintained in each treatment group. The nominal test concentrations were 0.43, 0.94, 2.07, 4.55 and 10 mg/L. Additionally a negative control was included. Samples of test solutions were taken at test initiation (0 hour) and at test termination (96 hours) for analysis of 3-methylsulfinyl-5-trifluoromethylpyridine-2-carboxylic acid. Samples were analysed by high performance liquid chromatography (HPLC) using UV detection. Recoveries were in the range of 80 – 120% of nominal. Thus, biological results after 72 hours and 96 hours are based on nominal concentrations. The study does not fulfil one of the validity criteria of the current version of OECD 201 guideline. There were no noticeable changes in cell morphology in any of the tested concentrations when compared to the control. There was evidence of cell aggregation in all treatment levels including the controls. Because aggregation was observed in all treatment levels it was not considered to be treatment-related. In this study *Navicula pelliculosa* has been exposed to five test concentrations of M-05 (AE 1344122). The 72 and 96-hour E_bC_{50} and E_rC_{50} values, are > 10 mg/L, the highest measured concentration tested. The 72 and 96-hour NOAEC for each growth parameter is10 mg/L.

I. MATERIAL AND METHODS:

Test material	M-05 (3-methylsulfinyl-5-trifluoromethylpyridine-2-carboxylic acid) AE 1344122 Batch No: YG3228 Specification: not reported Purity 98.8 %
Guideline(s) adaptation	None specified
Test species	*Navicula pelliculosa* UTEX 667-
Culturing conditions	Algal cells used in this test were obtained from cultures that had been actively growing in culture medium for at least two weeks prior to test initiation. The culture was last transferred to fresh medium three days prior to test initiation. The algal cells were cultured and tested in freshwater algal medium
Test solutions	Nominal concentrations: 0.43 - 0.94 - 2.07 - 4.55 - 10 mg /L Corresponding mean measured concentration: 0.46 - 0.97 - 2.12 - 4.71 - 10 mg/L Controls: culture medium (freshwater algal medium)
Replication	No. of vessels per concentration (replicates): 8 No. of vessels per control (replicates): 4
Exposure	Static Total exposure duration: 96 hours
Initial cells density	1×10^4 cells/mL in each test group
Test conditions	Temperature: 24.0 - 24.4°C Photoperiod: continuous light Light intensity at surface of test vessels: 3890 to 4460 lux pH: 7.3 – 7.9 Growth medium same as culture medium: Yes Type of light: cool white fluorescent tubes
Parameters Measured / Observations	Samples were collected at approximately 24-hour intervals during the 96-hour exposure and were held for a maximum of three days under refrigerated conditions sufficient to inhibit growth until cell counts could be performed. pH was measured at study start and end. Temperature was measured continuously in the environmental chamber and twice daily in a vessel adjacent to the test vessels.
Sampling for chemical analysis	Samples of test solutions were taken at test initiation (0 hour) and at test termination (96 hours) for analysis of test substance. Samples were analysed by high performance liquid chromatography (HPLC) using UV detection.
Data analysis	The calculation of cell densities, areas under the growth curve (biomass), growth rates and percent inhibition values, as well as all statistical analyses, were conducted using "The SAS System for Windows", Version 8.02. Non-linear regression was used to calculate EC_{50} values and their corresponding 95% confidence intervals. The data were evaluated for normality and homogeneity of variance (p = 0.05) using the Shapiro-Wilk's and Levene's tests, respectively. The treatment groups then were compared to the negative control using Dunnett's test (p = 0.05). If the assumptions of normality and homogeneity of variances were not met an attempt was made to correct the condition by log transformation of the data. If the data failed the assumptions of normality and/or homogeneity of variances and transformations would not correct the problem Dunnett's test was still used to make the comparisons. The results of the statistical analyses, as well as an evaluation of the concentration-response pattern, were used to determine the NOAEC relative to each parameter at 72 and 96 hours.

II. RESULTS AND DISCUSSION:

Validity criteria (OECD 201, 2011)	Required	Obtained
Increase of biomass in the control cultures	16	115
Mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3) in the control cultures	≤ 35%	69 %
Coefficient of variation of average specific growth rates in replicate control cultures	≤ 10%	1.2 %

Analytical results:

Recoveries were 100 - 112% of nominal concentration (see table below). The biological results are based on nominal concentrations of M-05 (AE 1344122). No residues of M-05 (AE 1344122) were measured in the control above the limit of quantification (0.2 mg/L).

Nominal Concentration (mg /L)	Arithmetic mean Measured Concentration (mg /L)	0-hour % Nominal	96-hour % Nominal
0.43	0.46	112	102
0.94	0.97	106	102
2.07	2.12	104	100
4.55	4.71	106	101
10	10	107	102

Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Biological results:

Biomass

Nominal concentrations (mg /L)	Inhibition % at 24h	Inhibition % at 48 h	Inhibition % at 72 h*	Inhibition % at 96 h*
0.43	-4.5	-3.0	-1.7	-0.20
0.94	-1.9	1.3	1.5	0.50
2.07	10	-5.7	-6.0	-4.3
4.55	-6.7	-15	-10	-7.3
10	-4.1	-7.0	-5.1	-4.3

*No statistically significant differences ($p>0.05$) at 72-hours and 96-hours from the negative control replicates using Dunnett's test

Algae growth rate

Nominal concentrations (mg /L)	Inhibition % at 24h	Inhibition % at 48 h	Inhibition % at 72 h*	Inhibition % at 96 h*
0.43	1.5	-1.1	0.40	0.29
0.94	-0.23	0.85	0.22	-0.57
2.07	3.9	-2.5	-0.64	-0.30
4.55	-2.5	-3.6	-0.48	-0.93
10	-1.3	-1.8	-0.31	-1.1

*No statistically significant differences (p > 0.05) at 72-hours and 96-hours from the negative control replicates using Dunnet`s test

III. CONCLUSIONS:

The study is not considered to be valid. The endpoints based on nominal concentrations are:

E_rC_{50} 72 hours, 96 hours (95 % CI)	**> 10 mg /L (not applicable)**
E_bC_{50} 72 hours, 96 hours (95 % CI)	**> 10 mg /L (not applicable)**
NOEC: highest concentration without effects (based on biomass and growth rate)	10 mg /L

Assessment and conclusion by applicant:

The study is not acceptable and should not be used in risk assessment.

Data Point:	KCA 8.2.6.2/07
Report Author:	[redacted]
Report Year:	2015
Report Title:	Toxicity of fluopicolide technical to the saltwater diatom Skeletonema costatum during a 96 hour exposure
Report No:	007SRLS14C39
Document No:	M-533278-01-1
Guideline(s) followed in study:	EU Directive 91/414/EEC; Regulation (EC) No. 1107/2009; US EPA OCSPP 850.4500; OCSPP Guideline 850.4500 (2012), OECD Guideline 201 (2006). The afore-mentioned guidelines were harmonized for various test parameters (i.e. temperature, light, etc.) to achieve optimal environmental conditions for the test organism. Scientific discretion was implemented where guideline parameters do not fully converge
Deviations from current test guideline:	Method: none Study: Current Guidelines: OECD 201 (2011), OCSPP 850.4500 (2012) The validity is assessed according to criteria of both OECD 201 and OCSPP 850.4500. The test conditions are compared to OCSPP 850.4500 only since no information is available in OECD 201 regarding marine species. There is a slight deviation of light range: 4490-5100 lux instead of 3655-4945 lux recommended. However, all validity criteria are met so this deviation has no influence on the study results.
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive summary

A toxicity study was performed with saltwater diatom (*Skeletonema costatum*) under static conditions for 96 hours. The following nominal (mean measured) concentrations of fluopicolide were included in the study: 0.00147 (0.00139), 0.00470 (0.00463), 0.0150 (0.0135), 0.0481 (0.0446), and 0.154 (0.147) mg a.s./L. Additionally a control and solvent control was included. Number of replicates: 4 per toxicant level and 6 in the controls. Cell density counts were conducted daily in the control and all treatments till the end of the test. Algal cells were inspected with the help of a light microscope. Sampling was done on day 0 from batch preparation for each level and on day 3 and 4 from composite samples from each level. The analysis was performed by Liquid Chromatography-Mass Spectrometry/Mass Spectrometry (LC-MS/MS). Recoveries were in the range of 80 - 120% of nominal. Thus, biological results after 72 hours and 96 hours are based on nominal concentrations. The study meets the validity criteria of the current version of OECD 201 guideline. Cells exposed to all treatment levels tested and the controls were observed to be normal. The 72-hour E_rC_{50} value is 73.0 µg a.s./L with LOEC and NOEC values of 1.47 and < 1.47 µg a.s./L, respectively.

I. MATERIAL AND METHODS:

Test material	Fluopicolide (technical) Batch No: ETFP000273 Spec No: 10200001644401 Purity: 100.5% w/w
Guideline(s) adaptation	None specified
Test species	Saltwater diatom (*Skeletonema costatum*)
Culturing conditions	In-house 3-day old batch culture held under test conditions.
Test solutions	Nominal concentrations: 0.00147, 0.00470, 0.0150, 0.0481 and 0.154 mg a.s./L Corresponding arithmetic mean measured concentrations (0 – 96 h): 0.00139, 0.00463, 0.0135, 0.0446, 0.147 mg a.s./L Controls: water and solvent controls (dimethylformamide at 0.1 µL/mL) Evidence of undissolved material: No precipitates
Replication	No. of vessels per concentration (replicates): 4 No. of vessels per control (replicates): 6 No. of vessels per solvent control (replicates): 6
Exposure	Static Total exposure duration: 96 hours
Initial cell density	approx. 1×10^4 cells/mL at test initiation
Test conditions	Temperature: 20.4-21.1°C Photoperiod: 14 hours light, 10 hours dark Light intensity: 4490-5100 lux pH of controls (0 – 72 h): 8.2 to 8.3, range for test concentrations: 8.2-8.6 Salinity: 3‰ Growth medium same as culture medium: Yes, enriched saltwater medium Type of light: artificial
Parameters Measured / Observations	Temperature was measured continuously during the test. Salinity and pH were measured on day 0, 3 and 4. Cell density counts were conducted daily in the control and all treatments till the end of the test. Algal cells were inspected via light microscope.
Sampling for chemical analysis	Sampling on day 0 from batch preparation for each level and on day 3 and 4 from composite samples from each level. The analysis was performed by Liquid Chromatography-Mass Spectrometry/Mass Spectrometry (LC-MS/MS).
Data analysis	Shapiro-Wilks Test and Bartlett Equality of Variance Test were used to check data for normality and homogeneity, respectively. ANOVA followed by Wilcoxon/Bonferroni Adj. Test was used to determine the NOEC and LOEC. EC estimates were done by linear interpolation. Statistical software used was CETIS v1.8.7.4 Controls were pooled for the statistical analysis since they were not significantly different

II. RESULTS AND DISCUSSION:

Validity criteria (OECD 201, 2011)	Required	Obtained
Biomass increase in the control cultures over 72 h	16	19
Mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3) in the control cultures	≤ 35%	16%
Coefficient of variation of average specific growth rates during the 72-hour test period in replicate control cultures	≤ 10%	1.9%
Additional validity criteria acc. to OCSPP 850.4500 (2012)	**Required**	**Obtained**
Biomass increase in the control cultures over 96 h	30	85
Coefficient of variation for average specific growth rates during the 0 to 96-hour test period in replicate control cultures	< 15%	0.7%
Coefficient of variation for control yield during the 0 to 96-hour test period in replicate control cultures	< 15%	3.3%

Analytical results:

Recoveries were in the range of 80 – 120% of nominal (see table below). Thus, biological results after 72 hours and 96 hours are based on nominal concentrations. No residues of fluopicolide were measured in the controls above the limit of quantification (0.1 µg/L).

Nominal Concentration (mg a.s./L)	Arithmetic mean measured concentrations after 96 hours (mg a.s./L)	% of nominal concentrations*		
		0-hour	72-hour	96-hour
0.00147	0.00139	105	92	86
0.00470	0.00463	108	103	85
0.0150	0.0135	87	91	93
0.0481	0.0446	95	97	85
0.154	0.147	93	99	93

Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Biological results:

Cells exposed to all treatment levels tested and the controls were observed to be normal.

72 hours

Nominal concentration (mg a.s./L)	Mean growth rate (h^{-1})	Inhibition of average specific growth rate (%)
Control	0.042151	-
Solvent control	0.041840	-
0.00147	0.040592	3.3*
0.00470	0.040472	3.6*
0.0150	0.039976	4.8*
0.0481	0.037346	11.1*
0.154	-0.032106	176*

* Treatment group was significantly reduced when compared to pooled controls (Wilcoxon/Bonferroni Adj test; $p \leq 0.05$)

96 hours

Nominal concentration (mg a.s./L)	Mean growth rate (h^{-1})	Inhibition of average specific growth rate (%)
Control	0.046575	-
Solvent control	0.046364	-
0.00147	0.045636	1.8*
0.00470	0.044677	3.9*
0.0150	0.044795	3.6*
0.0481	0.040747	12.3*
0.154	-0.016859	136.3*

* Treatment group was significantly reduced when compared to pooled controls (Wilcoxon/Bonferroni Adj test; $p \leq 0.05$)

Nominal concentration (mg a.s./L)	Yield (cells/mL × 10^4)	Inhibition of yield (%)
Control	86.50	-
Solvent control	84.75	-
0.00147	79.00	7.7*
0.00470	71.94	16.0*
0.0150	72.75	15.8*
0.0481	49.00	42.8*
0.154	-0.98	101.1*

* Treatment group was significantly reduced when compared to pooled controls (Wilcoxon/Bonferroni Adj test; $p \leq 0.05$)

Nominal concentration (mg a.s./L)	Area under the growth curve	Inhibition of biomass integral (%)
Control	1684.9	-
Solvent control	1650.3	-
0.00147	1513.5	9.2*
0.00470	1405.4	15.7*
0.0150	1400.0	16.0*
0.0481	1047.6	37.2*
0.154	-49.4	103.0*

* Treatment group was significantly reduced when compared to pooled controls (Wilcoxon/Bonferroni Adj test; $p \leq 0.05$)

III. CONCLUSIONS:

The study meets the validity criteria and the 72- and 96-hour endpoints based on nominal concentrations are given in the table below.

E_rC_{50} 72 hours (95% C.I.):	0.073 mg a.s./L (0.0667-0.0798 mg a.s./L)
E_rC_{20} 72 hours (95% C.I.):	0.0538 mg a.s./L (0.0522– 0.0557 mg a.s./L)
E_rC_{10} 72 hours (95% C.I.):	0.0424 mg a.s./L (0.0330 – 0.0517 mg a.s./L)
E_rC_{05} 72 hours (95% C.I.):	0.016 mg a.s./L (0.00023-0.027 mg a.s./L)
LOE_rC 72 hours: lowest concentration with significant effect compared to the control	0.00147 mg a.s./L
NOE_rC 72 hours: highest concentration without significant effect compared to the control	< 0.00147 mg a.s./L
E_rC_{50} 96 hours (95% C.I.):	0.0803 mg a.s./L (0.08.6 – 0.102 mg a.s./L)
E_rC_{20} 96 hours (95% C.I.):	0.0547 mg a.s./L (0.0523 – 0.0593 mg a.s./L)
E_rC_{10} 96 hours (95% C.I.):	0.0392 mg a.s./L (0.0353 – 0.0428 mg a.s./L)
E_rC_{05} 96 hours (95% C.I.):	0.020 mg a.s./L (0.016-0.023 mg a.s./L)
LOE_rC 96 hours: lowest concentration with significant effect compared to the control	0.00147 mg a.s./L
NOE_rC 96 hours: highest concentration without significant effect compared to the control	< 0.00147 mg a.s./L
E_yC_{50} 96 hours (95% C.I.):	0.0612 mg a.s./L (0.0568 – 0.0651 mg a.s./L)
E_yC_{20} 96 hours (95% C.I.)	0.0205 mg a.s./L (0.0162 – 0.0236 mg a.s./L)
E_yC_{10} 96 hours (95% C.I.)	0.00241 mg a.s./L (0.000571 – 0.00410 mg
LOE_yC 96 hours: lowest concentration with significant effect compared to the control	0.00147 mg a.s./L
NOE_yC 96 hours: highest concentration without significant effect compared to the control	< 0.00147 mg a.s./L

E_bC_{50} 96 hours (95% C.I.):	0.0687 mg a.s./L (0.0654 – 0.0717 mg a.s./L)
E_bC_{20} 96 hours (95% C.I.)	0.0212 mg a.s./L (0.0152 – 0.0256 mg a.s./L)
E_bC_{10} 96 hours (95% C.I.)	0.00185 mg a.s./L (0.000807 – 0.00362 mg
LOE_bC 96 hours: lowest concentration with significant effect compared to the control	0.00147 mg a.s./L
NOE_bC 96 hours: highest concentration without significant effect compared to the control	< 0.00147 mg a.s./L

Assessment and conclusion by applicant:

The study is reliable and the 72h-E_rC_{50} of 0.073 mg a.s./L can be used in risk assessment.

The NOErC is < 0.00147 mg a.s./L based on 3.3% or 1.8% of effects at 0.00147 mg a.s/L at 72 and 96h, respectively. The biological relevance of such a low level of effects is questionable. Moreover, very similar inhibition, 3.8 and 3.9% are observed at the next concentration. Therefore, this is representative of biological variability.

Bayer performed 18 other studies showing effects in the same laboratory between December 2003 and July 2018. 17 of these studies showed at least 5.0% of effects at the LOEC. Only one performed in April 2015 (the study performed just after the study on fluopicolide) showed a similar profile with significant effects at 2.1 and 3.3% of inhibition. The median values for the inhibition at the LOEC were 14% and 11% at 72 and 96h respectively (ranges: 3.3 – 67.9% at 72h and 2.1 – 53.8 at 96h). This demonstrates that the NOEC determined in the study on fluopicolide, was artificially low.

Consequently, EC_{05} values were calculated in the report and are considered more biologically relevant than the NOEC. The EC_{05} at 72h is 0.016 mg a.s./L, this endpoint is relevant for the PBT assessment.

Data Point:	KCA 8.2.6.2/08
Report Author:	[redacted]
Report Year:	2020
Report Title:	Fluopicolide (AEC638206): A 96-hour toxicity test with the freshwater diatom (Navicula pelliculosa)
Report No:	149P-120
Document No:	M-678011-01-1
Guideline(s) followed in study:	OECD 201 (2011); OCSPP 850.4500 (2012)
Deviations from current test guideline:	Method: none ; Study: Current Guideline: OECD 201 (2011) The pH in the control solution increased by greater than 1.5 units over the course of the study. The pH of the negative control solution was 9.8 at 72 hours and 9.0 at 96 hours. Although the pH in the control groups increased by greater than 1.5 units after 72 hours of exposure, the increases in pH documented for this study are commonly observed for this diatom species and all control validity criteria were achieved despite the increase in pH.
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The aim of the study was to determine the toxicity of fluopicolide (AE C638206) to the freshwater diatom (*Navicula pelliculosa*) expressed as NOEC, LOEC and EC_X for growth rate and yield. *Navicula pelliculosa* with an initial cells density of 10,000 cells/mL were exposed to fluopicolide for 96 hours. Nominal test concentrations were 0 (control and solvent control), 2.0, 5.5, 15, 43, and 120 µg a.s./L. The cell density in each replicate was counted at 24-hour intervals. The water samples were analysed with LC-MS/MS. Measured concentrations ranged from 91.0 to 103% of nominal concentrations. After 96 hours the mean measured concentrations were 1.8, 5.3, 15, 43 and 118 µg a.s./L. No control and solvent control contaminations were detected. The reported results were based on 0-96 hour mean measured concentrations of fluopicolide. The 72-hour and 96-hour E_rC_{50} were calculated to be 121 µg a.s./L and >118 µg a.s./L, respectively. The 72-hour and 96-hour NOE_rC were both determined to be 43 µg a.s./L.

I. MATERIAL AND METHODS:

Test material	Fluopicolide (AEC638206) Batch code: AE C638206-01-27 Purity: 98.7% Specification: 102000016444
Guideline(s) adaptation	None
Test species	Freshwater diatom (*Navicula pelliculosa*)
Culturing conditions	Algal cells used in this test have been actively growing in culture medium under the same environmental conditions as used in this test for at least two weeks prior to test initiation.
Test solutions	Test medium: freshwater AAP medium with silica constituents Nominal concentrations: 0 (control and solvent control, 2.0, 5.5, 15, 43, and 120 µg a.s./L Corresponding mean measured concentrations 0–96 hours: < LOQ (control and solvent control), 1.8, 5.3, 15, 43 and 118 µg a.s./L Control: untreated test medium Solvent control: *N,N*-dimethylformamide (0.1 mL/L) Evidence of undissolved material: No particulates or surface-slicks were observed in any experimental group.
Replication	No. of vessels per concentration (replicates): 4 No. of vessels per control (replicates): 8 No. of vessels per solvent control (replicates): 4
Exposure	Test type: static Total exposure duration: 96 hours
Initial cells density	Approx. 10,000 cells/mL cells/mL. Algal cells for this study were taken from a culture that had been transferred to fresh medium three days prior to test initiation.
Test conditions	Temperature: 22.0–22.5 °C Photoperiod: continuous light Light intensity: 3880–4280 lux Type of light: cool white fluorescent lighting pH: 7.3–9.8 pH in controls: 7.3 – 9.8 (over 72 h)
Parameters Measured Observations	Cell density was determined at 24-hour intervals using an electronic particle counter. Cells were also assessed for atypical morphology (e.g., changes in cell shape, size or color), aggregation or flocculation, and adherence to the test chamber. The pH of the medium in each treatment and control group was measured at test initiation, at 72 hours, and at exposure termination. The temperature of a container of water adjacent to the test chambers in the environmental chamber was measured continuously. Light intensity was measured at test solution level at nine locations surrounding the test flasks at test initiation.
Sampling for chemical analysis	Samples were collected at approximately 0, 72, and 96 hours. At test initiation (0 hour), samples were collected for each treatment and control group prior to distribution into test chambers. At 72 hours, samples were collected from surrogate replicates included in each treatment and control group. At test termination (96 hours), the remaining replicates from each respective treatment and control group were pooled and then sampled. The samples were analysed with by high performance liquid chromatography with tandem mass spectrometric detection (LC/MS/MS)

Data analysis	Average specific growth rate (r), area under the growth curve (biomass) and yield (y), were calculated for each test flask. EC_x-values: E_rC_x and E_yC_x values and their corresponding 95% confidence intervals were calculated at 72 and 96 hours of exposure, when possible, using non-linear regression with treatment response (growth rate, area under the growth curve and yield) and exposure concentration data (0–96 hour mean measured concentrations). NOEC and LOEC values: The 72- and 96-hour growth rate and yield data were evaluated for normality and homogeneity of variance ($\alpha = 0.01$) using Shapiro Wilk's and Levene's tests, respectively. The treatment group means for the datasets which met assumptions of normality and homogeneity of variance were compared to the pooled control using Dunnett's test ($\alpha = 0.05$). A non-parametric test, Wilcoxon test with Bonferroni-Holm correction ($\alpha = 0.05$) was used to evaluate the treatment group means for the datasets which did not meet the assumptions of normality or homogeneity of variance.

II. Results and Discussion:

Validity criteria:

Validity criteria (OECD 201, 2011)	Required	Obtained
Biomass in the control within the 72-hour test period	≥ 16	269
Mean coefficient of variation for section-by-section specific growth rates in the control within the 72-hour test period	≤ 35%	27.6%
Coefficient of variation of average specific growth rates within 72-hour test period in control replicates	≤ 10%	1.9%

Analytical results:

Recoveries ranged from 91.0 to 101% of nominal concentrations after 72 h and from 92.4 to 100% of nominal concentrations after 96 h (see table below). Results of the study are based on mean measured fluopicolide concentrations.

No residues of fluopicolide were measured in the control and solvent control samples above the limit of quantification (1.25 µg a.s./L).

Nominal conc. [µg a.s./L]	Day 0 (New)		Day 3 (Old)		Day 4 (Old)		Mean measured conc. [µg a.s./L]	Mean % of nominal
	Measured conc. [µg a.s./L]	% of nominal	Measured conc. [µg a.s./L]	% of nominal	Measured conc. [µg a.s./L]	% of nominal		
2.0	1.87	93.7	1.82	91.0	1.85	92.4	1.8	92.3
5.5	5.20	94.6	5.23	95.1	5.47	99.5	5.3	96.4
15	15.4	103	15.2	101	15.1	100	15	101
43	42.9	99.8	43.3	101	42.8	99.6	43	100
120	118	98.1	120	100	117	97.1	118	98.3

Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Biological results:

There were no noticeable changes in cell morphology in any treatment group when compared to the control replicates during the microscopic examinations of the cells. Cells present in all fluopicolide treatment groups and in the solvent control on days 1, 2, 3, and 4, appeared normal when compared to cells present in the negative control. Adherence of cells to the test chambers was not observed in any of the experimental groups. Flocculation or aggregation of cells was not observed in the control groups or any of the Fluopicolide treatment groups.

% Inhibition of growth rate and yield

Mean measured concentration [µg a.s./L]	Growth rate inhibition [%]		Yield inhibition [%]	
	72 h	96 h	72 h	96 h
1.8	0	0	1	-1
5.3	-4	0	-29	1
15	0	0	-1	-2
43	1	-1	6	-6
118	48 a)	32 b)	93 a)	85 b)

a) Treatment group mean was significantly reduced (Dunnett's test; $p < 0.05$) when compared to the pooled control response.
b) Treatment group mean was significantly reduced (Wilcoxon/Bonferroni-Holm test; $p < 0.05$) when compared to the pooled control response.

III. CONCLUSIONS:

The endpoints based on mean measured concentrations are:

Results – 0 to 72 hours	
E_rC_{50} - 72 hours (95% CI):	**121 µg a.s./L* (118 to 124)**
E_rC_{20} - 72 hours (95% C.I.):	79 µg a.s./L (71 to 89)
E_rC_{10} - 72 hours (95% C.I.):	64 µg a.s./L (54 to 76)
LOEC - 72 hours: lowest concentration with an effect (based on growth rate)	118 µg a.s./L
NOEC - 72 hours: highest concentration without an effect (based on growth rate)	43 µg a.s./L
E_yC_{50} - 72 hours (95% CI):	68 µg a.s./L (58 to 79)
E_yC_{20} - 72 hours (95% C.I.):	50 µg a.s./L (40 to 62)
E_yC_{10} - 72 hours (95% C.I.):	42 µg a.s./L (32 to 55)
LOEC - 72 hours: lowest concentration with an effect (based on yield)	118 µg a.s./L
NOEC - 72 hours: highest concentration without an effect (based on yield)	43 µg a.s./L

Results – 0 to 96 hours	
E_rC_{50} - 96 hours (95% CI):	**>118 µg a.s./L (NA)**
E_rC_{20} - 96 hours (95% C.I.):	113 µg a.s./L (113 to 114)
E_rC_{10} - 96 hours (95% C.I.):	110 µg a.s./L (110 to 111)
LOEC - 96 hours: lowest concentration with an effect (based on growth rate)	118 µg a.s./L
NOEC - 96 hours: highest concentration without an effect (based on growth rate)	43 µg a.s./L
E_yC_{50} - 96 hours (95% CI):	105 µg a.s./L (103 to 106)
E_yC_{20} - 96 hours (95% C.I.):	95 µg a.s./L (94 to 96)
E_yC_{10} - 96 hours (95% C.I.):	90 µg a.s./L (89 to 91)
LOEC - 96 hours: lowest concentration with an effect (based on yield)	118 µg a.s./L
NOEC - 96 hours: highest concentration without an effect (based on yield)	43 µg a.s./L

CI = confidence interval; NA = not applicable
* Extrapolated value. The 72-hour E_rC_{50} estimate is greater than the highest concentration tested, however, the estimated was considered to be sensible based on evaluation of the dose response and the precision of the 95% confidence interval.

Assessment and conclusion by applicant:

The study is reliable and the 72h-E_rC_{50} of 0.121 mg a.s./L can be used in risk assessment.

Data Point:	KCA 8.2.6.2/09
Report Author:	[redacted]
Report Year:	2020
Report Title:	BCS-AB43478: A 96-hour toxicity test with the freshwater diatom (Navicula pelliculosa)
Report No:	149P-121
Document No:	M-678012-01-1
Guideline(s) followed in study:	OECD 201 (2011); OCSPP 850.4500 (2012)
Deviations from current test guideline:	Method: none; Study: Current Guideline: OECD 201 (2011) The pH of the negative control solution was 9.4 at 72 hours and 9.6 at 96 hours. Although the pH in the control groups increased by greater than 1.5 units after 72 hours of exposure, the increases in pH documented for this study are commonly observed for this diatom species and all control validity criteria were achieved despite the increase in pH.
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The aim of the study was to determine the toxicity of M-02 (BCS-AB43478) to the freshwater diatom (*Navicula pelliculosa*) expressed as NOEC, LOEC and EC_x for growth rate and yield. *Navicula pelliculosa* with an initial cells density of 10,000 cells/ml were exposed to M-02 (BCS-AB43478) for 96 hours. Nominal test concentrations were 0 (control), 1.0, 2.6, 6.4, 16, 40 and 100 mg p.m./L. The cell density in each replicate was counted at 24-hour intervals. The water samples were analysed with LC-MS/MS. Measured concentrations ranged from 97.4 to 113% of nominal concentrations. After 96 hours the mean measured concentrations were 1.1, 2.7, 6.6, 16, 42 and 107 mg p.m./L. No control contaminations were detected. The reported results were based on 0-96 hour mean measured concentrations of M-02 (BCS-AB43478). The 72-hour and 96-hour E_rC_{50} were calculated to be 74 mg p.m./L and 106 mg p.m./L, respectively. The 72-hour and 96-hour NOE_rC were both determined to be 42 mg p.m./L.

I. MATERIAL AND METHODS:

Test material	M-02 (BCS-AB43478) Batch code: AE C657188-PU-02 Purity: 99.9%
Guideline(s) adaptation	None
Test species	Freshwater diatom (*Navicula pelliculosa*)
Culturing conditions	Algal cells used in this test have been actively growing in culture medium under the same environmental conditions as used in this test for at least two weeks prior to test initiation.
Test solutions	Test medium: freshwater AAP medium with silica constituents Nominal concentrations: 0 (control), 1.0, 2.6, 6.4, 16, 40 and 100 mg p.m./L Corresponding mean measured concentrations 0–96 hours: <LOQ (control), 1.1, 2.7, 6.6, 16, 42 and 107 mg p.m./L Controls: untreated test medium Evidence of undissolved material: All test solutions appeared clear and colourless, with no particulates or surface-slicks visible.
Replication	No. of vessels per concentration (replicates): 4 No. of vessels per control (replicates): 8
Exposure	Test type: static Total exposure duration: 96 hours
Initial cells density	Approx. 10,000 cells/mL cells/mL Algal cells for this study were taken from a culture that had been transferred to fresh medium three days prior to test initiation
Test conditions	Temperature: 21.0–23.0 °C Photoperiod: continuous light Light intensity: 3910–4640 lux Type of light: cool white fluorescent lighting pH: 3.7–9.8, pH in controls: 7.2-8.9 (over 72 hr) Introduction of BCS-AB43478 to the test medium at a nominal concentration of 100 mg p.m./L significantly reduced the pH of the test medium. The pH was not adjusted in order to provide the most conservative estimate of toxicity and the most realistic exposure scenario. Physical toxicity is highly likely at these pH levels, however, this was considered to be a treatment related effect since the test substance caused the reduction in pH.
Parameters Measured / Observations	Cell density was determined at 24-hour intervals using an electronic particle counter. Cells were also assessed for atypical morphology (e.g., changes in cell shape, size or color), aggregation or flocculation, and adherence to the test chamber. The pH of the medium in each treatment and control group was measured at test initiation, at 72 hours, and at exposure termination. The temperature of a container of water adjacent to the test chambers in the environmental chamber was measured continuously. Light intensity was measured at test solution level at nine locations surrounding the test flasks at test initiation
Sampling for chemical analysis	Samples were collected at approximately 0, 72, and 96 hours. At test initiation (0 hour), samples were collected for each treatment and control group prior to distribution into test chambers. At 72 hours, samples were collected from surrogate replicates included in each treatment and control group. At test termination (96 hours), the remaining replicates from each respective treatment and control group were pooled and then sampled. The samples were analysed with by high performance liquid chromatography with tandem mass spectrometric detection (LC/MS/MS)

Data analysis	Average specific growth rate (r), area under the growth curve (biomass) and yield (y), were calculated for each test flask. EC_x-values: E_rC_x and E_yC_x values and their corresponding 95% confidence intervals were calculated at 72 and 96 hours of exposure, when possible, using non-linear regression with treatment response (growth rate, area under the growth curve and yield) and exposure concentration data (0–96 hour mean measured concentrations). NOEC and LOEC values: The 72- and 96-hour growth rate and yield data were evaluated for normality and homogeneity of variance ($\alpha = 0.01$) using Shapiro Wilk's and Levene's tests, respectively. The treatment group means for the datasets which met assumptions of normality and homogeneity of variance were compared to the control using Dunnett's test ($\alpha = 0.05$). A non-parametric test, Jonckheere-Terpstra Step-Down Trend test ($\alpha = 0.05$) was used to evaluate the treatment group means for the datasets which did not meet the assumptions of normality or homogeneity of variance

II. Results and Discussion:

Validity criteria:

Validity criteria (OECD 201, 2011)	Required	Obtained
Biomass in the control within the 72-hour test period	≥ 16	323
Mean coefficient of variation for section-by-section specific growth rates in the control within the 72-hour test period	≤ 35%	25.5%
Coefficient of variation of average specific growth rates within 72-hour test period in control replicates	≤ 10%	0.60%

Analytical results:

Recoveries ranged from 104 to 113% of nominal concentrations after 72 h and from 97.4 to 113% of nominal concentrations after 96 h (see table below). Results of the study are based on mean measured concentrations of M-02 (BCS-AB43478).

No residues of M-02 (BCS-AB43478) were measured in the control samples above the limit of quantification (0.500 mg p.m./L).

Nominal conc. [mg p.m./L]	Day 0 (New)		Day 3 (Old)		Day 4 (Old)		Mean measured conc. [mg p.m./L]	Mean % of nominal
	Measured conc. [mg p.m./L]	% of nominal	Measured conc. [mg p.m./L]	% of nominal	Measured conc. [mg p.m./L]	% of nominal		
1.0	1.04	104	1.13	113	1.13	113	1.1	110
2.6	2.60	99.8	2.74	105	2.78	107	2.7	104
6.4	6.35	99.3	6.74	105	6.75	105	6.6	103
16	15.6	97.5	16.7	104	15.6	97.4	16	99.8
40	40.9	102	44.6	111	41.6	104	42	106
100	104	104	112	112	104	104	107	107

Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Biological results:

Cells present in the 1.1, 2.7, 6.6, 16, and 42 mg p.m./L treatment groups on days 1, 2, 3, and 4, appeared normal when compared to cells present in the negative control. Lysed cells were observed in the 107 mg p.m./L treatment group on days 1-4. Adherence of cells to the test chambers was not observed in any of the experimental groups. Aggregation of cells was observed in the control and the 1.1, 2.7, 6.6, 16, and 42 mg p.m./L treatment groups. This is a normal observation for this diatom species, and this was not considered to be a treatment related effect since it was also noted in the control. A lack of aggregation in the 107 mg p.m./L treatment group was considered to be a treatment related effect but was also related to reduced cell densities relative to the other experimental groups.

% Inhibition of growth rate and yield

Mean measured conc. [mg p.m./L]	Growth rate inhibition [%]		Yield inhibition [%]	
	72 h	96 h	72 h	96 h
1.1	0	1	1	3
2.7	0	2	-3	11 b)
6.6	0	1	-3	6
16	0	0	-2	2
42	1	0	5	0
107	100 a)	80 a)	100 a)	99 b)

a) Treatment group mean was significantly reduced (Jonckheere-Terpstra Step-Down Trend test; $p = 0.05$) when compared to the control response.
b) Treatment group mean was significantly reduced (Dunnett's test; $p = 0.05$) when compared to the control response.

III. CONCLUSIONS:

The endpoints based on mean measured concentrations are:

Results – 0 to 72 hours	
E_rC_{50} - 72 hours (95% CI):	**74 mg p.m./L (74 to 74)**
E_rC_{20} - 72 hours (95% C.I.):	54 mg p.m./L (54 to 55)
E_rC_{10} - 72 hours (95% C.I.):	48 mg p.m./L (47 to 49)
LOEC - 72 hours: lowest concentration with an effect (based on growth rate)	107 mg p.m./L
NOEC - 72 hours: highest concentration without an effect (based on growth rate)	42 mg p.m./L
E_yC_{50} - 72 hours (95% CI):	72 mg p.m./L (70 to 75)
E_yC_{20} - 72 hours (95% C.I.):	51 mg p.m./L (46 to 55)
E_yC_{10} - 72 hours (95% C.I.):	44 mg p.m./L (35 to 49)
LOEC - 72 hours: lowest concentration with an effect (based on yield)	107 mg p.m./L
NOEC - 72 hours: highest concentration without an effect (based on yield)	42 mg p.m./L

Results – 0 to 96 hours	
E_rC_{50} - 96 hours (95% CI):	106 mg p.m./L (106 to 106)
E_rC_{20} - 96 hours (95% C.I.):	89 mg p.m./L (88 to 90)
E_rC_{10} - 96 hours (95% C.I.):	86 mg p.m./L (85 to 87)
LOEC - 96 hours: lowest concentration with an effect (based on growth rate)	107 mg p.m./L
NOEC - 96 hours: highest concentration without an effect (based on growth rate)	42 mg p.m./L
E_yC_{50} - 96 hours (95% CI):	106 mg p.m./L (106 to 106)
E_yC_{20} - 96 hours (95% C.I.):	83 mg p.m./L (81 to 85)
E_yC_{10} - 96 hours (95% C.I.):	45 mg p.m./L (42 to 48)
LOEC - 96 hours: lowest concentration with an effect (based on yield)	107 mg p.m./L
NOEC - 96 hours: highest concentration without an effect (based on yield)	42 mg p.m./L

CI = confidence interval

Assessment and conclusion by applicant:

The study is reliable and the 72h-E_rC_{50} of 74 mg p.m./L for M-02 can be used in risk assessment.

Data Point:	KCA 8.2.6.2/10
Report Author:	[redacted]
Report Year:	2020
Report Title:	BCS-AA65784: A 96-hour toxicity test with the freshwater Diatom (Navicula pelliculosa)
Report No:	EBAC0075
Document No:	M-678377-01-1
Guideline(s) followed in study:	OECD 201 (2011); OCSPP 850.4500 (2012)
Deviations from current test guideline:	Method: none; Study: Current Guideline: OECD 201 (2011) The pH of the negative control solution was 9.4 at 72 hours and 9.6 at 96 hours. Although the pH in the control groups increased by greater than 1.5 units after 72 hours of exposure, the increases in pH documented for this study are commonly observed for this diatom species and all control validity criteria were achieved despite the increase in pH.
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The aim of the study was to determine the toxicity of M-01 (BCS-AA65784) to the freshwater diatom (*Navicula pelliculosa*) expressed as NOEC, LOEC and EC_X for growth rate and yield. *Navicula pelliculosa* with an initial cells density of 10,000 cells/ml were exposed to M-01 (BCS-AA65784) for 96 hours. Nominal test concentrations were 0 (control), 0.32, 1.0, 3.2, 10, 32 and 100 mg p.m./L. The cell density in each replicate was counted at 24-hour intervals. The water samples were analysed with LC-MS/MS. Measured concentrations ranged from 92.6 to 103% of nominal concentrations. After 96 hours the mean measured concentrations were 0.31, 0.97, 3.2, 9.6, 30 and 97 mg p.m./L. No control contaminations were detected. The reported results were based on 0-96 hour mean measured concentrations of M-01 (BCS-AA65784). The 72-hour and 96-hour E_rC_{50} were calculated to be 92 mg p.m./L and > 97 mg p.m./L, respectively. The 72-hour and 96-hour NOE_rC were both determined to be 30 mg p.m./L.

I. Material and Methods:

Test material	M-01 (BCS-AA65784) Batch code: AE C653711 00 1B96 001 CAS No.: 2008-58-4 Purity: 96.2%
Guideline(s) adaptation	None
Test species	Freshwater diatom (*Navicula pelliculosa*)
Culturing conditions	Algal cells used in this test have been actively growing in culture medium under the same environmental conditions as used in this test for at least two weeks prior to test initiation.
Test solutions	Test medium: freshwater AAP medium with silica constituents Nominal concentrations: 0 (control), 0.32, 1.0, 3.2, 10, 32 and 100 mg p.m./L Corresponding mean measured concentrations 0-96 hours: < LOQ (control), 0.31, 0.97, 3.2, 9.6, 30 and 97 mg p.m./L Controls: untreated test medium Evidence of undissolved material: All test solutions appeared clear and colourless. No particulates or surface-slicks were visible.
Replication	No. of vessels per concentration (replicates): 4 No. of vessels per control (replicates): 8
Exposure	Test type: static Total exposure duration: 96 hours
Initial cells density	Approx. 10,000 cells/mL cells/mL Algal cells for this study were taken from a culture that had been transferred to fresh medium three days prior to test initiation
Test conditions	Temperature: 21.3–22.1 °C Photoperiod: continuous light Light intensity: 3800–4690 lux Type of light: cool-white fluorescent lighting pH: 7.2–9.8
Parameters Measured / Observations	Cell density was determined at 24-hour intervals using an electronic particle counter. Cells were also assessed for atypical morphology (e.g., changes in cell shape, size or color), aggregation or flocculation, and adherence to the test chamber. The pH of the medium in each treatment and control group was measured at test initiation, at 72 hours, and at exposure termination. The temperature of a container of water adjacent to the test chambers in the environmental chamber was measured continuously. Light intensity was measured at test solution level at nine locations surrounding the test flasks at test initiation
Sampling for chemical analysis	Samples were collected at approximately 0, 72, and 96 hours. At test initiation (0 hour), samples were collected for each treatment and control group prior to distribution into test chambers. At 72 hours, samples were collected from surrogate replicates included in each treatment and control group. At test termination (96 hours), the remaining replicates from each respective treatment and control group were pooled and then sampled. The samples were analysed with by high performance liquid chromatography with tandem mass spectrometric detection (LC/MS/MS)

Data analysis	Average specific growth rate (r), area under the growth curve (biomass) and yield (y), were calculated for each test flask. EC_x-values: E_rC_x, , and E_yC_x values and their corresponding 95% confidence intervals were calculated at 72 and 96 hours of exposure, when possible, using non-linear regression with treatment response (growth rate, area under the growth curve and yield) and exposure concentration data (0–96 hour mean measured concentrations).EC_x-values: E_rC_x and E_yC_x values and their corresponding 95% confidence intervals were calculated at 72 and 96 hours of exposure, when possible, using non-linear regression with treatment response (growth rate, area under the growth curve and yield) and exposure concentration data (0-96 hour mean measured concentrations). NOEC and LOEC values: The 72- and 96-hour growth rate and yield data were evaluated for normality and homogeneity of variance ($\alpha = 0.01$) using Shapiro Wilk's and Levene's tests, respectively. The treatment group means for the datasets which met assumptions of normality and homogeneity of variance were compared to the pooled control using Dunnett's test ($\alpha = 0.05$). A non-parametric test, Wilcoxon test with Bonferroni-Holm correction ($\alpha = 0.05$) was used to evaluate the treatment group means for the datasets which did not meet the assumptions of normality or homogeneity of variance.

II. Results and Discussion:

Validity criteria:

Validity criteria (OECD 201, 2011)	**Required**	**Obtained**
Biomass in the control within the 72-hour test period	≥ 16	259
Mean coefficient of variation for section-by-section specific growth rates in the control within the 72-hour test period	≤ 35%	13.1%
Coefficient of variation of average specific growth rates within 72-hour test period in control replicates	≤ 10%	1.5%

Analytical results:

Recoveries ranged from 92.6 to 99.5% of nominal concentrations after 72 h and from 93.7 to 97.9% of nominal concentrations after 96 h (see table below). Results of the study are based on mean measured concentrations of M-01 (BCS-AA65784).

No residues of M-01 (BCS-AA65784) were measured in the control samples above the limit of quantification (0.100 mg p.m./L).

Nominal conc. [mg p.m./L]	**Day 0 (New)**		**Day 3 (Old)**		**Day 4 (Old)**		**Mean measured conc. [mg p.m./L]**	**Mean % of nominal**
	Measured conc. [mg p.m./L]	**% of nominal**	**Measured conc. [mg p.m./L]**	**% of nominal**	**Measured conc. [mg p.m./L]**	**% of nominal**		
0.32	0.314	98.2	0.312	97.5	0.301	94.2	0.31	97
1.0	0.993	99.3	0.957	95.7	0.953	95.3	0.97	97
3.2	3.29	103	3.13	97.8	3.06	95.6	3.2	100
10	9.74	97.4	9.62	96.2	9.54	95.4	9.6	96
32	30.1	94.1	29.6	92.6	30.0	93.7	30	94
100	94.5	94.5	99.5	99.5	97.9	97.9	97	97

Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Biological results:

There were no noticeable changes in cell morphology in any treatment group when compared to the control replicates during the microscopic examinations of the cells. Cells present in all M-01 (BCS-AA65784) treatment groups on days 1, 2, 3, and 4, appeared normal when compared to cells present in the negative control. Adherence of cells to the test chambers was not observed in any of the experimental groups. Flocculation or aggregation of cells was not observed in the control groups or any of the M-01 (BCS-AA65784) treatment groups.

% Inhibition of growth rate and yield

Mean measured conc. [mg p.m./L]	Growth rate inhibition [%]		Yield inhibition [%]	
	72 h	96 h	72 h	96 h
0.31	-2	0	-11	-1
0.97	-2	1	-10	4
3.2	2	0	11	-3
9.6	0	0	2	-2
30	3	0	16 [b)]	1
97	53 [a)]	31 [a)]	95 [b)]	84 [b)]

a) Treatment group mean was significantly reduced (Wilcoxon/Bonferroni-Holm test; $p < 0.05$) when compared to control response.
b) Treatment group mean was significantly reduced (Dunnett's test; $p < 0.05$) when compared to the control response.

III. Conclusions:

The endpoints based on mean measured concentrations are:

Results – 0 to 72 hours	
E_rC_{50} - 72 hours (95% CI):	**92 mg p.m./L (89 to 95)**
E_rC_{20} - 72 hours (95% C.I.):	55 mg p.m./L (49 to 61)
E_rC_{10} - 72 hours (95% C.I.):	42 mg p.m./L (35 to 49)
LOEC - 72 hours: lowest concentration with an effect (based on growth rate)	97 mg p.m./L
NOEC - 72 hours: highest concentration without an effect (based on growth rate)	30 mg p.m./L
E_yC_{50} - 72 hours (95% CI):	46 mg p.m./L (36 to 58)
E_yC_{20} - 72 hours (95% C.I.):	31 mg p.m./L (25 to 36)
E_yC_{10} - 72 hours (95% C.I.):	26 mg p.m./L (17 to 30)
LOEC - 72 hours: lowest concentration with an effect (based on yield)	30 mg p.m./L
NOEC - 72 hours: highest concentration without an effect (based on yield)	9.6 mg p.m./L

Results – 0 to 96 hours	
E_rC_{50} - 96 hours (95% CI):	**> 97 mg p.m./L (NA)**
E_rC_{20} - 96 hours (95% C.I.):	81 mg p.m./L (77 to 85)
E_rC_{10} - 96 hours (95% C.I.):	64 mg p.m./L (58 to 71)
LOEC - 96 hours: lowest concentration with an effect (based on growth rate)	97 mg p.m./L
NOEC - 96 hours: highest concentration without an effect (based on growth rate)	30 mg p.m./L
E_yC_{50} - 96 hours (95% CI):	66 mg p.m./L (62 to 71)
E_yC_{20} - 96 hours (95% C.I.):	48 mg p.m./L (42 to 54)
E_yC_{10} - 96 hours (95% C.I.):	40 mg p.m./L (35 to 47)
LOEC - 96 hours: lowest concentration with an effect (based on yield)	97 mg p.m./L
NOEC - 96 hours: highest concentration without an effect (based on yield)	30 mg p.m./L

CI = confidence interval; NA = not applicable

Assessment and conclusion by applicant:

The study is reliable and the 72h-E_rC_{50} of 92 mg p.m./L can be used in the M-01 (AE C653711 / BCS-AA65784) risk assessment.

Data Point:	KCA 8.2.6.2/11
Report Author:	[redacted]
Report Year:	2017
Report Title:	Solubility/stability study of BCS-AX86048 in algae test media
Report No:	EBAC0022
Document No:	M-611528-01-1
Guideline(s) followed in study:	none
Deviations from current test guideline:	No guideline available
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive summary

The objective of this study is to determine the technical feasibility of a study with the algae *Navicula* considering the known hydrolytic properties of the substance M-03 (BCS-AX86048 / AE 0608000) The solubility and stability of the test item were assessed in the usual conditions (medium type, duration, temperature, pH) of the OECD 201 test with diatoms. The 2 most common algae growth media i.e. AAP and OECD, both supplemented with silicates were used in this test. The initial pH of the media was 7.5 and 8.1 for AAP and OECD medium, respectively. Concentrations of 10 and 100 µg/L were tested, these concentrations were chosen based on the concentration range of the test on fluopicolide. Samples were taken after 10 seconds of stirring (t0), after 10, 30, 240 minutes and on day 1, 2, 3 and 4. The metabolite M03 was quantified by HPLC-MS/MS, the limit of quantification was set at 0.0625 µg/L. It was possible to solubilize the test item in *Navicula* test media with the help of DMF stock solutions, however, the test item is very unstable and (after 30 min only 20 to 27% of the nominal concentrations remain in the systems. As the algae test is performed in static conditions, it is not possible to carry out the test with the substance M-03 (BCS-AX86048 / AE 0608000) over 72h as requested by the OECD 201 guideline.

I. Material and Methods:

Test material	M-03 (BCS-AX86048, AE 0608000) Batch code: AE 060800 00 1B97 0001 Purity: 96.9 % w/w
Test solutions	10 and 100 µg/L in AAP and OECD media supplemented with silicates. Solvent: 0.1 µL/mL of dimethylformamide Test solutions: normal appearance
Exposure	4 days
Test conditions	As set in OECD 201 guideline for *Navicula*
Sampling for chemical analysis	After 10 sec of stirring (t0), 10, 30, 240 minutes and on day 1, 2, 3 and 4. The test item was quantified by HPLC-MS/MS.

II. RESULTS AND DISCUSSION:

Analytical results:

Measured concentrations (in µg/L) found in the samples are presented in the table below.

Sampling time	OECD medium		AAP medium	
	Nominal concentration: 10 µg/L	Nominal concentration: 100 µg/L	Nominal concentration: 10 µg/L	Nominal concentration: 100 µg/L
0	10.7	94.8	10.8	107
10 min	6.09	64.6	6.86	64.3
30 min	2.07	20.3	2.59	26.9
240 min	< LOQ	< LOQ	< LOQ	< LOQ
Day 1	< LOQ	< LOQ	< LOQ	< LOQ
Day 2	< LOQ	< LOQ	< LOQ	< LOQ
Day 3	< LOQ	< LOQ	< LOQ	< LOQ
Day 4	< LOQ	< LOQ	< LOQ	< LOQ

LOQ = 0.0625 µg/L

Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

III. CONCLUSIONS:

It was possible to solubilize the test item in *Navicula* test media with the help of DMF stock solutions, however, the test item is very unstable and after 30 min only 20 to 27% of the nominal concentrations remain in the systems. As the algae test is performed in static conditions, it is not possible to carry out the test with the substance M-03 (BCS_AX86048 / AE 0608000) over 72h as requested by the OECD 201 guideline. The DT_{50} and DT_{90} are approximately 15 and 45 minutes, respectively (see table below).

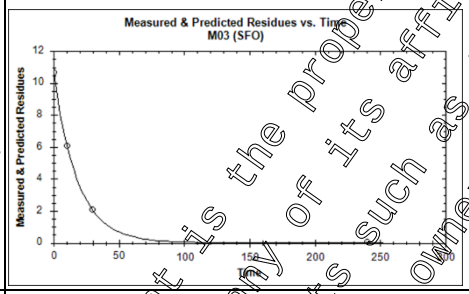
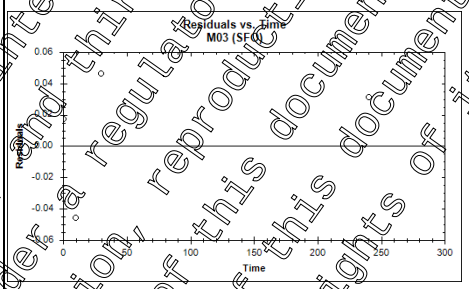
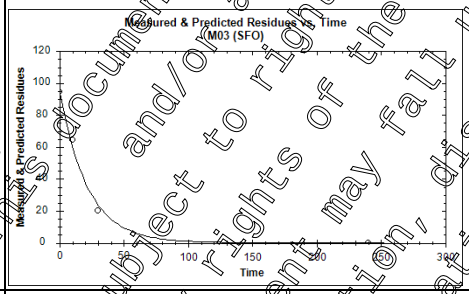
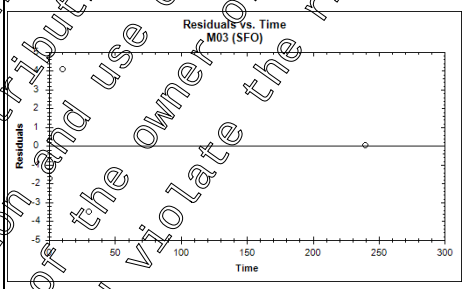
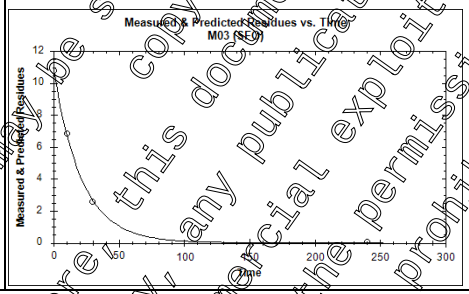
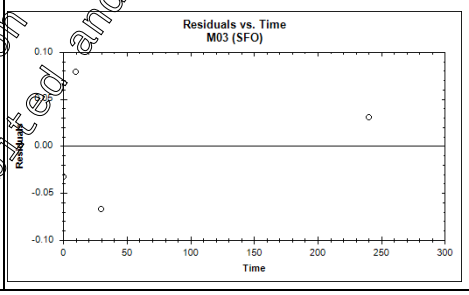
Test media	Kinetic model	M_0	Parameter (k, k1, k2, g, tb, α, β)	χ²-%-error	Prob > t	Lower CI	Upper CI	DT_{50} [minutes]	DT_{90} [minutes]
OECD 10 µg/L	**SFO**	**10.68**	**k 0.05545**	**0.64**	**7.60E-05**	**0.05411**	**0.05700**	**12.5**	**41.5**
OECD 10 µg/L	FOMC	10.70	α 18.78 β 328.22	0.33	n.r. n.r.	1.696 20.316	35.860 636.120	12.3	42.8
OECD 100 µg/L	**SFO**	**96.51**	**k 0.04670**	**5.13**	**0.005412**	**0.03710**	**0.05600**	**14.8**	**49.3**
OECD 100 µg/L	FOMC	96.51	α 1.679E+04 β 3.596E+05	6.41	n.r. n.r.	1.192E+04 3.594E+05	21665 359806	14.8	49.3
AAP 10 µg/L	**SFO**	**10.83**	**k 0.04684**	**0.91**	**0.000175**	**0.04513**	**0.04900**	**14.8**	**49.2**
AAP 10 µg/L	FOMC	10.83	α 9.876E+03 β 2.108E+05	1.14	n.r. n.r.	9.364E+03 2.108E+05	10387.9 210835.7	14.8	49.2

Test media	Kinetic model	M_0	Parameter (k, k1, k2, g, tb, α, β)	χ^2, %-error	Prob >t	Lower CI	Upper CI	DT_{50} [minutes]	DT_{90} [minutes]
AAP 100 µg/L	**SFO**	**106.3**	**k 0.04767**	**1.93**	**0.000780**	**0.04398**	**0.05100**	**14.5**	**48.3**
AAP 100 µg/L	FOMC	107.0	α 4.5585 β 84.713	0.22	n.r. n.r.	3.7377 67.644	5.379 101.782	13.9	55.7

N.B degradation rates (k) and DT_x values are presented in minutes.

Only four datapoints, thus evaluation of DFOP not possible. SFO considered acceptable for all datasets.

Plots and residuals for selected best fits.

Media Model Reference	Modelled vs observed	Residuals
OECD 10 µg/L SFO		
OECD 100 µg/L SFO		
AAP 10 µg/L SFO		

Assessment and conclusion by applicant:

The study is reliable and shows that a study on the most relevant test organisms (*Navicula*) is not technically feasible with metabolite M-03.

CA 8.2.7 Effects on aquatic macrophytes

Data Point:	KCA 8.2.7/01
Report Author:	[redacted]
Report Year:	2003
Report Title:	AE C638206 - 7-day toxicity test with duckweed (Lemna gibba)
Report No:	M-220201-01-2
Document No:	M-220201-01-2
Guideline(s) followed in study:	OECD: 221 (2000); USEPA (=EPA): OPPTS 850.4400 (1996)
Deviations from current test guideline:	Method: Deviations from current guideline SANCO/3029/99 rev.4: Limited sets of validation recoveries were analysed. However, the average recoveries were within the acceptable range of 70–140% and the RSD values were below 20%. The analytical method can be regarded as fit for purpose. Study: Current Guideline: OECD 221 (2006) The test was conducted with 15 fronds per replicates instead of 9-12 as in OECD 221 guideline, to be compliant with OCSPP 850.4400 guideline which requires 12 to 16 fronds. Since the validity criteria of the OECD guideline are met, this deviation had no impact on the study results.
Previous evaluation:	yes, evaluated and accepted in DAR (2003)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Data Point:	KCA 8.2.7/04
Report Author:	[redacted]
Report Year:	2018
Report Title:	Statement - Certificate of analysis for fluopicolide toxicity study on Lemna gibba (Hoberg, 2003; M-220201-01-1)
Report No:	M-634696-01-1
Document No:	M-634696-01-1
Guideline(s) followed in study:	--
Deviations from current test guideline:	Not applicable
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	Yes

Executive summary

The purpose of this study was to determine the effects of fluopicolide on the growth of the duckweed *Lemna gibba* under static conditions for 7 days. On days 3, 5 and at test termination (day 7), fronds were counted, and observations were made. At test termination, frond density for each replicate treatment and control vessel were determined in addition to dry weight. The nominal test concentrations were 0.16, 0.31, 0.63, 1.3, 2.5 and 5.0 mg a.s./L. Exposure solutions and QC samples were analysed for fluopicolide concentration using gas chromatography with electron capture detection (GC/ECD) Measured concentrations on day 0 and day 7 ranged between 64 and 98%. The highest concentration averaged 64% of nominal concentration which was believed to be the solubility limit of fluopicolide in 20X AAP medium. The results are based on arithmetic mean measured concentrations: 0.16, 0.28, 0.57, 1.0, 2.2 and 3.2 mg a.s./L. The study fulfils all validity criteria of the current version of OECD 221 guideline. On day 7, fronds exposed to all treatment levels tested and the controls were observed to be normal. Endpoints (Effect on mean frond number, effect on mean growth rate of frond number, and effect on mean dry weight) are based on arithmetic mean measured concentrations: EC_{50}: > 3.2 mg a.s./L, LOEC > 3.2 mg a.s./L and NOEC = 3.2 mg a.s./L.

I. Material and Methods:

Test material	Fluopicolide (AEC638206) Lot No. 2050190/PP241024/2 97.7 %
Guideline(s) adaptation	not specified
Test species	Duckweed (*Lemna gibba*) strain G3
Acclimation	Inoculum pre-culture, preparation 7 days before the start of the main test cultivation under the same conditions as in main test
Culturing conditions	20X AAP medium 6500 – 10000 lux temperature of 24 ± 2 °C pH 7.5 ± 0.1
Test solutions	Nominal concentrations: 0.16, 0.31, 0.63, 1.3, 2.5 and 5.0 mg a.s./L Mean measured concentrations: 0.16, 0.28, 0.57, 1.0, 2.2 and 3.2 mg a.s./L Control: untreated medium Solvent control: 0.1 mL/L Dimethylformamid (DMF) Evidence of undissolved material: not observed
Replication	No. of vessels per concentration (replicates): 3 No. of vessels per control (replicates): 3 No. of vessels per solvent control (replicates): 3
Organisms per replicate	No. of fronds per vessel: 15
Exposure	Static Total exposure duration: 7 days
Test conditions	vessels: 250 mL crystallizing dishes with 100 mL test solution Temperature: 23 °C to 24 °C Photoperiod: permanent light Light quality: fluorescent bulbs Light intensity: 7000 – 7800 lux pH: 7.7-8.9 Water hardness: not specified Dissolved oxygen: not specified Conductivity: not specified Growth medium: 20X AAP

Parameters Measured/ Observations	On days 3, 5 and at test termination (day 7), fronds were counted, and observations were made. At test termination, frond density for each replicate treatment and control vessel were determined. Fronds were counted and then removed from each vessel, blotted dry and transferred to pre-weighed aluminium pans. Fronds were dried in an oven at 67 to 69 °C for two days prior to dry weight determination. Temperature was measured continuously with a Fisher Scientific minimum/maximum thermometer located in a flask of water adjacent to the test vessels within the environmental chamber. Light intensity was measured with a General Electric type 214 light meter at 0 hour and at each subsequent 24-hour interval during the exposure period. The pH of all exposure solutions was measured at test initiation and at test termination.
Sampling for chemical analysis	On test day 0 (test initiation) and day 7, one sample was removed from each treatment and control solution to be analysed for fluopicolide concentration. Furthermore, three quality control (QC) samples were prepared at test initiation and termination and remained with the appropriate set of exposure solution samples throughout the analytical process. Exposure solutions and QC samples were analysed for fluopicolide concentration using gas chromatography with electron capture detection (GC/ECD) based on methodology validated at Springborn Smithers.
Data analysis	A t-test was conducted to statistically compare the growth rate of the control to the solvent control. For determination of the NOEC and LOEC, the data were first checked for normality using Shapiro-Wilks' Test and for homogeneity of variance using Bartlett's Test. If the data sets passed the tests for homogeneity and normality, then Williams' Test was used to determine the NOEC. If the data did not pass the tests for homogeneity and normality, then Kruskal-Wallis' Test was used to determine the NOEC. All statistical determinations were made at the 95% level of certainty, except in the case of Shapiro-Wilks and Bartlett's Tests, where the 99% level of certainty was applied. The EC_{05}, EC_{50} and EC_{90} values were calculated for frond densities, average growth rate and biomass at test termination. A computer program was used to perform both the statistical (LOEC and NOEC determinations) and EC_{05}, EC_{50} and EC_{90} calculations.

II. RESULTS AND DISCUSSION:

Validity criteria (OECD 221, 2006)	Required	Obtained
Doubling time	< 2.5 days	1.71 days

Analytical results:
Measured concentrations on day 0 and day 7 ranged between 64 and 98% and are presented below. The highest concentration averaged 64% of nominal concentration which was believed to be the solubility limit of AE C638206 in 20X AAP medium. The results are based on arithmetic mean measured concentrations.

Nominal Concentration (mg a.s./L)	Day 0 Measured Concentration (mg a.s./ L)	Day 7 Measured Concentration (mg a.s./ L)	Mean Measured Concentration[a] (mg a.s./ L)	Percent of nominal %
Control	< 0.0055	< 0.0055	-	-
Solvent control	< 0.0055	< 0.0055	-	-
0.16	0.16	0.15	0.16	98
0.31	0.29	0.28	0.28	91
0.63	0.53	0.60	0.57	90
1.3	0.97	1.1	1.0	81
2.5	2.4	1.9	2.2	87
5.0	3.2	3.2	3.2	64

[a] Calculated results are based on the original raw data and not the rounded results presented in this table

Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4 with minor acceptable exceptions only.

Biological results:

Observations

On day 7, fronds exposed to all treatment levels tested and the controls were observed to be normal.

Mean measured concentration [mg a.s./L]	Frond number on day 7[b] (SD)	7-day inhibition of frond number [a]	Mean growth rate for frond number (SD)	7-day inhibition of growth rate [a]	Mean dry weight (SD)	7-day inhibition of dry weight [a]
Control	257 (21)	NA	0.41 (0.01)	NA	0.044 (0.0069)	NAC
Solvent control	272 (42)	NA	0.42 (0.02)	NA	0.043 (0.0037)	NA
Pooled control	265 (31)	NA	0.41 (0.02)	NA	0.044 (0.005)	NA
0.16	260 (24)	1.8	0.41 (0.01)	0.0	0.045 (0.0042)	-3
0.28	278 (21)	-5.1	0.42 (0.01)	-2.4	0.048 (0.0065)	-10
0.57	294 (47)	-11	0.43 (0.02)	-4.9	0.046 (0.0089)	-6
1.0	272 (24)	-2.7	0.42 (0.01)	-2.4	0.042 (0.00082)	3
2.2	283 (23)	-6.9	0.42 (0.01)	-2.4	0.047 (0.0022)	-8
3.2	304 (37)	-15	0.43 (0.02)	-4.9	0.050 (0.0082)	-14

[a] Percent inhibition relative to pooled control, [b] Mean of 3 replicates, NA not applicable

III. CONCLUSIONS:

Endpoints were calculated based on arithmetic mean measured concentrations.
EC_{10} and EC_{20} cannot be calculated for this test because there is no dose response relationship.

The E_rC_{50} > 3.2 mg a.s./L and the NOE_rC of 3.2 mg a.s./L can be used in risk assessment. The calculated endpoints are not fully in line with the current OECD guideline, more specifically the yield of frond number and dry weight or the growth rate of dry weight are not available. They were not re-calculated since no effects were observed in this test, so they are all assumed to be > 3.2 mg a.s./L.

Endpoint (0-7 days)	Effect on mean frond number	Effect on mean growth rate of frond number	Effect on mean dry weight
EC_{50} (95% C.I.):	> 3.2 mg a.s./L (NA)	> 3.2 mg a.s./L (NA)	> 3.2 mg a.s./L (NA)
LOEC: lowest concentration with	> 3.2 mg a.s./L	> 3.2 mg a.s./L	> 3.2 mg a.s./L
NOEC: highest concentration	3.2 mg a.s./L	3.2 mg a.s./L	3.2 mg a.s./L

NA = not applicable

Assessment and conclusion by applicant:
The study is reliable and the E_rC_{50} > 3.2 mg a.s./L and the NOE_rC of 3.2 mg a.s./L can be used in risk assessment.

Data Point:	KCA 8.2.7/02
Report Author:	[redacted]
Report Year:	2003
Report Title:	AE C653711: A 7-day toxicity test with duckweed Lemna gibba G3
Report No:	149A-167A
Document No:	M-219725-01-2
Guideline(s) followed in study:	ASTM: 1415-91E (1991); OECD: 221 (2000); USEPA (EPA): OPPTS 850.4400 (1996)
Deviations from current test guideline:	Method: Deviations from current guideline SANCO/3029/99 rev.4: Limited sets of validation recoveries were analysed. However, the average recoveries were within the acceptable range of 70–110% and the RSD values were below 20%. The analytical method can be regarded as fit for purpose. Study: Current Guideline: OECD 221 (2006) The light intensity of 4500-5450 lux was lower than recommended in the OECD guideline 221, 2006 (6500-10000 lux) but was compliant with the OCSPP 850.4400 guideline (4200-6700 lux). Since the validity criteria are met, this deviation had no impact on the results of the test.
Previous evaluation:	yes, evaluated and accepted in DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Data Point:	KCA 8.2.7/03
Report Author:	[redacted]
Report Year:	2019
Report Title:	Statistical re-evaluation of a Lemna study performed with AE C653711 (BAM) (Desjardins et al. 2003; M-219725-01-1)
Report No:	M-664031-01-1
Document No:	M-664031-01-1
Guideline(s) followed in study:	--
Deviations from current test guideline:	Not applicable
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	Yes

Executive summary

Fronds of duckweed, *Lemna gibba* G3, were exposed to a geometric series of five test concentrations of M-01 (2,6-Dichlorobenzamide / AE C653711) and a negative control (culture medium) under static conditions for seven days. Three replicate test chambers were maintained in each treatment and control group. The nominal test concentrations were 13, 25, 50, 100 and 200 mg a.s./L. Growth, defined as an increase in the total number of fronds in each replicate test chamber, was determined through direct counts on Days 0, 3 and 5 during the test and at the end of the test (Day 7) for all treatment and control replicates. In addition, the total number of duckweed plants in each replicate test chamber was determined at test termination. Concentrations of M-01 (2,6-Dichlorobenzamide) in the samples were determined by high performance liquid chromatography with UV detection using an Agilent Model 1100 High Performance Liquid Chromatograph (HPLC) equipped with an Agilent Series 1100 Variable Wavelength Detector. The results of the study were based on mean measured test concentrations of 12, 25, 50, 101 and 203 mg a.s./L. The study fulfils all validity criteria of the current version of the OECD 221 guideline. In the highest treatment level, 100% of the test organisms were dead at the end of the study. Slight chlorotic effects (0.41-1.7%) were observed in the control and in the treatments with 12, 25 and 50 mg a.s./L. In the treatment with 101 mg a.s./L 23% of plants showed chlorosis. Slight signs of necrosis were observed in the control and the 50 mg a.s./L test concentration. In the treatment with 101 mg/L, 58% necrosis was observed. The E_bC_{50} are 97.6 mg a.s./L and 110.2 mg a.s./L for frond number and dry weight (biomass), respectively). The NOE$_b$C is 25.0 mg a.s./L for both frond number and dry weight.

I. MATERIAL AND METHODS:

Test material	M-01 (2,6-Dichlorobenzamide; AE C653711) Lot No. 48499A 98 %
Guideline(s) adaptation	not specified
Test species	Duckweed (*Lemna gibba*) strain G3
Acclimation	Lemna fronds had been actively growing in 20X AAP culture medium for at least two weeks prior to test initiation.
Culturing conditions	20X AAP medium pH 7.5 ± 0.1
Test solutions	Nominal concentrations: 13, 25, 50, 100 and 200 mg/L Mean measured concentrations: 12, 25, 50, 101 and 203 mg/L Control: untreated medium Evidence of undissolved material: not observed
Replication	No. of vessels per concentration (replicates): 3 No. of vessels per control (replicates): 3 No. of vessels per solvent control (replicates): 3
Organisms per replicate	No. of fronds per vessel: 12 No. of plants: 4
Exposure	Static Total exposure duration: 7 days

Test conditions	vessels: 250 mL glass beakers with 100 mL test solution Temperature: 24 °C to 25 °C Photoperiod: permanent light Light quality: warm white fluorescent lighting Light intensity: 4500 – 5450 lux pH: 7.5-8.9 Water hardness: not specified Dissolved oxygen: not specified Conductivity: not specified Growth medium: 20X AAP
Parameters Measured / Observations	Growth, defined as an increase in the total number of fronds in each replicate test chamber, was determined through direct counts on Days 0, 3 and 5 during the test and at the end of the test (Day 7) for all treatment and control replicates. In addition, the total number of duckweed plants in each replicate test chamber was determined at test termination. Observations of effects such as chlorosis, necrosis, dead fronds, root destruction and break-up of duckweed colonies were performed on Days 0, 3, and 5 during the test and at the end of the test (Day 7). Biomass (dry weight in milligrams) was determined at test termination. The temperature of a container of water adjacent to the test chambers in the environmental chamber was recorded twice daily during the test. Light intensity was measured at five locations surrounding the test chambers on Day 0. The pH of the medium in each treatment and control group was measured at test initiation and at test termination.
Sampling for chemical analysis	Samples of the test solutions were collected at test initiation and test termination. Samples at test initiation were collected from the individual batches of test solution prepared for each treatment and control group. At test termination, samples were collected from the pooled replicates from treatment groups and the control group. Concentrations of M-01 (AE C653711) in the samples were determined by high performance liquid chromatography with UV detection using an Agilent Model 1100 High Performance Liquid Chromatograph (HPLC) equipped with an Agilent Series 1100 Variable Wavelength Detector.
Data analysis	Day 7 EC_{50} values were determined using linear interpolation with treatment response (frond number, growth rate and biomass) and exposure concentration data. The Day 7 frond numbers, growth rates and biomass were evaluated for normality and homogeneity of variances ($p = 0.05$) using the Shapiro-Wilk's and Bartlett's tests, respectively. Treatment groups were compared to the control groups ($p = 0.05$) using analysis of variance (ANOVA) and Dunnett's t-test (5). The least significant difference (LSD) detected by ANOVA was 22 for frond number; 0.023 for growth rate; and 3.8 for biomass. However, the endpoints were re-calculated to meet the requirements of the current guideline.

II. RESULTS AND DISCUSSION:

Validity criteria (OECD 221, 2006)	Required	Obtained
Doubling time	< 2.5 days	1.9 days

Analytical results:

Nominal stock solution concentrations were 13, 25, 50, 100 and 200 mg a.s./L. The results of the study were based on mean measured test concentrations of 12, 25, 50, 101 and 203 mg a.s./L.

Nominal Concentration (mg/L)	Day 0 Measured Concentration (mg/ L)	Day 0 Percent of Nominal (%)	Day 7 Measured Concentration (mg/ L)	Day 7 Percent of Nominal (%)[2]	Mean Measured Concentration (mg/ L)	Percent of nominal %
Control	< LOQ[1]	-	< LOQ[1]	-	-	
13	12.1	93.3	12.3	94.8	12	92.3
25	24.8	99.2	25.2	101	25	100
50	49.4	98.8	50.5	101	50	100
100	98.5	98.5	103	103	101	101
200	200	100	206	103	203	102

[1] The limit of quantitation (LOQ) was 6.12 mg/L calculated as the product of the concentration of the lowest calibration standard (6.00 mg/L) and the dilution factor of the matrix blank samples (1.00) corrected for purity (98%).
[2] Results were generated using Excel 2000 in full precision mode. Manual calculations may differ slightly.

Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4 with minor acceptable exceptions only.

Biological results:

Observations

In the highest treatment level, 100% of the test organisms were dead at the end of the study. Slight chlorotic effects (0.41-1.7%) were observed in the control and in the treatments with 12, 25 and 50 mg/L. In the treatment with 101 mg/L, 23% of plants showed chlorosis. Slight signs of necrosis were observed in the control and the 50 mg/L test concentration. In the treatment with 101 mg/L, 58% necrosis was observed.

Mean measured concentration [mg/L]	Day 7 Frond number [a]	7-day inhibition of frond number	Day 0-7 Mean growth rate for frond number	7-day inhibition of growth rate	Day 0 – 7 Mean Biomass (mg)	7-day inhibition of dry weight
Control	155	-	0.365	-	23.4	-
12	157	-0.86	0.366	-0.25	24.1	-3.1
25	164	-5.8	0.374	-2.2	24.0	-2.7
50	135	13	0.346	5.3	17.4	25
101	40*	74	0.171*	53	7.93*	66
203	13*	91	0.015*	96	2.63*	89

[a] Mean of 3 replicates, NA not applicable
* Statistically significant difference on day 7 frond growth ($p<0.05$) from the control replicates using Dunnett's test.

To derive endpoints that meet current regulatory demands, the study with M-01 (AE C653711 also called BAM) and *Lemna gibba* by [redacted] (2003; M-219725-01-2) has been re-evaluated.

The original study report includes endpoint calculations and estimates for the following response and measurement variables:

- 7-day EC_{50} for frond number (80 mg/L), calculated based on frond count at the end of the test: endpoint not in accordance with OECD 221 (2006)
- 7-day E_rC_{50} frond number growth rate (97 mg/L): endpoint in accordance with OECD 221 (2006)
- 7-day EC_{50} for dry weight (80 mg/L), calculated based on dry weight at the end of the test: endpoint named 'biomass' in study report, not in accordance with OECD 221 (2006).
- 7-day NOEC values for frond number, frond number growth rate and 'biomass' (50, 50 and 25 mg/L, respectively). Only the frond number growth rate NOEC is in accordance with OECD 221 (2006)

This report provides a full statistical re-evaluation of the study. The following recalculations were performed to meet current data and guideline requirements:

- EC_{10}, EC_{20}, EC_{50}, NOEC values for the measurement variable frond number and the two response variables growth rate and yield
- EC_{10}, EC_{20}, EC_{50}, NOEC values for the measurement variable dry weight and the two response Variables growth rate and yield.

Dry weight was not determined at test start, therefore to be in accordance with OECD 221 (2006), it has been estimated from control data at the end of the test. It was then used to calculate yield and growth rate EC_{50} for dry weight. This response parameter is called biomass in the statistical report in appendix.

All recalculations were performed with the software ToxRat Professional Vers. 3.2.1, on the basis of nominal concentrations as for the originally reported endpoints. This approach is acceptable since all measured concentrations of M-01 (BAM) are in the range 80-120% of nominal concentrations.

The results of the recalculations based on nominal concentrations are presented in the table below. Details on the statistical re-evaluation are provided in the Appendix.
In the original report, EC_x were calculated according to binomial interpolation. Probit analysis is preferred in this re-assessment since the dose-response curve covers a large effect range. Therefore, the recalculated E_rC_{50} for frond number is slightly different form the original value: 97.6 vs 97 mg/L.

Similarly, for the NOEC determination, the statistical method used in this re-assessment (Williams Multiple Sequential t-test Procedure) is considered more appropriate than the approach (Dunnett's t-test) in the original report.

Variable	Endpoint type	Result (mg/L)	95% confidence interval (mg/L)
Frond number	E_yC_{10}	46.5	41.7 – 50.6
	E_yC_{20}	54.5	50.1 – 58.4
	E_yC_{50}	73.9	69.9 – 78.0
	NOE_yC	25.0	Not applicable
	E_rC_{10}	57.2	53.8 – 60.2
	E_rC_{20}	68.7	65.7 – 71.4
	E_rC_{50}	**97.6**	95.5 – 99.8
	NOE_rC	25.0	Not applicable
Dry weight (biomass)	E_yC_{10}	33.8	28.2 – 38.7
	E_yC_{20}	43.8	38.3 – 48.6
	E_yC_{50}	71.8	66.5 – 77.6
	NOE_yC	25.0	Not applicable
	E_rC_{10}	51.0	45.1 – 56.3
	E_rC_{20}	66.5	60.7 – 71.6
	E_rC_{50}	110.2	104.6 – 116.1
	NOE_rC	25.0	Not applicable

III. CONCLUSIONS:

Endpoints were calculated based on nominal concentrations. The lowest E_rC_{50} is 97.6 mg/L, obtained for the measurement variable frond number. For the second variable dry weight, an E_rC_{50} of 110.2 mg/L was calculated.

Endpoint (0-7 days)	Effect on mean frond number	Effect on Biomass (dry weight)
E_rC_{50} (95% C.I.):	97.6 mg/L (95.5 – 99.8 mg/L)	110.2 mg/L (104.6 – 116.1 mg/L)
NOE_rC	25.0 mg/L	25.0 mg/L

Assessment and conclusion by applicant:

The study is reliable. The E_rC_{50} of 97.6 mg/L and the NOE_rC based on frond number and dry weight of 25 mg/L can be used in risk assessment.

CA 8.2.8 Further testing on aquatic organisms

Data Point:	KCA 8.2.8/01
Report Author:	[redacted]
Report Year:	2010
Report Title:	Acute toxicity of fluopicolide to Xenopus laevis under static conditions
Report No:	EBACY001
Document No:	M-393869-01-1
Guideline(s) followed in study:	No formal English guideline exists for this test protocol. Methodologies from USEPA, OPPTS Guideline 850.1075 (1996), USEPA-FIFRA, 40 CFR, Part 158, Guideline No. 72-1 (1982), and OECD Guideline 203 (1992), were considered in the development of this protocol. Scientific discretion was implemented where guideline parameters do not fully converge.
Deviations from current test guideline:	Method: Deviations from current guideline SANCO/3029/99 rev.4: Limited sets of validation recoveries were analysed. However, the average recoveries were within the acceptable range of 70–110% and the RSD values were below 20%. The analytical method can be regarded as fit for purpose. Study: Not applicable
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive summary

An acute toxicity study was performed with fluopicolide (*Xenopus laevis*) under flow-through conditions for 48 hours. The following nominal (mean measured) concentrations were included in the study: 0.063 (0.052), 0.125 (0.115), 0.25 (0.23), 0.50 (0.51) and 1.0 (0.88) mg a.s./L. Additionally a control and solvent control was included. There were three replicates of 10 tadpoles in the control and each toxicant level. Survival (mortality) and sublethal behavioural effects were assessed after 6, 24 and 48 hours. No statistical calculations were necessary to determine the EC_{50} for this study. The NOEC and LOEC were empirically determined based upon observation data including lethal and sublethal effects. The mean measured recoveries ranged from 84 to 103% of the nominal test concentrations. Since the concentration of the test solutions was stable and within 80% of the nominal concentrations, the results of the study are based on the nominal test concentrations. The final results for the test are: 48-hour LC_{50} (95% C.I.): > 1.0 mg a.s./L (NAO), 48-hour LOEC: 0.25 mg a.s./L and 48-hour NOEC: 0.125 mg a.s./L.

I. MATERIAL AND METHODS:

Test material	Fluopicolide Batch: PF90195670 99.1%
Guideline(s) adaptation	No specific guideline is available. Methodologies from USEPA, OPPTS Guideline 850.1075, USEPA-FIFRA, 40 CFR, Part 158, Guideline No. 72-1, and OECD Guideline 203 were considered in the development of this protocol. Scientific discretion was implemented where guideline parameters do not fully converge.
Test species	African clawed frog - tadpoles (*Xenopus laevis*)
Acclimation	4 days acclimation Mortalities less than 5% during holding period, no treatments for disease Not fed for 24 hours prior to testing
Organism age/size at study initiation	Tadpoles Size: 15.1 +/- 0.9 mm
Test solutions	Nominal concentrations: 0.063, 0.125, 0.25, 0.50 and 1.0 mg a.s./L Mean measured concentrations: 0.052, 0.115, 0.23, 0.51, 0.88 mg a.s./L Control: Untreated medium: hard processed water (reverse osmosis water blended with spring water) Solvent control: Dimethylformamide (0.1 mL/L) Precipitates: None
Replication	No. of vessels per concentration (replicates): 3 No. of vessels per control (replicates): 3 No. of vessels per solvent control (replicates): 3
Organisms per replicate	No. of organisms per vessel: 10
Exposure	Static 48 h
Test Vessel Loading	10 tadpoles in a testing volume of 7 L.
Feeding during test	None
Test conditions	Temperature: 21.6 – 22.2 °C Photoperiod: 16 hours light, 8 hours dark, with a 30-min transition period Light intensity: 570 to 832 lux pH: 8.4 to 8.5 Water hardness: 170 to 186 mg/L Alkalinity range: 134 to 145 mg/L Conductivity range: 486 to 513 µmhos/cm Dissolved oxygen saturation (%): 91 to 102% saturation (8.1 to 8.9 mg/L)
Parameters Measured / Observations	Survival (mortality) and sublethal behavioural effects were assessed after 6, 24 and 48 hours. Temperature was measured hourly, dissolved oxygen and pH daily. Alkalinity, hardness and conductivity were measured on day 0 and day 2.
Sampling for chemical analysis	On test day 0 and day 2 analytical samples from each test level were taken and analysed with Liquid Chromatograph / Tandem Mass Spectrometry system (LC/MS/MS). Day 0 samples were taken from the batch prepared solutions and the day 2 sample were collected were composites of the three replicates at each test concentration.
Data analysis	No statistical calculations were necessary to determine the EC_{50} for this study. The NOEC and LOEC were empirically determined based upon observation data including lethal and sublethal effects.

II. RESULTS AND DISCUSSION:

Validity criteria as defined in the study plan	Required	Obtained
Mortality during domestication period	≤ 5%	< 5%
Mortality in the control	≤ 10%	6.7%
Dissolved oxygen content	≥ 5.8	≥ 8.1
pH during the test	Constant	8.4 – 8.5

Analytical results:

Measured concentrations on day 0 and day 2 ranged between 83 and 104% and are presented below. Therefore, results are based on nominal concentrations. No residues of fluopicolide were measured in the controls above the LOQ (Limit of Quantification = 0.006 mg a.s./L).

Nominal Concentration (mg a.s./L)	Day 0 % of nominal	Day 2 % of nominal	Mean Measured Concentration (mg a.s./L)	Percent of nominal %
0.063	84	83	0.052	84
0.125	89	94	0.115	92
0.25	93	94	0.23	93
0.50	102	104	0.51	103
1.0	88	89	0.88	88

Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4 with minor acceptable exceptions only.

Biological results:

Sublethal Observations			
Nominal Test Concentration (mg a.s./L)	number with sublethal effects/number of survivors		
	Hour 0	24 Hour	48 Hour
Control	0/30	0/30	0/28
Solvent Control	0/30	0/30	0/30
0.063	0/30	0/29	1/29 (1 OB)
0.125	0/30	0/30	0/29
0.25	0/30	0/28	0/27
0.50	23/30 (2 AS, 21 OB)	7/30 (7 OB)	15/29 (15 OB)
1.0	30/30 (30 OB)	29/29 (29 OB)	24/24 (24 OB + P)

AS = At surface, OB = On bottom, P = Pale color

Mortality	
Nominal test concentration (mg a.s./L)	**Percent Dead at 48 hours**
Control	6.7
Solvent Control	0.0
0.063	3.3
0.125	3.3
0.25	10
0.50	3.3
1.0	20

III. CONCLUSIONS:

The final results for the test are based on nominal concentrations of fluopicolide in the test system.

48 hour LC_{50} (95% C.I.)	> 1.0 mg a.s./L (NA)
48 hour LOEC based on sublethal effects	0.25 mg a.s./L
48 hour NOEC based on sublethal effects	0.125 mg a.s./L

Assessment and conclusion by applicant:

The study is reliable, and the LC_{50} > 1.0 mg a.s./L can be used in the fluopicolide risk assessment.

CA 8.3 Effect on arthropods

CA 8.3.1 Effects on bees

The following studies describing the toxicity to bees have been performed with technical fluopicolide or the straight formulation fluopicolide SC 486 according to current guidelines, guidance documents or the current understanding of the state-of-the-art of testing.

- Acute oral and contact toxicity to honeybees under laboratory conditions (OECD 213/OECD 214)
- Acute oral and contact toxicity to bumble bees under laboratory conditions (OECD 246/OECD 247)
- Chronic toxicity to adult honeybees under laboratory conditions (OECD 245)
- Chronic toxicity to honeybee larvae under laboratory conditions (OECD 239)
- Honey bee brood feeding test (Oomen *et al*., 1992)
- Honeybee brood – Semi-Field (OECD GD 75)

For fluopicolide metabolites studies on acute oral and contact toxicity to honeybees under laboratory conditions have been conducted.

The studies are summarized below and a full list of the relevant ecotoxicological endpoints for fluopicolide is presented in the following table.

Table 8.3.1- 1: Toxicity of fluopicolide (technical and formulated product) to bees

Test substance	Test species/ study type	Endpoint	References
Fluopicolide tech.	Honeybee, adult, acute, 72 h	LD_{50} – oral > 241 µg a.s./bee	[redacted] 2012; M-200452-03-1 KCA 8.3.1.1.1/01
	Honeybee, adult acute, 48 h	LD_{50} – oral > 107.3 µg a.s./bee LD_{50} – contact > 100 µg a.s./bee	[redacted] 2015; M-539964-01-1 KCA 8.3.1.1.1/02
	Honeybee, adult, acute, 72 h	LD_{50} – contact > 100 µg a.s./bee	[redacted] 2012; M-200506-03-1 KCA 8.3.1.1.2/01
	Honeybee larvae, chronic (emergence after 22 days follow repeated feeding)	NOED ≥ 60.1 µg a.s./larva	[redacted] 2018; M-615695-01-1 KCA 8.3.1.3/01
	Bumble bee, adult, acute, 48 h	LD_{50} – oral > 87.3 µg a.s./bumble bee	[redacted] 2015; M-519981-01-1 KCA 8.3.1.1.1/03
	Bumble bee, adult, acute 48 h	LD_{50} - contact > 100 µg a.s./bumble bee	[redacted] 2015; M-511408-01-1 KCA 8.3.1.1.2/02

Test substance	Test species/ study type	Endpoint	References
Fluopicolide SC 486	Honeybee, adult, 10 day feeding test	LDD_{50} > 132.68 µg a.s./bee/day	[redacted] 2016; M-552253-01-1 KCA 8.3.1.2/01
	Honeybee brood feeding test (Oomen *et al*., 1992)	No adverse effects were observed on the development of brood (eggs, young and old larvae) and on pupal mortality. Adult bee mortality in the test item treatment group appeared higher compared to the control group. However, since this observation was not consistent amongst replicates it is considered to be random and not of biological relevance. Overall, fluopicolide fed at a concentration of 1.33 g a.s./L sugar solution caused no adverse effects on honey bee colony performance including no indication for negative impacts on brood rearing success.	[redacted] 2016; M-545732-01-1 KCA 8.3.1.3/02
	Honeybee Brood Semi-Field (OECD GD 75)	Overall, no adverse effects on brood development, adult and pupal mortality, foraging activity, behaviour, colony development and strength after application of 331.6 g product/ha (corresponding to 133 g a.s./ha) onto flowering *Phacelia tanacetifolia* and bees foraging on the crop.	[redacted] 2016; M-547424-01-1 KCA 8.3.1.3/03
	Honeybee Brood Semi-Field (OECD GD 75)	Overall, no adverse effects on brood development, adult and pupal mortality, foraging activity, behaviour, colony development and strength after application of 133 g a.s./ha onto flowering *Phacelia tanacetifolia* and bees foraging on the crop.	[redacted] 2020; M-685049-01-1 KCA 8.3.1.3/04
Metabolite M-04 (AE C653711)	Honeybee, adult, acute, 48 h	LD_{50} – oral > 100 µg p.m./bee LD_{50} – contact > 80.8 µg p.m./bee	[redacted] 2016; M-571897-01-1 KCA 8.3.1.1.1/04
Metabolite M-02 (AE C657188)	Honeybee, adult, acute, 48 h	LD_{50} – oral > 110.9 µg p.m./bee LD_{50} – contact > 100.0 µg p.m./bee	[redacted] 2016; M-566365-01-1 KCA 8.3.1.1.1/05

p.m.: pure metabolite

CA 8.3.1.1 Acute toxicity to bees

CA 8.3.1.1.1 Acute oral toxicity

Data Point:	KCA 8.3.1.1.1/01
Report Author:	[redacted]
Report Year:	2012
Report Title:	Amendment no. 2 - Oral toxicity (LD50) to honey bees (Apis mellifera L.) Substance pure Code: AE C638206 00 1B99 0002
Report No:	CW00/090
Document No:	M-200452-03-1
Guideline(s) followed in study:	EPPO Guideline No. 170 (1992)
Deviations from current test guideline:	Current Guideline: OECD 213 (1998) Triazophos was used as reference item instead of dimethoate as recommended in the guideline. Only 3 test item doses were used with a spacing factor of 10 instead of 5 with a maximum spacing factor of 2.2 as recommended in the guideline. These deviations are not expected to have impacted the study results.
Previous evaluation:	yes, evaluated and accepted in DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The purpose of this study was to determine the acute oral toxicity of fluopicolide technical to the honeybee (*A. mellifera* L.) in the laboratory. Mortality of the bees was used as toxic endpoint.
Therefore, under laboratory conditions *Apis mellifera* worker bees were exposed by use of sucrose diet paste to mean measured doses of 2.61, 21.04, and 240.74 µg a.s./bee during a 5-hour feeding period. Furthermore, each test consisted of a control and a reference item group. Each treatment group consisted of 5 replicates (test units) with 10 bees per replicate. The tests were conducted in darkness, temperature was 24 - 26 °C and relative humidity was between 58 and 68 %. The number of dead bees in each cage was assessed 24, 48 and 72 hours after application. The oral LD_{50} value for the reference substance was calculated with the aid of SAS probit analysis. No mortality occurred during the test with the different test doses. Also, no mortality occurred in the control. In the test with the reference item the doses of 0.1101, 0.21614 and 0.69278 µg product/bee resulted in 5, 15 and 35 dead bees after 72 hours. The LD_{50} of the reference item was calculated to be 0.162 µg a.s./bee. All validity criteria of the test were met. The LD_{50} (72 h) for honeybees was > 241 µg a.s./bee in the oral toxicity test performed with fluopicolide.

I. MATERIAL AND METHODS:

Test item: Fluopicolide technical: 99.3% w/w, origin batch no.: R001737, Identification code: AE C638206 00 1B99 0002, Certificate of Analysis: C/030/2000 (dated 05 April 2000).
Test organism: female worker honeybees (*Apis mellifera*), obtained from a healthy and queen-right colony.
Under laboratory conditions *Apis mellifera* worker bees were exposed by use of sucrose diet paste to mean measured doses of 2.61, 21.04, and 240.74 µg a.s./bee during a 5-hour feeding period. Furthermore, each test consisted of a control and a reference item group. In the control an untreated sucrose diet paste was offered to the bees as food source. In the test AE F002960 00 EC 40 C667 (active ingredient: 41.1 % w/w triazophos, Batch no.: C07174065) was used as reference item; the reference item was tested at three different doses (0.1101, 0.21614 and 0.69278 µg product/bee).
Each treatment group consisted of 5 replicates (test units) with 10 bees per replicate. Test units were 12-13 cm high cylindrical test cages with a diameter of 5 cm.

For the reference item group 0.4 mL of a 50% sucrose solution containing the reference substance AE F002960 00 EC40 C667 (41.1 % w/w triazophos) in the three different concentrations 0.0006, 0.0012 and 0.0047% product were offered to the bees.
The tests were conducted in darkness, temperature was 24 - 26°C and relative humidity was between 58 and 68 %. The number of dead bees in each cage was assessed 24, 48 and 72 hours after application.
The oral LD_{50} value for the reference substance was calculated with the aid of SAS probit analysis.

Dates of experimental work: August 31, 2000 – September 03, 2000

II RESULTS AND DISCUSSION:

Biological findings

Test substance	Endpoint	
Fluopicolide	24-72 h LD_{50} [µg a.s./bee]	> 241
Reference item	72 h LD_{50} [µg product/bee]	0.394

Observations

No mortality occurred during the test with the different test doses. Also, no mortality occurred in the control. In the test with the reference item the doses of 0.11014, 0.21614 and 0.69278 µg product/bee resulted in 5, 15 and 35 dead bees after 72 hours.

	Total number of dead bees (and mortality in %) after		
	24 h	48 h	72 h
Control	0 (0)	0 (0)	0 (0)
Test item [µg a.s./bee]			
2.6122	0 (0)	0 (0)	0 (0)
21.038	0 (0)	0 (0)	0 (0)
240.740	0 (0)	0 (0)	0 (0)
Reference item [µg product/bee]			
0.11010	3 (6)	5 (10)	5 (10)
0.21614	15 (30)	15 (30)	15 (30)
0.69278	34 (68)	34 (68)	35 (70)

Validity criteria:
All validity criteria of the test were met.

Validity criteria (OECD 213, 1998)	Obtained in this study
Control mortality should not exceed 10 % at test end	Control: 0 %
LD_{50} of the reference item should be in the specified range (dimethoate: oral test 0.10 – 0.35 µg a.s./bee)	0.162 µg a.s./bee* (a.s. triazophos)

*0.394 µg product/bee × 41.1 % w/w triazophos
The reference item triazophos confirmed the sensitivity of the bees used in the test.

III. CONCLUSIONS:

The LD_{50} (72 h) for honeybees was > 241 µg a.s./bee in the oral toxicity test performed with fluopicolide.

Assessment and conclusion by applicant:
This study is considered reliable for risk assessment and the endpoint is:
LD_{50} oral (72 h) > 241 µg a.s./bee

Data Point:	KCA 8.3.1.1.1/02
Report Author:	[redacted]
Report Year:	2015
Report Title:	Fluopicolide tech.: Effects (Acute contact and oral) on honey bees (Apis mellifera L.) in the laboratory
Report No:	99581035
Document No:	M-539964-01-1
Guideline(s) followed in study:	Regulation (EC) No. 1107/2009 Directive 2003-01 (Canada/PMRA) US EPA OCSPP 850.3020, 850.supp. OECD 213 and 214 (1998)
Deviations from current test guideline:	Current Guidelines: OECD 213 (1998) and OECD 214 (1998) A 5 µL droplet was chosen in the contact toxicity test in deviation to the guideline recommendation of a 1 µL droplet, since a higher volume ensured a more reliable dispersion of the test item. This deviation is not expected to have impacted the study results.
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The purpose of this study was to determine the acute contact and oral toxicity of fluopicolide technical to the honeybee (*A. mellifera* L.) in the laboratory. Mortality of the bees was used as the toxic endpoint. Sublethal effects, such as changes in behaviour, were also assessed.
Therefore under laboratory conditions *Apis mellifera* worker bees were exposed for 48 hours to a single dose of 100.0 µg a.s. per bee, by topical application of 5 µL, in a contact limit test and to a single dose of 107.3 µg a.s. per bee by feeding in an oral limit test (value based on the actual intake of the test item after a feeding period of < 2 hours). Furthermore, each test consisted of a control, solvent control and a reference item group. Each treatment group consisted out of 5 replicates (test units) with 10 bees per replicate. The tests were conducted in darkness, temperature was 25 °C and humidity was between 50 and 78 %. Biological observations, including mortality and behavioural changes were recorded 4, 24 and 48 h after application. At the end of the contact toxicity test (48 hours after application), there was 6.0 % mortality at 100.0 µg a.s./bee. There was 2.0 % mortality in the water control group (water + 0.5 % Adhäsit) and no mortality occurred in the solvent control group (acetone). In the oral toxicity test, the actual intake of 107.3 µg a.s./bee led to no mortality after 48 hours. No mortality occurred in the water control group (50 % w/v sucrose solution = 500 g sucrose/L tap water) and in the solvent control group (50 % w/v sucrose solution containing 4 % acetone and 1 % Tween 80) at the end of the oral toxicity test (after 48 hours). The LD_{50} of the reference item was calculated to be 0.23 and 0.14 µg a.s./bee in the contact and oral test respectively. All validity criteria of the test were met. The LD_{50} (48 h) for honeybees was > 100 µg a.s./bee in the contact toxicity test and > 107.3 µg a.s./bee in the oral toxicity test performed with fluopicolide.

I. MATERIAL AND METHODS:

Test item: Fluopicolide technical: 98.8% w/w; origin batch no.: BCHR 1111-2-1, Material: Fluopicolide, technical, Specification No.: 102000016444. Article No.: 06032698, Certificate of Analysis No: AZ 20033.
Test organism: female worker honeybees (*Apis mellifera*), obtained from a healthy and queen-right colony, bred by IBACON.
Under laboratory conditions *Apis mellifera* worker bees were exposed for 48 hours to a single dose of 100.0 µg a.s. per bee, by topical application of 5 µL, in a contact limit test and to a single dose of 107.3 µg a.s. per bee by feeding in an oral limit test (value based on the actual intake of the test item). Furthermore, each test consisted of a control, solvent control and a reference item group. In the contact limit test, tap water containing 0.5 % Adhaesit and pure acetone were used as control and solvent control, respectively. In the oral limit test a 50 % w/v sucrose solution containing solvent (4 % acetone and 1% Tween) and 50 % w/v sucrose solution were used as solvent control and control, respectively. In both limit tests, BAS 152 11 I (active ingredient 400.9 g/L dimethoate, Batch no.: FRE-000926) was used as reference item. Each treatment group consisted out of 5 replicates (test units) with 10 bees per replicate. Test units were stainless steel cages with 8 cm × 6 cm × 4 cm (length × height × width).
The tests were conducted in darkness, temperature was 25°C and humidity was between 50 and 78 %. Biological observations, including mortality and behavioural changes were recorded 4, 24 and 48 h after application.
The software used to perform the statistical analysis was ToxRat Professional.

Dates of experimental work: April 13, 2015 – April 15, 2015

II RESULTS AND DISCUSSION:

Biological findings:

Test item	**Fluopicolide tech.**	
Test object	***Apis mellifera***	
Exposure	**Contact (solution in acetone)**	**Oral (sugar/ acetone + Tween 80/water solution)**
Dose [µg a.s./bee]	100.0	107.3
LD_{50} [µg a.s./bee]	> 100.0	> 107.3
LD_{20} [µg a.s./bee]	> 100.0	> 107.3
LD_{10} [µg a.s./bee]	> 100.0	> 107.3
NOED [µg a.s./bee]*	100.0	≥ 107.3

* The NOED was estimated using Fisher's Exact Test (pairwise comparison, one-sided greater, α = 0.05).

Observations

Contact test

At the end of the contact toxicity test (48 hours after application), there was 6.0 % mortality at 100.0 µg a.s./bee. There was 2.0 % mortality in the water control group (water + 0.5 % Adhäsit) and no mortality occurred in the solvent control group (acetone). During the first 4 hours 42.0 % bees were affected in the test item treated group. Thereafter no more behavioural abnormalities were found by the end of the test (48 hours).

Dosage [µg a.s./bee]	After 4 hours		After 24 hours		After 48 hours	
	Mortality mean %	Behavioral abnormalities mean %	Mortality mean %	Behavioral abnormalities mean %	Mortality mean %	Behavioral abnormalities mean %
Test item 100.0	4.0	42.0	4.0	0.0	6.0	0.0
Water	0.0	0.0	0.0	0.0	2.0	0.0
Solvent	0.0	0.0	0.0	0.0	0.0	0.0
Reference item 0.30	10.0	2.0	72.0	0.0	76.0	2.0
0.20	2.0	2.0	30.0	0.0	32.0	0.0
0.15	0.0	0.0	22.0	2.0	26.0	0.0
0.10	0.0	2.0	4.0	0.0	8.0	0.0

Results are averages from five replicates (ten bees each) per dosage / control
Water = CO_2/water-treated control, solvent = CO_2/solvent control

Oral test

In the oral toxicity test, the maximum nominal test level of fluopicolide tech. (i.e. 100 µg a.i./bee) corresponded to an actual intake of 107.3 µg a.i./bee. This dose level led to no mortality after 48 hours. No mortality occurred in the water control group (50 % w/v sucrose solution = 500 g sucrose/L tap water) and in the solvent control group (50 % w/v sucrose solution containing 4 % acetone and 1 % Tween 80) at the end of the oral toxicity test (after 48 hours). No test item induced behavioural effects were observed at any time in the oral toxicity test.

Dosage [µg a.s./bee]	After 4 hours		After 24 hours		After 48 hours	
	Mortality mean %	Behavioral abnormalities mean %	Mortality mean %	Behavioral abnormalities mean %	Mortality mean %	Behavioral abnormalities mean %
Test item 107.3	0.0	0.0	0.0	0.0	0.0	0.0
Water	0.0	0.0	0.0	0.0	0.0	0.0
Solvent	0.0	0.0	0.0	0.0	0.0	0.0
Reference item 0.32	54.0	32.0	100.0	0.0	100.0	0.0
0.16	0.0	18.0	66.0	26.0	78.0	8.0
0.08	0.0	0.0	2.0	2.0	4.0	0.0
0.06	0.0	0.0	0.0	0.0	0.0	0.0

Results are averages from five replicates (ten bees each) per dosage / control
Water = water control, solvent = solvent control

Validity criteria:
All validity criteria of the test were met.

Validity criteria (OECD 213, 1998 and 214, 1998)	Obtained in this study
Control mortality should not exceed 10 % at test end	Contact test Control: 2 % Solvent control: 0 % Oral test Control: 0 % Solvent control: 0 %
LD_{50} of the reference item should be in the specified range (contact test: 0.10 – 0.30 µg a.s./bee, oral test: 0.10 – 0.35 µg a.s./bee)	Contact test 0.23 µg a.s./bee Oral test 0.14 µg a.s./bee

III, CONCLUSIONS:

The LD_{50} (48 h) for honeybees was > 100 µg a.s./bee in the contact toxicity test and > 107.3 µg a.s./bee in the oral toxicity test performed with fluopicolide.

Assessment and conclusion by applicant:

This study is considered reliable for risk assessment and the endpoints are:

LD_{50} contact (48 h) > 100 µg a.s./bee

LD_{50} oral (48 h) > 107.3 µg a.s./bee

Data Point:	KCA 8.3.1.1.1/03
Report Author:	[redacted]
Report Year:	2015
Report Title:	Fluopicolide tech.: Effects (Acute oral) on bumblebees (Bombus terrestris L.) in the laboratory
Report No:	97621105
Document No:	M-519981-01-1
Guideline(s) followed in study:	(GLP compliant study based on van der Steen (2001) and OECD 214 (1998) with modifications and adaptions, Ring test bumblebee acute contact toxicity (ICPPR non-apis group, 2014))
Deviations from current test guideline:	Current Guideline: OECD 247 (2017) Analytical determination of the test item was not conducted, but the study was conducted before guideline implementation and no analytical dose verification was foreseen at that point in time. Moreover, since it is a limit test with a single dosing of the test item this deviation is not expected to have impacted the study results. The exposure duration was 6 hours and thus greater than the maximum 4 hours recommended by the guideline. The test was conducted before implementation of the guideline and the exposure duration of the ring test discussed at the time was 6 hours. This deviation is not expected to have impacted the study results.
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The purpose of this study was to determine the acute oral toxicity of fluopicolide tech. to the bumble bee (*Bombus terrestris* L.) in the laboratory. Mortality of the bumble bees was used as the toxic endpoint. Sublethal effects, such as changes in behaviour, were also assessed. Therefore, under laboratory conditions *Bombus terrestris* worker bumble bees were exposed for 6 hours to a single dose of 87.3 µg a.s. per bumble bee by feeding in an oral limit test. Furthermore, the test consisted of a control, solvent control and a reference item group. Each treatment group consisted out of 50 bumble bees with 1 bumble bee per test unit (replicate). The test was conducted in darkness, temperature was 24-25°C and humidity 50-67% during exposure. Biological observations, including mortality and sub-lethal effects were recorded 4, 24 and 48 h after application. The software used to perform the statistical analysis was ToxRat Pro Version 2.10. At test termination (48 hours) 6.0 % mortality occurred at 87.3 µg fluopicolide/bumblebee. No mortality occurred in the water control group (50 % w/v sucrose solution) and there was 2.0 % mortality in the solvent control group. The mortality in the reference item group was 84% after 48 hours at a dose of 3.4 µg dimethoate/bumble bee. All validity criteria of the test were met. The (48 h) oral LD_{50} was determined to be ≥ 87.3 µg a.s./bumble bee.

I. MATERIAL AND METHODS:

Test item: Fluopicolide technical: 100.5 % w/w (analytical), Origin Batch No.: ETFP000273, Customer Order No.: TOX 10747; Material: Fluopicolide, technical; Specification No.: 102000016444-01, Article No.: 06032698.
Test organism: female worker bumble bees (*B. terrestris*), obtained from a healthy and queen-right colony, bred by a commercial bumble bee breeding company (Biobest Belgium N.V.).
Under laboratory conditions *Bombus terrestris* worker bumble bees were exposed for 6 hours to a single dose of 87.3 µg a.s. per bumble bee by feeding in an oral limit test (value based on the actual intake of the test item). Furthermore, the test consisted of a control, solvent control and a reference item group.
In the oral limit test a 50 % w/v sucrose solution containing solvent (5 % acetone and 1 % Tween80) and 50% w/v sucrose solution were used as solvent control and control, respectively.
BAS 152 11 I EC (active ingredient 400.9 g/L dimethoate, Batch no.: FRE-000926) was used as reference item. Each treatment group consisted out of 50 bumble bees with 1 bumble bee per test unit (replicate). Test units were 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 test was conducted in darkness, temperature was 24-25°C and humidity 50-67% during exposure.
Biological observations, including mortality and sub-lethal effects were recorded 4, 24 and 48 h after application.
The software used to perform the statistical analysis was ToxRat Pro Version 2.10.

Dates of experimental work: February 17, 2015 – February 19, 2015

II RESULTS AND DISCUSSION:

Biological findings:

Test item	**Fluopicolide tech.**
Test object	***Bombus terrestris***
Exposure	**Oral**
Dose [µg a.s./bumble bee] based on recorded consumption	87.3
LD_{50} [µg a.s./bumble bee]	> 87.3
LD_{20} [µg a.s./bumble bee] *	> 87.3
LD_{10} [µg a.s./bumble bee] *	> 87.3
NOED [µg a.s./bumble bee] **	≥ 87.3

* Since no mortality above 10% occurred, the respective $LD_{x/20}$ values are assumed to be > 87.3 µg a.s./bumble bee
** The NOED was estimated using Fisher's Exact Test (pairwise comparison, one-sided greater, α = 0.05).

Observations

Oral test:
At test termination (48 hours) 6.0 % mortality occurred at 87.3 µg fluopicolide/bumble bee. No mortality occurred in the water control group (50 % w/v sucrose solution) and there was 2.0 % mortality in the solvent control group (50 % w/v sucrose solution containing 5 % acetone and 1 % Tween80).

Treatment Group	After 4 hours		After 24 hours		After 48 hours	
	Mortality mean %	Behavioral abnormalities mean %	Mortality mean %	Behavioral abnormalities mean %	Mortality mean %	Behavioral abnormalities mean %
Test item: 87.3 µg a.s./bumble bee	0.0	0.0	0.0	0.0	6.0	0.0
Control	0.0	0.0	0.0	0.0	0.0	0.0
Solvent Control	0.0	0.0	0.0	0.0	2.0	0.0
Reference item 3.1 µg dimethoate /bumble bee	0	80	78.0	4.0	84.0	0.0

Beh. abnormalities = Behavioural abnormalities, Mean = mean of 50 individuals per treatment group
Control = 50 % w/v sucrose solution; solvent control = 50 % w/v sucrose solution containing 5 % acetone + 1 % Tween80
Ref. = Reference item

Validity criteria:
All validity criteria of the test were met.

Validity criteria OECD 247 (2017)	Obtained in this study
Control mortality should not exceed 10 % at test end	Control: 0% Solvent control: 2 %
Mortality of the reference item should be > 50 % at test end	Reference item: 84 %

III. CONCLUSIONS:

The toxicity of fluopicolide tech. was tested in an acute oral toxicity test on bumble bees. The (48 h) oral LD_{50} was determined to be ≥ 87.3 µg a.s./bumble bee.

Assessment and conclusion by applicant:

This study is considered reliable for risk assessment and the endpoint is:

LD_{50} oral (48 h) ≥ 87.3 µg a.s./bumble bee

Data Point:	KCA 8.3.1.1.1/04
Report Author:	[redacted]
Report Year:	2016
Report Title:	AE C653711: Effects (Acute contact and oral) on honey bees (Apis mellifera L.) in the laboratory - Final report -
Report No:	114821035
Document No:	M-571897-01-1
Guideline(s) followed in study:	Regulation (EC) No. 1107/2009 Directive 2003-01 (Canada/PMRA) US EPA OCSPP 850.3020, 850.supp. OECD 213 and 214 (1998)
Deviations from current test guideline:	Current Guidelines: OECD 213 (1998) and OECD 214 (1998) A 5 µL droplet was chosen in the contact toxicity test in deviation to the guideline recommendation of a 1 µL droplet, since a higher volume ensured a more reliable dispersion of the test item. This deviation is not expected to have impacted the study results.
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The purpose of this study was to determine the acute contact and oral toxicity of M-01 (AE C653711) to the honeybee (*A. mellifera* L.) in the laboratory. Mortality of the bees was used as the toxic endpoint. Sublethal effects, such as changes in behavior, were also assessed. Therefore, under laboratory conditions *Apis mellifera* worker bees were exposed for 48 hours to a single dose of 100.0 µg p.m. per bee, by topical application of 5 µL, in a contact limit test and to a single dose of 80.8 µg p.m. per bee by feeding in an oral limit test. Furthermore, each test consisted of a control, solvent control and a reference item group. Each treatment group consisted out of 5 replicates (test units) with 10 bees per replicate. The tests were conducted in darkness, temperature was 24 - 25 °C and humidity was between 59 and 70 %. Biological observations, including mortality and behavioral changes were recorded 4, 24 and 48 h after application. The software used to perform the statistical analysis was ToxRat Professional. At the end of the contact toxicity test, no mortality occurred at 100 µg p.m./bee, in the control and in the solvent control. In the oral toxicity test, the actual intake of 80.8 µg p.m./bee led to a mortality of 12.0% after 48 hours. No mortality occurred in the water control group and in the solvent control group at the end of the oral toxicity test. The LD_{50} of the reference item was calculated to be 0.16 and 0.13 µg/bee in the contact and oral test, respectively. All validity criteria of the test were met. The LD_{50} (48 h) for honeybees was > 100 µg p.m./bee in the contact toxicity test and > 80.8 µg a.s./bee in the oral toxicity test performed with M-01 (AE C653711).

I. Material and Methods

Test item: M-01 (AE C653711), 96.2% w/w, origin batch no.: 08018ET, Material: M-01 (AE C653711), Certificate of Analysis No: AZ 20300.

Test organism: female worker honeybees (*Apis mellifera*), obtained from a healthy and queen-right colony, bred by IBACON.

Under laboratory conditions *Apis mellifera* worker bees were exposed for 48 hours to a single dose of 100.0 µg p.m. per bee, by topical application of 5 µL, in a contact limit test and to a single dose of 80.8 µg p.m. per bee by feeding in an oral limit test (value based on the actual intake of the test item after a maximum of 6 hours feeding period). Furthermore, each test consisted of a control, solvent control and a reference item group. In the contact limit test, tap water containing 0.5 % Adhaesit and pure acetone were used as control and solvent control, respectively. In the oral limit test a 50 % w/v sucrose solution containing solvent (4 % acetone and 1% Tween) and 50 % w/v sucrose solution were

used as solvent control and control, respectively. In both limit tests, BAS 152 11 I (active ingredient 420.3 g/L dimethoate, Batch no.: FRE-001226) was used as reference item. Each treatment group consisted out of 5 replicates (test units) with 10 bees per replicate. Test units were stainless steel cages with 8 cm × 6 cm × 4 cm (length × height × width).
The tests were conducted in darkness, temperature was 24 - 25°C and humidity was between 59 and 70 %. Biological observations, including mortality and behavioural changes were recorded 4, 24 and 48 h after application. The software used to perform the statistical analysis was ToxRat Professional.

Dates of experimental work: February 22, 2016 – February 24, 2016

II RESULTS AND DISCUSSION:

Biological findings:

Test item	**M-01 (AE C653711)**	
Test object	***Apis mellifera***	
Exposure	**Contact (solution in acetone)**	**Oral (sugar/ acetone + Tween 80/water solution)**
Dose [µg p.m./bee]	100.0	80.8
LD_{50} [µg p.m./bee]	> 100.0	< 80.8
LD_{20} [µg p.m./bee]	> 100.0	< 80.8
LD_{10} [µg p.m./bee]	> 100.0	< 80.8
NOED [µg p.m./bee]*	= 100.0	= 80.8

* The NOED was estimated using Fisher's Exact Test (pairwise comparison, one-sided greater, α = 0.05)

Observations

Contact test
At the end of the contact toxicity test (48 hours after application), there was 0.0 % mortality at 100.0 µg p.m./bee and no mortality occurred in the water control group (water + 0.5 % Adhäsit) and in the solvent control group (pure acetone), respectively.

Dosage	**After 4 hours**		**After 24 hours**		**After 48 hours**	
	Mortality mean %	**Behavioral abnormalities mean %**	**Mortality mean %**	**Behavioral abnormalities mean %**	**Mortality mean %**	**Behavioral abnormalities mean %**
Test item 100.0 µg p.m./bee	0.0	0.0	0.0	0.0	0.0	0.0
Water	0.0	0.0	0.0	0.0	0.0	0.0
Solvent	0.0	0.0	0.0	0.0	0.0	0.0
Reference item [µg a.s./bee]						
0.30	0.0	20.0	94.0	0.0	98.0	0.0
0.20	0.0	60	68.0	0.0	82.0	0.0
0.15	0.0	2.0	54.0	8.0	74.0	0.0
0.10	0.0	0.0	4.0	2.0	14.0	0.0

Results are averages from five replicates (ten bees each) per dosage / control
Water = water-treated control, solvent = acetone treated control

Oral test

In the oral toxicity test, the maximum nominal test level of M-01 (AE C653711) (*i.e.* 100 µg p.m./bee) corresponded to an actual intake of 80.8 µg p.m./bee. This dose level led to a mortality of 12.0 % after 48 hours.

No mortality occurred in the water control group (50 % w/v sucrose solution = 500 g sucrose/L tap water) and in the solvent control group (acetone (4%) and Tween 80 (1%)) at the end of the oral toxicity test (after 48 hours).

Dosage	After 4 hours		After 24 hours		After 48 hours	
	Mortality mean %	Behavioral abnormalities mean %	Mortality mean %	Behavioral abnormalities mean %	Mortality mean %	Behavioral abnormalities mean %
Test item 80.8 µg p.m./bee	4.0	72.0	12.0	0.0	12.0	12.0
Water	0.0	0.0	0.0	0.0	0.0	0.0
Solvent	0.0	0.0	0.0	0.0	0.0	0.0
Reference item [µg a.s./bee]						
0.34	36.0	40.0	98.0	0.0	100.0	0.0
0.17	0.0	22.0	72.0	6.0	82.0	4.0
0.08	0.0	0.0	14.0	6.0	20.0	4.0
0.06	0.0	0.0	0.0	0.0	0.0	0.0

Results are averages from five replicates (ten bees each) per dosage / control
Water = water control, solvent = acetone treated control

Validity criteria:

All validity criteria of the test were met.

Validity criteria (OECD 213, 1998 and 214, 1998)	Obtained in this study
Control mortality should not exceed 10 % at test end	Contact test Control: 0 % Solvent control: 0 % Oral test Control: 0 % Solvent control: 0 %
LD_{50} of the reference item should be in the specified range (contact test: 0.10 – 0.30 µg a.s./bee, oral test: 0.10 – 0.35 µg a.s./bee)	Contact test 0.16 µg a.s./bee Oral test 0.13 µg a.s./bee

III. CONCLUSIONS:

The LD_{50} (48 h) for honeybees was > 100 µg p.m./bee in the contact toxicity test performed with M-01 (AE C653711). The LD_{50} (48 h) for honeybees was > 80.8 µg p.m./bee in the oral toxicity test performed with M-01 (AE C653711).

Assessment and conclusion by applicant:

This study is considered reliable for risk assessment and the endpoints of M-01 are:

LD_{50} contact (48 h) > 100 µg p.m./bee

LD_{50} oral (48 h) > 80.8 µg p.m./bee

Data Point:	KCA 8.3.1.1.1/05
Report Author:	[redacted]
Report Year:	2016
Report Title:	AE C657188: Effects (Acute contact and oral) on honey bees (Apis mellifera L.) in the laboratory
Report No:	114811035
Document No:	M-566365-01-1
Guideline(s) followed in study:	Regulation (EC) No. 1107/2009 Directive 2003-01 (Canada/PMRA) US EPA OCSPP 850.3020, 850.supp. OECD 213 and 214 (1998)
Deviations from current test guideline:	Current Guidelines: OECD 213 (1998) and OECD 214 (1998) A 5 µL droplet was chosen in the contact toxicity test in deviation to the guideline recommendation of a 1 µL droplet, since a higher volume allows to test a higher application dose. This deviation is not expected to have impacted the study results.
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The purpose of this study was to determine the acute contact and oral toxicity of M-02 (AE C657188) to the honeybee (*A. mellifera* L.) in the laboratory. Mortality of the bees was used as the toxic endpoint. Sublethal effects, such as changes in behavior, were also assessed. Therefore under laboratory conditions *Apis mellifera* worker bees were exposed for 48 hours to a single dose of 100.0 µg p.m. per bee, by topical application of 5 µL, in a contact limit test and to a single dose of 110.9 µg p.m. per bee by feeding in an oral limit test (value based on the actual intake of the test item after a feeding period of 1 hour and 35 minutes). Furthermore, each test consisted of a control, solvent control and a reference item group. In the contact limit test, tap water containing 0.5 % Adhäsit and pure acetone were used as control and solvent control, respectively. In the oral limit test a 50 % w/v sucrose solution containing solvent (5 % acetone) and 50 % w/v sucrose solution were used as solvent control and control, respectively. Each treatment group consisted out of 5 replicates (test units) with 10 bees per replicate.
The tests were conducted in darkness, temperature was 27°C (mean) and humidity was between 56 and 64 %. Biological observations including mortality and behavioral changes were recorded 4, 24 and 48 h after application. The software used to perform the statistical analysis was ToxRat Professional. At the end of the contact toxicity test there was 6.0 % mortality at 100.0 µg p.m./bee. 0.0 % mortality occurred in the water control group and 2.0 % in the solvent control group, respectively. In the oral toxicity test, the actual intake of 110.9 µg p.m./bee led to no mortality after 48 hours. 4.0 % mortality occurred in the water control group and 2.0 % mortality occurred in the solvent group at the end of the oral toxicity test. The LD_{50} of the reference item was calculated to be 0.22 and 0.14 µg /bee in the contact and oral test respectively. All validity criteria of the test were met. The LD_{50} (48 h) for honeybees was > 100 µg p.m./bee in the contact toxicity test and > 110.9 µg a.s./bee in the oral toxicity test performed with M-02 (AE C657188).

I. MATERIAL AND METHODS

Test item: M-02 (AE C657188): 98.5% w/w; origin batch no.: SES 10250-1-1, Material: M-02 (AE C657188), Certificate of Analysis No: AZ 20206.
Test organism: female worker honeybees (*Apis mellifera*), obtained from a healthy and queen-right colony, bred by IBACON.
Under laboratory conditions *Apis mellifera* worker bees were exposed for 48 hours to a single dose of 100.0 µg p.m. per bee, by topical application of 5 µL, in a contact limit test and to a single dose of 110.9 µg p.m. per bee by feeding in an oral limit test (value based on the actual intake of the test item after a feeding period of 1 hour and 35 minutes). Furthermore, each test consisted of a control, solvent control and a reference item group. In the contact limit test, tap water containing 0.5 % Adhaesit and pure acetone were used as control and solvent control, respectively. In the oral limit test a 50 % w/v sucrose solution containing solvent (5 % acetone) and 50 % w/v sucrose solution were used as solvent control and control, respectively. In both limit tests, BAS 152 11 I (active ingredient 420.3 g/L dimethoate, Batch no.: FRE-001226) was used as reference item. Each treatment group consisted out of 5 replicates (test units) with 10 bees per replicate. Test units were stainless steel cages with 8 cm × 6 cm × 4 cm (length × height × width).
The tests were conducted in darkness, temperature was 27°C (mean) and humidity was between 56 and 64 %. Biological observations, including mortality and behavioral changes were recorded 4, 24 and 48 h after application.
The software used to perform the statistical analysis was ToxRat Professional.

Dates of experimental work: June 21, 2015 – June 23, 2015

II. RESULTS AND DISCUSSION:

Biological findings:

Test item	**M-02 (AE C657188)**	
Test object	***Apis mellifera***	
Exposure	**Contact (solution in acetone)**	**Oral (sugar/acetone /water solution)**
Dose [µg p.m./bee]	100.0	110.9
LD_{50} [µg p.m./bee]	> 100.0	> 110.9
LD_{20} [µg p.m./bee]	> 100.0	> 110.9
LD_{10} [µg p.m./bee]	> 100.0	> 110.9
NOED [µg p.m./bee]*	> 100.0	≥ 110.9

* The NOED was estimated using Fisher's Exact Test (pairwise comparison, one-sided greater, α = 0.05).
p.m. = pure metabolite

Observations

Contact test

At the end of the contact toxicity test (48 hours after application), there was 6.0 % mortality at 100.0 µg p.m./bee. 0.0 % mortality occurred in the water control group (water + 0.5 % Adhaesit) and 2.0% in the solvent control group (acetone), respectively.

Dosage	After 4 hours		After 24 hours		After 48 hours	
	Mortality mean %	Behavioural abnormalities mean %	Mortality mean %	Behavioural abnormalities mean %	Mortality mean %	Behavioural abnormalities mean %
Test item 100.0 µg p.m./bee	0.0	0.0	0.0	0.0	6.0	0.0
Water	0.0	0.0	0.0	0.0	0.0	0.0
Solvent	0.0	0.0	0.0	0.0	2.0	0.0
Reference item [µg a.s./bee]						
0.30	0.0	96.0	76.0	0.0	78.0	0.0
0.20	0.0	66.0	44.0	0.0	50.0	0.0
0.15	0.0	16.0	14.0	0.0	22.0	0.0
0.10	0.0	0.0	4.0	0.0	8.0	0.0

Results are averages from five replicates (ten bees each) per dosage / control
Water = water-treated control, solvent = acetone-treated control

Oral test

In the oral toxicity test, the maximum nominal test level of M-02 (AE C657188) (*i.e.* 100 µg p.m./bee) corresponded to an actual intake of 110.9 µg p.m./bee. This dose level led to no mortality after 48 hours. 4.0% mortality occurred in the water control group (50 % w/v sucrose solution = 500 g sucrose/L tap water) and 2.0 % mortality occurred in the solvent control group at the end of the oral toxicity test (after 48 hours).

Dosage	After 4 hours		After 24 hours		After 48 hours	
	Mortality mean %	Behavioural abnormalities mean %	Mortality mean %	Behavioural abnormalities mean %	Mortality mean %	Behavioural abnormalities mean %
Test item 110.9 µg p.m./bee	0.0	0.0	0.0	0.0	0.0	0.0
Water	0.0	0.0	0.0	0.0	4.0	0.0
Solvent	0.0	0.0	0.0	0.0	2.0	0.0
Reference item [µg a.s./bee]						
0.33	36.0	56.0	98.0	2.0	98.0	0.0
0.17	0.0	20.0	68.0	8.0	74.0	0.0
0.08	0.0	4.0	0.0	4.0	0.0	0.0
0.06	0.0	0.0	0.0	0.0	0.0	0.0

Results are averages from five replicates (ten bees each) per dosage / control
Water = water control, solvent = acetone treated control

Validity criteria:
All validity criteria of the test were met.

Validity criteria (OECD 213, 1998 and 214, 1998)	Obtained in this study
Control mortality should not exceed 10 % at test end	Contact test Control: 0.0 % Solvent control: 2.0 % Oral test Control: 4.0 % Solvent control: 2.0 %
LD_{50} of the reference item should be in the specified range (contact test: 0.10 – 0.30 µg a.s./bee, oral test: 0.10 – 0.35 µg a.s./bee)	Contact test 0.22 µg a.s./bee Oral test 0.14 µg a.s./bee

III, CONCLUSIONS:

The LD_{50} (48 h) for honeybees was > 100 µg p.m./bee in the contact toxicity test and > 110.9 µg p.m./bee in the oral toxicity test performed with M-02 (AE C657188).

Assessment and conclusion by applicant:

This study is considered reliable for risk assessment and the endpoints for M-02 are:

LD_{50} contact (48 h) > 100 µg p.m./bee

LD_{50} oral (48 h) > 110.9 µg p.m./bee

CA 8.3.1.1.2 Acute contact toxicity

Data Point:	KCA 8.3.1.1.2/01
Report Author:	[redacted]
Report Year:	2012
Report Title:	Amendment no. 2 to final study report - Contact toxicity (LD50) to honey bees (Apis mellifera L.) - Substance pure Code: AE C638206 00 1B99 0002
Report No:	CW00/065
Document No:	M-200506-03-1
Guideline(s) followed in study:	EPPO Guideline No. 170 (1992)
Deviations from current test guideline:	Current Guideline: OECD 214 (1998) Triazophos was used as reference item instead of dimethoate as recommended in the guideline. The test was conducted at a temperature of 22 – 28.5°C, outside the range of 25 ± 2°C recommended in the guideline. These deviations are not expected to have impacted the study results.
Previous evaluation:	yes, evaluated and accepted in DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The purpose of this study was to determine the acute contact toxicity of fluopicolide technical to the honeybee (*A. mellifera* L.) in the laboratory. Mortality of the bees was used as toxic endpoint. Therefore, under laboratory conditions *Apis mellifera* worker bees were exposed to the test item, reference item or control by topical application of a single dose of 1.0 µL to the ventral thorax. The solvent control bees were treated with 1.0 µL dimethylsulfoxide (DMSO). The five dose rates of the test substance prepared in DMSO were 1, 5, 10, 50 and 100 µg a.s./bee. Each treatment group consisted of 5 replicates (test units) with 10 bees per replicate. The tests were conducted in darkness, temperature was 22 – 28.5°C and relative humidity was between 53 and 70 %. The number of dead bees in each cage was assessed 24, 48 and 72 hours after application. Contact LD_{50} values were calculated with the aid of SAS probit – analysis. After 72 hours between 3 and 8 dead bees were found in the five test concentrations. In the control 3 dead bees were found after 72 hours. After 72 hours 44 bees were dead in the highest concentration with the reference substance. The LD_{50} of the reference item was calculated to be 0.113 µg /bee. All validity criteria of the test were met. The LD_{50} (72 h) for honeybees was > 100 µg a.s./bee in the contact toxicity test performed with fluopicolide.

I. Material and Methods

Test item: Fluopicolide technical: 99.3% w/w, origin batch no.: R001737, Identification code: AE C638206 00 1B99 0002, Certificate of Analysis: C/030/2000 (dated 05 April 2000).
Test organism: female worker honeybees (*Apis mellifera*), obtained from a healthy and queen-right colony.
Under laboratory conditions *Apis mellifera* worker bees were exposed to the test item, reference item or control by topical application of a single dose of 1.0 µL to the ventral thorax. The solvent control bees were treated with 1.0 µL dimethylsulfoxide (DMSO). The five dose rates of the test substance prepared in DMSO were 1, 5, 10, 50 and 100 µg a.s./bee. The reference item (triazophos 41.1% w/w) prepared in water was tested in 3 dose rates of 0.2, 0.3 and 0.4 µg product/bee. Before application, the bees were slightly anaesthetized with CO_2.
Each treatment group consisted of 5 replicates (test units) with 10 bees per replicate. Test units were 12-13 cm high cylindrical test cages with a diameter of 5 cm.
The tests were conducted in darkness, temperature was 22 – 28.5°C and relative humidity was between 53 and 70 %. The number of dead bees in each cage was assessed 24, 48 and 72 hours after application.

Contact LD_{50} values were calculated with the aid of SAS probit – analysis.

Dates of experimental work: August 09, 2000 – August 12, 2000

II. RESULTS AND DISCUSSION:

Biological findings

Test substance	Endpoint	
Fluopicolide	24-72 h LD_{50} [µg a.s./bee]	> 100
Reference item	72 h LD_{50} [µg product/bee]	0.274

Observations

One mortality occurred after 24 h in the highest test concentration. After 48 hours between 0 and 3 dead bees were found in the four highest test concentrations. After 72 hours between 3 and 8 dead bees were found in the five-test concentration. In the control no mortality occurred after 48 hours and 3 dead bees were found after 72 hours. The test with the reference item resulted in high mortalities in the two highest test concentrations after 24 hours. After 72 hours 44 bees were dead in the highest concentration with the reference substance.

	Total number of dead bees (and mortality in %) after		
	24 h	**48 h**	**72 h**
Control	0 (0)	0 (0)	3 (3)
Test item [µg a.s./bee]			
1	0	0	3
5	0	3	8
10	0	2	4
50	0	0	5
100	1	2	6
Reference item [µg product/bee]			
0.2	2	3	5
0.3	26	27	30
0.4	42	44	44

Validity criteria:

All validity criteria of the test were met.

Validity criteria (OECD 214, 1998)	Obtained in this study
Control mortality should not exceed 10 % at test end	Control: 3 %
LD_{50} of the reference item should be in the specified range (dimethoate: contact test: 0.10 – 0.30 µg a.s./bee)	0.113 µg a.s./bee* (a.s. triazophos)

*0.274 µg product/bee × 41.3 % w/w triazophos

The reference item triazophos confirmed the sensitivity of the bees used in the test.

III. CONCLUSIONS

The LD_{50} (72 h) for honeybees was > 100 µg a.s./bee in the contact toxicity test performed with fluopicolide.

Assessment and conclusion by applicant:

This study is considered reliable for risk assessment and the endpoint is:

LD_{50} contact (72 h) > 100 µg a.s./bee

Data Point:	KCA 8.3.1.1.2/02
Report Author:	[redacted]
Report Year:	2015
Report Title:	Fluopicolide tech.: Effects (Acute contact) on bumblebees (Bombus terrestris L.) in the laboratory
Report No:	88641105
Document No:	M-511408-01-1
Guideline(s) followed in study:	(GLP compliant study based on van der Steen (2001) and OECD 214 (1998) with modifications and adaptions, Ring test bumblebee acute contact toxicity (ICPPR non-apis group, 2014))
Deviations from current test guideline:	Current Guideline: OECD 246 (2017) A 5 µL droplet was chosen in deviation to the guideline recommendation of a 2 µL droplet, since a higher volume ensured a more reliable dispersion of the test item and allows a higher application dose. Analytical determination of the test item was not conducted, but the study was conducted before guideline implementation and no analytical dose verification was foreseen at that point in time. Since it is a limit test with a single dosing of the test item this deviation is not expected to have impacted the study results.
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The purpose of this study was to determine the acute contact toxicity of fluopicolide tech. to the bumble bee (*Bombus terrestris* L.) in the laboratory. Mortality of the bumble bees was used as the toxic endpoint. Sublethal effects, such as changes in behaviour, were also assessed.
Therefore, under laboratory conditions *Bombus terrestris* worker bumble bees were exposed to 100 µg a.s. per bumble bee by topical application. Furthermore, the test consisted of a control, solvent control and a reference item group. Each treatment group consisted out of 50 bumble bees with 1 bumble bee per test unit (replicate). The test was conducted in darkness, temperature was 24-26°C and humidity was 50-63 %. Biological observations, including mortality and sub-lethal effects were recorded 4, 24 and 48 h after application. The NOED was estimated using Fisher Exact Test. At test termination no mortality occurred at 100 µg fluopicolide tech. a.s per bumble bee. 2.0 % mortality occurred in the water control group and there was no mortality in the solvent control group (acetone). The mortality in the reference item group was 64% after 48 hours at a dose of 12 µg dimethoate/bumble bee. All validity criteria of the test were met. The 48 hr contact LD_{50} was determined to be > 100 µg a.s./bumble bee.

I. Material and Methods

Test item: Fluopicolide, technical; 100.5 % w/w (analytical), Origin Batch No.: ETP000273, Customer Order No.: TOX 10747-00; Material: Fluopicolide, technical; Specification No.: 102000016444-01, Article No.: 06032698.
Test organism: female worker bumble bees (*B. terrestris*), obtained from a healthy and queen-right colony, bred by a commercial bumble bee breeding company (Biobest Belgium N.V.).
Under laboratory conditions *Bombus terrestris* worker bumble bees were exposed to 100 µg a.s. per bumble bee by topical application (contact limit test).
Furthermore, the test consisted of a control, solvent control and a reference item group. In the contact limit test tap water containing 0.1% v/v Triton X-100 was used.
BAS 152 11 I EC (active ingredient 400.9 g/L dimethoate, Batch no.: FRE-000926) was used as reference item. Each treatment group consisted out of 50 bumble bees with 1 bumble bee per test unit (replicate).
Test units were 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 test was conducted in darkness, temperature was 24-26°C and humidity was 50-63 %. Biological observations, including mortality and sub-lethal effects were recorded 4, 24 and 48 h after application. The NOED was estimated using Fisher Exact Test (pairwise comparison, one-sided greater, $\alpha = 0.05$).

Dates of experimental work: December 02, 2014 – December 04, 2014

II. RESULTS AND DISCUSSION:

Biological findings:

Test item	**Fluopicolide tech.**
Test object	***Bombus terrestris***
Exposure	**Contact (solution in acetone)**
Dose [µg a.s./bumble bee]	100
LD_{50} [µg a.s./bumble bee]	> 100
LD_{20} [µg a.s./bumble bee]*	> 100
LD_{10} [µg a.s./bumble bee]*	> 100
NOED [µg a.s./bumble bee]**	100

* Since no mortality above 10% occurred in the test, the respective LD_{50}'s values are assumed to be > 100 µg a.s./bumble bee

** The NOED was estimated using Fisher's Exact Test (pairwise comparison with control, one-sided greater, $\alpha = 0.05$).

Observations

Contact test:

At test termination (48 hours after treatment) no mortality occurred at 100 µg fluopicolide tech. a.s per bumble bee. 2.0 % mortality occurred in the water control group (water containing 0.1% v/v Triton X-100) and there was no mortality in the solvent control group (acetone).

Treatment Group	**After 4 hours**		**After 24 hours**		**After 48 hours**	
	Mortality mean %	**Behavioral abnormalities mean %**	**Mortality mean %**	**Behavioral abnormalities mean %**	**Mortality mean %**	**Behavioral abnormalities mean %**
Test item 100 µg a.s./bumble bee	0.0	0.0	0.0	0.0	0.0	2.0
Water control	0.0	0.0	2.0	0.0	2.0	0.0
Solvent Control	0.0	0.0	0.0	0.0	0.0	0.0
Reference item 12 µg dimethoate/bumble bee	0.0	18.0	28.0	72.0	64.0	36.0

Mean = mean of 50 individuals per treatment group
Water control = tap water containing 0.1% Triton X-100
Solvent control = acetone

Validity criteria:

All validity criteria of the test were met.

Validity criteria (OECD 246, 2017)	**Obtained in this study**
Control mortality should not exceed 10 % at test end	Control: 2.0 % Solvent control: 0.0 %
Mortality of the reference item should be ≥ 50 % at test end	Reference item: 64 %

III. CONCLUSIONS

The toxicity of fluopicolide tech. was tested in an acute contact toxicity test on bumble bees. The (48 h) contact LD_{50} value was determined to be > 100 µg a.s./bumble bee.

Assessment and conclusion by applicant:

This study is considered reliable for risk assessment and the endpoint is:

LD_{50} contact (48 h) > 100 µg a.s./bumble bee

CA 8.3.1.2 Chronic toxicity to bees

Data Point:	KCA 8.3.1.2/01
Report Author:	[redacted]
Report Year:	2016
Report Title:	Fluopicolide SC 486 G - Assessment of effects on the adult honey bee, Apis mellifera L., in a 10 day chronic feeding test under laboratory conditions
Report No:	S15-00366
Document No:	M-552253-03-1
Guideline(s) followed in study:	No specific guidelines available. Based on Kling, A. and Schmitzer, S. (2015)
Deviations from current test guideline:	Method: No deviation. Study: Current Guideline: OECD 245 (2017) The relative humidity was 36.7% for a short period of 2 hours, below the range of 50 – 70% recommended in the guideline. This deviation is not expected to have impacted the study results.
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary:

The purpose of this study was to determine chronic oral toxicity of fluopicolide SC 486 (486 g/L) to the honey bee (*A. mellifera* L.) for a period of ten days. Mortality of the bees was used as the toxic endpoint. Sublethal effects, such as changes in behaviour, were also assessed.

Therefore, under laboratory conditions 50 newly hatched worker bees divided into 5 replicates, containing 10 test organisms were exposed for 10 days to a concentration of 3000 mg a.s./kg food of the test item treated sugar solutions *ad libitum*. An untreated control and a reference item with the same number of replicates and bees were included in this study. In addition, 4 additional cages were filled with ~ 3-4 mL of pure 50 % (w/v) aqueous sucrose solution and weighed daily for the determination of the evaporation. Mortality and behavioural abnormalities were assessed every day throughout the 10 days exposure period. The test conditions during the study were 31.7 - 34.6 °C temperature, 36.7 – 64.2% relative humidity and 24 h darkness. At test end, 10 days following start of exposure, 2.0 % mortality occurred in the untreated water control. At 132.68 µg a.s./bee/day 4.0 % mortality occurred. This effect was statistically significant. The reference item at 0.02 µg/bee per day caused 100 % mortality at day 10. All validity criteria were met in this study. The LDD_{50} value (10 days) was determined to be > 132.68 µg a.s./bee per day. The LC_{50} value (10 days) was determined to be > 3000 mg a.s./kg feeding solution.

I. MATERIAL AND METHODS

Test material: Fluopicolide SC 486 G (486 g/L); Short code: FLC SC 486; Fluopicolide: 40.1 % w/w, 486.5 g/L (analysed); Batch ID.: 2014-014197; Sample description: TOX10894-00, Specification No.: 102000011893; density: 1.213 g/mL.
The chronic effects of the test item Fluopicolide SC 486 on the honey bee, *Apis mellifera* L. were assessed in a 10 days continuous oral feeding test in the laboratory (limit test).
Under laboratory conditions 50 newly hatched worker bees (*Apis mellifera* L., 1 to 2 days old) divided into 5 replicates, containing 10 test organisms were exposed for 10 days to a concentration (3000 mg a.s./kg food [ppm]) of the test item treated sugar solutions *ad libitum*. An untreated control (50 % w/v sucrose solution) and a reference item (0.9 mg dimethoate/kg feeding solution [ppm]) with the same number of replicates and bees were included in this study. Mortality and behavioural abnormalities were assessed every day throughout the 10 days exposure period.
The test conditions during the study were 31.7 – 34.6 °C temperature, 36.7 – 64.2% relative humidity and 24 h darkness.

Dates of work: June 09, 2015 to June 23, 2015

II RESULTS AND DISCUSSION:

Analytical results:
The actual concentrations of fluopicolide in the feeding solutions were in a range of 95% - 106%. Therefore, the concentrations of the test item in the diet were confirmed and the endpoints are based on nominal concentrations.

Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Biological results:
The test item was daily administered to the bees in a sugar solution at the following concentrations: 3000 mg a.s./kg sugar solution. This concentration led to a daily mean dose of 132.68 µg a.s./bee per day after 10 days.

At test end, 10 days following start of exposure, 2.0 % mortality occurred in the untreated water control (50 % w/v sucrose solution). At 3000 mg a.s./kg (corresponding to 132.68 µg a.s./bee/day) 4.0 % mortality occurred. This effect was statistically significant (Fisher`s Exact Test, $\alpha = 0.05$). In the test item treatment group no remarkable behavioural abnormalities were observed. The reference item (dimethoate) at a concentration of 0.9 mg dimethoate/kg sugar solution corresponding to 0.02 µg a.s./bee per day caused 100 % mortality at day 10.

Chronic oral toxicity of Fluopicolide SC 486 to young honey bees (laboratory test)

Test Object		*Apis mellifera carnica*	
Treatment Group	Concentration [mg a.s./kg]	Dose Level[1)] [µg a.s./bee/day]	Mortality at day 10[2)] [% Mean]
Fluopicolide SC 486 (486 g/L)	3000	132.68	4.0
Water control	0.0	0.0	2.0 (-2.1)[3]
Reference Item (dimethoate)	0.9	0.02	100.0

Endpoint at test termination (day 10)			
LC_{50}	**LDD_{50}**	**NOEC** [4]	**NOEDD** [4]
> 3000 mg a.s./kg	**> 132.68 µg a.s./bee/day**	**3000 mg a.s./kg**	**132.68 µg a.s./bee/day**

[1)] mean dose per bee per day; dose measured based on consumed feeding solution
[2)] Mortality at study termination 10 days after start of first feeding
[3)] Mortality corrected with the corresponding control mortality according to Schneider-Orelli, O. (1947); a negative value means lower mortality in the test item treatment compared to the control group
[4)] Fisher's Exact Test with Bonferroni Correction (one-sided greater, $\alpha = 0.05$)

The determination of LC_{10}/LD_{10} and LC_{20}/LD_{20} values has not been possible in this study since it was performed as a limit test and due to the low mortality observed in the test item.

Validity criteria:
All validity criteria were met in this study.

Validity criteria (OECD 245, 2017)	**Obtained in this study**
Average mortality at test end in control: ≤15%	2.0%
Average mortality at test end in toxic reference item: ≥50%	100%

III. CONCLUSIONS

The chronic toxicity of Fluopicolide FS 486 (486 g/L) was tested over 10 days on adult honeybees. The LDD_{50} value (10 days) was determined to be > 132.68 µg a.s./bee per day. The LC_{50} value (10 days) was determined to be > 3000 mg a.s./kg feeding solution.

Assessment and conclusion by applicant:

This study is considered reliable for risk assessment and the endpoints are:

LDD_{50} oral (10 days) > 132.68 µg a.s./bee per day

LC_{50} oral (10 days) > 3000 mg a.s./kg feeding solution

CA 8.3.1.3 Effects on honeybee development and other honeybee life stages

Data Point:	KCA 8.3.1.3/01
Report Author:	[redacted]
Report Year:	2018
Report Title:	Fluopicolide - Honey bee (Apis mellifera L.) 22 day larval toxicity test (Repeated exposure) - Final report
Report No:	S17-00177
Document No:	M-615695-01-1
Guideline(s) followed in study:	Regulation (EC) No 1107/2009 (2009) Directive 2003-01 (Canada/PMRA) US EPA OCSPP 850.SUPP OECD (2016): Series on Testing and Assessment Number 239: Guidance Document on Honey Bee (Apis mellifera) Larval Toxicity Test, Repeated Exposure
Deviations from current test guideline:	Method: Deviations from current guideline SANCO/3029/99 rev.4: Limited sets of validation recoveries were analysed. However, the average recoveries were within the acceptable range of 70–110% and the RSD values were below 20%. The analytical method can be regarded as fit for purpose. Study: Current Guideline: OECD GD 239 (2016) The deviations from the recommended humidity ranges recorded for more than 2 hours on day 8, 9 and 18 are not expected to have impacted the study results.
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The purpose of this study was to determine the chronic toxicity ($ED_{10/20/50}$, $EC_{10/20/50}$, NOED/NOEC and LOED/LOEC for adult emergence up to day 22) of the test item fluopicolide applied to the honey bee, *Apis mellifera* L., larvae in an *in vitro* test after repeated oral application. The test item was administered to the larvae at a constant concentration in the diet according to their growth at a range of five increasing doses. Cumulative mortalities of honey bee larvae treated with the test item were assessed daily from day 4 to day 8. Cumulative mortalities during the pupation phase were assessed on day 15 and on day 22. All mortalities were compared to the control. The adult emergence rate was assessed on day 22. On day 8, larval mortality was 6.3 % in the control group and 8.3 % in the solvent control group. Larval mortality in the reference item group was 100 %. The larval mortality was 8.3, 8.3, 12.5, 14.6 and 22.9 % at cumulative doses of 3.76, 7.52, 15.0, 30.0 and 60.1 µg fluopicolide/larva per developmental period, respectively. On day 22, the adult emergence rate in the control and solvent control group was 83.3 and 79.2 %. Consequently, validity criteria for the control groups and the reference item group were met and the test was considered valid. The adult emergence rates were 81.3, 79.2, 72.9, 70.8 and 60.4 % at cumulative doses of 3.76, 7.52, 15.0, 30.0 and 60.1 µg fluopicolide/larva per developmental period, respectively.

The NOEC for adult emergence on day 22 was determined as ≥ 390 mg fluopicolide/kg diet, equivalent to a NOED of ≥ 60.1 µg fluopicolide/larva per developmental period. The EC_{10} for adult emergence on day 22 was determined as 175 mg fluopicolide/kg diet, equivalent to an ED_{10} of 27.0 µg fluopicolide/larva per developmental period. The EC_{20} for adult emergence on day 22 was determined as 316 mg fluopicolide/kg diet, equivalent to an ED_{20} of 48.7 µg fluopicolide/larva per developmental period. The EC_{50}/ED_{50} for larval mortality on day 22 could not be calculated due to the lack of inhibition in emergence above 50 %. However, the EC_{50} is empirically considered to be > 390 mg fluopicolide/kg diet, equivalent to an ED_{50} of > 60.1 µg fluopicolide/larva per developmental period.

I. MATERIAL AND METHODS

Test item: Fluopicolide; Batch No.: FP805026, Sample description: TOX203703-01, Specification No.: 10200001644-01, Analysed purity a.s.: 98.7 % w/w, Certificate No.: AZ 21472.
Test species: Honey bee (*Apis mellifera carnica* (Pollmann)), synchronized first instar (L1, one day old) larvae originating from healthy (free of clinical symptoms of any disease) and queen-right bee colonies. The larvae were taken from hives that had not received treatments with chemical substances for at least one month. The test was conducted at Eurofins Agroscience Services Ecotox GmbH Neulingen-Göbrichen, Nordweg 10, 75245 Neulingen-Göbrichen, Germany.
Test design: Dose response test with a duration of 22 days from grafting on day 1 to the final assessment on day 22. From day 3 until day 6 of the test, five different concentrations of fluopicolide diluted in the larval food (aqueous yeast/sugar solution mixed with royal jelly 1:1 (w/w)) were fed to larvae of the test item groups and one single concentration of the reference item dimethoate was fed to the larvae of the reference item group with diet B or C. After the applications, no additional feeding of the larvae took place.
The analysed purity was considered for the calculation of the test item and reference item concentrations; the daily feeding volume increased from 20 µL to 50 µL diet per larva over the application period. The cumulative feeding volume from day 3 until day 6 of 140 µL diet per larva and the density of the diet (1.1 g/cm^3) were considered for the calculation of the cumulative doses per larva. A control group was included in the test and exposed for the same period of time under identical exposure conditions to the water treated artificial diet. Each treatment group consisted of 48 larvae from three different colonies (each colony representing a replicate). Assessment of larval mortality was performed during the larval phase from day 4 until day 8, assessment of mortality during pupation was performed on day 15 and day 22. Other observations and any other adverse effects were qualitatively recorded to aid in the interpretation of mortality in comparison to the solvent control group. Assessment of adult emergence was performed on day 22. The presence of uneaten food was qualitatively recorded on day 8.
Test concentrations: One control group; one solvent control group; 5 test item groups with 24.4, 48.8, 97.5, 195 and 390 mg fluopicolide/kg diet, equivalent to cumulative doses of 3.76, 7.52, 15.0, 30.0 and 60.1 µg fluopicolide/larva per developmental period; 1 dimethoate reference item group with 48 mg dimethoate/kg diet, equivalent to a cumulative dose of 7.39 µg dimethoate/larvae per developmental period.

Dates of work: August 09, 2000 – August 12, 2000

II. RESULTS AND DISCUSSION:

Analytical results:
The mean measured concentrations of the test item in the larval diet were within ± 20% of nominal for each test item group. Therefore, the concentrations of the test item in the larval diet were confirmed and the endpoints are based on nominal concentrations.

Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4 with minor acceptable exceptions only.

Biological results:
On day 8, larval mortality was 6.3 % in the control group and 8.3 % in the solvent control group. Larval mortality in the reference item group was 100%. The larval mortality was 8.3, 8.3, 12.5, 14.6 and 22.9 % in the test item groups of 24.4, 48.8, 97.5, 195 and 390 mg fluopicolide/kg diet, equivalent to cumulative doses of 3.76, 7.52, 15.0, 30.0 and 60.1 µg fluopicolide/larva per developmental period, respectively.

On day 22, the adult emergence rate in the control and solvent group was 83.3% and 79.2%. The adult emergence rates were 81.3, 79.2, 72.9, 70.8 and 60.4% in the test item groups of 24.4, 48.8, 97.5, 195 and 390 mg fluopicolide/kg diet, equivalent to cumulative doses of 3.76, 7.52, 15.0, 30.0 and 60.1 µg fluopicolide/larva per developmental period, respectively. Compared to the control group the adult emergence rate on day 22 was not statistically significantly different in any test item group (Multiple Chi²-test with Bonferroni-Holm adjustment, one-sided greater, α = 0.05).
During the assessments of mortality and emergence no other test item related observations such as deviating sizes, appearances and malformations of the test organisms were made.
On day 8, uneaten food was observed in the control groups and all test item groups.
Results for larval mortality until day 8, as well as for adult emergence on day 22, including the corresponding endpoints are presented in the following table.

Mortality and other observations of larvae in the repeated exposure toxicity test

Treatment Group	Concentration		Cumulative Dose		Larval Mortality on Day 8		Adult Emergence on Day 22 [a]	
					[%]	Corrected [%]	[%]	Inhibition [b] [%]
Control	---	---	---	---	6.3	---	83.3	---
Solvent Control	---	---	---	---	8.3	---	79.2	---
Test item (Fluopicolide)	24.4	[mg fluopicolide/ kg diet] [c]	3.76	[µg fluopicolide/ larva per developmental period] [c, d]	8.3	0.0	81.3	-2.7
	48.8		7.52		8.3	0.0	79.2	0.0
	97.5		15.0		12.5	4.6	72.9	8.0
	195		30.0		14.6	6.9	70.8	10.6
	390		60.1		22.9	15.9	60.4	23.7
Reference Item (Dimethoate)	48.0	[mg dimethoate/ kg diet] [c]	7.39	[µg dimethoate/ larva per developmental period] [c, d]	100	100	---	---

[a] Statistical evaluation of non-emergence
[b] Negative values indicate higher emergence compared to the solvent control group.
[c] Based on the analysed purity
[d] Based on the cumulative feeding volume from day 3 until day 6 of 140 µL diet/larva and a density of the diet of 1.1 g/cm3

Calculated endpoints of the repeated exposure larvae toxicity test

Treatment	Endpoint: Successful adult emergence	Up to day 22
Test item doses	ED_{50} [µg a.s./larva]	> 60.1 b
	ED_{20} [µg a.s./larva]	48.7
	ED_{10} [µg a.s./larva]	27.0
	LOED [µg a.s./larva]	n.d. a
	NOED [µg a.s./larva]	≥ 60.1
Test item concentrations	EC_{50} [mg a.s./kg food]	> 390 b
	EC_{20} [mg a.s./kg food]	316
	EC_{10} [mg a.s./kg food]	175
	LOEC [mg a.s./kg food]	n.d. a
	NOEC [mg a.s./kg food]	≥ 390

[a] The LOEC/LOED could not be determined due to the lack of statistically significant effects (Multiple Chi²-test with Bonferroni-Holm adjustment, one-sided greater, α = 0.05
[b] The EC_{50}/ED_{50} could not be calculated due to the lack of inhibition in emergence > 50%, but can be regarded as above the highest concentration/dose tested.

Validity criteria:
All validity criteria were met in this study.

Validity criteria (OECD GD 239, 2016)	Obtained in this study
Cumulative larval mortality from day 3 to 8 in control/solvent control: ≤ 15%	6.3% / 8.3%
Mean adult emergence rate on day 22 in control/solvent control: ≥ 70%	83.3% / 79.2%
For reference item dimethoate larval mortality at day 8: ≥ 50%	100%

III. CONCLUSIONS

In a repeated exposure honeybee larval feeding toxicity study with fluopicolide and a duration of 22 days, the NOED (emergence) was determined to be ≥ 60.1 µg a.s./larva, equivalent to a NOEC of ≥ 390 mg a.s./kg food.

Assessment and conclusion by applicant:

This study is considered reliable for risk assessment and the endpoints are:

NOED (emergence) ≥ 60.1 µg a.s./larva

NOEC (emergence) ≥ 390 mg a.s./kg food

Data Point:	KCA 8.3.1.3/02
Report Author:	[redacted]
Report Year:	2016
Report Title:	Fluopicolide SC 486 (486 g/L): Effects on honey bee brood (Apis mellifera L.) - Brood feeding test
Report No:	98781031
Document No:	M-545732-01-1
Guideline(s) followed in study:	Oomen P.A., de Ruijter, A. & van der Steen, J., 1992 Regulation (EC) No. 1107/2009 Directive 2003-01 (Canada/PMRA) US EPA OCSPP not applicable
Deviations from current test guideline:	Current Guideline: Oomen at al. (1992) No deviations
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The purpose of the honey bee brood feeding study was to evaluate potential effects of Fluopicolide SC 486 on brood development and mortality of adult worker honey bees, *Apis mellifera* L. (Hymenoptera: Apidae). Therefore, a bee brood test was conducted under natural field conditions with a control, 3.23 g test item and the reference item. Four bee colonies were used per treatment group. Each colony contained two magazines with 11 frames each. The mean strength of the colonies per treatment group, one day before application ranged between 14805 and 16211 adult bees. At the start of the experiment, each colony had 8 – 15 brood combs containing eggs, larvae and capped cells and a sufficient amount of honey and pollen. 1-2-year-old healthy sister queens were used. Ontogenesis of a defined number of honey bee eggs, young- and old larvae were observed for a period of 21 days following the application for each treatment group and colony. Mortality of adult bees, pupae and larvae was also assessed between 3 days before to 21 days after application. Statistical evaluation was done for mortality and brood termination rates using ToxRat Professional. No effect of the test item on the development of eggs and young larvae was observed. The mean termination rate of eggs and the mean development success of larvae was slightly but not significantly higher in the treatment than in the control. The mean termination rate of old larvae was lower in the test item treatment group than in the control group. Adult bee mortality in the test item group was significantly higher when compared to the control group. This was interpreted as random fluctuation and as not biologically relevant in relation to colony strength. No effects of the test item on honey bee pupae and larvae were observed. The reference item treatment resulted in a statistically significant increase of unsuccessful egg, young- and old larvae development and thus, confirmed the sensitivity of the test system and the validity of the test conditions. According to the results of this study the administration of the test item Fluopicolide SC 486 at a concentration of 1.33 g fluopicolide/L to honey bee colonies did not adversely affect honey bee colonies and bee brood development.

I. MATERIAL AND METHODS

Test material: Fluopicolide SC 486 (486 g/L), Specification No.: 1020000118893, Supplier Batch No.: 2014-014197, Sample description: TOX10894-00, density: 1.213 g/mL, content of active ingredient: 40.1% w/w, 486.5 g/L (analysed).

Test species: *Apis mellifera carnica* L. Honey bee colonies, maintained according to normal beekeeping practice, by ibacon's responsible beekeeper. No varroacide has been used in the colonies for at least 4 weeks prior to the experimental starting date. Colonies were well fed and queen-right, each colony occupied two magazines ("Deutsch Normalmaß, DN") with 11 frames each. At the start of the experiment, each colony had 8 - 15 brood combs containing eggs, larvae and capped cells and a sufficient amount of honey and pollen. The colonies were assembled at the same time with healthy queens in order to guarantee uniform bee material in all treatments. 1- 2 year-old sister queens were used. The colonies contained a mean of about 14805 - 16211 adult honey bees. All colonies were equipped with a dead bee trap at the entrance.

Test design: The study included three treatments: Control (C, 1 L ready-to-use sugar syrup (Apiinvert) per colony, Test item (T, 3.23 g test item (Fluopicolide SC 486 in 1 L sugar solution (ready-to-use syrup, Apiinvert)), equivalent to an active substance concentration of 1.33 g fluopicolide/L sugar solution, Reference item (R, 3.2 g reference item (Insegar) in 1 L ready-to-use sugar syrup (Apiinvert), equivalent to a nominal concentration of 0.8 g fenoxycarb/L.
For all treatments, 4 replicates (colonies) were set up 13 days before treatment (DAT -13). The treatment administration was conducted during the afternoon. The ready prepared sugar solutions were offered per colony in a feeding trough. The trough was put into an empty magazine on top of the populated bee magazines. Food uptake was complete 42 hours after application. The colonies were monitored from day -3 to day 22 after application.

Test conditions during the experiment: The experimental phase of this study took place at a settled, constant weather period with sunny and moderate warm days. Mean temperatures over the course of the study ranged from 12.7 °C to 24.7 °C. Rain occurred only on a few occasions and mostly at the end of the experiment.

Dates of work: May 29, 2015 – June 24, 2015

II. RESULTS AND DISCUSSION:

Validation of the study

Control Brood Termination Rates:
The mean brood termination rates of eggs, young larvae and old larvae in the control group were: 11.5, 6.8 and 10.2%, respectively. The corresponding historic mean brood termination rates are: 23.8, 17.4 and 7.7%. Thus, the presented control brood termination rates were seen as within normal values compared with historic data.

Control Mortality and Effects on Brood:
Mean control mortality of the adult bees from day 0 after application to day 21 differed between 0.5 and 25.3 dead bees. As the overall mean mortality in the control group after application was low (8.3 dead bees/colony/day), this value can be empirically regarded to be within the range of normal mortality levels of colonies of the employed size under field conditions. In addition, a mean of 1.0 dead pupae per colony per day were found during the 21 days post-application period. This value can be considered to represent a biologically typical number of dead pupae over a period of 21 days.

Reference Item Mortality and Effects on Brood

There was a high number of impacted bee brood, which resulted in 89.5% mean loss of the initial observed cells (98.0% eggs, 94.5% young larvae and 76% old larvae stages, respectively). The termination rates of the different brood stages were statistically significantly higher compared to the control. Thus, the reference item values were on an absolute scale sufficiently high to demonstrate the sensitivity of the test system and the validity of the test conditions.

Effects of Fluopicolide SC 486 G (486 g/L) on honey bee brood

Test item	Fluopicolide SC 486 (486 g/L)		
Test species	Honey bees *(Apis mellifera* L.) (complete colonies)		
Exposure	*via* treated sugar solution		
Treatment	Untreated control	Fluopicolide SC 486 (486 g/L)	Reference Item (Insegar, a.i. = fenoxycarb)
Rate per L sugar solution [product][1)]		3.32 g Test item/L	3.2 g/L
Rate per L sugar solution [a.s.][1)]		1.33 g fluopicolide/L	0.8 g a.s./L
Termination rate of the eggs [%] [2)]	11.5 %	15.3 % (n.s.)	98.0 % (*)
Termination rate of the young larvae [%] [2)]	6.8 %	9.8 % (n.s.)	94.5 % (*)
Termination rate of the old larvae [%] [2)]	10.2 %	6.5 % (n.s.)	76.0 % (*)
Mean brood termination rate over all stages	9.5 %	10.5 % (n.s.)	89.5 % (*)
Mean mortality of worker bees/colony/day during pre-application phase [3)] during the entire post-application phase [3)]	9.1 8.3	10.9 (n.s.) 31.0 (*)	9.8 (n.s.) 32.5 (*)
Mean mortality of pupae/colony/day during pre-application phase [4)] during the entire post-application phase [4)]	0.1 1.0	0.3 (n.s.) 1.5 (n.s.)	0.0 (n.d.) 5.2 (*)
Mean number of bees before application [5)]	15075	14805	16211

Statistics: n.s. = not statistically significant compared to the control; * = statistically significant compared to the control; n.d. = not determined (due to "0" response); Student or Welch t-test, $\alpha = 0.05$, pairwise comparison, two-sided (before application), one-sided greater (after application)

1) test and reference item were mixed in sugar solution
2) mean termination rate of 4 colonies per treatment group
3) mean number of dead honeybees per day and colony found in dead bee traps
4) mean number of dead pupae/larvae per day and colony found in dead bee traps
5) mean number of bees per colony

In-hive worker mortality (dead bee traps):

The overall daily mean worker bee mortality observed before provisioning of the feeding solutions (DAT -3 to DAT 0) was low and ranged from 9.1 to 10.9 amongst the three treatments. No statistically significant differences between treatments were detected.

The overall daily mean worker bee mortality after provisioning of the feeding solutions (DAT 0 – 21) was 8.3, 31.1 and 32.5 in the control, test item and reference item treatment, respectively. Statistically significant differences were detected between control and test item and between control and reference item.

Pupal and larval mortality:
The overall daily mean pupal and larval mortality observed before provisioning of the feeding solutions (DAT -3 – 0) was 0.1, 0.3 and 0.0 pupae/colony in the control, test item and reference item, respectively. No statistically significant differences between treatments were detected.

The overall daily mean pupal and larval mortality after application (day 0 – 21) of all treatments was 1.0, 1.6 and 5.2 in the control, test item and reference item treatment, respectively. As compared to the control, there was a statistically significant increase of pupae mortality in the reference item treatment throughout the entire post-application phase, but not in the test item treatment.

Behaviour:
No behavioural impairments were noted at any time in any of the test or reference item treatment groups until test end. Also, no behavioural abnormalities were observed in the control group.

Uptake of feeding solutions:
Feeding solutions were fully collected from the feeders within 24-48 hours in the control and test item treatment and within 24 hours in the reference treatment.

Colony strength:
The mean colony strength before treatment administration (DAT -1) was 15075, 14805 and 16211 bees/colony in the control, test item and reference item treatment, respectively.

Brood Termination Rate:
The mean Brood Termination Rate of the control, test item and reference item treatment, respectively, at the last assessment (BFD 22) were 11.5%, 15.3% and 98.0% for eggs. For young larvae the mean brood termination rate of the control, test item and reference item, respectively at the last assessment were (BFD 22) 6.8%, 9.8% and 94.5% (young larvae). For old larvae the mean brood termination rate at the last assessment (BFD 16) was 10.2%, 6.5% and 76.0%. The mean brood termination rates over all stages were 9.5% for the control, 10.5% for the test item and 89.5% for the reference item. Brood Termination Rates of eggs, young, old larvae and over all stages did not significantly differ between test item and control replicates.

As compared to the control, in the reference item treatment, Brood Termination Rate was statistically significantly higher for eggs, young larvae (from BFD 22 onwards), as well as for old larvae (both from BFD 16 onwards). Also, the Brood Termination Rate over all stages was statistically significantly higher in the reference item treatment than in the control. This indicates that the test system was sufficiently sensitive to detect potential effects of plant protection products on honey bee brood.

III. CONCLUSIONS

No effect on the development of eggs was observed after consumption of the test item treated sugar solution. The mean termination rate of eggs in the test item treatment group was slightly higher with a mean of 15.3 % compared to 11.5 % in the control group. This difference was not statistically significant compared to the control group.

There was also no effect on the development of young larvae after consumption of the test item *via* treated sugar solution. The development success of the young larvae in the test item treatment group resulted in a mean termination rate of 9.8 % compared to 6.8 % in the control group. This difference was not statistically significant compared to the control group.

The mean termination rate of old larvae was lower in the test item treatment group (6.5 %) when compared to the values of the control group (10.2 %). Accordingly, this was not statistically significant different. Thus, there was no effect on the development of old larvae following the consumption of the test item via treated sugar solution.

Adult bee mortality in the test item treatment group was higher (mean of 31.0 dead bees per day) when compared to the control group (8.3 dead bees per day). Although this difference was statistically significantly different to the control it is interpreted to be a random fluctuation since peaks in mortality occurred in two test item replicates only and since comparable peaks in mortality occurred also in two of the control replicates. Overall this low mortality rate is not biological relevant, considering colony strength of about 12000 to 17000 bees per colony.

No effects of the test item on honey bee pupae and larvae were observed.
The reference item treatment (Insegar, a.s. = fenoxycarb) resulted in a statistically significant increase of unsuccessful egg, young- and old larvae development and thus confirmed the sensitivity of the test system and the validity of the test conditions.

Overall, it can be concluded according to the results of this study that the administration of Fluopicolide SC 486 (486 g/L) fortified sugar syrup at a concentration of 1.33 g fluopicolide/L to honey bee colonies did not adversely affect honey bee colonies and bee brood development.

Assessment and conclusion by applicant:

This study is considered reliable for risk assessment and the conclusions are:

No statistically significant effect on the development of eggs. Brood termination rate for eggs (BFD 22) of 15.3 %.

No statistically significant effect on the development of young larvae. Brood termination rate for young larvae (BFD 22) of 9.8 %.

No statistically significant effect on the development of old larvae. Brood termination rate for old larvae (BFD 22) of 6.5 %.

Statistically significant difference on daily dult bee mortality considered as random fluctuation and biologically irrelevant.

Fluopicolide SC 486 (486 g/L) applied as fortified sugar syrup at a concentration of 1.33 g fluopicolide/L to honey bee colonies did not adversely affect honey bee colonies and bee brood development.

Data Point:	KCA 8.3.1.3/03
Report Author:	[redacted]
Report Year:	2016
Report Title:	Fluopicolide SC 486 (486 g/L): Effects on honey bee brood (Apis mellifera L.) under semi-field conditions - Tunnel Test - Final report
Report No:	98781033
Document No:	M-547124-01-1
Guideline(s) followed in study:	OECD No. 75 (2007) and OEPP/EPPO No. 170 (4) (2010) Regulation (EC) No. 1107/2009 Directive 2003-01 (Canada/PMRA) US EPA OCSPP not applicable
Deviations from current test guideline:	Current Guidelines: OECD 75 (2007) and EPPO 170 (4) (2010) No deviations
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The purpose of the study was to investigate potential side effects of a spray application of Fluopicolide SC 486 (486 g/L) on the honeybee (*Apis mellifera* L.) under semi-field conditions by following the OECD Guidance Document No. 75 (2007), with methodological improvements by the AG Bienenschutz (2011). Therefore, tunnels were set up on a ca. 75 m² plot of *Phacelia tanacetifolia*. Small bee colonies were introduced to the tunnels 3 days before the application. One honey bee colony was used per tunnel. Colonies contained 11 combs with honey, pollen and brood. The mean strength of the colonies per treatment group, one day before the application, was similar and ranged between 6514 and 6953 adult bees per colony. The preliminary brood check indicated healthy colonies with all brood stages present and a minimum reserve of food. The test item, water and a reference item were applied on the whole plot of plants in two operations, with foraging bees present. The trial was carried out using four tunnels (i.e. replicates) for the test item treatment, the control and the reference item treatment, respectively. The confined exposure phase of the honey bees inside the treated crop was 7 days following the test item application. Ontogenesis of the bees from egg to adult workers, behavioural abnormalities, mortality of adult bees and pupae, the condition of the colonies and the bee brood development were assessed. No biological relevant adverse effects on mortality of pupae were observed. Worker bee mortality, foraging activity, behaviour, nectar- and pollen storage as well as queen survival was not affected. No effects on colony development, colony strength or bee brood were observed. The observed, characteristic brood effects of the reference item showed that the test conditions allowed a profound detection of effects on immature honey bee life stages. Based on the results of this study, it can be concluded that Fluopicolide SC 486 does not adversely affect honey bees and honey bee brood when applied at a concentration of 132 g fluopicolide/ha during honey bees actively foraging on a bee-attractive, flowering crop.

I. MATERIAL AND METHODS

Test item: Fluopicolide SC 486 (486 g/L): Fluopicolide: 40.1 % w/w (analytical); supplier batch No: 2014-014197; Sample Description: TOX10894-00; Specification No.: 102000011893; density: 1.213 g/mL (20°C).
Test species were honeybees (*Apis mellifera L.*; Hymenoptera, Insecta); small honeybee colonies containing 6514 – 6953 bees each and 5 brood combs containing all brood stages and an appropriate amount of nectar and pollen. Colonies were free of obvious bee diseases and were in a queenright state.
A plot of *Phacelia tanacetifolia* with an effective crop area of ca. 75 m² (2 × 36 m²) was prepared for each tunnel (20 m long, 5.5 m wide and 2.5 m high) and each plot constituted one replicate. For each treatment group (control, test item and reference item), 4 tunnels/replicates were set up, resulting in 12 tunnels in total. Per tunnel one honeybee colony was used. The bee colonies were placed into the tunnels with *Phacelia* (BBCH 65) three days before application. After 7 days of exposure all bee colonies were transferred to an area with no pesticide application and main crops flowering.
Applications of the test item Fluopicolide SC 486 (486 g/L), control and reference item (Insegar WG, 250 g/kg fenoxycarb) were conducted by spraying the whole area of plants within the tunnel at full flowering of the crop (BBCH 65) with confined honey bees during bee flight. Control tunnels were sprayed with water (400 L/ha), test item tunnels received 133 g a.s. in 400 L tap water/ha (corresponding to 273 mL product/ha) and reference item tunnels received 300 g a.s. in 400 L tap water/ha (corresponding to nominal 1200 g Insegar/ha).

The following endpoints were assessed:

- Mortality assessment: Dead worker bees were assessed in dead-bee traps and by collecting dead bees from gauze strips.
- Foraging activity: Numbers of bees foraging on flowering plants were recorded.
- Behavioural abnormalities of the bees were recorded.
- Colony conditions: The status of the brood (eggs, young and old larvae, closed brood) and the status of pollen/nectar stores in the colonies was estimated in a quantitative manner (in percentage to the different brood stages on each comb).
- The strength of the colonies was also estimated in a quantitative manner.
- The development of bee brood (ontogenesis of eggs) was evaluated for appropriate amount of eggs (250) from each colony.

Statistical evaluation was done for mortality, foraging activity, colony strength, brood termination rate and brood indices using Shapiro-Wilk's test, Levene's test (check for homogeneity of variance), Student or Welch t-test (pairwise comparison); software: TQX Rat Professional, Version 2.10.05, ®ToxRat Solutions GmbH).

Dates of experimental work: June 10, 2015 – June 11, 2015

II. RESULTS AND DISCUSSION:

Mortality of the adult bees (worker bees)

Mean worker bee mortality in dead-bee traps was comparable between all treatment groups during pre-exposure, i.e. 32, 32.6 and 23.9 dead bees/colony/day in the control, test item treatment and the reference item group, respectively.

During the exposure phase the average control mortality (53.1 dead bees/colony/day) was higher than the average mortality in the test item group (38.8 dead bees/colony/day) and the average mortality in the group with the reference item (43.6 dead bees/colony/day).

During the post-exposure phase the mortality in the test item group and the reference item group (4.0 dead bees/colony/day for both groups) was slightly higher than in the control (2.8 dead bees/colony/day).

There was no significant difference ($p > 0.05$) in the mean worker bee mortality between control and test item and control and reference item considering the pre-application phase (day -2 to 0), the exposure phase (day 0 – 7) and the post-exposure phase (day 8 to 27).

Mortality of pupae

No dead pupae were found in the control treatment before application. In the test and reference item group 0.25 dead pupae/day/colony and 1.33 dead pupae/day/colony were found, respectively.

Mean pupae mortality during exposure phase in the control, test item and reference group was 0.19, 0.41 and 0.16 dead pupae/day/colony, respectively.

During the post-exposure phase 0.05 dead pupae/day/colony were found in the control, 0.41 dead pupae/day/colony in the test item group and 2.17 dead pupae/day/colony were found in the group with the reference item. Both figures during post-application, i.e. for the test item group and the reference item group were statistically different from the control.

Effects of Fluopicolide SC 486 G (486 g/L) on honey bee brood under semi-field conditions

Parameter	Treatment group[1]		
	Control	Fluopicolide SC 486 (486 g/L) [133 g a.s./ha]	Reference Item Insegar [300 g a.s./ha]
Mean mortality of worker bees / colony / day [n] during			
pre-application phase [2]	32.0 ± 26.1	32.6 ± 17.6 (n.s.)	23.9 ± 13.3 (n.s.)
exposure phase in the tunnels [2]	53.1 ± 17.4	38.8 ± 12.4 (n.s.)	43.6 ± 18.5 (n.s.)
phase outside the tunnels [3]	2.8 ± 2.1	4.0 ± 4.4 (n.s.)	4.0 ± 2.3 (n.s.)
overall after application	17.1 ± 24.8	13.9 ± 17.6 (n.s.)	15.3 ± 20.6 (n.s.)
Mean mortality of larvae and pupae [n] during			
pre-application phase [4]	0.00 ± 0.00	0.25 ± 0.43 (n.s.)	1.33 ± 2.31 (n.s.)
exposure phase in the tunnels [4]	0.19 ± 0.29	0.41 ± 0.61 (n.s.)	0.16 ± 0.23 (n.s.)
phase outside the tunnels [5]	0.05 ± 0.10	0.35 ± 0.71 (*) [a]	2.98 ± 3.58 (*)
overall after application	0.09 ± 0.18	0.37 ± 0.67 (*) [a]	2.17 ± 3.27 (*)
Mean foraging activity / m² / colony / day [n] during			
pre-application phase	15.4 ± 8.1	12.6 ± 9.2 (n.s.)	14.7 ± 5.3 (n.s.)
exposure phase in the tunnels	24.8 ± 9.4	23.7 ± 11.6 (n.s.)	23.9 ± 9.1 (n.s.)
Mean brood termination rate [%] [6]	13.2 (n.s.)	24.2 (n.s.)	63.4 (*)

1) each with four tunnels (replicate)
2) mean number of dead honey bees per day and colony found in dead bee traps and on gauze strips in the tunnels
3) mean number of dead honey bees per day and colony found in dead bee traps, only
4) mean number of dead pupae/larvae per day and colony found in dead bee traps and on gauze strips in the tunnels
5) mean number of dead pupae/larvae per day and colony found in dead bee traps, only
6) at BFD23Statistic: Student or Welch t-test, α=0.05, pairwise; before application: two-sided; after application: one-sided greater (mortality and termination rate), one-sided smaller (foraging activity).
n.s. = not statistically significant compared to the control; * = statistically significant compared to the control

[a]: the statistically significant higher mortalities was caused by 1 out of the 4 colonies only.

Behaviour

No test item related behavioural abnormalities occurred at any time during the whole assessment period (up to day 27). No behavioural abnormalities were observed in the control group and in the reference item group.

Foraging activity

Overall daily mean foraging activity observed before application was similar in all treatments with 15.4, 12.6 and 14.7 bees in the control, test item and reference item group, respectively.
During the exposure phase on average 24.8, 23.7 and 23.9 were recorded for control, test item and reference item, respectively. No statistically significant differences were observed between control and test item and between control and reference item.

	Control	Test Item	Reference Item
Mean foraging activity / m^2 / day during pre-application phase	15.4	12.6 n.s.	14.7 n.s.
Mean foraging activity / m^2 / day during exposure phase in the tunnels	24.8	23.7 n.s.	23.9 n.s.

n.s. = not significant compared to the control

Colony condition

At the beginning of the trial all colonies to be used for the test were very similar, all queens (or eggs) and brood stages (eggs, larvae and closed brood) was found in all colonies as an indication of healthy colonies. Moreover, the amount of food reserves (uncontaminated nectar and pollen) was sufficient to ensure colony viability and brood status but also allowed that enough space was available for exposure of the brood to new food sources.
All queens and/or a sufficient presence of eggs were found in the test item treated colonies during all following brood checks indicating that the queens were alive and healthy.
At the end of the 7th day after application, the hives were relocated from their tunnels. In general, the test item treated colonies developed in the same manner as the control colonies. Compared to the control, a similar amount of brood could be found during the assessments with no indication of a test item related effect. All test item treated colonies remained vital with increasing bee numbers and healthy brood. There was no indication of any effect of the test item on the condition of the bee colonies.
In contrast to this, the development of the larvae and pupae in the reference item colonies was distinctly decreased after the application.

Brood termination rate

The mean brood termination rates (BTR) on BFD -23 were 13.2%, 24.2% and 63.4% for control, test item and reference item colonies, respectively. The statistical analysis showed no significant difference in the brood termination rates of colonies exposed to the control and test item ($p > 0.05$). In contrast, a statistically significant effect on the brood termination rate was detected in the comparison between the control and reference item colonies ($p < 0.05$).

	Control	Test Item	Reference Item
Brood Termination Rate (BTR) [%]	13.2	24.2 n.s.	63.4*

n.s. = not significant compared to the control, * = significant compared to the control ($p < 0.05$)

Brood index

Following the labelling of the egg stages, mean brood indices of the test item group indicated a continuous brood development with values slightly lower when compared to the control group between BFD +5 to BFD +23. The mean brood indices in the test item group were 2.5, 3.1, 3.0 and 3.8 at BFD +5, BFD +9, BFD +15 and BFD +23, respectively compared with 2.8, 3.6, 3.5 and 4.3 in the control group. This was not statistically significantly different compared to the control ($p > 0.05$). Accordingly, no adverse effects of the test item on brood development have been observed throughout the study following the labelling of the egg stage up to day 22 after application (BFD + 23). After treatment with the reference item Insegar (a.s.: fenoxycarb) following the labelling of the eggs, the mean Brood Indices were statistically significant lower compared to the control indices (> 0.05).

Treatment Group	BFD +5	BFD +9	BFD +15	BFD +23
Control	2.8	3.6	3.5	4.3
Test Item	2.5 n.s.	3.1 n.s.	3.0 n.s.	3.8 n.s.
Reference Item	1.4*	1.6 n.s.	1.5*	1.8*

n.s. = not significant compared to the control, * = significant compared to the control ($p < 0.05$)

Brood compensation index

The mean brood compensation indices in the test item group were 2.5, 3.1, 3.1 and 4.1 at BFD +5, BFD +9, BFD +15 and BFD +23, respectively compared with 2.8, 3.6, 3.5 and 4.4 in the control. This slight difference was not statistically significant compared to the control ($p > 0.05$). Accordingly, no adverse effects of the test item on brood development have been observed throughout the study, following the labelling of the egg stage up to day 22 after application (BFD +23). The high termination rate of the marked cells after treatment with the reference item Insegar (a.s. fenoxycarb) is also reflected by the statistically significantly lower Brood Compensation Indices in the reference item group when compared to the control ($p > 0.05$). Brood Compensation Indices for the reference item were 1.6, 1.7, 2.0, and 3.2 at BFD +5, BFD +9, BFD + 15 and BFD +23.

Treatment Group	BFD +5	BFD +9	BFD +15	BFD +23
Control	2.8	3.6	3.5	4.4
Test Item	2.5 n.s.	3.1 n.s.	3.1 n.s.	4.1 n.s.
Reference Item	1.6*	1.7 n.s.	2.0*	3.2*

n.s. = not significant compared to the control, * = significant compared to the control ($p < 0.05$)

Validation of the study:

Foraging activity:

Mean flight densities in all experimental groups shortly before application were 22.4 bees per m² in the control tunnels, 19.8 bees per m² in the test item tunnels and 20.3 bees per m² in the reference item tunnels.

Control Mortality:

Mean control mortality from day 0 after application to day 7 (gauze strips + traps) varied between 25.0 and 77.0 dead bees per tunnel. As the overall mean mortality after application until day 7 (53.1 dead bees/tunnel/day) was comparable to the level before the application (32.0 dead bees/tunnel/day), it can be regarded to be within regular mortality levels under semi-field conditions. In particular, the majority of the dead bees was found on the gauze strips (i.e. older foragers), reflecting the natural dynamics of the colony. Mortality at the hive (in the traps) was consistently low, indicating that colonies were healthy and adapted to the tunnel conditions.

Reference Item Mortality and Effects on Brood:

There was a high number of impacted bee brood, which resulted in 63.4 % loss of the initial observed eggs. This was statistically significant compared to the control.
Sufficient exposure was additionally verified by a high number of dead pupae mainly found from day 13 onwards. In total, 243 dead pupae were found in all 4-reference item treated colonies following the application of the reference item (24 times higher compared to the control value). The mean number of dead pupae found in the reference item treatment was statistically significantly higher compared to the control. The considerable effects on honey bee brood as observed in the reference item treatment, demonstrated the sensitivity of the test system to detect effects on immature honey bee life stages.

III. CONCLUSIONS

To assess the potential effects of Fluopicolide SC 486 (486 g/L) on honey bee colonies including brood development, 0.273 L (331.6 g) product in 400 L tap water/ha (corresponding to 133 g fluopicolide/ha), tap water for the control and a reference item were applied to a full-flowering and highly bee-attractive crop (i.e. *Phacelia tanacetifolia*) under semi-field (tunnel) conditions during bee-flight.
No biological relevant adverse effects on mortality of pupae were observed. Worker bee mortality, foraging activity, behaviour, nectar- and pollen storage as well as queen survival was not affected.
No effects on colony development, colony strength or bee brood were observed.
Based on the results of this study, it can be concluded that Fluopicolide SC 486 (486 g/L) does not adversely affect honey bees and honey bee brood when applied at a rate 0.273 L (331.6 g) product in 400 L tap water/ha (corresponding to 133 g fluopicolide/ha) during honey bees actively foraging on a bee-attractive, flowering crop.
The observed, characteristic brood effects of the reference item Insegar (a.s. fenoxycarb) in terms of typicality, time of occurrence and extent, showed that the prevailing test conditions allowed for a profound detection of effects on immature honey bee life stages.

Assessment and conclusion by applicant:

This study is considered reliable for risk assessment and the conclusions are:

No statistically significant effect on the development of bee brood. Brood termination rate for eggs (BFD 23) of 24.2 %.

No biological relevant adverse effects on mortality of pupae were observed. Worker bee mortality, foraging activity, behaviour, nectar- and pollen storage as well as queen survival was not affected.
No effects on colony development, colony strength or bee brood were observed.

Fluopicolide SC 486 (486 g/L) does not adversely affect honey bees and honey bee brood when applied at a rate 0.273 L (331.6 g) product in 400 L tap water/ha (corresponding to 133 g fluopicolide/ha), during honey bees actively foraging on a bee-attractive, flowering crop.

Data Point:	KCA 8.3.1.3/04
Report Author:	[redacted]
Report Year:	2020
Report Title:	Assessment of side effects of fluopicolide SC 480 G on the honeybee (Apis mellifera L.) in the semi-field after one application on Phacelia tanacetifolia in 2018
Report No:	B180AMS
Document No:	M-685049-01-1
Guideline(s) followed in study:	OECD Guidance Document No. 75 (2007) and current recommendations of the AG Bienenschutz (Pistorius J et al., 2012) OEPP/EPPO Guideline No. 170(4) (2010)
Deviations from current test guideline:	Current Guidelines: OECD 75 (2007) and EPPO 170 (4) (2010) No deviations
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The purpose of the study was to investigate potential side effects of a spray application of Fluopicolide SC 480 G (482.8 g/L) on the honeybee (*Apis mellifera* L.) under semi-field conditions by following the OECD Guidance Document No. 75 (2007), with methodological improvements by the AG Bienenschutz (2012). The study included three treatment groups with four replicates (tunnels) each; one tap water treated control group (C), one test item group (T) and one reference item group (R). For the test item 3 additional replicates were used for analytical verification. Therefore, tunnels were set up on an area of 100 m², covered by about 85.5 m² *Phacelia tanacetifolia*. Small bee colonies were introduced to the tunnels 4 days before the application. After application the bees were exposed for 8 days. The post-exposure period was 21 days. One honey bee colony was used per tunnel. Colonies contained 10 combs with honey, pollen and brood. The mean strength of the colonies per treatment group, one day before the application, was similar and ranged between 6338 and 6468 adult bees per colony. The preliminary brood check indicated healthy colonies with all brood stages present and a minimum reserve of food. All treatments were applied at full-flowering (BBCH 64-65) with honeybees actively foraging on the crop. The target application rate of the test item Fluopicolide SC 480 G was 133 g a.s./ha (actual average rate applied 131.80 g a.s./ha). Tap water was applied in the control group and Insegar 25 WG was applied at a target rate of 300 g fenoxycarb/ha in the reference item group. The spray volume was 300 L/ha in all treatment groups.

Ontogenesis of the bees from egg to adult workers, behavioural abnormalities, flight intensity, mortality of adult bees and pupae, the condition of the colonies and the bee brood development were assessed. No test item related adverse effects on mortality of pupae were observed. Worker bee mortality, foraging activity, behaviour, nectar- and pollen storage were not affected. No effects on colony development, colony strength or bee brood development were observed. Based on the results of this study, it can be concluded that Fluopicolide SC 480 does not adversely affect honey bees and honey bee brood when applied at a nominal concentration of 133 g fluopicolide/ha during honey bees actively foraging on a bee-attractive, flowering crop.

Analytical verification confirmed that the spray solutions were prepared correctly and confirmed that the honeybees were adequately exposed to the test item.

I. MATERIAL AND METHODS

Test item: Fluopicolide SC 480 (482.8 g/L analysed): Fluopicolide: 39.8 % w/w (analytical); supplier batch No: 2015-008261; Sample Description: TOX20609-00; Specification No.: 102000011893; density: 1.213 g/mL).
The aim of the study was to evaluate potential side effects of a spray application of Fluopicolide SC 480 G on honeybee (*Apis mellifera* L.) brood under confined semi-field conditions by following the OECD guidance document No. 75 (2007), with methodological improvements by the AG Bienenschutz (Pistorius J. *et al*., 2012). The crop used was full-flowering *Phacelia tanacetifolia*, the study was conducted in a site near Mézin, Lot-et-Garonne, SW-France.
The study included three treatment groups with four replicates (tunnels) each: one tap water treated control group (C), one test item group (T) and one reference item group (R). For the test item 3 additional replicates were used for analytical verification.
All treatments were applied at full-flowering (BBCH 64-65) with honeybees actively foraging on the crop. The target application rate of the test item Fluopicolide SC 480 G was 133 g a.s./ha (actual average rate applied 131.80 g a.s./ha). Tap water was applied in the control group and Insegar 25 WG was applied at a target rate of 300 g fenoxycarb/ha in the reference item group. The spray volume was 300 L/ha in all treatment groups.
The initial mean colony size assessed during bee flight per treatment group was in the range of 6338 to 6468 bees. The bee colonies were placed in the tunnels on the 22nd of April 2018 in the late evening and remained in the tunnels for 12 consecutive days. On the 4th of May 2018 the colonies were relocated to the monitoring site. Thus, there was a pre-exposure period of 4 days, an exposure period of 8 days and a post-exposure period of 21 days. The colonies were assessed twice during the confined phase, and four times after the end of the confined phase.

The following endpoints were assessed:

- Total and mean number of dead bees (worker and pupae + larvae separately) on linen sheets in tunnels and in the dead bee traps before as well as after the start of exposure in C, T and R.

- Flight intensity (mean number of forager bees/m² phacelia) before and after the start of exposure in C, T and R.

- Behaviour of the bees in the crop and around the hive.

- Condition of the colonies (colony strength and area of the different brood stages and food storage per colony at each assessment date).

- Development of the bee brood assessed in individual brood cells. For this particular assessment, 200 individually marked egg cells per colony were selected.

- Determination of residue loads in pollen and nectar from collected forager bees as well as the active ingredient contents in the test item solutions.

The data from all treatment groups were tested for normality using the Shapiro-Wilks Test ($\alpha = 0.05$) and for equality of error of variances using Levene's Test. The data of mortality of worker bees were compared to the control pooled by period with ANOVA analysis followed by Dunnett's t-Test, $\alpha = 0.05$. In case ANOVA assumptions were not met, analysis was done with a non-parametric test (Mann-Whitney U-test, $\alpha=0.05$). Adult mortality at each assessment moment and mortality of pupae + larvae were analysed with the Mann-Whitney U-test, $\alpha = 0.05$.

Flight intensity was statistically compared using Dunnett's t-Test ($\alpha = 0.05$) with the data pooled for the pre-exposure period, the exposure day and the whole exposure period. Data from each assessment day was compared to the control group with the Mann-Whitney U-test, $\alpha = 0.05$.

Brood indices, compensation indices and termination rates from the different treatment groups were statistically compared to the control group using Dunnett's t-Test, α=0.05. Colony strength and number of cells containing food were compared between control and treatment groups using the Dunnett's t-Test (α = 0.05). Cells containing brood were compared to the control using with the Mann-Whitney U-test, α = 0.05. All statistical analyses and data representation with box plots were conducted using IBM SPSS statistics Version 23 for Windows. Data calculations and graphical representation of the findings were performed with Excel 2016.

Dates of experimental work: April 22, 2018 – May 25, 2018

II. Results and Discussion

Mortality of adult bees (worker bees)

Throughout the period before exposure, mortality of adult bees across all future treatment groups was similar indicating comparable acclimatization of the colonies to restricted conditions in the tunnels. On the application day and during the entire exposure period from day 0 until day 7 after application, mortality of adult bees across all treatment groups was similar, indicating no effect of the test item (Dunnett's t-Test, pooled data, α = 0.05).

The number of dead adult bees in the test item treatment did not differ statistically from the control treatment group in the monitoring period 8DAA to 28DAA and over the entire post application period 0-28DAA, Dunnett's t-Test, pooled data, α = 0.05). The number of adult bees in the R group did not show statistically significant differences with the control group over these periods.

Mortality (adult worker bees)

Treatment group		Mean Control (C)	STD	Mean Test item (T)	STD	Mean Reference item (R)	STD
Daily mean mortality (dead worker bees/colony) ± STD	4DBA to 0DBA	27.70	13.91	33.65	12.40	19.45	7.93
	0DAA	10.00	2.16	15.75	5.44	10.25	3.40
	0DAA to 7DAA	20.04	7.82	22.42	8.11	21.91	10.88
	8DAA to 28DAA	13.56	3.97	12.71	5.41	23.39	12.07
	0DAA to 28DAA	15.35	3.16	15.39	3.69	22.98	6.35

DAA: days after application; DBA: days before application; STD: standard deviation of the daily mean mortality of the 4 replicates

Mortality of pupae

The number of dead pupae and larvae observed before start of the exposure was similar between the control and the T group and lower in the R group in comparison to the control (P=0.020, Mann-Whitney U-test, pooled data). However, on 1DBA to 0DBA no statistically significant differences were observed for pupae mortality between the C and the R group, which ensured comparable starting conditions. During the exposure period (0 to 7DAA) in the tunnels the number of dead pupae and larvae in the T and R treatments did not show a statistically significant difference compared to the control treatment (Mann-Whitney U-test, pooled data, α = 0.05). Data analysis of the mortality from each assessment moment showed a statically significant increase of the number of dead pupae+larvae in the T treatment group on 4DAA in comparison with the C group (P = 0.047). This was considered as transient without biological relevance and the mortality values between the T treatment group and the C group were therefore comparable during this period.

Over the monitoring period after the exposure phase of the study (8-28DAA) and over the entire post application period (0-28DAA), the test item treatment T did not show statistically significant differences in comparison with the control (P = 0.773 and P = 1.000 respectively, Mann-Whitney U-test, pooled data).

Pupae and larvae mortality in the reference item treatment was statistically significant over the period of 8DAA to 28DAA (P = 0.021, Mann-Whitney U-Test pooled data) and over the entire post application period of 0-28DAA (P = 0.021). These effects are expected after exposure of bees to this reference substance confirming exposure and sensitivity of the test system to detect harmful effects. Overall, no test-item related adverse effects on adult bee or pupal mortality were observed.

Mortality (larvae & pupae)

Treatment group		Mean Control (C)	STD	Mean Test item (T)	STD	Mean Reference item (R)	STD
Daily mean mortality (dead larvae & pupae /colony) ± STD	4DBA to 0DBA	1.80	1.08	1.20	0.93	0.70[1)]	0.62
	0DAA	0.00	0.00	0.50	0.58	0.25	0.50
	0DAA to 7DAA	0.70	0.65	1.85	1.91	0.75	0.79
	8DAA to 28DAA	0.83	0.78	0.62	0.85	26.40[2)]	20.71
	0DAA to 28DAA	0.80	0.70	0.96	0.66	19.33[2)]	16.02

DAA: days after application; DBA: days before application; STD: standard deviation of the daily mean mortality of the 4 replicates

[1)] Statistically significantly lower than the C group (P = 0.020, Mann-Whitney U-test, pooled data)

[2)] Statistically significantly higher than the C group (P = 0.021, Mann-Whitney U-test, pooled data)

Flight intensity

The observed foraging intensity was similar across all treatment groups before exposure (4DBA and 0DBA). No significant differences were found between future treatment groups and the control group (Dunnett's t-Test, pooled data, α = 0.05). On the application day just before the water application, the average number of foraging bees was 11.5 in the C, 10.0 in the T treatment and 11.3 in the R treatment. Individual tunnels with less than 10 bees/m^2 at the initial assessment moment were reassessed before the application and fulfilled the criterion of ≥10 bees/m^2.

On the day of application (0DAA) no statistically significant differences of the mean number of forager bees were observed between the test item group and the control group (P = 0.388, Dunnett's t-Test, pooled data). The R group showed a statistically significant reduction of the number of foraging bees on the application day (P = 0.022).

Over the entire exposure period from 0DAA to 7DAA foraging activity was similar in all treatment groups and no test item and reference item related effects occurred (P = 0.617 for T and P = 0.455 for R, Dunnett's t-Test, pooled data).

Thus, no relevant test-item related adverse effects on flight intensity were observed.

Flight intensity

Treatment group		**Control (C)**	**Test item (T)**	**Reference Item (R)**
Daily mean flight intensity (bees/m^2) ± STD	4DBA to 0DBA	10.1 ± 3.4	9.1 ± 3.6	10.6 ± 3.8
	0DBA[1)]	11.5 ± 0.9	10.0 ± 2.5	11.3 ± 1.2
	0DAA	11.2 ± 1.5	10.1 ± 1.5	8.3 ± 2.2[2)]
	0DAA to 7DAA	13.1 ± 6.4	11.8 ± 6.0	11.4 ± 6.3

DAA: days after application; DBA: days before application; STD: standard deviation of the daily mean flight intensity of the 4 replicates;
[1)] Assessment before the application;
[2)] Statistically significantly lower than the C group (P = 0.022, Dunnett's t-Test, pooled data)

Behaviour of the bees

During the assessments of flight intensity and once the colonies were in the monitoring site the behaviour of the honeybees in the crop and around the hive was assessed. No abnormal behaviour was observed at any of the assessment moments during the study period. No behavioural abnormalities related to the test item occurred.

Development of honeybee brood in individual cells

In the control group C, successful development was observed in the majority of the marked brood cells, indicating a healthy development of the brood. The mean termination rate at the end of the observation period (BFD +22) was at 20%. Throughout the study period the mean values of the brood and compensation indices in the C group increased from 1 to 4.10 and 4.52 respectively.

In the test item treatment group T the observed mean termination rate at the end of the observation period (BFD +22) was 17%. This termination rate did not result on a statistically significant difference compared to the control group (P = 0.900, Dunnett's t-Test). The mean brood and compensation indices were 4.27 and 4.64 respectively at BFD+22. There were not statistically significant differences with the control group (P= 0.841 and P = 0.710 respectively).

The mean termination rate in the reference item group R was 22% at BFD +22. This was not statistically significantly different than the control group (P = 0.917 Dunnett's t-Test). The mean values of the brood and compensation indices at the end of the observation period were comparable to those of the C group and no statistically significant differences were observed (P = 0.841 and P = 0.994 respectively). These findings show that exposure to the reference item did not show the strong negative effect observable on individually marked cell level, in contrast to what was seen in pupae mortality. However, historical data show that replicates with low brood termination rate often display an increased pupal mortality, which is the case in this study, indicating that there was sufficient exposure of the honey bees and therefore the test system was suitable to detect potential effects on the bee brood (Pistorius et al. 2012).

In summary, the brood development of eggs, followed on individually marked cells indicated similar mean brood and compensation indices and a similar mean termination rate in comparison with the control group and these differences were not statistically significantly different in any case.

Strenght of the colonies

The overall development of colony strength (number of bees per hive) of all treatment groups showed fluctuations in a typical and normal range. At the start of the test the colony strength of all future treatment groups was comparable to the control group and no statistically significant differences were observed between the treatment groups and the control group (Dunnett's t-Test, α = 0.05). The mean number of bees in the control and the treatment groups T and R showed a similar trend throughout the study period and no statistically significant differences in comparison with the control group were observed at any of the assessment points.

Development of the Brood Area

The mean amount of brood in the colonies (sum of cells containing eggs, larvae, and pupae) was assessed. At the start of the test the brood area of all future treatment groups was comparable to the control group.

The mean number of brood stages in the control and the test item treatment group showed a similar trend from the first to the last assessment. No statistically significant differences between the test item treatment and the control were detected in the post-application assessments (Mann-Whitney U-Test, α = 0.05). No effects were observed in the R group.

Development of the Food Storage Area

The mean amount of food stores in the colonies (sum of cells containing nectar and pollen) was assessed. No statistically significant treatment-related effects on the food storage area were observed in the T group in comparison with the C (Dunnett t-test, α = 0.05). No effects were observed in the R group. Thus, no treatment related adverse effects on the development of the food storage area were detected.

Residue analysis

Analysed concentration of fluopicolide, M-01 (AE C653711) and M-02 (AE C657188) in samples of nectar and pollen collected by honeybees are presented in the table below.

Residues of fluopicolide, M-01 (AE C653711) and M-02 (AE C657188) in samples of nectar and pollen

Sample ID	Matrix	Treatment group	Timing	Fluopicolide [mg/kg]	M-01 (AE C653711) [mg/kg]	M-02 (AE C657188) [mg/kg]
B180AMS-1-NB-Te-D0E	Nectar	Te	0DAA	0.37	<0.01	<0.01
B180AMS-1-NB-Tf-D0E		Tf		0.35	<0.01	<0.01
B180AMS-1-NB-Tg-D0E		Tg		0.31	<0.01	<0.01
		Mean		**0.34**	**<0.01**	**<0.01**
B180AMS-1-NB-Te-D1E	Nectar	Te	1DAA	0.29	<0.01	<0.01
B180AMS-1-NB-Tf-D1E		Tf		0.23	<0.01	<0.01
B180AMS-1-NB-Tg-D1E		Tg		0.084	<0.01	<0.01
		Mean		**0.20**	**<0.01**	**<0.01**
B180AMS-1-PL-Te-D0E	Pollen	Te	0DAA	23	0.013	<0.01
B180AMS-1-PL-Tf-D0E		Tf		24	0.012	<0.01
B180AMS-1-PL-Tg-D0E		Tg		18	0.010	<0.01
		Mean		**22**	**0.012**	**<0.01**
B180AMS-1-NB-Te-D1E	Pollen	Te	1DAA	7.9	<0.01	<0.01
B180AMS-1-NB-Tf-D1E		Tf		6.0	<0.01	<0.01
B180AMS-1-NB-Tg-D1E		Tg		4.5	<0.01	<0.01
		Mean		**6.1**	**<0.01**	**<0.01**

LOQ = Limit of Quantification = 0.01 mg/kg (= 10 µg/kg = 10 ppb) for fluopicolide, M-01 (AE C653711) and M-02 (AE C657188)
LOD = Limit of Detection = 0.003 mg/kg (= 3 µg/kg = 3 ppb) for fluopicolide, M-01 (AE C653711) and M-02 (AE C657188)
DAA = Days after application

Analysed concentration of fluopicolide in spray solutions are presented in the table below.

Sample ID	Matrix	Treatment	Timing	Fluopicolide [mg/kg]*	Fluopicolide [%]**	Mean Fluopicolide [%]**
B180AMS-1-SO-Ta-	Spray solution	T	0DAA	444	100	102
B180AMS-1-SO-Tb-				460	104	
B180AMS-1-SO-Tc-				448	101	
B180AMS-1-SO-Td-				448	101	
B180AMS-1-SO-Te-				449	101	
B180AMS-1-SO-Tf-				463	105	
B180AMS-1-SO-Tg-				443	100	
Mean				**451**		

Fluopicolide nominal content: 443 mg/kg
** Concentration [%] = ((Concentration [mg/kg]) / (Concentration nominal [mg/kg])) *100; unrounded values were used.
DAA = Days after application

III. CONCLUSIONS

Fluopicolide SC 480 G was applied nominally at 133 g a.s./ha at full-flowering *Phacelia tanacetifolia*, during daily honeybee foraging activity. The effects on honeybee colonies under confined conditions considering mortality, flight intensity, behaviour, colony strength, amount of brood and food, and brood cell development were evaluated.
No test item related adverse effects on mortality were observed during the exposure phase and once the colonies were placed in the monitoring site (0DAA to 28DAA).
Over the entire exposure period from 0DAA to 7DAA the foraging activity was similar between control and test item group. Thus, no test item related adverse effects on flight intensity were observed.
No unusual behavioural was observed during the study period in the test item treated group.
The colony strength in the control and the treatment group T showed a similar trend throughout the study period and no differences between the control and test item groups were observed.
The test item did not show any difference compared to the control on the amount of brood and food, measured as mean number of cells covered with the different brood stages and of the food storage.
The mean brood and compensation indexes and the brood termination rate in the test item treated group showed similar values in comparison to control group.
Analytical verification confirmed that the spray solutions were prepared correctly and confirmed that the honeybees were adequately exposed to the test item.

Assessment and conclusion by applicant:

This study is considered reliable for risk assessment and the conclusions are:

No statistically significant effect on the development of bee brood. Brood termination rate for eggs of 17 % (BFD 22).

No test item related adverse effects on mortality of pupae were observed. Worker bee mortality, foraging activity, behaviour, nectar- and pollen storage were not affected. No effects on colony development, colony strength or bee brood development were observed.

Fluopicolide SC 480 (482.8 g/L) does not adversely affect honey bees and honey bee brood when applied at a rate of 0.307 L (372.8 g) product in 300 L tap water/ha (corresponding to 131.80 g fluopicolide/ha), during honey bees actively foraging on a bee-attractive, flowering crop.

CA 8.3.1.4 Sub-lethal effects

There is no particular study design /test guideline to assess "sub-lethal effects" in honeybees. However, in each laboratory study as well as in any higher-tier study, sub-lethal effects, if occurring, are described and reported.

CA 8.3.2 Effects on non-target arthropods other than bees

Studies on non-target arthropods have been performed with the representative formulation and are presented in the respective Document MCP, Section 10.3.2. Additionally, studies on non-target arthropods have been conducted with the solo formulation fluopicolide SC 480 and are presented in this document, MCA, Section 8.3.2.1.

CA 8.3.2.1 Effects on Aphidius rhopalosiphi

Data Point:	KCA 8.3.2.1/01
Report Author:	[redacted]
Report Year:	2003
Report Title:	Acute dose-response toxicity (LR50) of AE C638206 00 SC40 A2 to the cereal aphid parasitoid Aphidius rhopalosiphi (Destefani-Perez) under laboratory conditions
Report No:	C035110
Document No:	M-218217-01-1
Guideline(s) followed in study:	IOBC: Mead-Briggs et al 2000
Deviations from current test guideline:	Current Guideline: Mead-Briggs et al. (2000) No deviations
Previous evaluation:	yes, evaluated and accepted DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The objective of this laboratory study was to investigate the lethal and sublethal toxicity of Fluopicolide SC 480 G on the parasitoid wasp *Aphidius rhopalosiphi* when exposed on a treated glass surface. The test item was applied on glass plates at rates of 170, 255, 383, 574 and 861-mL product/ha in 200 L deionised water/ha. The effects of the test item on the parasitoid wasp *Aphidius rhopalosiphi* were compared to those of a deionised water treated control. Dimethoate EC 400 (0.3 ml product/ha in 200 L/ha of water) was used as toxic reference treatment. Mortality of 30 adult wasps, not older than 48 h at study start (3 replicates with 10 wasps (7 female and 3 male wasps) per test group, was assessed 2, 24 and 48 h after exposure. For the assessment of the effects on the relative fecundity of surviving wasps, if possible, a minimum of 15 females per treatment group were taken from the test units after 48 h. They were individually confined to an acrylic cylinder containing untreated aphid-infested wheat plants. After 24 hours, the wasps were removed, and the plants were maintained under controlled conditions for further 10 days before the number of aphid mummies was assessed (for each individual wasp). All validity criteria were met. The LR_{50} (median lethal rate) was estimated to be > 861 mL product/ha (equivalent to > 419 g a.s./ha). No significant sublethal effects were observed up to the rate of 861 mL product/ha (equivalent to 419 g a.s./ha).

I. MATERIAL AND METHODS:

The fungicide Fluopicolide SC 480 G (AE C63820600 SC40 A2), purity: 487 g/L fluopicolide, specification: Lot No.: OP220823) was tested under laboratory conditions after residual contact exposure of adults of the cereal parasitoid *Aphidius rhopalosiphi* to spray residues with rates of 170 – 255 – 383 – 574 and 861 ml product/ha in 200 L deionized water/ha applied onto glass plates. The control was treated with deionized water (200 L/ha) only and Dimethoate EC 400 (0.3 ml product/ha in 200 L/ha of water) was used as toxic reference treatment.
The sprayed and for one-hour dried glass plates were assembled with an aluminium frame to build a cage. Afterwards 7 females and 3 males of *Aphidius rhopalosiphi* were added to each cage. Three replicate cages for the control and each treatment, and one cage for the reference item were prepared. During the mortality test, the wasps were fed with aqueous fructose solution (25 % w/v).
For the assessment of the effects on the relative fecundity of surviving wasps, if possible, a minimum of 15 females per treatment group were taken from the test units after 48 h. They were individually confined to an acrylic cylinder containing untreated aphid-infested wheat plants. After 24 hours, the wasps were removed, and the plants were maintained under controlled conditions for further 10 days before the number of aphid mummies was assessed.
The number of surviving wasps and the number of parasitised aphids (mummies) were recorded over a period of 14 days. From these data the endpoints mortality after 48 hours and fecundity over a 24 h oviposition period were calculated.

Dates of experimental work: June 17, 2003 – July 01, 2003

II. RESULTS AND DISCUSSION:

Summary of effects of Fluopicolide SC 480 G on *Aphidius rhopalosiphi*

Test item	Fluopicolide SC 480 G (AE C638206 00 SC40 A2)			
Test object	***Aphidius rhopalosiphi*** (Destefani-Perez)			
Exposure	Dried spray deposits onto glass plates			
Treatment	Mortality after 48 hours [%]	Reproduction mean number of mummies/ female	Relative to control [%]	Reduction relative to control [%]
Control	0	15.9	-	-
Application rate [ml product/ha]	Corrected mortality [%]			
170	0	16.1	101.3	0 (+ 1.3)
255	3.3	15.4	96.9	3.1
383	0	14.9	93.7	6.3
574	3.3	15.6	98.1	1.9
861	23	13.4	84.3	15.7
LR_{50} [CL 95 %]	**not determinable (above the 861 ml/ha)**			
Reference item Dimethoate EC 400 0.3 ml product/ha	100	not assessed	-	-

In the test item groups, no significant difference in mortality compared to the control group was observed. Because of no or negligible mortality in all test item treatment groups, a calculation of the LR_{50} was not possible. The LR_{50} has to be regarded being above the highest tested application rate of the test item (861 ml product/ha equivalent to 419 g a.s./ha). No statistically significant difference in reproduction (mean number of mummies/female) was observed in all test item groups, when compared to the control group. The toxic reference treatment resulted in 100 % corrected mortality within 24 hours.

Validity criteria:

Validity Criteria (Mead-Briggs et al., 2000)	Recommended	Obtained
Control Mortality	≤ 13%	0%
Toxic reference mortality (according to study protocol)	> 50%	100%
Reproduction rate in control:	≥ 5 mummies per female ≤ 2 females producing 0 mummies	15.9 mummies per female 1 female with 0 mummies

III. CONCLUSIONS:

The LR_{50} (median lethal rate) of fluopicolide SC 480G (AE C638206 00 SC40 A2) to the cereal aphid parasitoid *Aphidius rhopalosiphi* was > 861 ml product/ha (equivalent to > 419 g a.s./ha). No significant sublethal effects were observed up to the rate of 861 ml product/ha (equivalent to 419 g a.s./ha).

Assessment and conclusion by applicant:

This study is considered reliable for risk assessment and the endpoint is:

LR_{50} > 861 mL product/ha

CA 8.3.2.2 Effects on Typhlodromus pyri

Data Point:	KCA 8.3.2.2/01
Report Author:	[redacted]
Report Year:	2003
Report Title:	Acute dose-response toxicity (LR50) of AE C638206 00 SC40 A2 to predatory mite Typhlodromus pyri (Scheuten) under laboratory conditions
Report No:	C035109
Document No:	M-218216-01-1
Guideline(s) followed in study:	IOBC: Bluemel et al 2000
Deviations from current test guideline:	Current Guideline: Bluemel et al. (2000) No deviations
Previous evaluation:	yes, evaluated and accepted DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The objective of this laboratory study was to investigate the lethal and sublethal toxicity of fluopicolide SC 480 G to the predatory mite *Typhlodromus pyri* when exposed to a treated glass surface. The test item was applied onto glass plates at rates of 170, 255, 383, 574 and 861 product/ha in 200 L deionised water/ha. The effects of the test item on the predatory mite *Typhlodromus pyri* were compared to those of a deionised water treated control. Dimethoate EC 400 (0.3 mL product/ha in 200 L/ha of water) was used as toxic reference treatment. Protonymphs of *T. pyri* were exposed in 5 replicates of 20 mites (per treatment group) to the spray residues of the test item, reference item and control, respectively. During the assessments the predatory mites were fed with pollen (*Pinus negra* and *Betula pendula*). The number of surviving, dead and escaped predatory mites and the number of eggs laid per viable female per evaluation period were recorded over a period of 14 days. All validity criteria were met. The LR_{50} of fluopicolide SC 480 G to *Typhlodromus pyri* was 642 mL product/ha with 95% confidence limits ranging from 591 to 698 mL product/ha.

I. Material and Methods:

The fungicide fluopicolide SC 480 G (AE C638206 00 SC40 A2) (purity: 487 g/L AE C638206; specification: Lot No.: OP220823) was tested under laboratory conditions on protonymphs of the predatory mite *T. pyri* (Scheuten) with rates of 170 - 255 - 383 - 574- and 861-mL product/ha in 200 L deionized water/ha applied onto glass plates. The control was treated with deionized water (200 L/ha). Dimethoate EC 400 (10 mL product/ha in 200 L/ha of water) was used as a toxic reference treatment.

Protonymphs of *T. pyri* were exposed in 5 replicates of 20 mites (per treatment group) to the spray residues of the test item, reference item and control, respectively. During the assessments the predatory mites were fed with pollen (*Pinus negra* and *Betula pendula*). The number of surviving, dead and escaped predatory mites and the number of eggs laid per viable female per evaluation period were recorded over a period of 14 days. From these data the endpoints mortality and effect on reproduction were calculated. The dose-response relationship regarding mortality and effect on reproduction were calculated.

Dates of experimental work: June 16, 2003 – June 30, 2003

II. RESULTS AND DISCUSSION:

Summary of effects of Fluopicolide SC 480 G on *Typhlodromus pyri*

Test item	Fluopicolide SC 480 G (AE C638206 00 SC40 A2)			
Test object	*Typhlodromus pyri* (Scheuten)			
Exposure	Dried spray deposits onto glass plates			
Treatment	Mortality after 7 days [%]	Reproduction		
		mean number of eggs/ female	Relative to control [%]	Reduction relative to control [%]
Control	2	5.15	-	-
Application rate [ml product/ha]	Corrected mortality [%]			
170	1	5.75	111.7	0 (+ 4.4)
255	1	5.05	98.1	1.9
383	14.3*	5.47	106.2	0 (+ 6.2)
574	52.0*	4.97	96.5	3.5
861	65.3*	Not assessed**		
LR_{50} [CL 95 %]	**642 ml product/ha (lower CL: 591) (upper CL: 698)**			
Reference item Dimethoate EC 400 10 ml product/ha	100	Not assessed**	-	-

* Statistically significantly different from the control ($p < 0.05$)
** Reproduction was not assessed because the corrected mortality was not ≤ 50%

There were statistically significant differences in mortality (caused by a repellent effect a high number of mites escaped) in the 383, 574 and 861 mL product/ha test item treatment groups compared to control group. No statistically significant differences in reproduction were found in the test item treatment groups, which were tested, compared to the control group.

Validity criteria:

Validity criteria (Bluemel et al., 2000)	**Recommended**	**Obtained**
Control Mortality	≤ 20%	2%
Toxic reference mortality (according to study protocol)	> 50%	100%
Reproduction rate in control:	≥ 4 eggs per female	5.15 eggs per female

III. CONCLUSIONS:

The LR_{50} (median lethal rate) of fluopicolide SC 480 G (AE C638206 00 SC40 A2) to *Typhlodromus pyri* was 642 mL product/ha with 95% confidence limits ranging from 591 to 698 mL product/ha.

Assessment and conclusion by applicant:

This study is considered reliable for risk assessment and the endpoint is:

LR_{50} = 642 mL product/ha

CA 8.4 Effects on non-target soil meso and macrofauna

CA 8.4.1 Earthworm, sub-lethal effects

Table 8.4.1- 1: Ecotoxicological endpoints – Earthworm reproduction studies with active substance fluopicolide and its soil metabolites

Test substance	Test species, test design	Ecotoxicological endpoint	Reference
Fluopicolide	*Eisenia fetida* reproduction 56 d, mixed	NOEC 31.25 mg a.s./kg dws* EC_{10} calculation not possible	[redacted] 2003; M-218270-01-1; KCA 8.4.1/05
M-01 (AE C653711)	*Eisenia fetida* reproduction 56 d, mixed	NOEC 250 mg p.m./kg dws EC_{10} calculation not possible	[redacted] 2003; M-218219-01-1; KCA 8.4.1/06
M-02 (AEC657188)	*Eisenia fetida* reproduction 56 d, mixed	NOEC ≥ 100 mg p.m./kg dws EC_{10} calculation not possible	[redacted] 2016; M-558329-01-1; KCA 8.4.1/08
M-03 (AE0608000)	*Eisenia fetida* reproduction 56 d, mixed	NOEC ≥ 50 mg p.m./kg dws* EC_{10} calculation not possible	[redacted] 2016; M-557552-01-1; KCA 8.4.1/07

dws = dry weight soil, a.s. = active substance; p.m. = pure metabolite
*Endpoint corrected by a factor of 2 due to lipophilic substance (log P_{OW} > 2)

Acute earthworm studies

Data Point:	KCA 8.4.1/01
Report Author:	[redacted]
Report Year:	2002
Report Title:	Acute Toxicity of AE C638206 Technical to the Earthworm, Eisenia fetida
Report No:	B003665
Document No:	M-240680-01-1
Guideline(s) followed in study:	OECD 207 (1984)
Deviations from current test guideline:	Current Guideline: OECD 207 (1984) No deviations
Previous evaluation:	yes, evaluated and accepted in DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The purpose of this study was to assess the effect of fluopicolide technical (AE C638206) on survival of the earthworm *Eisenia fetida* during exposure to an artificial soil at 5 different application rates. Adult earthworms (*Eisenia fetida*), between 364 – 539 g/worm were exposed for 14 days in groups of 40 (four replicates of 10 worms per concentration) to control, solvent control, 62.5, 125, 250, 500 and 1000 mg test item/kg dry weight mixed into artificial soil. The assessment of the number of surviving earthworms and observations were made on day 7 and day 14. After 14 days of exposure to fluopicolide technical (AE C638206) the mortality was 0% in the control treatments and 2.5% in the vehicle control treatments. The control and vehicle control survival were within the acceptability range specified by the protocol. Mortality in the treated soils was 0% in treatments ≤ 250 mg/kg and 2.5% in both the 500 and 1000 mg/kg treatments. The 7-day and 14-day LC_{50} values for earthworm survival are both > 1000 mg/kg of dry soil.

I. Material and Methods:

Test item: Fluopicolide (AE C638206), Batch No.: 2050190/PP241024/2, Sample No: 12671, purity: 97.1%. Adult earthworms (*Eisenia fetida*), between 364 – 539 g/worm were exposed for 14 days in groups of 40 (four replicates of 10 worms per concentration) to 62.5, 125, 250, 500 and 1000 mg test item/kg dry weight mixed into artificial soil, containing 70% U.S. silica sand, 20% kaolin clay and 10% sphagnum peat. Calcium carbonate was added to the soil to adjust the pH to a range of approximately 6.0 ± 0.5. The assessment of the number of surviving earthworms and observations were made on day 7 and day 14. Earthworms were weighed initially and at end of the test. Control: untreated, solvent control; acetone as solvent.

Dates of work: December 14, 2001 – December 28, 2001

II. RESULTS AND DISCUSSION:

Biological findings:

Effects on mortality and growth of the earthworms are shown in the following tables.

Test item **Test object** **Exposure**	**Fluopicolide (AE C638206)** ***Eisenia fetida*** **Artificial soil**
	Mortality
	[mg test item/kg soil d.w.]
LC_{50}	> 1000

	Control	**Solvent control**	**Fluopicolide (AE C638206) [mg test item/kg soil d.w.]**				
			62.5	**125**	**250**	**500**	**1000**
% Mortality of adult worms after 14 days	0	2.5	0	0	0.0	2.5	2.5
Biomass change in % (change in fresh weight after 14 days relative to initial fresh weight)	6.15	0.86	-6.09	-9.53	-16.0	-15.7	-10.7

Validity criteria:

The validity criteria of the test according to OECD guideline 207 were fulfilled.

Validity criteria (OECD 207, 1984)	**Recommended**	**Obtained**
Mortality of the adults in the control	≤ 10 %	0 %
Average loss of biomass in the control	≤ 20 %	6.15 %

III. CONCLUSIONS:

Fluopicolide showed no effects on survival of the earthworm *Eisenia fetida* in artificial soil at the highest treatment at 1000 mg test item/kg soil d.w. The 14-day LC_{50} for the test material to earthworms (*Eisenia fetida*) based on nominal test concentrations was > 1000 mg/kg soil d.w.

Assessment and conclusion by applicant:
The endpoint from this study is concluded to be LC_{50} > 1000 mg/kg dry weight soil.

However, acute earthworm studies are no longer a data requirement and are therefore not considered in the risk assessment.

Data Point:	KCA 8.4.1/02
Report Author:	[redacted]
Report Year:	2001
Report Title:	2,6-dichlorobenzamide (BAM): Acute toxicity to earthworms (Eisenia foetida)
Report No:	C034061
Document No:	M-234309-01-1
Guideline(s) followed in study:	OECD 207 (1984)
Deviations from current test guideline:	Current Guideline: OECD 207 (1984) No deviations
Previous evaluation:	yes, evaluated and accepted in DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The purpose of this study was to assess the effect of M-01 (2,6-dichlorobenzamide) on survival of the earthworm *Eisenia fetida* during an exposure into an artificial soil at 5 different application rates. Earthworms were exposed for 14 days in groups of 40 (four replicates of 10 worms per concentration) to control, solvent control and concentrations of 100, 180, 320, 560 and 1000 mg test item/kg dry weight mixed into artificial soil, containing 69% industrial quartz sand, 20% kaolin clay, 10% sphagnum peat and 1% calcium carbonate. The assessment of the number of surviving earthworms and observations were made on day 7 and day 14. The mortality of earthworms was 0% in the control and 100% in the highest treatments. The weight change of the earthworms ranged between -12.2 to -2.5% in the treated groups and was -9.52% in the control. The 14-day LC_{50} for the test material to earthworms (*Eisenia fetida*) based on nominal test concentrations is 750 mg/kg soil d.w. The No Observed Effect Concentration is 320 mg/kg soil d.w.

I. Material and Methods:

Test item: M-01 (2,6-dichlorobenzamide), Batch No.: FUX001000/FUN81G02C, purity: 99.5%.
Earthworms were exposed for 14 days in groups of 40 (four replicates of 10 worms per concentration) to concentrations of 100, 180, 320, 560 and 1000 mg test item/kg dry weight mixed into artificial soil, containing 69% industrial quartz sand, 20% kaolin clay, 10% sphagnum peat and 1% calcium carbonate. The assessment of the number of surviving earthworms and observations were made on day 7 and day 14. Earthworms were weighed initially and at end of the test. Toxic standard: 2-chloroacetamide, separate study at concentrations of 5.6, 10, 18, 32 and 56 mg/kg soil d.w.; control: untreated, solvent control: none.

Dates of work: April 24, 2001 – May 8, 2001

II. RESULTS AND DISCUSSION:

Biological findings:

Effects on mortality and growth of the earthworms are shown in the following tables.

Test item **Test object** **Exposure**	**M-01 (2,6-dichlorobenzamide)** ***Eisenia fetida*** **Artificial soil**
	Mortality
	[mg test item/kg soil d.w.]
NOEC	320
LOEC	560
LC_{50}	750

	Control	**M-01 (2,6-dichlorobenzamide) [mg test item/kg soil d.w.]**				
		100	**180**	**320**	**560**	**1000**
% Mortality of adult worms after 14 days	0	0	0	0	0	100
Biomass change in % (change in fresh weight after 14 days relative to initial fresh weight)	- 9.52	-16.7	-12.2	-14.6	-22.5	-*

*Not determinable due to 100% mortality after 14 days

The mortality of earthworms was 0% in the control and 100% in the highest treatments. The weight change of the earthworms ranged between -12.2 to -22.5% in the treated groups and was -9.52% in the control.

Validity criteria:

The validity criteria of the test according to OECD guideline 207 were fulfilled.

Validity criteria (OECD 207, 1984)	**Recommended**	**Obtained**
Mortality of the adults in the control	≤ 10 %	0 %
Average loss of biomass in the control	≤ 20 %	9.52 %

To verify the sensitivity of the test system, the reference item 2-chloroacetamide was tested at concentrations of 5.6, 10, 18, 32 and 56 mg/kg soil d.w. The result of this positive control study gave a 14-day LC_{50} for 2-chloroacetamide of 23 mg/kg soil d.w.

III. CONCLUSIONS:

M-01 (2,6-dichlorobenzamide) showed effects on survival of the earthworm *Eisenia fetida* in artificial soil at the highest treatment at 1000 mg test item/kg soil d.w. The 14-day LC_{50} for the test material to earthworms (*Eisenia fetida*) based on nominal test concentrations was 750 mg/kg soil d.w. The No Observed Effect Concentration was 320 mg/kg soil d.w.

Assessment and conclusion by applicant:

The endpoint from this study is concluded to be LC_{50} = 750 mg/kg dry weight soil.

However, acute earthworm studies are no longer a data requirement and are therefore not considered in the risk assessment.

Data Point:	KCA 8.4.1/03
Report Author:	[redacted]
Report Year:	2003
Report Title:	Acute toxicity (14 days) of AE C657188 to the earthworm Eisenia fetida in artificial soil
Report No:	C035115
Document No:	M-218222-01-1
Guideline(s) followed in study:	ISO: 11268 part 1 (1993); OECD: 207 (1984)
Deviations from current test guideline:	Current Guideline: OECD 207 (1984) No deviations
Previous evaluation:	yes, evaluated and accepted DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The purpose of this study was to assess the effect of M-02 (AE C657188) on survival of the earthworm *Eisenia fetida* during an exposure into an artificial soil at 5 different application rates. Earthworms were exposed for 14 days in groups of 40 (four replicates of 10 worms per concentration) to control, solvent and concentrations of 63, 125, 320, 500 and 1000 mg test item/kg dry weight mixed into artificial soil, containing 69.5% fine quartz sand, 20% kaolin clay, 10% sphagnum peat and 0.5% calcium carbonate. The assessment of the number of surviving earthworms and observations were made on day 7 and day 14. The mortality of earthworms was 0% in the control and all tested treatments. The weight change of the earthworms ranged between -9.5 to -13.5% in the treated groups and was -8.2% in the control. M-02 (AE C657188) showed no effects on survival of the earthworm *Eisenia fetida* in artificial soil at the highest treatment at 1000 mg test item/kg soil d.w. The 14-day LC_{50} for the test material to earthworms (*Eisenia fetida*) based on nominal test concentrations is > 1000 mg/kg soil d.w. The No Observed Effect Concentration is 1000 mg/kg soil d.w

I. Material and Methods:

Test item: M-02 (AE C657188), Batch No.: RAW244055/1, purity: 97.2%. Earthworms were exposed for 14 days in groups of 40 (four replicates of 10 worms per concentration) to concentrations of 63, 125, 320, 500 and 1000 mg test item/kg dry weight mixed into artificial soil, containing 69.5% fine quartz sand, 20% kaolin clay, 10% sphagnum peat and 0.5% calcium carbonate. The assessment of the number of surviving earthworms and observations were made on day 7 and day 14. Earthworms were weighed initially and at end of the test. Toxic standard: 2-chloroacetamide, control: untreated, solvent control: none.

Dates of work: June 04, 2003 – June 23, 2003

II. RESULTS AND DISCUSSION:

Biological findings:

Effects on mortality and growth of the earthworms are shown in the following tables.

Test item **Test object** **Exposure**	**M-02 (AE C657188)** ***Eisenia fetida*** **Artificial soil**
	Mortality
	[mg test item/kg soil d.w.]
NOEC	≥ 1000
LOEC	> 1000
LC_{50}	> 1000

	Control	**M-02 (AE C657188) [mg test item/kg soil d.w.]**				
		63	**125**	**250**	**500**	**1000**
% Mortality of adult worms after 14 days	0	0	0	0	0	0
Biomass change in % (change in fresh weight after 14 days relative to initial fresh weight)	- 8.2	-14.0	-9.5	-10.2	-12.8	-13.5

The mortality of earthworms was 0% in the control and all tested treatments. The weight change of the earthworms ranged between -9.5 to -13.5% in the treated groups and was -8.2% in the control.

Validity criteria:

The validity criteria of the test according to OECD guideline 207 were fulfilled.

Validity criteria (OECD 207, 1984)	**Recommended**	**Obtained**
Mortality of the adults in the control	≤ 10 %	0 %
Average loss of biomass in the control	≤ 20 %	8.2 %

To verify the sensitivity of the test system, the reference item 2-chloroacetamide was tested. In the most recent test with the toxic standard compound 2-chloroacetamide the LC_{50} after 14 days was determined as 26.8 mg/kg.

III. CONCLUSION:

M-02 (AE C657188) showed no effects on survival of the earthworm *Eisenia fetida* in artificial soil at the highest treatment at 1000 mg test item/kg soil d.w. The 14-day LC_{50} for the test material to earthworms (*Eisenia fetida*) based on nominal test concentrations was > 1000 mg/kg soil d.w. The No Observed Effect Concentration was 1000 mg/kg soil d.w.

Assessment and conclusion by applicant:

The endpoint from this study is concluded to be LC_{50} > 1000 mg/kg dry weight soil.

However, acute earthworm studies are no longer a data requirement and are therefore not considered in the risk assessment.

Data Point:	KCA 8.4.1/04
Report Author:	[redacted]
Report Year:	2003
Report Title:	Acute toxicity (14 days) of AE 0608000 to the earthworm Eisenia fetida in artificial soil
Report No:	C035116
Document No:	M-218223-01-1
Guideline(s) followed in study:	ISO: 11268 part 1 (1993); OECD: 207 (1984)
Deviations from current test guideline:	Current Guideline: OECD 207 (1984) No deviations
Previous evaluation:	yes, evaluated and accepted DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The purpose of this study was to assess the effect of M-03 (AE 0608000) on survival of the earthworm *Eisenia fetida* during an exposure into an artificial soil at 5 different application rates. Earthworms were exposed for 14 days in groups of 40 (four replicates of 10 worms per concentration) to control and concentrations of 63, 125, 320, 500 and 1000 mg test item/kg dry weight mixed into artificial soil, containing 69.5% fine quartz sand, 20% kaolin clay, 10% sphagnum peat and 0.5% calcium carbonate. The assessment of the number of surviving earthworms and observations were made on day 7 and day 14. The mortality of earthworms was 0% in the control and all tested treatments. The weight change of the earthworms ranged between -5.4 to -10.3% in the treated groups and was -5.3% in the control. M-03 (AE 0608000) showed no effects on survival of the earthworm *Eisenia fetida* in artificial soil at the highest treatment at 1000 mg test item/kg soil d.w. The 14-day LC_{50} for the test material to earthworms (*Eisenia fetida*) based on nominal test concentrations was > 1000 mg/kg soil d.w. The No Observed Effect Concentration was 1000 mg/kg soil d.w.

I. Material and Methods:

Test item: M-03 (AE 0608000), Batch No.: M0Y4622M, purity: 96.9%. Earthworms were exposed for 14 days in groups of 40 (four replicates of 10 worms per concentration) to control and concentrations of 63, 125, 320, 500 and 1000 mg test item/kg dry weight mixed into artificial soil, containing 69.5% fine quartz sand, 20% kaolin clay, 10% sphagnum peat and 0.5% calcium carbonate. The assessment of the number of surviving earthworms and observations were made on day 7 and day 14. Earthworms were weighed initially and at end of the test. Toxic standard: 2-chloroacetamide, control: untreated, solvent control: none.

Dates of work: May 20, 2003 – June 06, 2003

II. RESULTS AND DISCUSSION:

Biological findings:

Effects on mortality and growth of the earthworms are shown in the following tables.

Test item **Test object** **Exposure**	**M-03 (AE 0608000)** ***Eisenia fetida*** **Artificial soil**
	Mortality
	[mg test item/kg soil d.w.]
NOEC	≥ 1000
LOEC	> 1000
LC_{50}	> 1000

	Control	**M-03 (AE 0608000) [mg test item/kg soil d.w.]**				
		63	**125**	**250**	**500**	**1000**
% Mortality of adult worms after 14 days	0	0	0	0	0	0
Biomass change in % (change in fresh weight after 14 days relative to initial fresh weight)	- 5.3	-7.0	-5.4	-5.6	-8.1	-10.3

The mortality of earthworms was 0% in the control and all tested treatments. The weight change of the earthworms ranged between -5.4 to -10.3% in the treated groups and was -5.3% in the control.

Validity criteria:

The validity criteria of the test according to OECD guideline 207 were fulfilled.

Validity criteria (OECD 207, 1984)	**Recommended**	**Obtained**
Mortality of the adults in the control	≤ 10 %	0 %
Average loss of biomass in the control	≤ 20 %	5.3 %

To verify the sensitivity of the test system, the reference item 2-chloroacetamide was tested. In the most recent test with the toxic standard compound 2-chloroacetamide the LC_{50} after 14 days was determined as 26.8 mg/kg.

III. CONCLUSION:

M-03 (AE 0608000) showed no effects on survival of the earthworm *Eisenia fetida* in artificial soil at the highest treatment at 1000 mg test item/kg soil d.w. The 14-day LC_{50} for the test material to earthworms (*Eisenia fetida*) based on nominal test concentrations was > 1000 mg/kg soil d.w. The No Observed Effect Concentration was 1000 mg/kg soil d.w.

Assessment and conclusion by applicant:

The endpoint from this study is concluded to be LC_{50} > 1000 mg/kg dry weight soil.

However, acute earthworm studies are no longer a data requirement and are therefore not considered in the risk assessment.

Data Point:	KCA 8.4.1/05
Report Author:	[redacted]
Report Year:	2003
Report Title:	AE C638206 technical: Effects on survival, growth and reproduction on the earthworm Eisenia fetida tested with 5 percent peat in the test substrate
Report No:	C035163
Document No:	M-218270-01-1
Guideline(s) followed in study:	BBA: VI, 2-2 (1994); ISO: 11268-2 E (1998)
Deviations from current test guideline:	Current Guideline: OECD 222 (2004) The pH was greater than 6.0 ± 0.5 on day 56 (6.76). The number of replicates in the control was 4 instead of 8. These deviations are not expected to have impacted the study results.
Previous evaluation:	yes, evaluated and accepted DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The purpose of this study was to assess the effect of fluopicolide technical (AE C638206) on survival, growth, and reproduction of the earthworm *Eisenia fetida* during an exposure into an artificial soil at 5 different application rates. 240 adult earthworms *Eisenia fetida* (9 months old, 4 × 10 animals per control and test item group) were exposed in an artificial soil to the application rates of 0 (control), 15.63, 31.25, 62.50, 125 and 250 mg a.s./kg weight soil. After 28 days, the number of surviving animals and their weight alteration was determined. After further 28 days, the numbers of juveniles were determined.
All validity criteria were met. No statistically significant effects ($p < 0.05$) are observed in mortality and reproduction of treated earthworms and hence the NOEC based on mortality (28 days) and the NOEC based on reproduction (56 day) is 250 mg a.s./kg dry soil. The NOEC for growth (28 days) is 62.5 mg a.s./kg dry soil based on statistically significant biomass changes seen at higher treatment rates.

I. Material and Methods:

Test substance: Fluopicolide (AE C638206 technical; batch AEC638206 00 1C96 0001; OP2050046 of purity 96.1%). 240 adult earthworms *Eisenia fetida* (9 months old, 4 × 10 animals per control and test item group) were exposed in an artificial soil to the application rates of 0 (control), 15.63, 31.25, 62.50, 125 and 250 mg a.s./kg weight soil. Artificial soil composition was 73-74% quartz sand, 20% kaolin clay, 5% sphagnum peat, 1% dried organism manure and 0.2-1% calcium carbonate. The artificial soil was prepared by mixing the dry components intensely in a laboratory mixer. The test vessels were kept in a temperature-controlled room at 20 ± 2°C under a 16-hour light to 8-hour darkness photoperiod and a light intensity at light period between approximately 400-800 lux. The test item was mixed into the soil.
After 28 days, the number of surviving animals and their weight alteration was determined. They adult earthworms were then removed from the artificial soil. After further 28 days, the numbers of juveniles were determined.

The most recent reference test with carbendazim (360 g a.s./L; trade name “Derosal flüssig”) was performed from October to December 2002 with the following 3 application rates: 1.25, 2.50 and 5.00 mg a.s./kg dry weight soil. The test ensured that the laboratory test conditions were adequate and verified that the response of the test organisms did not change significantly over time. No statistically significant effects ($p < 0.05$) were observed in mortality and reproduction of treated earthworms and hence the NOEC based on mortality (28 days) and the NOEC based on reproduction (56 day) is 250 mg a.s./kg

dry soil. The NOEC for growth (28 days) was 62.5 mg a.s./kg dry soil based on statistically significant biomass changes seen at higher treatment rates.

Dates of experimental work: January 09, 2003 – March 06, 2003

II. RESULTS AND DISCUSSION:

No mortality of adult earthworms was observed at any application rate of the test item in the study.

The growth of the adult worms was significantly different to the control at the application rates of 125 and 250 mg a.s./kg dry weight soil. No significantly different values of the growth to the control were observed at the application rates of 15.63, 31.25 and 62.50 mg a.s./kg dry weight soil.

The number of juveniles was not significantly reduced at any application rate.

Summary of effects of fluopicolide on mortality and changes in body weight of the adults after an exposure period of 28 days and the number of juveniles after 56 days.

Test substance	Control	Fluopicolide (AE C638206)				
Test concentrations (mg a.s./kg dry weight soil)	--	15.63	31.25	62.50	125	250
Mortality of adults after 28 days (%)	0	0	0	0	0	0
Mean change of body weights of the adults from day 0 to day 28 ± St. Dev. (%)	+ 97.2 ±2.3	83.4 ±18.4	+ 78.3 ± 12.4	+ 76.9 ± 12.7	+ 74.8 ± 7.6	+ 75.5 ± 8.4
Statistical comparison to the control*	--	n.s.	n.s.	n.s.	s.	s.
Mean number of juveniles per surviving adult ± St. Dev.	10.4 ± 1.4	11.6 ± 2.5	10.2 ± 1.6	12.8 ± 2.0	11.4 ± 0.6	13.8 ± 3.0
Statistical comparison to the control**	--	n.s.	n.s.	n.s.	n.s.	n.s.

* Result of a Dunnett's multiple t-test, one sided smaller, $\alpha = 0.05$
**Result of a Williams multiple sequential t-test, one sided smaller, $\alpha = 0.05$
s.: mean value statistically significantly different compared to the control ($p<0.05$)
n.s.: mean value not statistically significantly different compared to the control ($p \geq 0.05$)

The calculation of an ECx curve for reproduction was not possible due to the lack of a significant dose-response relationship.

Results of the most recent test with the reference substance (carbendazim 360 g a.s./L): The growth of adult earthworms was significantly reduced at the application rate of 5 mg a.s./kg dry substrate. The reproduction rate was significantly reduced at the application rates of 2.5 and 5 mg a.s./kg dry substrate. The survival of the adults was not affected.

Validity criteria:

Validity criteria OECD 222 (2004)	Recommended	Obtained
Adult control mortality	≤ 10%	0 % (after 4 weeks)
Number of juveniles per control replicate	≥ 30	109, 90, 121, 96
Coefficient of variation of reproduction in the control	≤ 30%	13.3 %

III. CONCLUSION:

No statistically significant effects ($p < 0.05$) were observed in mortality and reproduction of treated earthworms and hence the NOEC based on mortality (28 days) and the NOEC based on reproduction (56 day) is 250 mg a.s./kg dry soil. The NOEC for growth (28 days) was 62.5 mg a.s./kg dry soil based on statistically significant biomass changes seen at higher treatment rates.

Assessment and conclusion by applicant:

This study is considered reliable for risk assessment and the endpoint is:

NOEC = 62.5 mg a.s./kg dws

Data Point:	KCA 8.4.1/06
Report Author:	[redacted]
Report Year:	2003
Report Title:	Effect of AE C653711 on reproduction and growth of earthworms Eisenia fetida in artificial soil
Report No:	C035112
Document No:	M-218219-01-1
Guideline(s) followed in study:	BBA: VI 2-2, 1994, ISO: 11268 part 2 (1998)
Deviations from current test guideline:	Current Guideline: OECD 222 (2004) The number of replicates in the control was 4 instead of 8 as recommended by the guideline. This deviation is not expected to have impacted the study results.
Previous evaluation:	yes, evaluated and accepted DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The purpose of this study was to investigate the effects of M-01 (2,6-dichlorobenzamide (AE C653711)) on survival, growth and reproduction of the earthworm *Eisenia fetida* during an exposure into an artificial soil at 5 different application rates. Under laboratory conditions *Eisenia fetida* (40 worms per treatment group and control) were exposed to the following concentrations of AE C653711: 0, 31, 63, 125, 250 and 500 mg/kg artificial soil. After 28 days, the number of surviving animals and their weight alteration was determined. After further 28 days, the number of juveniles was determined. Endpoints were mortality, growth and reproduction.

All validity criteria were met. During the 4 weeks of exposure one adult worm died at the test item concentration of 31 mg/kg soil dry weight and two worms died at 250 mg/kg soil dry weight. The body weights in the test item treated group increased by 38.9 % to 50.8 %. The reproduction ranged from 205 to 274 juvenile worms in the groups treated with test item. M-01 (AE C653711) did not show significant effects on mortality and growth of the earthworm *Eisenia fetida* up to the concentration of 500 mg/kg soil dry weight, *i.e.* the highest concentration tested. Reproduction of *Eisenia fetida* was not significantly affected up to and including the concentration of 250 mg/kg soil dry weight but at 500 mg/kg soil dry weight a statistically significant reduction of reproduction was observed. The no-observed-effect-concentration (NOEC) determined in this study is 250 mg/kg dry artificial soil.

I. MATERIAL AND METHODS:

Test substance: M-01 (2,6-dichlorobenzamide (AE C653711 technical; batch No: 8808018; purity 97.0%)) was tested in a laboratory study lasting eight weeks. The control was untreated and moistened with deionised water. Under laboratory conditions *Eisenia fetida* (40 worms per treatment group and control) were exposed to the following concentrations of M-01 (AE C653711): 0, 31, 63, 125, 250 and 500 mg/kg artificial soil. Endpoints were mortality, growth and reproduction. Artificial soil composition was approximately 69.5% fine quartz sand, 20% kaolin clay, 10% sphagnum peat and approximately 0.5% calcium carbonate to adjust the pH. The test vessels were kept in a temperature-controlled room at 18 – 21°C under a 16-hour light to 8-hour darkness photoperiod and a light intensity between approximately 422 – 527 lux. The test item was mixed into the soil.
After 28 days, the number of surviving animals and their weight alteration was determined. They adult earthworms were then removed from the artificial soil. After further 28 days, the number of juveniles was determined. The most recent reference test with Derosal SC 360 (active ingredient: carbendazim) was performed from August to October 2002. The test ensured that the laboratory test conditions were adequate and verified that the response of the test organisms did not change significantly over time.

Dates of experimental work: March 18, 2003 – May 16, 2003

II. RESULTS AND DISCUSSION:

Observations:

During the 4 weeks of exposure one adult worm died at the test item concentration of 31 mg/kg soil dry weight and two worms died at 250 mg/kg soil dry weight which was not significantly different compared to the control (Fisher Exact test, a =0.05).

The body weights in the test item treated group increased by 38.9 % to 50.8 %. None of the weight changes was significantly different compared to the control group where weight increase was 37.4 % (Dunnett-test, a = 0.05).

The reproduction ranged from 205 to 274 juvenile worms in the groups treated with test item. Up to and including the concentration of 250 mg/kg soil dry weight the reproduction was not significantly different compared to the control, where 281 juvenile worms were found (Bonferroni-t test, a = 0.05). At the concentration of 500 mg/kg soil dry weight the number of juvenile earthworms was statistically significantly reduced compared to the control (Bonferroni-t test, a = 0.05).

Test item	M-01 (AE C653711)					
Test species	*Eisenia fetida*					
Exposure	Test item mixed into the soil					
	Control	31 mg/kg	63 mg/kg	125 mg/kg	250 mg/kg	500 mg/kg
Mean Mortality + St. Dev. [%][1]	0 ± 0	2.5 ± 5	0 ± 0	0 ± 0	5.0 ± 5.8	0 ± 0
Mean body weight change + St. Dev. [%][1]	37.4 ± 7.7	50.8 ± 8.9	44.8 ± 6.2	44.9 ± 7.9	44.9 ± 8.9	38.9 ± 4.7
Mean reproduction of juveniles + St. Dev.	281 ± 23	259 ± 25	239 ± 21	274 ± 37	257 ± 69	205 ± 25*

St. Dev. = Standard deviation
*significantly different compared to the control

A calculation of a valid ECx curve for reproduction was not possible due to the lack of a significant dose-response relationship.

The most recent toxic standard test showed statistically significant effects on reproduction at a concentration of 1.6 mg carbendazim/kg artificial soil (dry weight); the EC_{50} for reproduction was calculated as 1.9 mg carbendazim/kg soil dry weight.

Validity criteria:

Validity criteria OECD 222 (2004)	Recommended	Obtained
Adult control mortality	≤ 10%	0 % (after 4 weeks)
Number of juveniles per control replicate	≥ 30	281 (mean)
Coefficient of variation of reproduction in the control	≤ 30%	8%

III. CONCLUSION:

M-01 (AE C653711) did not show significant effects on mortality and growth of the earthworm *Eisenia fetida* up to the concentration of 500 mg/kg soil dry weight, *i.e.* the highest concentration tested. Reproduction of *Eisenia fetida* was not significantly affected up to and including the concentration of 250 mg/kg soil dry weight but at 500 mg/kg soil dry weight a statistically significant reduction in number of juveniles was observed. The no-observed-effect-concentration (NOEC) determined in this study was 250 mg/kg dry artificial soil.

Assessment and conclusion by applicant:

This study is considered reliable for risk assessment and the endpoint is:

NOEC = 250 mg/kg dws

Data Point:	KCA 8.4.1/07
Report Author:	[redacted]
Report Year:	2016
Report Title:	AE 0608000 (BCS-AX86048): Effects on survival, growth and reproduction of the earthworm Eisenia fetida tested in artificial soil
Report No:	E 312 04864-8
Document No:	M-537757-01-1
Guideline(s) followed in study:	International Standards ISO 11268-2: 1998(E): "Soil quality - Effects of pollutants on earthworms (Eisenia fetida) - Part 2: Determination of effects on reproduction", July 1998. OECD 222: April 13, 2004: "OECD Guideline for the Testing of Chemicals - Earthworm Reproduction Test (Eisenia fetida / Eisenia andrei)"
Deviations from current test guideline:	Current Guideline: OECD 222 (2004) The temperature increased up to 22.7 °C, out of the range of 20°C ± 2 °C recommended by the guideline. This deviation is not expected to have impacted the study results.
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The purpose of this study was to investigate the effects of M-03 (AE0608000) on survival, growth and reproduction of the earthworm *Eisenia fetida* during exposure to an artificial soil at one application rate. Under laboratory conditions *Eisenia fetida* (80 worms per treatment group) were exposed to the following concentration of M-03 (AE0608000) which was mixed into the soil: 100 mg/kg dry weight artificial soil. Endpoints were mortality, growth and reproduction.
All validity criteria were met. After 28 days, the number of surviving animals and their weight alteration was determined. The no-observed-effect-concentration (NOEC) determined in this study is ≥ 100 mg/kg soil dry weight.

I. Material and Methods:

Test substance: M-03 (AE0608000 (BCS-AX86048)); batch No: SES 12767-19-2; purity 99.4%) was tested in a laboratory study lasting eight weeks. The control was untreated and moistened with deionised water. Under laboratory conditions *Eisenia fetida* (80 worms per treatment group) were exposed to the following concentration of M-03 (AE0608000) which was mixed into the soil: 100 mg/kg dry weight artificial soil. Endpoints were mortality, growth and reproduction. Artificial soil composition was approximately 70% industrial quartz sand, 20% kaolin clay and 10% sphagnum peat. Calcium carbonate for the adjustment to pH 6.0 ± 0.5 was added. The test vessels were kept in a temperature-controlled room at 20 ± 2°C under a 16-hour light to 8-hour darkness photoperiod and a light intensity between 400 – 800 lux. The test item was mixed into the soil.
After 28 days, the number of surviving animals and their weight alteration was determined. They were then removed from the artificial soil. After further 28 days, the number of juveniles was determined. The most recent reference test with the reference test item (360 g carbendazim/L) was performed from August 25 to November 19. The test ensured that the laboratory test conditions were adequate and verified that the response of the test organisms did not change significantly over time.

Dates of experimental work: January 18, 2016 - March 21, 2016

II. Results and Discussion:

Observations:

During the 4 weeks of exposure no adult earthworm died in the control and in the treatment with 100 mg /kg dry weight artificial soil.

The body weights in the test item treated group increased in average by 65 %. In the control group the increase was in average 61%. The weight change in the treatment was not significantly different from the control (STUDENT t-test, two-sided, a = 0.05).

The mean reproduction was approximately 190 worms per test vessel in the control. In the treatment group a slightly lower reproduction was found, i.e. 170 worms per test vessel. No statistically significant differences concerning the number of juveniles relative to the control was observed (STUDENT t-test, one-sided smaller, α = 0.05).

Test item	M-03 (AE0608000)	
Test species	*Eisenia fetida*	
Exposure	Test item mixed into the soil	
	Control	100 mg/kg
Mean Mortality	0	0
Mean change of body fresh weight of the adults from day 0 to day 28 + St. Dev. [%]	61.03 ± 6.99	65.0 ± 6.59
Mean number of juveniles per test vessel after 56 days + St. Dev.	190.4 ± 24.6	169.5 ± 26.5

St. Dev. = Standard deviation

A calculation of an ECx curve for reproduction was not possible as only one concentration was tested.

The most recent toxic standard test showed statistically significant effects on reproduction at the test concentrations of 2.5 and 5.0 mg carbendazim/kg artificial soil dry weight.

Validity criteria:

Validity criteria (OECD 222, 2004)	Recommended	Obtained
Adult control mortality	≤ 10%	0 %
Number of juveniles per control replicate	≥ 30	157 to 234
Coefficient of variation of reproduction in the control	≤ 30%	12.91 %

III. CONCLUSIONS:

M-03 (AE0608000) did not show significant effects on mortality, growth and reproduction of the earthworm *Eisenia fetida* at the limit concentration of 100 mg/kg soil dry weight. The no-observed-effect-concentration (NOEC) determined in this study was therefore ≥ 100 mg/kg soil dry weight.

Assessment and conclusion by applicant:

This study is considered reliable for risk assessment and the endpoint is:

NOEC ≥ 100 mg/kg dws

Data Point:	KCA 8.4.1/08
Report Author:	[redacted]
Report Year:	2016
Report Title:	Pyridyl carboxylic acid (BCS-AB43478): Effects on survival, growth and reproduction of the earthworm Eisenia fetida tested in artificial soil
Report No:	EBACN058
Document No:	M-558329-01-1
Guideline(s) followed in study:	OECD 222 (2004), ISO 11268-2 (1998); US EPA OCSPP not applicable
Deviations from current test guideline:	Current Guideline: OECD 222 (2004) No deviations
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The purpose of this study was to investigate the effects of M-02 (AEC657188) on survival, growth and reproduction of the earthworm *Eisenia fetida* during an exposure into an artificial soil at five different application rates. Under laboratory conditions *Eisenia fetida* (80 worms for the control and 40 worms per treatment group) were exposed in an artificial soil to the application rates of 0, 10, 18, 32, 56 and 100 mg/kg d.w. artificial soil. After 28 days, the number of surviving animals and their weight alteration was determined. After further 28 days, the number of juveniles was determined.
All validity criteria were met. No-Observed-Effect-Concentration (NOEC) was determined to be ≥ 100 mg metabolite/kg soil dry weight, and the Lowest-Observed-Effect-Concentration (LOEC) was determined to be >100 mg/kg soil dry weight.

I. MATERIAL AND METHODS:

Test substance: M-02 (AEC657188 (BCS-AB43478)); batch code: AE C657188-PU-01, SES 10250-1-1; purity 98.5%) was tested in a laboratory study lasting eight weeks. The control was untreated and moistened with deionised water. Under laboratory conditions *Eisenia fetida* (80 worms for the control and 40 worms per treatment group) were exposed in an artificial soil to the application rates of 0, 10, 18, 32, 56 and 100 mg/kg d.w. artificial soil. Endpoints were mortality, growth and reproduction. Artificial soil composition was approximately 69.5% industrial quartz sand, 20% kaolin clay, 10% sphagnum peat and 0.5% calcium carbonate. The test vessels were kept in a temperature-controlled room at 18 – 21.9 °C under a 16-hour light to 8-hour darkness photoperiod and a light intensity of 580 lux. The test item was mixed into the soil.

After 28 days, the number of surviving animals and their weight alteration was determined. They were then removed from the artificial soil. After further 28 days, the number of juveniles was determined. As a reference item Maypon Flow (Carbendazim, SC 500) was tested in a seprate study at concentrations of 5 and 10 mg product/kg soil dry weight.

Dates of experimental work: March 23, 2016 – May 18, 2016

II. RESULTS AND DISCUSSION:

Observations:

During the 4 weeks of exposure one adult worm died at the test item concentration of 10 mg/kg soil dry weight and two worms died at 56 mg/kg soil dry weight which was not significantly different compared to the control (Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm, $\alpha = 0.05$).

The weight change of the adult worms ranged between 23.6 and 26.5% in the treated groups and 25.0% in the control group. The test item caused no statistically significant change in biomass compared to the control groups at any concentration tested (Williams-t-test, $\alpha = 0.05$, one-sided smaller).

No statistically significant effect (Williams-t-test, $\alpha = 0.05$, one-sided smaller) on the number of juveniles was found for any concentration tested.

Test item	M-02 (AEC657188)					
Test species	*Eisenia fetida*					
Exposure	Test item mixed into the soil					
	Control	Treatment (mg/kg soil d.w.)				
		10	18	32	56	100
Mortality [%]	1.3	2.5	0	0	5.0	0
Mean body weight change + St. Dev.	25.0 ± 3.4	24.4 ± 4.8	25.6 ± 2.5	23.6 ± 2.4	26.5 ± 4.4	24.9 ± 2.4
Number of juveniles per replicate after 8 weeks	136.9 ± 20.9	141.0 ± 24.9	130.3 ± 24.3	144.0± 28.1	138.5 ± 11.5	132.8 ± 10.0
Reproduction compared to control (%)	100	103.0	95.2	105.2	101.2	97.0

A calculation of a valid ECx curve for reproduction was not possible due to the lack of a significant dose-response relationship.

In the most recent study with Maypon Flow (Carbendazim, SC 500) the number of juveniles was reduced by 39 and 96% at concentrations of 5 and 10 mg product/kg soil dry weight after 8 weeks of test duration when compared to control (mean number of juveniles = 148).

Validity criteria:

Validity criteria (OECD 222, 2004)	Recommended	Obtained
Adult control mortality	≤ 10%	1.3 %
Number of juveniles per control replicate	≥ 30	109 to 168
Coefficient of variation of reproduction in the control	≤ 30%	15.31 %

III. CONCLUSION:

M-02 (AEC657188) showed no statistically significant effects on survival, growth and reproduction of the earthworm *Eisenia fetida* in an artificial soil up to and including 100 mg/kg soil dry weight, i.e. the highest concentration tested.
Therefore, the No-Observed-Effect-Concentration (NOEC) was determined to be ≥ 100 mg metabolite/kg soil dry weight, and the Lowest-Observed-Effect-Concentration (LOEC) was determined to be > 100 mg metabolite/kg soil dry weight.

Assessment and conclusion by applicant:

This study is considered reliable for risk assessment and the endpoint is:

NOEC ≥ 100 mg/kg dws

CA 8.4.2 Effects on non-target soil mesoand macrofauna (other than earthworms)

Table 8.4.2- 1: **Ecotoxicological endpoints – Collembola and soil mites reproduction studies with active substance fluopicolide and its soil metabolites**

Test substance	Test species, test design	Ecotoxicological Endpoint	Reference
Collembola, reproduction			
Fluopicolide	*Folsomia candida* reproduction 28 d, mixed	NOEC 31.25 mg a.s./kg dws* EC_{10} 40.44 mg a.s./kg dws*	[redacted] 2003; M-241194-01-1 KCA 8.4.2.1/01 EC_{10} calculation: [redacted] 2020; M-679537-01-1
M-01 (AE C653711)	*Folsomia candida* reproduction 28 d, mixed	NOEC ≥ 25 mg p.m./kg dws EC_{10} calculation not possible	[redacted] 2003; M-241193-01-1 KCA 8.4.2.1/02
M-02 (AE C657188)	*Folsomia candida* reproduction 28 d, mixed	NOEC ≥ 100 mg p.m./kg dws EC_{10} calculation not possible	[redacted] 2016; M-558332-01-1 KCA 8.4.2.1/04
M-03 (AE 0608000)	*Folsomia candida* reproduction 28 d, mixed	NOEC ≥ 50 mg p.m./kg dws* EC_{10} calculation not possible	[redacted] 2016; M-558337-01-1 KCA 8.4.2.1/03
Soil mites, reproduction			
Fluopicolide	*Hypoaspis aculeifer* reproduction 14 d, mixed	NOEC ≥ 500 mg a.s./kg dws* EC_{10} calculation not possible	[redacted] 2016; M-548042-01-1 KCA 8.4.2.1/05
M-01 (AE C653711)	*Hypoaspis aculeifer* reproduction 14 d, mixed	NOEC ≥ 100 mg p.m./kg dws EC_{10} calculation not possible	[redacted] 2015; M-538626-01-1 KCA 8.4.2.1/06
M-02 (AE C657188)	*Hypoaspis aculeifer* reproduction 14 d, mixed	NOEC ≥ 100 mg p.m./kg dws EC_{10} calculation not possible	[redacted] 2016; M-557987-01-1 KCA 8.4.2.1/07
M-03 (AE 0608000)	*Hypoaspis aculeifer* reproduction 14 d, mixed	NOEC ≥ 50 mg p.m./kg dws*#	# Calculated endpoint, assuming a 10-fold higher toxicity than the active substance fluopicolide

dws = dry weight soil, a.s. = active substance, p.m. = pure metabolite
* Endpoint corrected by a factor of 2 due to lipophilic substance (log P_{OW} > 2)

CA 8.4.2.1 Species level testing

Data Point:	KCA 8.4.2.1/01
Report Author:	[redacted]
Report Year:	2003
Report Title:	Effects of AE C638206 on Reproduction of the Collembola Folsomia candida in Artificial Soil
Report No:	15021016
Document No:	M-241194-01-1
Guideline(s) followed in study:	ISO 11267 ISO Soil Quality - Inhibition of reproduction of Collembola (Folsomia Candida) by soil pollutants, 1999
Deviations from current test guideline:	Current Guideline: OECD 232 (2016) The number of replicates in the control were 5 instead of 8 as recommended by the guideline. This deviation is not expected to have impacted the study results.
Previous evaluation:	yes, evaluated and accepted DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Data Point:	KCA 8.4.2.1/08
Report Author:	[redacted]
Report Year:	2020
Report Title:	Statistical re-evaluation (non-glp) of the Folsomia candida reproduction study with fluopicolide (Klein, 2003; M-241194-01-1) using the Probit analysis
Report No:	M-679537-01-1
Document No:	M-679537-01-1
Guideline(s) followed in study:	--
Deviations from current test guideline:	Not applicable
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	Yes

Executive Summary

The purpose of the study was to determine the effects of fluopicolide technical (AE C638206) on reproduction of the Collembola *Folsomia candida* in artificial soil. 10 (age of 10-12 days) collembolans per replicate were exposed to control (water treated), solvent control (acetone) and treatments with 62.5, 125, 250, 500 and 1000 mg/kg dry soil in a first assay. The assay was conducted under controlled environmental conditions. Test containers were reusable glass vessels (volume: 100 mL; diameter: 5 cm), closed tightly by plastic-lids to avoid water evaporation from the artificial soil. Due to unusually high reproduction recorded in controls in the first assay a second assay was conducted at nominal concentrations of 15.6, 31.3 and 62.5 mg/kg dry soil. Mortality and reproduction were determined after 28 days.

All validity criteria were met. Based on the effects observed on reproduction, it is concluded, that the overall NOEC for the study is determined to be 62.5 mg a.s./kg artificial soil dry weight. Thus, the overall LOEC is determined to be 125 mg a.s./kg artificial soil dry weight. The probit analysis revealed an EC_{10} = 32.88 mg a.s./kg dws, an EC_{20} = 134.60 mg a.s./kg dws, and an EC_{50} = 1995.40 mg a.s./kg.

I. MATERIAL AND METHODS:

Test item: Fluopicolide (AE C638206 00 1C99 0005, Batch No: 2050190//PP241024/2). Test organisms: *Folsomia candida* (Collembola: Isotomidae), age of 10-12 days. 10 collembolans per replicate were exposed to control (water treated), solvent control (acetone) and treatments with 62.5, 125, 250, 500 and 1000 mg/kg dry soil in a first assay. Due to unusually high reproduction recorded in controls in the first assay a second assay was conducted at nominal concentrations of 15.6, 31.3 and 62.5 mg/kg dry soil. Both assays were conducted under controlled environmental conditions at 19-21°C and 590-660 lux in the 1st experiment and 18-21°C and 430-550 lux in the second experiment with a light regime of 16 h light and 8 hours dark. There was one additional container per test item and control for measurement of pH and humidity. Test containers were reusable glass vessels (volume: 100 mL; diameter: 5 cm), closed tightly by plastic-lids to avoid water evaporation from the artificial soil, filled with approximately 30 g ± 5% wet weight artificial soil. Artificial soil contained 10% Sphagnum peat, 20% Kaolin clay, approximately 0.5% Calcium carbonate and approximately 69.5% fine quartz-sand. During the study, the test organisms were fed with granulated dry yeast. Mortality and reproduction were determined after 28 days. The pH and water content were measured at start and finish of the study for each concentration. The software used to perform the statistical analysis was Systat Version 9.0.

Dates of experimental work: November 13, 2002 – February 04, 2003

II. RESULTS AND DISCUSSION:

Analytical results:

1st experiment

Test item concentration[1]	pH		Water content (%)		WHCmax [2]	
	Start	End	Start	End	Start	End
Control	5.6	5.7	36%	34%	55%	52%
Solvent control	5.7	5.6	36%	33%	54%	51%
62.5	5.7	5.7	36%	34%	54%	52%
125	5.7	5.7	36%	35%	54%	53%
250	5.7	5.6	36%	34%	54%	52%
500	5.7	5.7	36%	34%	54%	51%
1000	5.7	5.8	36%	34%	54%	52%

[1] mg a.s./kg soil dry weight
[2] % WHC_{max} = percent of maximum water holding capacity

2nd experiment

Test item concentration[1]	pH		Water content (%)		WHCmax [2]	
	Start	End	Start	End	Start	End
Control	6.2	5.8	36%	33%	54%	51%
Solvent control	6.2	5.7	36%	33%	54%	51%
15.6	6.1	5.7	36%	33%	55%	51%
31.3	6.0	5.6	36%	33%	55%	51%
62.5	6.0	5.6	36%	34%	54%	51%

[1] mg a.s./kg soil dry weight
[2] % WHC_{max} = percent of maximum water holding capacity

Biological results:

1st experiment

	Water control	Solvent control	Pooled control	AE C638206 [mg/kg artificial soil dry weight]				
				62.5	125	250	500	1000
Mortality[1]	4%	6%	5%	6%	12%	2%	10%	6%
	± 5.5%	± 5.5%	± 5.3%	± 5.5%	± 4.5%	± 4.5%	± 10%	± 5%
Statistical analysis	-	n.s.[2]	-	n.s.[2]	n.s.[2]	n.s.[2]	n.s.[2]	n.s.[2]
NOEC	1000 mg/kg							
LOEC	> 1000 mg/kg							
LC_{50}	Data not appropriate for calculation							
Reproduction[1]	916	909	912	753	703	647	598	585
	45	128	97	97	53	84	48	81
% of (pooled) control	-	99	-	83	77	71	66	62
Statistical analysis	-	n.s.[4]		*[3]	*[3]	*[3]	*[3]	*
NOEC	< 62.5							
LOEC	62.5							
EC_{50}	Data not appropriate for calculation							

[1] Mean ± standard deviation (SD)
[2] Fisher Exact-Test, α = 0.05, two-sided
[3] Williams Test, α = 0.05, one-sided smaller
[4] Student-t test, α = 0.05, one-sided smaller
- = not applicable
n.s. = not significantly different compared to pooled water and acetone controls
* = significantly different compared to pooled water and acetone controls

2nd experiment

	Water control	Solvent control	Pooled control	Fluopicolide (AE C638206 [mg/kg artificial soil dry])		
				15.6	31.3	62.5
Mortality	8%	4%	6%	8%	20%	6%
	± 8.4%	± 5.5%	± 7.0%	± 8.4%	± 7.1%	± 5.5%
Statistical analysis	-	n.s.[2]	-	n.s.[2]	#[2]	n.s.[2]
NOAEC	62.5 mg/kg					
LOEC	> 62.5 mg/kg					
LC_{50}	Data not appropriate for calculation					
Reproduction[1]	755	645	700	704	606	684
	± 84	± 107	± 108	± 71	± 89	± 65
% of (pooled) control	-	85	-	101	87	98
Statistical analysis		n.s.[4]	-	n.s.[3]	n.s.[3]	n.s.[3]
NOAEC	< 62.5 mg/kg soil					
LOEC	> 62.5 mg/kg soil					
EC_{50}	Data not appropriate for calculation					

[1] Mean number of juveniles per replicate ± standard deviation (SD)
[2] Fisher Exact-Test, α = 0.05, two-sided
[3] Williams Test, α = 0.05, one-sided smaller
[4] Student-t test, α = 0.05, one-sided smaller
- = not applicable
n.s. = not significantly different compared to pooled water and acetone controls
# Significantly different compared to pooled water and acetone controls

ECx evaluation for reproduction

ECx were statistically re-evaluated based on the number of juveniles of *F. candida* provided in this study report (Table 7 and Table 8, original study report (M-241194-01-1), page 25). Details are given in a separate report ([redacted] 2020; M-679537-01-1). From each run separately, all mean data referring to the number of juveniles at day 28 in a test item treatment group was corrected as a percentage of the mean pooled control (water and solvent control were pooled). Both runs were then combined and a probit analysis was performed in order to derive EC_{10}, EC_{20} and EC_{50}-values for the % effect on the number of juveniles of *F. candida*. The concentration of 62.5 mg/kg was tested in both runs, hence, the average of both means was used at 62.5 mg/kg (overall mean of 90.14 % as a mean of 97.71 % and 82.57 % at 62.5 mg/kg). This re-analysis was performed using the software ToxRatPro 3.2.1.

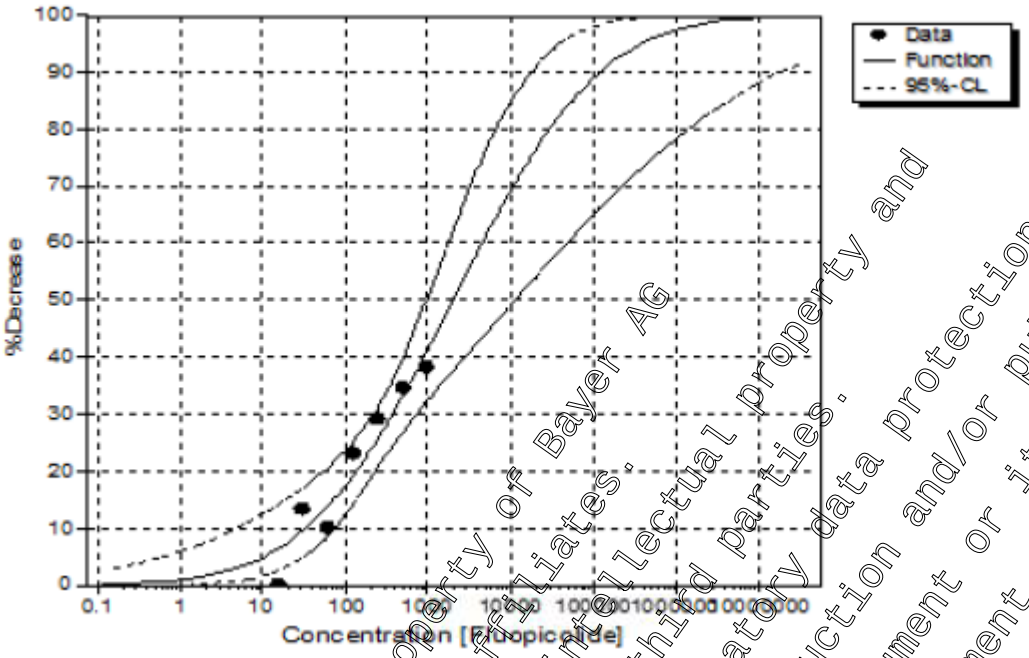
Percentage of the mean number of juveniles of *F. candida* transformed from the results presented in the study report at different test item treatment concentration of the test item in soil [mg a.s./kg dws] at day 28.

Test item treatment	Pooled Control	15.6 mg/kg	31.3 mg/kg	62.5 mg/kg	125 mg/kg	250 mg/kg	500 mg/kg	1000 mg/kg
% of control	100 %	100.57 %	86.57 %	90.14 %	77.08 %	70.94 %	69.57 %	61.95 %

The probit analysis revealed an EC_{10} = 32.88 mg a.s./kg dws, an EC_{20} = 134.60 mg a.s./kg dws, and an EC_{50} = 1995.40 mg a.s./kg. The dose-response curve from the probit analysis does fit to the most relevant data; the calculated EC_{10} = 32.88 mg a.s./kg dws is considered reliable. This is supported by $p(CHI^2)$ = 1.000 and p(F)= 0.002 indicating a sufficiently robust EC_x-calculation.

EC_{10}, EC_{20}, and EC_{50}-values (with confidence limits) for % effect on number of juveniles compared to the control

	EC_{10}	EC_{20}	EC_{50}
Value [mg a.s./kg dws]	32.883	134.604	1995.397
Lower 95%-cl	4.008	54.282	947.186
Upper 95%-cl	73.228	227.396	11301.761



Dose-response relationship for percentage (%) of the number of juveniles of *F. candida* with Fluopicolide concentrations in soil compared to the control following the probit analysis ($\chi^2(CHI)^2 = 1.000$; $p(F)=.0.002$.

The EC_{10}, EC_{20} and EC_{50} values were calculated being 32.88 mg a.s./kg dws, 134.60 mg a.s./kg dws and 1995.40 mg a.s./kg, respectively.

Toxic reference test:

The most recent toxic standard test showed statistically significant effects on reproduction at concentrations of ≥ 50 mg Betosip/kg artificial soil (dry weight), the EC_{50} for reproduction was calculated to be 135 mg Betosip/kg soil (dry weight). Mortality was statistically significantly higher than the control at 400 mg Betosip/kg artificial soil (dry weight), the LC_{50} for mortality was calculated as 285.1 mg Betosip/kg soil (dry weight).

Validity criteria:

All validity criteria were met in this study.

Validity criteria (OECD 232, 2016)	Obtained in this study
Mean adult mortality ≤ 20%	< 20% in 1st and 2nd experiment
Mean number of juveniles/replicates ≥ 100	> 100 in 1st and 2nd experiment
Coefficient of variation calculated for the number of juveniles per replicate ≤ 30%	< 30% in 1st and 2nd experiment

III. CONCLUSION:

Based on the effects observed on reproduction, it is concluded, that the overall NOEC for the study is determined to be 62.5 mg a.s./kg artificial soil dry weight. Thus, the overall LOEC is determined to be 125 mg a.s./kg artificial soil dry weight. The EC_{10}, EC_{20} and EC_{50} values for reproduction were calculated being 32.88 mg a.s./kg dws, 134.60 mg a.s./kg dws and 1995.40 mg a.s./kg, respectively.

Assessment and conclusion by applicant:

This study is considered reliable for risk assessment. A calculated EC_{10} = 32.88 mg a.s./kg dws is considered as the relevant endpoint for the risk assessment.

Data Point:	KCA 8.4.2.1/02
Report Author:	[redacted]
Report Year:	2003
Report Title:	Effects of AE C653711 on Reproduction of the Collembola Folsomia candida in Artificial Soil
Report No:	15031016
Document No:	M-241193-01-1
Guideline(s) followed in study:	ISO 11267 ISO Soil Quality - Inhibition of reproduction of Collembola (Folsomia Candida) by soil pollutants, 1999
Deviations from current test guideline:	Current Guideline: OECD 232 (2016) The number of replicates in the control were 5 instead of 8 as recommended by the guideline. This deviation is not expected to have impacted the study results.
Previous evaluation:	yes, evaluated and accepted DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The purpose of the study was to determine the effects of M-01 (AE C653711) on reproduction of the Collembola *Folsomia candida* in artificial soil. Test organisms: *Folsomia candida* (Collembola: Isotomidae), age of 10-12 days. 10 collembolans per replicate were exposed to control (water treated), solvent control (acetone) and treatments with 6.25, 12.5, 25, 50 and 100 mg/kg artificial soil (dry weight). The assay was conducted under controlled environmental conditions. Test containers were reusable glass vessels (volume: 100 mL; diameter: 5 cm) and contained artificial soil. Mortality and reproduction were determined after 28 days.
All validity criteria were met. In this study, M-01 (AE C653711) causes no mortality and no adverse effect on reproduction of *Folsomia candida* at concentrations up to and including 25 mg/kg artificial soil dry weight (NOEC). The LOEC for both endpoints is 50 mg/kg artificial soil dry weight.

I. MATERIAL AND METHODS:

Test item: M-01 (AE C653711 00 1B97 0001, Batch No: 8808018; purity: 97.0% w/w). Test organisms: *Folsomia candida* (Collembola: Isotomidae), age of 10-12 days. 10 collembolans per replicate were exposed to control (water treated), solvent control (acetone) and treatments with 6.25, 12.5, 25, 50 and 100 mg/kg artificial soil (dry weight). The assay was conducted under controlled environmental conditions at 19-21°C and 480-650 lux with a light regime of 16 h light and 8 hours dark. There was one additional container per test item and control for measurement of pH. Test containers were reusable glass vessels (volume: 100 mL; diameter: 5 cm), closed tightly by plastic-lids to avoid water evaporation from the artificial soil, filled with approximately 30 g ± 1.5g fresh weight artificial soil. Artificial soil contained 10% Sphagnum peat, 20% Kaolin clay, approximately 0.5% Calcium carbonate and approximately 69.5% fine quartz-sand.

Feeding: After the introduction of the test organisms, approximately 2 mg (half of a small spatula) of granulated dry yeast (Dr. Oetker) were spread over the soil surface at test start. After 14 days granulated dry yeast was added ad libitum.

Mortality and reproduction were determined after 28 days. At day 28 after application, water content and pH of one additional test container (prepared at test start and without Collembola) per treatment group and control were determined according to DIN 19683 and DIN 19684 (CaCl$_2$). The software used to perform the statistical analysis was Systat Version 9.0.

Dates of experimental work: November 18, 2002 – December 17, 2002

II. RESULTS AND DISCUSSION

Analytical results:

Test item concentration[1]	pH		Water content (%)[3]		WHC_{max} [2, 3]	
	Start	End	Start	End	Start	End
Control	5.9	5.8	35%	34%	54%	51%
Solvent control	5.8	5.9	35%	33%	54%	51%
6.25	5.8	5.9	35%	34%	54%	52%
12.5	5.8	6.1	35%	34%	54%	52%
25	5.8	5.8	35%	34%	54%	52%
50	5.8	5.7	35%	34%	54%	51%
100	5.8	5.7	35%	34%	54%	51%
minimum	5.8	5.7	35%	33%	54%	51%
maximum	5.9	6.1	35%	34%	54%	52%

[1] mg a.s./kg artificial soil dry weight
[2] % WHC_{max} = percent of maximum water holding capacity (WHC_{max} = 65.47% dry weight)
[3] the results represent rounded values calculated on the exact raw data

Biological results:

mg/kg soil dry weight nominal conc.	Adult mortality (%)	Mean number of juveniles/test vessel ± SD	Reproduction (% of pooled controls)	Significance
Control	4	677 ± 92	-	
Solvent control	4	769 ± 124	114	n.s.[1]
6.25	6	834 ± 144	115	n.s.[2]
12.5	6	679 ± 83	94	n.s.[2]
25	8	683 ± 46	94	n.s.[2]
50	16	548 ± 100	76	*[2]
100	6	571 ± 128	79	*[2]

[1] Student t-Test, α = 0.05, one-sided greater
[2] Williams Test, α = 0.05, one-sided smaller

A valid ECx curve for reproduction could not be calculated due to the lack of a significant dose-response relationship.

Toxic reference test:

The most recent toxic standard test showed statistically significant effects on reproduction at concentrations > 50 mg Betosip/kg artificial soil dry weight; the EC_{50} for reproduction was calculated as 135 mg Betosip/kg soil dry weight.

Mortality was statistically significantly higher than the control at 400 mg Betosip/kg artificial soil dry weight, the LC_{50} for mortality was calculated as 285.1 mg Betosip/kg soil dry weight.

Validity criteria:

All validity criteria were met in this study.

Validity criteria (OECD 232, 2016)	Obtained in this study
Mean adult mortality ≤ 20%	4% ± 5.5%
Mean number of juveniles/replicate ≥ 100	> 677 ± 92
Coefficient of variation calculated for the number of juveniles per replicate ≤ 30%	16%

III. Conclusion:

In this study, M-01 (AE C653711) caused no mortality and no adverse effect on reproduction of *Folsomia candida* at concentrations up to and including 25 mg/kg artificial soil dry weight (NOEC). The LOEC for both endpoints was 50 mg/kg artificial soil dry weight.

Assessment and conclusion by applicant:

This study is considered reliable for risk assessment. The NOEC of 25 mg/kg for reproduction is the relevant endpoint for the risk assessment.

Data Point:	KCA 8.4.2.1/03
Report Author:	[redacted]
Report Year:	2016
Report Title:	AE 0608000 (BCS-AX86048): Effects on mortality and reproduction of the collembolan species Folsomia candida tested in artificial soil
Report No:	EBACN060
Document No:	M-558337-01-1
Guideline(s) followed in study:	OECD 232 (2009): OECD Guideline for testing of chemicals No. 232 (adopted 7 September 2009): Collembolan reproduction test in soil; US EPA OCSPP not applicable
Deviations from current test guideline:	Current Guideline: OECD 232 (2016) The illumination was 12 h light and 12 h dark and not 16 h light and 8 h dark as recommended by the guideline. This deviation is not expected to have impacted the study results.
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The purpose of the study was to determine the effects of M-03 (AE 0608000) on reproduction of the Collembola *Folsomia candida* in artificial soil. *Folsomia candida* (Collembola: Isotomidae), age of 9-12 days. 10 collembolans per replicate were exposed to control (water treated), and treatments with 10, 18, 32, 56 and 100 mg/kg dry weight soil. The assay was conducted under controlled environmental conditions in glass vessels (volume: 150 ml) covered with a lid. Mortality and reproduction were determined after 28 days. All validity criteria were met. In this study M-03 (AE 0608000) caused no mortality and no adverse effect on reproduction of *Folsomia candida* at concentrations up to and including 100 mg/kg soil dry weight (NOEC). The LOEC for both endpoints are > 100 mg /kg soil dry weight.

I. Material and Methods:

Test item: M-03 (AE 0608000 (BCS-AX86048)), Batch Code: AE 0608000-01-01; purity: 99.4% w/w.
Test organisms: *Folsomia candida* (Collembola: Isotomidae), age of 9-12 days. 10 collembolans per replicate were exposed to control (water treated), and treatments with 10, 18, 32, 56 and 100 mg p.m./kg dry weight soil. The assay was conducted under controlled environmental conditions at 18.9 -21.6°C and 520 lux with a light regime of 12h light and 12 hours dark. Test containers were reusable glass vessels (volume: 150 mL) covered with a lid; surface area of soil: 18.9 cm^2, filled with 30 g wet weight artificial soil. Artificial soil contained 5% sphagnum peat, 20% kaolin clay, 74.7% industrial quartz sand und 0.3% calcium carbonate. Feeding: After the introduction of the test organisms and after 14 days, approximately 2 mg of granulated dry yeast were spread over the soil surface.
Mortality and reproduction were determined after 28 days. The pH and water content of the test substrate were determined at the start and at the end of the test in the control and each treatment.

Dates of experimental work: February 26, 2016 – March 25, 2016

II. RESULTS AND DISCUSSION:

Analytical results:

Water content, water holding capacity (WHC) and pH in the control and treatments

Water content (g/100 g soil dry weight)		WHC (%)		pH	
Start	**End**	**Start**	**End**	**Start**	**End**
25.0 – 25.1	24.3 – 24.8	59.1 - 59.3	57.4 - 58.6	6.04 – 6.13	5.84 – 5.93

Biological results:

Test item Test object Exposure	M-03-(AE 0608000 (BCS-AX86048)) *Folsomia candida* Artificial soil						
mg pure metabolite/kg dry weight artificial soil (nominal concentrations)	Adult mortality (%)	Significance (**)	Mean number of juveniles per test vessel ± standard deviation			Reproduction (% of control)	Significance (*)
Control	1.3		1166	±	104		
10	2.5		1135	±	77	97	-
18	5.0	-	1183	±	144	101	-
32	2.5	-	1169	±	121	100	-
56	5.0		1177	±	82	101	-
100	5.0		1149	±	135	99	-
						Mortality	**Reproduction**
NOEC (mg pure metabolite/kg dry weight artificial soil)						≥ 100	≥ 100
LOEC (mg pure metabolite/kg dry weight artificial soil)						> 100	> 100

The calculations were performed with unrounded values
(*) = Williams-t-test, one-sided smaller, α = 0.05, + = significant, - = not significant
(**) = Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm, one-sided greater, α = 0.05, + = significant, - = not significant.

The calculation of an EC$_x$ curve for reproduction was not possible due to the lack of a significant dose-response relationship.

Toxic reference test:

In the most recent study with the reference item boric acid the EC_{50} for reproduction was determined to be 103 mg/kg dry weight soil. The LC_{50} was determined to be 162 mg /kg dry weight soil. The NOEC for mortality and for reproduction was determined to be 67 and 44 mg/kg soil dry weight, respectively. The EC_{50} value for reproduction was close to the value of 100 mg/kg soil dry weight as stated in OECD 232 (2009). The EC_{50} therefore showed that the test system was sensitive.

Validity criteria:

All validity criteria were met in this study.

Validity criteria according to OECD 232 (2016)	Obtained in this study
Mean adult mortality ≤ 20%	1.3%
Mean number of juveniles/replicate ≥ 100	1166
Coefficient of variation calculated for the mean number of juveniles < 30%	9.0%

III. CONCLUSION:

In this study, M-03 (AE 0608000) caused no mortality and no adverse effect on reproduction of *Folsomia candida* at concentrations up to and including 100 mg/kg soil dry weight (NOEC). The LOEC for both endpoints was > 100 mg/kg soil dry weight.

Assessment and conclusion by applicant:

This study is considered reliable for risk assessment. The NOEC of ≥ 100 mg/kg for reproduction is the relevant endpoint for the risk assessment.

Data Point:	KCA 8.4.2.1/04
Report Author:	[redacted]
Report Year:	2016
Report Title:	Pyridyl carboxylic acid (BCS-AB43478): Effects on mortality and reproduction of the collembolan species Folsomia candida tested in artificial soil
Report No:	EBACN056
Document No:	M-558332-01-1
Guideline(s) followed in study:	OECD 232 (2009): OECD Guideline for testing of chemicals No. 232 (adopted 7 September 2009): Collembolan reproduction test in soil, US EPA OCSPP not applicable
Deviations from current test guideline:	Current Guideline: OECD 232 (2016) The illumination was 12 h light and 12 h dark and not 16 h light and 8 h dark as recommended by the guideline. This deviation is not expected to have impacted the study results.
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The purpose of the study was to determine the effects of M-02 (AE C657188) on reproduction of the Collembola *Folsomia candida* in artificial soil. *Folsomia candida* (Collembola: Isotomidae), age of 9-12 days. 10 collembolans per replicate were exposed to control (water treated), and treatments with 10, 18, 32, 56 and 100 mg p.m./kg dry weight soil. The assay was conducted under controlled environmental conditions in glass vessels (volume: 450 mL) covered with a lid. Mortality and reproduction were determined after 28 days.

All validity criteria were met. In this study, M-02 (AE C657188) caused no mortality and no adverse effect on reproduction of *Folsomia candida* at concentrations up to and including 100 mg p.m. /kg soil dry weight (NOEC). The LOEC for both endpoints is > 100 mg p.m./kg soil dry weight.

I. Material and Methods:

Test item: M-02 (AE C657188 (BCS-AB43478)), Batch Code: AE C657188-PU-01, Origin Batch No.: SES 10250-1-1, CAS No.: 80194-68-9, LIMS No.: 1518969, purity: 98.5 % w/w.
Test organisms: *Folsomia candida* (Collembola: Isotomidae), age of 9-12 days. 10 collembolans per replicate were exposed to control (water treated), and treatments with 10, 18, 32, 56 and 100 mg p.m./kg dry weight soil. The assay was conducted under controlled environmental conditions at 18.2 -21.9°C and 620 lux with a light regime of 12h light and 12 hours dark. Test containers were reusable glass vessels (volume: 150 mL) covered with a lid; surface area of soil: 18.9 cm², filled with 30 g wet weight artificial soil. Artificial soil contained 5% sphagnum peat, 20% kaolin clay, 74.7% industrial quartz sand und 0.3% calcium carbonate. Feeding: After the introduction of the test organisms and after 14 days, approximately 2 mg of granulated dry yeast were spread over the soil surface.
Mortality and reproduction were determined after 28 days. The pH and water content of the test substrate were determined at the start and at the end of the test in the control and each treatment. In this study, M-02 (AE C657188) caused no mortality and no adverse effect on reproduction of *Folsomia candida* at concentrations up to and including 100 mg p.m. /kg soil dry weight (NOEC). The LOEC for both endpoints was > 100 mg p.m./kg soil dry weight.

Dates of experimental work: April 14, 2016 – May 12, 2016

II. Results and Discussion:

Analytical results:

Water content, water holding capacity (WHC) and pH in the control and treatments

Water content (g/100 g soil dry weight)		WHC (%)		pH	
Start	End	Start	End	Start	End
24.9 – 25.0	24.4 - 24.8	58.9 - 59.1	57.7 - 58.6	6.05 – 6.09	5.79 – 5.84

Biological results:

Test item Test object Exposure	M-02 (AE C657188 (BCS-AB43478)) *Folsomia candida* Artificial soil						
mg pure metabolite/kg dry weight artificial soil (nominal concentrations)	Adult mortality (%)	Significance (**)	Mean number of juveniles per test vessel ± standard deviation			Reproduction (% of control)	Significance (*)
Control	2.5		808	±	79	-	
10	2.5	-	814	±	46	101	-
18	0.0	-	826	±	94	102	-
32	2.5	-	828	±	81	102	-
56	2.5	-	806	±	41	100	-
100	2.5	-	816	±	134	101	-
						Mortality	**Reproduction**
NOEC (mg pure metabolite/kg dry weight artificial soil) LOEC (mg pure metabolite/kg dry weight artificial soil)						≥ 100 > 100	≥ 100 > 100

The calculations were performed with unrounded values
(*) = Williams t-test, one-sided smaller, α = 0.05, + = significant, - = not significant
(**) = Multiple Sequentially-rejective Fisher Test After Bonferroni-Holm, one-sided greater, α = 0.05, + = significant, - = not significant

The calculation of an ECx curve for reproduction was not possible due to the lack of a significant dose-response relationship.

Toxic reference test:

In the most recent study with the reference item boric acid the EC_{50} for reproduction was determined to be 103 mg/kg dry weight soil. The LC_{50} was determined to be 162 mg /kg dry weight soil. The NOEC for mortality and for reproduction was determined to be 67 and 44 mg/kg soil dry weight respectively. The EC_{50} value for reproduction was close to the value of 100 mg/kg soil dry weight as stated in OECD 232 (2009). The EC_{50} therefore showed that the test system was sensitive.

Validity criteria:

All validity criteria were met in this study.

Validity criteria (OECD 232, 2016)	**Obtained in this study**
Mean adult mortality ≤ 20%	2.5%
Mean number of juveniles/replicate ≥ 100	808
Coefficient of variation calculated for the mean number of juveniles < 30%	9.8%

III. CONCLUSIONS:

In this study, M-02 (AE C657188) caused no mortality and no adverse effect on reproduction of *Folsomia candida* at concentrations up to and including 100 mg p.m. /kg soil dry weight (NOEC). The LOEC for both endpoints was > 100 mg p.m./kg soil dry weight.

Assessment and conclusion by applicant:

This study is considered reliable for risk assessment. The NOEC of ≥100 mg/kg for reproduction is the relevant endpoint for the risk assessment.

Data Point:	KCA 8.4.2.1/05
Report Author:	[redacted]
Report Year:	2016
Report Title:	Fluopicolide a.s. (BCS-AM59797): Influence on mortality and reproduction of the soil mite species Hypoaspis aculeifer tested in artificial soil
Report No:	E 428 4833-2
Document No:	M-548042-01-1
Guideline(s) followed in study:	OECD 226 from October 03, 2008: OECD guideline for the Testing of Chemicals - Predatory mite (Hypoaspis (Geolaelaps) aculeifer) reproduction test in soil
Deviations from current test guideline:	Current Guideline: OECD 226 (2016) No deviations
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The purpose of this study was to assess the effect of fluopicolide a.s. (BCS-AM59797) on mortality and reproduction of the soil mite species *Hypoaspis aculeifer* tested during an exposure of 14 days in artificial soil comparing control and treatment. Ten adults, fertilized, female *Hypoaspis aculeifer* per replicate (8 replicates for the control group and 4 replicates for each treatment group) were exposed to control and treatments. Concentrations of 18, 32, 56, 100, 178, 316, 562 and 1000 mg a.s./kg artificial soil dry weight were tested. After a period of 14 days, the surviving adults and the living juveniles were extracted by applying a temperature gradient using a MacFadyen-apparatus. All *Hypoaspis aculeifer* were counted under a binocular.
All validity criteria were met. The $NOEC_{reproduction}$ and $LOEC_{reproduction}$ of fluopicolide were determined to be ≥ 1000 mg a.s./kg artificial soil dry weight and > 1000 mg a.s./kg artificial soil dry weight, respectively. Since there are no adverse effects on mortality and reproduction, no EC_{10}/EC_{20} calculation was possible.

I. Material and Methods:

Fluopicolide a.s. (BCS-AM59797) (analytical findings: 98.8 % w/w; batch code: AE C638206-01-26; customer order no.: TOX10889-00; specification no.: 102000016444; origin batch no.: BCHR 1111-2-1; certificate no.: AZ20033); purity: 98.8%. Ten adults, fertilized, female *Hypoaspis aculeifer* per replicate (8 replicates for the control group and 4 replicates for each treatment group) were exposed to control and treatments. Concentrations of 18, 32, 56, 100, 178, 316, 562 and 1000 mg a.s./kg artificial soil dry weight were tested. During the test, the *Hypoaspis aculeifer* were fed with nematodes bred on watered oat flakes. During the study a temperature of 20 ± 2 °C and light regime of 400 – 800 Lux, 16 h light: 8 h dark was applied. The artificial soil was prepared according to the guideline with the following constituents (percentage distribution on dry weight basis): 75 % fine quartz sand, 5 % Sphagnum peat, air dried and finely ground, 20 % Kaolin clay. After a period of 14 days, the surviving adults and the living juveniles were extracted by applying a temperature gradient using a MacFadyen-apparatus. Extracted mites were collected in a fixing solution (20% ethylene glycol, 80 % deionised water; 2 g detergent/L fixing solution were added). All *Hypoaspis aculeifer* were counted under a binocular. In the control group no mortality of adult *Hypoaspis aculeifer* was observed.
Concerning the number of juveniles statistical analysis (William's-t test, one-sided smaller, $\alpha = 0.05$) revealed no significant difference between control and any treatment group. Since there were no adverse effects on mortality and reproduction, no EC_{10}/EC_{20} calculation was possible.

Dates of experimental work: November 20, 2015 – December 11, 2015

II. RESULTS AND DISCUSSION:

Experimental conditions:
All values were within the range recommended by the guideline.

[mg a.s./ kg dry weight artificial soil]	pH		% water content			% of WHC_{max}	
	Start	End	Start	End	% deviation	Start	End
Control	5.65	5.55	19.52	19.45	0.4	47.46	47.25
18	5.63	5.56	19.25	19.20	0.2	46.64	46.50
32	5.60	5.68	19.31	18.81	2.7	46.82	45.33
56	5.59	5.55	19.36	19.20	0.8	46.99	46.51
100	5.56	5.57	19.02	19.30	1.4	45.98	46.80
178	5.56	5.57	19.22	19.02	1.1	46.56	45.96
316	5.51	5.56	19.64	19.02	3.3	47.83	45.96
562	5.51	5.53	19.22	19.00	1.2	46.56	45.90
1000	5.51	5.58	18.98	19.60	3.2	45.85	47.71

Biological results:
In the control group no mortality of adult *Hypoaspis aculeifer* was observed.
Concerning the number of juveniles statistical analysis (William's-t test, one-sided smaller, $\alpha = 0.05$) revealed no significant difference between control and any treatment group. Since there were no adverse effects on mortality and reproduction, no EC_{10}/EC_{20} calculation was possible.

Test item	Fluopicolide a.s.			
Test Object	*Hypoaspis aculeifer*			
Exposure	Artificial Soil			
[mg a.s./kg dry weight artificial soil]	% mortality (adults)	Mean number of juveniles per test vessel ± standard dev.	Reproduction (% of control)	Significance (*)
Control	0	233.0 ± 31.6		-
18	10.0	234.5 ± 32.1	100.6	-
32	6.7	217.7 ± 43.7	93.4	-
56	2.5	250.3 ± 12.1	107.4	-
100	5.0	282.3 ± 27.6	121.1	-
178	7.5	262.5 ± 48.6	112.7	-
316	5.0	279.8 ± 29.8	120.1	-
562	7.5	289.0 ± 22.3	124.0	-
1000	2.5	260.0 ± 15.0	111.6	-
$NOEC_{reproduction}$ [mg a.s./kg dry weight artificial soil] > 1000				
$LOEC_{reproduction}$ [mg a.s./kg dry weight artificial soil] > 1000				

Calculations were done with un-rounded values.
(*)=William's-t.-test one sided smaller; α=0.05; "-": non-significant; "+": significant

The calculation of an ECx-curve for reproduction was not possible due to the lack of a significant dose-response relationship.

Validity criteria:
All validity criteria were met.

Validity criteria (OECD 226, 2016)	Obtained in this study
Mean adult mortality should not exceed 20 % at the end of the test	0 %
Mean number of juveniles per replicate should be at least 50 (with 10 mites introduced)	233
Coefficient of variation calculated for the number of juveniles per replicate should not be higher than 30 %	13.6 %

Toxic reference test:
In a separate study (Maria Ivonne Larnaudie Lopez, LAR/HR-O-21/15, November 09, 2015) performed with the reference item dimethoate at test concentrations 1.0, 1.8, 3.2, 5.6 and 10.0 mg dimethoate/kg dry weight artificial soil, the LC_{50} was calculated to be 1.9 mg a.s./kg for mortality. The NOEC was calculated to be 3.2 mg a.s./kg and the LOEC was 5.6 mg a.s./kg. Since variances of the data were homogenous Williams-t test $\alpha = 0.05$, one-sided smaller was used. Dimethoate EC 400 G showed an EC_{50} of 5.36 mg a. s./kg (99 % confidence limits from 4.75 mg a. s./kg to 5.68 mg a. s./kg) for reproduction. This is in the recommended range of the guideline and demonstrates the sensitivity of the test system.

III. Conclusion:

The $NOEC_{reproduction}$ and $LOEC_{reproduction}$ of fluopicolide were determined to be ≥ 1000 mg a.s./kg artificial soil dry weight and > 1000 mg a.s./kg artificial soil dry weight, respectively. Since there were no adverse effects on mortality and reproduction, no EC_{10}/EC_{20} calculation was possible.

Assessment and conclusion by applicant:

This study is considered reliable for risk assessment. The NOEC = 1000 mg a.s./kg dws is the relevant endpoint for the risk assessment.

Data Point:	KCA 8.4.2.1/06
Report Author:	[redacted]
Report Year:	2015
Report Title:	AE C653711 (BCS-AA65784): Influence on mortality and reproduction of the soil mite species Hypoaspis aculeifer tested in artificial soil
Report No:	E 428 4712-8
Document No:	M-538626-01-1
Guideline(s) followed in study:	EU Directive 91/414/EEC Regulation (EC) No. 1107/2009 US EPA OCSPP: not applicable OECD 226 (2008)
Deviations from current test guideline:	Current Guideline: OECD 226 (2016) No deviations
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The purpose of this study was to assess the effect of M-01 (AE C653711 (BCS-AA65784)) on mortality and reproduction of the soil mite species *Hypoaspis aculeifer* tested during an exposure of 14 days in artificial soil comparing control and treatment. A concentration of 100 mg/kg artificial soil dry weight was tested. After a period of 14 days, the surviving adults and the living juveniles were extracted by applying a temperature gradient using a MacFadyen-apparatus. All *Hypoaspis aculeifer* were counted under a binocular. In the control group 3.8 % of the adult *Hypoaspis aculeifer* died which is below the allowed maximum of ≤ 20 % mortality.
All validity criteria were met. Concerning the number of juveniles statistical analysis (Student-t test, one-sided smaller, $\alpha = 0.05$) revealed no significant difference between control and any treatment group. The $NOEC_{reproduction}$ and $LOEC_{reproduction}$ of fluopicolide were determined to be ≥ 100 mg/kg artificial soil dry weight and > 100 mg/kg artificial soil dry weight, respectively.

I. Material and Methods:

M-01 (AE C653711 (BCS-AA65784)) (analytical findings: 96.2 % w/w; batch code: M-01 (AE C653711 001B96 0001, origin batch no.: 08018ET, certificate no.: AZ17535)), purity: 96.2%. Ten adults, fertilized, female *Hypoaspis aculeifer* per replicate (8 replicates for the control and the treatment group) were exposed to control and treatments. A concentration of 100 mg/kg artificial soil dry weight was tested. During the test *Hypoaspis aculeifer* were fed with nematodes bred on watered oat flakes. During the study a temperature of 20 ± 2 °C and light regime of 400 – 800 Lux, 16 h light: 8 h dark was applied. After a period of 14 days, the surviving adults and the living juveniles were extracted by applying a temperature gradient using a MacFadyen-apparatus. Extracted mites were collected in a fixing solution (20% ethylene glycol, 80 % deionised water; 2 g detergent/L fixing solution were added). All *Hypoaspis aculeifer* were counted under a binocular.
All validity criteria were met. In the control group 3.8 % of the adult *Hypoaspis aculeifer* died which is below the allowed maximum of ≤ 20 % mortality. Concerning the number of juveniles statistical analysis (Student-t test, one-sided smaller, $\alpha = 0.05$) revealed no significant difference between control and any treatment group. The $NOEC_{reproduction}$ and $LOEC_{reproduction}$ of fluopicolide were determined to be ≥ 100 mg/kg artificial soil dry weight and > 100 mg/kg artificial soil dry weight, respectively.

Dates of experimental work: February 20, 2015 – March 12, 2015

II. RESULTS AND DISCUSSION:

Experimental conditions:
All values were within the range recommended by the guideline.

[mg/ kg dry weight artificial soil]	pH		% water content			% of WHC_{max}	
	Start	End	Start	End	% deviation	Start	End
Control	5.64	5.54	19.06	18.78	1.5	49.88	48.97
100	5.75	5.58	19.06	18.32	3.8	49.86	47.60

Biological results:
In the control group 3.8 % of the adult *Hypoaspis aculeifer* died which is below the allowed maximum of ≤ 20 % mortality.
Concerning the number of juveniles statistical analysis (Student-t test, one-sided smaller, α = 0.05) revealed no significant difference between control and any treatment group.

Test item	M-01 (AE C653711)			
Test Object	*Hypoaspis aculeifer*			
Exposure	Artificial Soil			
[mg/kg dry weight artificial soil]	% mortality (adults)	Mean number of juveniles per test vessel ± standard dev.	Reproduction (% of control)	Significance (*)
Control	3.8	241.9 ± 29.0	-	-
100	5.0	266.3 ± 31.5	110.1	
$NOEC_{reproduction}$ [mg/kg dry weight artificial soil]	≥ 100			
$LOEC_{reproduction}$ [mg/kg dry weight artificial soil]	> 100			

Calculations were done with un-rounded values.
(*)=Welch-t-test for inhomogeneous variances, one sided smaller; α=0.05; "-": non-significant; "+": significant

The calculation of an ECx curve for reproduction was not possible as only one concentration was tested.

Validity criteria:
All validity criteria were met.

Validity criteria (OECD 226, 2016)	Obtained in this study
Mean adult mortality should not exceed 20 % at the end of the test	3.8 %
Mean number of juveniles per replicate should be at least 50 (with 10 mites introduced)	241.9
Coefficient of variation calculated for the number of juveniles per replicate should not be higher than 30 %	12.0 %

Toxic reference test:
In a separate study (Maria Ivonne Larnaudie Lopez, LAR/HR-O-16/14, January 05, 2015) performed with the reference item dimethoate at test concentrations 1.0, 1.8, 3.2, 5.6 and 10.0 mg dimethoate/kg dry weight artificial soil, the LC_{50} was calculated to be 2.51 mg/kg (95 % confidence limits from 0.85 mg/kg to 3.30 mg/kg) for mortality. The NOEC was calculated to be 3.2 mg/kg and the LOEC was 5.6 mg/kg. Since variances of the data were homogenous Williams-t test α = 0.05, one-sided smaller. Dimethoate EC 400E G showed an EC_{50} of 5.47 mg/kg (95 % confidence limits from 4.09 mg/kg to 7.30 mg/kg) for reproduction. This is in the recommended range of the guideline and demonstrates the sensitivity of the test system.

III. CONCLUSION:

The $NOEC_{reproduction}$ and $LOEC_{reproduction}$ of M-01 (AE C653711) were determined to be ≥ 100 mg/kg artificial soil dry weight and > 100 mg/kg artificial soil dry weight, respectively.

Assessment and conclusion by applicant:

This study is considered reliable for risk assessment. The NOEC ≥ 100 mg/kg is the relevant endpoint for the risk assessment.

Data Point:	KCA 8.4.2.1/07
Report Author:	[redacted]
Report Year:	2016
Report Title:	Pyridyl carboxylic acid (BCS-AB43478): Effects on mortality and reproduction of the predatory mite Hypoaspis aculeifer tested in artificial soil
Report No:	EBACN057
Document No:	M-557987-01-1
Guideline(s) followed in study:	OECD 226 (2008): Predatory mite (Hypoaspis (Geolaelaps) aculeifer) reproduction test in soil; US EPA OCSPP: not applicable
Deviations from current test guideline:	Current Guideline: OECD 226 (2016) No deviations
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The purpose of this study was to assess the effect of M-02 (AE C657188) on mortality and reproduction of the soil mite species *Hypoaspis aculeifer* tested during an exposure of 14 days in artificial soil comparing control and treatment. Ten adult soil mites (females) *Hypoaspis aculeifer* per replicate (8 replicates for the control and the treatment) were exposed to control and treatments. A concentration of 100 mg/kg artificial soil dry weight was tested. After a period of 14 days, the surviving adults and the living juveniles were extracted by applying a temperature gradient using a MacFadyen-apparatus. Extracted mites were collected in a fixing solution and counted.

All validity criteria were met. In the control group 1.3 % of the adult *Hypoaspis aculeifer* died which is below the allowed maximum of ≤ 20 % mortality. Concerning the number of juveniles statistical analysis revealed no significant difference between control and any treatment group.

I. MATERIAL AND METHODS:

M-02 (AE C657188), (batch code: C657188-PU-01; origin batch no.: SES 10250-1-1, certificate AZ 20206, CAS No.: 80194-68-9, LIMS No.: 1518969, purity: 98.5%. Ten adult soil mites (females) *Hypoaspis aculeifer* per replicate (8 replicates for the control and the treatment) were exposed to control and treatments. A concentration of 100 mg/kg artificial soil dry weight was tested. During the test, *Hypoaspis aculeifer* were fed every 2-3 days with cheese mites *Tyrophagus putrescentiae.* During the study a temperature of 19.8 – 20.4 °C and light regime of 450 Lux, 16 h light: 8 h dark was applied. The artificial soil was prepared according to the guideline with the following constituents (percentage distribution on dry weight basis): 74.7 % fine quartz sand, 5 % Sphagnum peat, air dried and finely ground, 20 % Kaolin clay and 0.2% calcium carbonate. After a period of 14 days, the surviving adults and the living juveniles were extracted by applying a temperature gradient using a MacFadyen-apparatus. Extracted mites were collected in a fixing solution and counted. The $NOEC_{reproduction}$ and

$LOEC_{reproduction}$ of fluopicolide were determined to be ≥ 100 mg /kg artificial soil dry weight and > 100 mg/kg artificial soil dry weight, respectively.

Dates of experimental work: March 18, 2016 – April 20, 2016

II. RESULTS AND DISCUSSION:

Experimental conditions:
All values were within the range recommended by the guideline.

[mg/ kg dry weight artificial soil]	pH		% water content			% of WHC_{max}	
	Start	End	Start	End	% deviation	Start	End
Control	5.8	5.4	18.37	17.77	3.3	47.79	48.10
100	5.8	5.4	18.49	18.08	2.2	46.24	47.04

Biological results**:**
In the control group 1.3 % of the adult *Hypoaspis aculeifer* died which is below the allowed maximum of ≤ 20 % mortality.
Concerning the number of juveniles statistical analysis (Fisher's Exact Binominal Test, one-sided greater, α = 0.05) revealed no significant difference between control and any treatment group.

Test item	M 02 (AE C657188)			
Test Object	*Hypoaspis aculeifer*			
Exposure	Artificial Soil			
[mg/kg dry weight artificial soil]	% mortality (adults)	Mean number of juveniles per test vessel ± standard dev.	Reproduction (% of control)	Significance (*)
Control	1.3	324 ± 38		
100	0	336 ± 13	104	-
$NOEC_{reproduction}$ [mg a.s./kg dry weight artificial soil]	≥ 100			
$LOEC_{reproduction}$ [mg a.s./kg dry weight artificial soil]	> 100			

Calculations were done with un-rounded values.
(*)= Fisher's Exact Binominal Test, one-sided greater, α = 0.05, + = significant, - = not significant

The calculation of an ECx curve for reproduction was not possible as only one concentration was tested.

Validity criteria:
All validity criteria were met.

Validity criteria (OECD 226, 2016)	Obtained in this study
Mean adult mortality should not exceed 20 % at the end of the test	1.3 %
Mean number of juveniles per replicate should be at least 50 (with 10 mites introduced)	324.3
Coefficient of variation calculated for the number of juveniles per replicate should not be higher than 30 %	11.8 %

Toxic reference test:
In a separate study (L. Schulz, BioChem project No. R151048001S, February 24, 2015) performed with the reference item dimethoate at a test concentration 100 mg dimethoate/kg dry weight artificial soil, the EC_{50} was calculated to be 6.7 mg/kg soil d.w. The results of the reference test demonstrate the sensitivity of the test item.

III. CONCLUSION:

The $NOEC_{reproduction}$ and $LOEC_{reproduction}$ of M-02 (AE C657188) were determined to be ≥ 100 mg/kg artificial soil dry weight and > 100 mg /kg artificial soil dry weight, respectively.

Assessment and conclusion by applicant:

This study is considered reliable for risk assessment. The NOEC ≥ 100 mg/kg is the relevant endpoint for the risk assessment.

CA 8.5 Effects on nitrogen transformation

Table 8.5 - 1: Effects of fluopicolide and its metabolites on soil nitrogen transformation

Test substance	Test species, test design	Ecotoxicological Endpoint	Reference
N-transformation			
Fluopicolide	Study duration, 28 days	no unacceptable effects at an application rate of 1.330 kg a.s./ha (1.77 mg a.s./kg dws)	[redacted] 2003; M-230023-01-1 KCA 8.5/01
M-01 (AE C653711)	Study duration, 28 days	no unacceptable effects at an application rate of 0.690 kg p.m./ha (0.92 mg p.m./kg dws)	[redacted] 2004; M-235991-01-1 KCA 8.5/03
M-01 (AE C653711)	Study duration, 14 days	no unacceptable effects at an application rate of 2.900 kg p.m./ha (3.8 mg p.m./kg dws)	[redacted] 1996; M-234312-01-1 KCA 8.5/02
M-02 (AE C657188)	Study duration, 28 days	no unacceptable effects at an application rate of 1.421 kg p.m./ha (1.89 mg p.m./kg dws)	[redacted] 2016; M-557910-01-1 KCA 8.5/06
M-03 (AE C0608000, (BCS-AX86048)	Study duration, 28 days	no unacceptable effects at an application rate of 2.083 kg p.m./ha (2.78 mg p.m./kg dws)	[redacted] 2016; M-558852-01-1 KCA 8.5/07

a.s. = active substance, dws = dry weight soil; p.m. = pure metabolite

Data Point:	KCA 8.5/01
Report Author:	[redacted]
Report Year:	2003
Report Title:	AE C638206 technical ingredient: Determination of effects on nitrogen transformation in soil
Report No:	C031645
Document No:	M-230023-01-1
Guideline(s) followed in study:	OECD 216 (2000)
Deviations from current test guideline:	Current Guideline: OECD 216 (2000) The sand content was 78% and not between 50 and 75% as recommended by the guideline. This deviation is not expected to have impacted the study results.
Previous evaluation:	yes, evaluated and accepted in DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The purpose of this study was to determine the effects of fluopicolide technical on the activity of soil microflora with regard to nitrogen transformation in a laboratory test. A silty sand soil was exposed for 28 days to concentrations of 0.18 and 1.84 mg test item/kg dry weight soil and a control. Each treatment consisted of 3 replicates. Application rates were equivalent to 0.138 kg test item/ha (corresponding to 0.133 kg test item/ha) and 1.38 kg test item/ha (corresponding to 1.33 kg test item/ha). The nitrogen transformation was determined in soil enriched with lucerne meal (concentration in soil 0.5 %). NH_4-nitrogen, NO_3- and NO_2-nitrogen were determined by an autoanalyzer at different sampling intervals (0, 7, 14 and 28 days after treatment). A reference item is not required by the guideline. Nevertheless, sodium chloride was used as a reference. The test item fluopicolide technical caused no effect > 25% on nitrogen transformation at the test doses of 0.18 mg test item/kg dry weight soil and 1.84 mg test item/kg dry weight soil. Fluopicolide technical causes no adverse effects (difference to control < 25 %, OECD 216) on the soil nitrogen transformation (expressed as NO_3-N-production rate) at the end of the 28-day incubation period.

I. Material and Methods:

Test item: Fluopicolide technical ingredient, batch No.: GP2050046, development No.: 3000312102, analysed purity: 96.1 % w/w. A silty sand soil was exposed for 28 days to concentrations of 0.18 and 1.84 mg test item/kg dry weight soil and a control. Each treatment consisted of 3 replicates. Application rates were equivalent to 0.138 kg test item/ha (corresponding to 0.133 kg test item/ha) and 1.38 kg test item/ha (corresponding to 1.33 kg test item/ha). The nitrogen transformation was determined in soil enriched with lucerne meal (concentration in soil 0.5 %). NH_4-nitrogen, NO_3- and NO_2-nitrogen were determined by an autoanalyzer at different sampling intervals (0, 7, 14 and 28 days after treatment). A reference item is not required by the guideline. Nevertheless, sodium chloride was used as a reference. The test conditions were: Soil held in the dark at 20 ± 2 °C, about 40% water capacity and pH values of 5.5 - 5.8. The pH-values in the soil used in the test were measured at test start (after application) and at the final sampling on day 28. Homogeneity of variances was determined by F-test (significance level 5%). Depending on the results of the F-test, the appropriate T-tests were performed.

II. RESULTS AND DISCUSSION:

The test item fluopicolide technical caused no effect > 25% on nitrogen transformation at the test doses of 0.18 mg test item/kg dry weight soil and 1.84 mg test item/kg dry weight soil.

Time interval (days)	Control			0.18 mg test item/kg soil dry weight equivalent to 0.138 kg test item/ha				1.84 mg test item/kg soil dry weight equivalent to 1.38 kg test item/ha			
	Nitrate-N [1]			Nitrate-N [1]			% difference to control	Nitrate-N [1]			% difference to control
0-7	- 2.25	±	0.07	-2.23	±	0.01	1	-2.06	±	0.08	9
7-14	1.11	±	0.03	1.05	±	0.02	5	1.17	±	0.16	5
14-28	1.02	±	0.02	1.07	±	0.03	5	1.09	±	0.09	7

Rate: Nitrate-N in mg/kg soil dry weight/day, mean of 3 replicates and standard deviation

In a separate study (non-GLP) with the same agricultural soil as used for this study, 16 g NaCl/kg dry weight soil had a distinct and long-term (> 28 days) influence on microbial mineralization of nitrogen.

Validity criteria:
All validity criteria were met in this study.

Validity criteria (OECD 216, 2000)	Obtained in this study
The coefficient of variation in the control for NO_3-N ≤ 15 %	≤ 11 %

III. CONCLUSION:

Fluopicolide technical caused no adverse effects (difference to control < 25 %, OECD 216) on the soil nitrogen transformation (expressed as NO_3-N-production rate) at the end of the 28-day incubation period. The study was performed in a field soil at concentrations up to 1.84 mg test item/kg soil dry weight (corresponding to 1.77 mg a.s./kg soil dry weight), which are equivalent to application rates up to 1.38 kg test item/ha (corresponding to 1.33 kg a.s./ha).

Assessment and conclusion by applicant:

This study is considered reliable for risk assessment. The relevant endpoint for the risk assessment is 1.77 mg a.s./kg dry weight soil.

Data Point:	KCA 8.5/02
Report Author:	[redacted]
Report Year:	1996
Report Title:	Effect of 2,6-dichlorobenzamide on the activity of soil microflora: Short term respiration and nitrogen minieralization
Report No:	C034063
Document No:	M-234312-01-1
Guideline(s) followed in study:	SETAC: Chapter 4, 1995
Deviations from current test guideline:	Current Guideline: OECD 216 (2000) The exposure period was only 14 days instead of 28 days as recommended by the guideline. The sand content was 88.2 % and not between 50 and 75%. Also, no information on variation in the control was provided.
Previous evaluation:	yes, evaluated and accepted DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Supportive only

Executive Summary

The purpose of this study was to determine the effects of M-01 (2,6-dichlorobenzamide (BAM)) on the activity of soil microflora with regard to carbon and nitrogen transformation in a laboratory test. A sandy soil, with a low organic carbon content (<1%), provided by the "Landwirtschaftlicher Untersuchungsanstalt" (Speyer, Germany) was used. The effects of the test compound M-01 (2,6-dichlorobenzamide) were investigated at two nominal concentrations: 6 and 0.12 mg/kg dry weight soil. The measured test concentrations of 2,6-Dichlorobenzamide were 3.8 mg and 0.19 mg 2,6-Dichlorobenzamide/kg dry soil. Dinoseb acetate was used as reference compound. This standard was tested at a dose rate of 2 mg dinoseb acetate/100 g dry soil. The test substance and reference compound were weighed and dis-solved in acetone. These acetonic solutions were mixed with sand, whereafter the acetone was evaporated slowly under reduced pressure. The addition of M-01 (2,6-dichlorobenzamide), coated on the sand was verified by extraction with methanol and analysis by HPLC. As nitrogen source, 0.5 g Lucerne meal was added. The reference compound dinoseb acetate showed an increased level of soil respiration (51% deviation versus control after 2 weeks). Dinoseb acetate also showed a significant effect (> 25%) on the nitrogen mineralisation (after two weeks a 962% increase of NH_4^+ and a 45% reduction of NO_3- compared to the control). The test compound M-01 (2,6-dichlorobenzamide) showed no effect at both test concentrations on the soil respiration (< 25% effect). The study was terminated after two weeks as no noticeable effect was found for the test compound M-01 (2,6-dichlorobenzamide) on the soil respiration and nitrification. During the 14-day experiments, M-01 (2,6-dichlorobenzamide) tested at the measured concentrations of 3.8 mg/0.19 mg M-01 (2,6-dichlorobenzamide)/kg dry soil had no influence (effects <25%) on soil respiration, soil ammonia and soil nitrate contents.

I. MATERIAL AND METHODS:

Test item: M-01 (2,6-dichlorobenzamide), batch No.: FUX00100/FUN81G02C, purity: 99%.

A sandy soil, with a low organic carbon content (<1%), provided by the "Landwirtschaftlicher Untersuchungsanstalt" (Speyer, Germany) was used. The effects of the test compound M-01 (2,6-dichlorobenzamide) were investigated at two nominal concentrations: 6 and 0.12 mg/kg dry weight soil. Dinoseb acetate was used as reference compound. This standard was tested at a dose rate of 2 mg dinoseb acetate/100 g dry soil. The test substance and reference compound were weighed and dis-solved in acetone. These acetonic solutions were mixed with sand, whereafter the acetone was evaporated slowly under reduced pressure.
The addition of M-01 (2,6-dichlorobenzamide) coated on the sand was verified by extraction with methanol and analysis by HPLC. The recoveries were 61% and 155% of the high and low nominal addition level, which resulted in the measured concentrations of 0.38 mg and 19 µg M-01 (2,6-dichlorobenzamide)/0.5 g sand respectively. The low recovery at the high addition level (61%) may have been caused by partial volatilization of the test compound during the coating process. The high recovery at the low addition level (155%) is most likely the result of cross-contamination, as erlenmeyer flasks receiving the high dose rate were treated first.
Half a gram sand coated with either M-01 (2,6-dichlorobenzamide) (high and low level), dinoseb acetate or acetone only (control) was mixed with 100 g pre-incubation soil. The final test concentrations of M-01 (2,6-dichlorobenzamide) were, therefore, 6.0 mg (measured 3.8 mg) and 0.12 mg (measured 0.19 mg) M-01 (2,6-dichlorobenzamide)/kg dry soil.

As nitrogen source, 0.5 g Lucerne meal was added. The soil samples were incubated in a thermostatic room at 20 + 1°C in the dark.
Within 6 hours and after 2 weeks, respiration and nitrogen mineralisation were determined in three soil samples per test condition.

Dates of work: October 15, 1996 – October 30, 1996

II. RESULTS AND DISCUSSION:

The reference compound dinoseb acetate showed an increased level of soil respiration (51% deviation versus control after 2 weeks). Dinoseb acetate also showed a significant effect (> 25%) on the nitrogen mineralisation (after two weeks a 962% increase of NH_4^+ and a 45% reduction of NO_3^- compared to the control).
The test compound M-01 (2,6-dichlorobenzamide) showed no effect at both test concentrations on the soil respiration (<< 25% effect). The study was terminated after two weeks as no noticeable effect was found for the test compound M-01 (2,6-dichlorobenzamide) on the soil respiration and nitrification.

Test substance (concentrations)	Effects on soil respiration as a function of time	
	Percentage deviation compared to the control (%)	
	After 6 hours incubation	After 14 days incubation
Control	--	--
M-01 (2,6-dichlorobenzamide) (0.19 mg/kg)	0.2	**1.8**
M-01 (2,6-dichlorobenzamide) (3.8 mg/kg)	-3.3	**3.4**
Reference (20 mg/kg)	-7.1	51.2

Test substance (concentrations)	Effects on nitrogen transformation as a function of time					
	Percentage deviation compared to the control (%)					
	After **6 hours** incubation			After **14 days** incubation		
	NH_4^+	NO_3^-	NO_2^-	NH_4^+	NO_3^-	NO_2^-
Control	--	--	--	--	--	--
M-01 (2,6-dichlorobenzamide) (0.19 mg/kg)	-2.7	-7.8	8.4	**-18**	**4.7**	**-25**
M-01 (2,6-dichlorobenzamide) (3.8 mg/kg)	4.7	-5.8	5.6	**-4.6**	**-5.4**	**-26**
Reference (20 mg/kg)	30	1.2	40	962	-45.4	4296

From the results on nitrogen transformation, it is clear that M-01 (2,6-dichlorobenzamide) tested at the two nominal concentrations of 3.8 and 0.19 mg/kg dry soil, has no effect on the soil contents of ammonia and nitrate (<25%) after 6 hours and 14 days of incubation.
With regard to soil nitrite contents, the absolute levels of nitrite in the controls were very low compared to the nitrate content (<0.2%). Due to these low levels, the variation in the percentage deviation from the controls is relatively high. After 2 weeks incubation, the effect was -25 and -26% for both M-01 (2,6-dichlorobenzamide) doses. Compared with the effects of the reference compound and taking into account the high variation, this effect is considered to be not significant.

III. Conclusion:

During the 14-day experiments, M-01 (2,6-dichlorobenzamide) tested at the measured dose concentrations of 3.8 mg and 0.19 mg M-01 (2,6-dichlorobenzamide)/kg dry soil had no influence (effects <25%) on soil respiration, soil ammonia and soil nitrate contents.

Assessment and conclusion by applicant:

The study design deviates significantly from the standard OECD 2016 test protocol, i.e. study duration. Therefore, the study is not further considered in the risk assessment.

Data Point:	KCA 8.5/03
Report Author:	[redacted]
Report Year:	2004
Report Title:	Metabolite AE C653711 (AE C653711 00 1B96 0001): Determination of effects on nitrogen trandformation in soil
Report No:	C044264
Document No:	M-235991-01-1
Guideline(s) followed in study:	OECD 216 (2000)
Deviations from current test guideline:	Current Guideline: OECD 216 (2000) The sand content was 78.1 % and not between 50 and 75% as recommended by the guideline. This deviation is not expected to have impacted the study results.
Previous evaluation:	yes, evaluated and accepted in DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities.
Acceptability/Reliability:	Yes

Executive Summary

The purpose of this study was to determine the effects of M-01 (AE C653711) on the activity of soil microflora with regard to nitrogen transformation in a laboratory test. A loamy sand soil was exposed for 28 days to concentrations of 0.09 and 0.92 mg test item/kg dry weight soil and a control.
Each treatment consisted of 3 replicates. Application rates were equivalent to 0.069 kg test item/ha and 0.69 kg test item/ha. The nitrogen transformation was determined in soil enriched with lucerne meal (concentration in soil 0.5 %). NH_4-nitrogen, NO_3- and NO_2-nitrogen were determined by an autoanalyzer at different sampling intervals (0, 7, 14 and 28 days after treatment). A reference item is not required by the guideline. Nevertheless, sodium chloride was used as a reference. The test item M-01 (AE C653711) caused no effect > 25% on nitrogen transformation at the test doses of 0.09 mg test item/kg dry weight soil and 0.92 mg test item/kg dry weight soil. M-01 (AE C653711) causes no adverse effects (difference to control < 25 %, OECD 216) on the soil nitrogen transformation (expressed as NO_3-N-production rate) at the end of the 28-day incubation period.

I. Material and Methods:

Test item: M-01 (AE C653711), batch No.: 08018ET, analysed purity: 96.2% w/w. A loamy sand soil was exposed for 28 days to concentrations of 0.09 and 0.92 mg test item/kg dry weight soil and a control. Each treatment consisted of 3 replicates. Application rates were equivalent to 0.069 kg test item/ha and 0.69 kg test item/ha. The nitrogen transformation was determined in soil enriched with lucerne meal (concentration in soil 0.5 %). NH_4-nitrogen, NO_3- and NO_2-nitrogen were determined by an autoanalyzer at different sampling intervals (0, 7, 14 and 28 days after treatment). A reference item is not required by the guideline. Nevertheless, sodium chloride was used as a reference. The test conditions were: Soil held in the dark at 20 ± 2 °C, about 40% water capacity and pH values of 5.5 - 5.9. The pH-values in the soil used in the test were measured at test start (after application) and at the final sampling on day 28. Homogeneity of variances was determined by F-test (significance level 5%). Depending on the results of the F-test, the appropriate T-tests were performed.

II. RESULTS AND DISCUSSION:

The test item M-01 (AE C653711) caused no effect > 25% on nitrogen transformation at the test doses of 0.09 mg test item/kg dry weight soil and 0.92 mg test item/kg dry weight soil.

Time interval (days)	Control			0.09 mg test item/kg soil dry weight equivalent to 0.069 kg test item/ha				0.92mg test item/kg soil dry weight equivalent to 0.69 kg test item/ha			
	Nitrate-N [1]			Nitrate-N [1]			% difference to control	Nitrate-N [1]			% difference to control
0-7	-1.59	±	0.06	-1.64	±	0.05	3	-1.47	±	0.07	8
7-14	0.79	±	0.12	0.72	±	0.03	9	0.68	±	0.06	13
14-28	1.01	±	0.02	0.99	±	0.05	2	0.97	±	0.15	4

Rate: Nitrate-N in mg/kg soil dry weight/day, mean of 3 replicates and standard deviation.

In a separate study with the same agricultural soil as used for this study, 16 g NaCl/kg dry weight soil had a distinct and long-term (> 28 days) influence on microbial mineralization of nitrogen.

Validity criteria:
All validity criteria were met in this study.

Validity criteria (OECD 216, 2000)	Obtained in this study
The coefficient of variation in the control for NO_3-N ≤ 15 %	≤ 8 %

III. CONCLUSION:

M-01 (AE C653711) caused no adverse effects (difference to control < 25 %, OECD 216) on the soil nitrogen transformation (expressed as NO_3-N-production rate) at the end of the 28-day incubation period. The study was performed in a field soil at concentrations up to 0.92 mg test item/kg soil dry weight, which are equivalent to application rates up to 0.69 kg test item/ha.

Assessment and conclusion by applicant:

This study is considered reliable for risk assessment. The relevant endpoint for the risk assessment is 0.92 mg a.s./kg dry weight soil.

Data Point:	KCA 8.5/04
Report Author:	[redacted]
Report Year:	2003
Report Title:	AE C638206 technical ingredient: Determination of effects on carbon transformation in soil
Report No:	C031644
Document No:	M-230021-01-1
Guideline(s) followed in study:	OECD 217 (2000)
Deviations from current test guideline:	Current Guideline: OECD 217 (2000) Not evaluated
Previous evaluation:	yes, evaluated and accepted in DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The purpose of this study was to determine the effects of fluopicolide on the activity of soil microflora with regard to carbon transformation in a laboratory test. A silty soil was exposed for 28 d to concentrations of 0.18 and 1.84 mg fluopicolide/kg dry weight soil (application rates were equivalent to 0.138 and 1.38 kg AE C638206 tech./ha. Glucose was added to the soil samples to induce maximum respiration rate (3 g/kg dry weight soil). No adverse effects of fluopicolide technical on carbon transformation in soil were observed at both test concentrations (0.18 mg test item/kg dry weight soil and 1.84 mg test item/kg soil dry weight soil) after 28 days. Differences from the control of 8.35% (test concentration 0.18 mg test item/kg dry weight soil) and 5.75% (test concentration 1.84 mg test item/kg dry weight soil) are measured at the end of the 28-day incubation period. Fluopicolide technical causes no adverse effects (deviation from control < 25 %, OECD 217) on the soil carbon transformation at the end of the 28-day incubation period. The study is performed in a field soil at concentrations up to 1.84 mg test item/kg soil dry weight, which are equivalent to application rates up to 1.38 kg test item/ha.

I. Material and Methods:

Test item: Fluopicolide technical (AE C638206 00 1C96 0001, development No.: 3000312102, batch No.: OP2050046), analytical findings: 96.1% w/w.
A silty soil was exposed for 28 d to concentrations of 0.18 and 1.84 mg fluopicolide/kg dry weight soil (application rates were equivalent to 0.138 and 1.38 kg AE C638206 tech./ha. Glucose was added to the soil samples to induce maximum respiration rate (3 g/kg dry weight soil).

Dates of experimental work: March 18, 2003 – May 16, 2003

II. Results and Discussion:

No adverse effects of fluopicolide technical on carbon transformation in soil were observed at both test concentrations (0.18 mg test item/kg dry weight soil and 1.84 mg test item/kg soil dry weight soil) after 28 days. Differences from the control of 8.35% (test concentration 0.18 mg test item/kg dry weight soil) and 5.75% (test concentration 1.84 mg test item/kg dry weight soil) were measured at the end of the 28 day incubation period.

Sampling date	Control	0.18 mg test item/kg dws equiv. to 0.138 kg test item/ha		1.84 mg test item/kg soil dws equiv. to 1.38 kg test item/ha	
	[mg CO_2 / hour / kg dws]	[mg CO_2 / hour / kg dws]	% difference to control#	[mg CO_2 / hour / kg dws]	% difference to control#
0	192.71	180.16	6.51	190.28	1.26
7	197.91	181.72	8.18	187.69	5.16
14	187.49	173.38	7.53	187.42	4.84
28	147.02	134.74*	8.35	138.57	5.75

* Significant difference between treated and untreated soil samples (t-test with 5% probability of error)
\# Exact Values not given in study report; calculated on the basis of the mg CO_2 / hour / kg dws values given in this table.
dws = dry weight soil

In a separate study the reference item sodium chloride was used as a reference standard. In tests (non-GLP) with the agricultural soil described above, 16 g NaCl/kg dry weight soil had distinct and long-term (> 28 days) influences on microbial mineralization of carbon.

Validity criteria:

The validity criteria of the test according to OECD guideline 217 were fulfilled.

Validity criteria (OECD 217, 2000)	Recommended	Obtained
Coefficients of variation in the control	≤ 15%	2.2 – 3.4 %

III. Conclusion:

Fluopicolide technical caused no adverse effects (deviation from control < 25 %, OECD 217) on the soil carbon transformation at the end of the 28-day incubation period. The study was performed in a field soil at concentrations up to 1.84 mg test item/kg soil dry weight, which are equivalent to application rates up to 1.38 kg test item/ha.

Assessment and conclusion by applicant:

This study is considered reliable. The endpoint is 1.84 mg/kg dry weight soil.

However, studies on microbial carbon transformation are no longer a data requirement. Therefore, this study will not be further considered in the risk assessment.

Data Point:	KCA 8.5/05
Report Author:	[redacted]
Report Year:	2004
Report Title:	Metabolite AE C653711 (AE C653711 00 1B96 0001): Determination of effects on carbon transformation in soil
Report No:	C044266
Document No:	M-235993-01-1
Guideline(s) followed in study:	OECD 217 (2000)
Deviations from current test guideline:	Current Guideline: OECD 217 (2000) Not evaluated
Previous evaluation:	yes, evaluated and accepted in DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The purpose of this study was to determine the effects of M-01 (AE C653711) on the activity of soil microflora with regard to carbon transformation in a laboratory test. A loamy sand soil was exposed for 28 d to concentrations of 0.09 and 0.92 mg M-01 (AE C653711)/kg dry weight soil (application rates were equivalent to 0.069 and 0.69 kg M-01 (AE C653711)/ha. Glucose was added to the soil samples (2 g/kg dry weight soil) to induce maximum respiration rate. No adverse effects of fluopicolide technical on carbon transformation in soil were observed at both test concentrations (0.09 mg test item/kg soil d.w. and 0.92 mg test item/kg dry weight soil) after 28 days. Differences from the control of 8.09% (test concentration 0.09 mg test item/kg dry weight soil) and 6.90% (test concentration 0.92 mg test item/kg soil dry weight) were measured at the end of the 28-day incubation period. M-01 (AE C653711) causes no adverse effects (deviation from control < 25 %, OECD 217) on the soil carbon transformation at the end of the 28-day incubation period. The study is performed in a field soil at concentrations up to 0.92 mg test item/kg soil dry weight, which are equivalent to application rates up to 0.69 kg test item/ha.

I. Material and Methods:

Test item: M-01 (AE C653711, batch No.: 08018ET), analytical findings: 96.2% w/w. A loamy sand soil was exposed for 28 d to concentrations of 0.09 and 0.92 mg M-01 (AE C653711)/kg dry weight soil (application rates were equivalent to 0.069 and 0.69 kg M-01 (AE C653711)/ha. Glucose was added to the soil samples (2 g/kg dry weight soil) to induce maximum respiration rate.

Dates of work: July 28, 2004 – August 25, 2004

II. Results and Discussion:

No adverse effects of M-01 (AE C653711) technical on carbon transformation in soil were observed at both test concentrations (0.09 mg test item/kg soil d.w. and 0.92 mg test item/kg dry weight soil) after 28 days. Differences from the control of 8.09% (test concentration 0.09 mg test item/kg dry weight soil) and 6.90% (test concentration 0.92 mg test item/kg soil dry weight) were measured at the end of the 28 day incubation period.

Sampling date	Control	0.09 mg test item/kg soil d.w. equiv. to 0.069 kg test item/ha		0.92 mg test item/kg soil d.w. equiv. to 0.69 kg test item/ha	
	[mg CO_2 / hour / kg dws]	[mg CO_2 / hour / kg dws]	% difference to control#	[mg CO_2 / hour / kg dws]	% difference to control#
0	351.2	324.9*	7.49	328.7	6.41
7	398.8	361.9	7.16	351.7	9.77
14	343.0	307.4	10.38	320.9	6.44
28	286.9	263.7	8.09	267.1	6.90

* Significant difference between treated and untreated soil samples (t-test with 5% probability of error)
#Exact Values not given in study report; calculated on the basis of the mg CO_2 / hour / kg dws values given in this table

Validity criteria:

The validity criteria of the test according to OECD guideline 217 were fulfilled.

Validity criteria (OECD 217, 2000)	Recommended	Obtained
Coefficients of variation in the control	≤ 15 %	2.4 – 3.2 %

III. Conclusion:

M-01 (AE C653711) caused no adverse effects (deviation from control < 25 %, OECD 217) on the soil carbon transformation at the end of the 28-day incubation period. The study was performed in a field soil at concentrations up to 0.92 mg test item/kg soil dry weight, which are equivalent to application rates up to 0.69 kg test item/ha.

Assessment and conclusion by applicant:

This study is considered reliable. The endpoint is 0.92 mg/kg dry weight soil. However, studies on microbial carbon transformation are no longer a data requirement. Therefore, this study will not be further considered in the risk assessment.

Data Point:	KCA 8.5/06
Report Author:	[redacted]
Report Year:	2016
Report Title:	Pyridyl carboxylic acid (BCS-AB43478): Effects on the activity of soil microflora (Nitrogen transformation test)
Report No:	EBACN059
Document No:	M-557910-01-1
Guideline(s) followed in study:	OECD 216; adopted January 21, 2000, OECD Guideline for the Testing of Chemicals, Soil Microorganisms: Nitrogen Transformation.
Deviations from current test guideline:	Current Guideline: OECD 216 (2000) No deviations
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The purpose of this study was to determine the effects of M-02 (AE C657188) on the activity of soil microflora with regard to nitrogen transformation in a laboratory test. A loamy sand soil (DIN 4220) was exposed for 28 days to 0.33 and 1.89 mg/kg soil dry weight and a control. Each treatment consisted of 3 replicates. Application rates were equivalent to 0.248 kg/ha and 1.421 kg/ha. The nitrogen transformation was determined in soil enriched with lucerne meal (concentration in soil 0.5 %). NH_4-nitrogen, NO_3- and NO_2-nitrogen were determined by an autoanalyzer at different sampling intervals (0, 7, 14 and 28 days after treatment). A reference item is not required by the guideline. Nevertheless, Dinoterb was used as a reference. The test item M-02 (AE C657188) causes a temporary stimulation of the daily nitrate rate at the tested concentrations of 0.33 and 1.89 mg /kg soil dry weight at time interval 7-14 days after application. However, no adverse effect of AE C657188 on nitrogen transformation in soil could be observed at both tested concentrations at the end of the test, 28 days after application (time interval 14-28). M-02 (AE C657188) causes no adverse effects (difference to control < 25 %, OECD 216) on the soil nitrogen transformation (expressed as NO_3-N-production rate) at the end of the 28-day incubation period. The study is performed in a field soil at concentrations up to 1.89 mg /kg soil dry weight, which are equivalent to application rates up to 1.421 kg/ha.

I. Material and Methods:

Test item: M-02 (AE C657188, batch No.: AE C657188-PU-01, origin batch code: SES 10250-1-1, LIMS No.: 1508969, CAS No.: 80194-68-9, Certificate No.: AZ 20206, analysed purity: 98.5%).
A loamy sand soil (DIN 4220) was exposed for 28 days to 0.33 and 1.89 mg /kg soil dry weight. and a control. Each treatment consisted of 3 replicates. Application rates were equivalent to 0.248 kg /ha and 1.421 kg /ha. The nitrogen transformation was determined in soil enriched with lucerne meal (concentration in soil 0.5 %). NH_4-nitrogen, NO_3- and NO_2-nitrogen were determined by an autoanalyzer at different sampling intervals (0, 7, 14 and 28 days after treatment). A reference item is not required by the guideline. Nevertheless, dinoterb was used as a reference. The test conditions were: Soil held in the dark at 19.6 - 21.6 °C, with 44.92 to 48.30% water capacity and pH values of 6.0 - 6.1. The pH-values in the soil used in the test were measured at test start (after application) and at the final sampling on day 28. A statistical evaluation of the test results was performed by means of a 2-sided Student-t-test (for homogeneous variances at 5% significance level).

II. RESULTS AND DISCUSSION:

The test item M-02 (AE C657188) caused a temporary stimulation of the daily nitrate rate at the tested concentrations of 0.33 and 1.89 mg /kg soil dry weight at time interval 7-14 days after application. However, no adverse effect of AE C657188 on nitrogen transformation in soil could be observed at both tested concentrations at the end of the test, 28 days after application (time interval 14-28).

Time interval (days)	Control			0.33 mg /kg soil dry weight equivalent to 0.248 kg /ha				1.89 mg /kg soil dry weight equivalent to 1.421 kg /ha			
	Nitrate-N [1]			Nitrate-N [1]			% difference to control	Nitrate-N [1]			% difference to control
0-7	3.31	±	0.04	3.16	±	0.23	- 4.6	3.14	±	0.47	-5.3
7-14	1.01	±	0.23	1.44	±	0.29	+ 42.9	1.56	±	0.42	54.7
14-28	1.06	±	0.13	0.82	±	0.06	-22.1[s]	0.91	±	0.05	-13.5

Rate: Nitrate-N in mg/kg soil dry weight/day, mean of 3 replicates and standard deviation
The calculations were performed with unrounded values
s. = statistically significantly different to control (Student-t-test for homogenous variances, 2-sided, $p \leq 0.05$)

In a separate study with the same agricultural soil as used for this study the reference item Dinoterb caused an effect of – 37.0% and + 37.6% on the nitrogen transformation in a field soil at the tested concentrations of 6.8 and 27.0 mg Dinoterb per kg soil dry weight respectively, 28 days after application (time interval 14-28) and thus demonstrates the sensitivity of the test system.

Validity criteria:
All validity criteria were met in this study.

Validity criteria (OECD 216, 2000)	Obtained in this study
The coefficient of variation in the control for NO_3-N ≤ 15 %	≤ 2.3 %

III. CONCLUSION:

M-02 (AE C657188) caused no adverse effects (difference to control < 25 %, OECD 216) on the soil nitrogen transformation (expressed as NO_3-N-production rate) at the end of the 28-day incubation period. The study was performed in a field soil at concentrations up to 1.89 mg/kg soil dry weight, which are equivalent to application rates up to 1.421 kg/ha.

Assessment and conclusion by applicant:

This study is considered reliable for risk assessment. The relevant endpoint for the risk assessment is 1.89 mg a.s./kg dry weight soil.

Data Point:	KCA 8.5/07
Report Author:	[redacted]
Report Year:	2016
Report Title:	AE 0608000 (BCS-AX86048): Effects on the activity of soil microflora (nitrogen transformation test)
Report No:	16 10 48 022 N
Document No:	M-555852-01-1
Guideline(s) followed in study:	OECD 216; adopted January 21, 2000, OECD Guideline for the Testing of Chemicals, Soil Microorganisms: Nitrogen Transformation; US EPA OCSPP not applicable
Deviations from current test guideline:	Current Guideline: OECD 216 (2000) No deviations
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary:
The purpose of this study was to determine the effects of M-03 (AE 0608000) on the activity of soil microflora with regard to nitrogen transformation in a laboratory test. A loamy sand soil was exposed for 28 days to concentrations of 0.56 and 2.78 mg/kg dry weight soil and a control. Each treatment consisted of 3 replicates. Application rates were equivalent to 0.417 kg/ha and 2.083 kg/ha. The nitrogen transformation was determined in soil enriched with lucerne meal (concentration in soil 0.5 %). NH_4-nitrogen, NO_3- and NO_2-nitrogen were determined by an autoanalyzer at different sampling intervals (0, 7, 14 and 28 days after treatment). A reference item is not required by the guideline. Nevertheless, Dinoterb was used as a reference. The test item AE 0608000 caused no effect > 25% on nitrogen transformation at the test doses of 0.56 mg/kg dry weight soil and 2.78 mg/kg dry weight soil. M-03 (AE C0608000 (BCS-AX86048)) causes no adverse effects (difference to control < 25 %, OECD 216) on the soil nitrogen transformation (expressed as NO_3-N-production rate) at the end of the 28-day incubation period. The study is performed in a field soil at concentrations up to 2.78 mg/kg soil dry weight, which are equivalent to application rates up to 2.083 kg/ha.

I. Material and Methods:

Test item: M-03 (AE 0608000), batch No.: SES-12767-19-2, analysed purity: 99.4% w/w. A loamy sand soil was exposed for 28 days to concentrations of 0.56 and 2.78 mg/kg dry weight soil and a control. Each treatment consisted of 3 replicates. Application rates were equivalent to 0.417 kg/ha and 2.083 kg/ha. The nitrogen transformation was determined in soil enriched with lucerne meal (concentration in soil 0.5 %). NH_4-nitrogen, NO_3- and NO_2-nitrogen were determined by an autoanalyzer at different sampling intervals (0, 7, 14 and 28 days after treatment). A reference item is not required by the guideline. Nevertheless, dinoterb was used as a reference. The test conditions were: Soil held in the dark at 18.8 – 20.7 °C, with 46.12 to 49.54% water capacity and pH values of 5.7 - 5.8. The pH-values in the soil used in the test were measured at test start (after application) and at the final sampling on day 28. Homogeneity of variances was determined by means of a 2-sided Student-t-test (significance level 5%). A statistical evaluation of the test results was performed by means of a 2-sided Student-t-test.

II. RESULTS AND DISCUSSION:

The test item M-03 (AE 0608000) caused no effect > 25% on nitrogen transformation at the test doses of 0.56 mg/kg dry weight soil and 2.78 mg/kg dry weight soil.

Time interval (days)	Control			0.56 mg/kg soil dry weight equivalent to 0.417 kg/ha				2.78 mg/kg soil dry weight equivalent to 2.083 kg/ha			
	Nitrate-N [1]			Nitrate-N [1]			% difference to control	Nitrate-N [1]			% difference to control
0-7	4.30	±	0.18	4.35	±	0.14	1.2	4.37	±	0.32	1.8
7-14	2.23	±	0.15	2.17	±	0.08	-3.0	1.99	±	0.28	-11.1
14-28	1.42	±	0.26	1.39	±	0.20	-2.0	1.36	±	0.24	-4.0

Rate: Nitrate-N in mg/kg soil dry weight/day, mean of 3 replicates and standard deviation
The calculations were performed with unrounded values

In a separate study with the same agricultural soil as used for this study the reference item dinoterb caused a stimulation of nitrogen transformation of +37.0% and +37.6% at 6.8 and 27 mg Dinoterb per kg soil dry weight, respectively determined 28 days after application (time interval 14-28).

Validity criteria:
All validity criteria were met in this study.

Validity criteria (OECD 216, 2000)	Obtained in this study
The coefficient of variation in the control for NO_3-N ≤ 15 %	≤ 3.4 %

III. CONCLUSION:

M-03 (AE C0608000 (BCS-AX86048)) caused no adverse effects (difference to control < 25 %, OECD 216) on the soil nitrogen transformation (expressed as NO_3-N production rate) at the end of the 28-day incubation period. The study was performed in a field soil at concentrations up to 2.78 mg kg soil dry weight, which are equivalent to application rates up to 2.083 kg/ha.

Assessment and conclusion by applicant:

This study is considered reliable for risk assessment. The relevant endpoint for the risk assessment is 2.78 mg/kg dry weight soil.

CA 8.6 Effects on terrestrial non-target higher plants

CA 8.6.1 Summary of screening data

Not necessary as guideline GLP studies conducted with the representative formulation for fluopicolide for terrestrial non-target plants are available (see Point KCP 10.6.2). A study with technical fluopicolide formulated as a WP has been conducted and is presented in section 8.7/02.

CA 8.6.2 Testing on non-target plants

Studies on terrestrial non-target plants (seedling emergence and vegetative vigour) conducted with the representative formulation for fluopicolide are presented under Point KCP 10.6.2. Studies on terrestrial non-target plants conducted with the solo formulation Fluopicolide SC 40 and with the fluopicolide metabolite M-01 (2,6-dichlorobenzamide) are presented below.

Data Point:	KCA 8.6.2/01
Report Author:	[redacted]
Report Year:	2004
Report Title:	Tier 1 seedling emergence and vegetative vigor nontarget phytotoxicity study using AE C638206 SC40
Report No:	C039705
Document No:	M-227139-01-1
Guideline(s) followed in study:	USEPA (=EPA): FIFRA, J, 122-1 (1986)
Deviations from current test guideline:	Current Guidelines: OECD 208 (2006) and OECD 227 (2006) The temperature ranged from 14 to 39°C. This is outside the proposed range of 22°C ± 10 °C. The humidity ranged from 12 – 103%. This is outside the range of 70% ± 25%. The light intensity was not reported. Planting density was higher than recommended by the current guideline (more plants per pot, smaller pots). These deviations are not expected to have impacted the study results.
Previous evaluation:	yes, evaluated and accepted in DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The purpose of this specific study is to evaluate the effect of fluopicolide SC 40 at an application rate of 133 g a.s./ha on the seedling emergence and vegetative vigour of ten plant species representing 8 different plant families. In the seedling emergence and vegetative vigour test for all test plants, 4 replicates with 10 plants per replicate were used giving a total of 40 plants per treatment and control.
Assessments were made for emergence, plant survival, growth, shoot dry weight and visual phytotoxicity. The analysis of fluopicolide in the initial test item stock solution revealed a measured concentration of approximately 100% of nominal.
There were no significant adverse effects in the seedling emergence and vegetative vigour test for any of the ten crops tested after the treatment with Fluopicolide SC 40 at an application rate of 133 g a.s./ha.

I. MATERIAL AND METHODS:

Test item: Fluopicolide SC40, CAS number: 239110-15-7, Batch No.: AE C638206 SC40: OP220233, analysed content of active ingredient: 485 g/L.

Test species: Six dicotyledonous and four monocotyledonous species representing 8 different plant families (EPPO code): *Fagopyrum esculentum* (FAGES), *Helianthus annus* (HELAN), *Cucumis sativus (*CUMSA*), Brassica rapa (*BRSRR*), Glycine max (GLXMA), Lycopersicon esculentum* (LYPES), *Zea mays* (ZEAMA), *Allium cepa* (ALLCE), *Lolium perenne* (LOLPE), *Triticum aestivum* (TRZAW).

Seedling emergence

For the six dicotyledonous plants, two seeds per pot with five pots per replicate were planted. For the four monocotyledonous plants, five seeds per pot with two pots per replicate were planted. Per treatment level for all test plants 4 replicates were used, giving a total of 40 plants. Growing pots were filled with a sandy loam. For the seeding of the monocotyledonous and dicotyledonous plants pots with 9 and 10.5 cm in diameter were used, respectively. Prior to spray application, each pot was randomly assigned to treatment and replicate. After spray application of 133 g a.s./ha to the soil surface, pots were placed on a capillary irrigation mat in the green house.

Following application, the pots were maintained under greenhouse conditions. The photoperiod throughout the study was approximately 16 hours light and 8 hours dark.

The measured minimum and maximum greenhouse temperatures were 17 and 39°C, the minimum and maximum relative humidity was 17 and 103%.

The number of emerged seedlings, the number of surviving plants and the phytotoxicity ratings were recorded on day 8, 14 and 21. At day 21 after treatment, all seedlings were cut at soil level for height and weight measurement.

Vegetative vigour

In order to reach the 2-4 leaf stage at the start of testing the selected non-target terrestrial plant species were sown in a sandy loam and were grown in plastic pots. Planting conditions were the same as in the seedling emergence study described above. After the application of 133 g a.s./ha with a laboratory spraying chamber to canopy height of the test plants, the pots were impartially arranged, by replicate in the greenhouse.

In the greenhouse the plants were kept under 16 hours light and 8 hours dark. The measured minimum and maximum greenhouse temperatures were 14 and 42°C, the minimum and maximum relative humidity was 12 and 101%. After the 21-day survival and phytotoxicity observations, all seedlings were cut at soil level for height and weight measurements.

Statistical evaluation

Means were calculated for all parameters in the seedling emergence and vegetative vigour test at study termination. A significant adverse effect was defined as greater than or equal to 25% inhibition or 25% damage for any endpoint as compared to the controls. Computerized spreadsheets (Excel, 1997) were used to calculate the percent effect for seedling emergence, seedling survival, visual phytotoxicity, plant height and dry weight.

II. RESULTS AND DISCUSSION:

Validity criteria:
The germination rate of the seeds used in this study was ≥ 70%. Emergence was only reported for the seedling emergence part of the study but is assumed to be similar for the plants used for the vegetative vigour part of the study.
The validity criterion of at least 90% survival of the plants during the study period was achieved for the untreated controls for all species tested.
The control seedlings of each species did not exhibit visible phytotoxic effects (e.g. chlorosis, necrosis, wilting, leaf and stem deformations).

Analytical findings:
The analysis of fluopicolide content in the spray solutions revealed measured concentrations of approximately 100% of nominal.

Biological findings:
The ten plant species treated pre- and post-emergence at a dose rate of 133 g a.s./ha showed no herbicidal effects ≥ 25%. Detailed results are shown in the following tables.

Seedling emergence:

Effects of the test item on emergence and survival

	Mean % Emergence on day 21			Mean % Survival on day 21		
Species	**Control**	**133 g a.s./ha**	**Inhibition ≥ 25%**	**Control**	**133 g a.s./ha**	**Inhibition ≥ 25%**
Fagopyrum esculentum	93	95	no	100	100	no
Zea mays	95	95	no	100	97	no
Cucumis sativus	100	100	no	100	100	no
Glycine max	100	100	no	100	100	no
Helianthus annus	93	95	no	100	100	no
Lycopersicon esculentum	83	80	no	100	100	no
Allium cepa	78	90	no	100	100	no
Lolium perenne	93	95	no	100	100	no
Brassica rapa	100	100	no	100	100	no
Triticum aestivum	97	97	no	100	100	no

Effects of the test item on shoot height and dry weight

	Mean shoot height (cm) on day 21			Mean dry weight (g) on day 21		
Species	**Control**	**133 g a.s./ha**	**Inhibition ≥ 25%**	**Control**	**133 g a.s./ha**	**Inhibition ≥ 25%**
Fagopyrum esculentum	53.4	49.9	no	17.788	16.169	no
Zea mays	81.3	78.0	no	22.655	22.563	no
Cucumis sativus	23.9	23.6	no	16.753	16.681	no
Glycine max	25.9	26.0	no	9.047	9.189	no
Helianthus annus	32.5	36.1	no	14.726	18.570	no
Lycopersicon esculentum	19.9	19.0	no	3.763	3.300	no
Allium cepa	14.5	15.7	no	0.117	0.162	no
Lolium perenne	31.1	28.9	no	1.411	1.399	no
Brassica rapa	15.5	15.4	no	5.363	5.628	no
Triticum aestivum	31.2	31.0	no	3.024	3.297	no

Phytotoxicity rating of the test item

	Phytotoxicity rating (%) on day 21		
Species	**Control**	**133 g a.s./ha**	**Damage ≥ 25%**
Fagopyrum esculentum	0	3	no
Zea mays	0	3	no
Cucumis sativus	0	0	no
Glycine max	1	3	no
Helianthus annus	3	1	no
Lycopersicon esculentum	0	1	no
Allium cepa	3	0	no
Lolium perenne	0	0	no
Brassica rapa	1	3	no
Triticum aestivum	0	0	no

Vegetative vigour

Effects of the test item on survival

	Mean % Survival on day 21		
Species	**Control**	**133 g a.s./ha**	**Inhibition ≥ 25%**
Fagopyrum esculentum	100	100	no
Zea mays	100	100	no
Cucumis sativus	100	100	no
Glycine max	100	100	no
Helianthus annus	100	100	no
Lycopersicon esculentum	100	100	no
Allium cepa	100	100	no
Lolium perenne	100	100	no
Brassica rapa	100	100	no
Triticum aestivum	100	100	no

Effects of the test item on shoot height and dry weight

	Mean shoot height (cm) on day 21			Mean dry weight (g) on day 21		
Species	**Control**	**133 g a.s./ha**	**Inhibition ≥ 25%**	**Control**	**133 g a.s./ha**	**Inhibition ≥ 25%**
Fagopyrum esculentum	105.1	104.8	no	54.709	54.295	no
Zea mays	90.6	89.7	no	46.280	46.227	no
Cucumis sativus	44.4	46.7	no	63.429	62.624	no
Glycine max	37.2	37.3	no	27.805	27.431	no
Helianthus annus	52.9	52.9	no	39.255	40.838	no
Lycopersicon esculentum	39.2	39.4	no	44.013	44.300	no
Allium cepa	18.9	19.6	no	0.795	0.890	no
Lolium perenne	34.9	33.4	no	6.273	5.343	no
Brassica rapa	13.0	13.0	no	7.434	8.150	no
Triticum aestivum	31.5	30.5	no	7.140	5.861	no

Phytotoxicity rating of the test item

	Phytotoxicity rating (%) on day 21		
Species	**Control**	**133 g a.s./ha**	**Damage ≥ 25%**
Fagopyrum esculentum	0	0	no
Zea mays	0	0	no
Cucumis sativus	0	0	no
Glycine max	0	0	no
Helianthus annus	0	0	no
Lycopersicon esculentum	0	0	no
Allium cepa	0	0	no
Lolium perenne	0	0	no
Brassica rapa	0	0	no
Triticum aestivum	0	0	no

III. CONCLUSIONS:

In a Tier 1 seedling emergence and vegetative vigour study fluopicolide SC 40 was tested under greenhouse conditions for effects on emergence, survival, growth and shoot dry weight of ten non-target terrestrial plant species. There were no significant adverse effects greater than 25% in the seedling emergence and vegetative vigour test for any of the ten crops tested after the treatment with fluopicolide SC 40 at an application rate of 133 g a.s./ha.

Assessment and conclusion by applicant:

In this Tier 1 seedling emergence and vegetative vigour study, no adverse effects > 50% on emergence, survival, growth and shoot dry weight of ten non-target terrestrial plant species after the treatment with fluopicolide SC 40 at an application rate of 133 g a.s./ha were detected. The study is considered reliable.

Data Point:	KCA 8.6.2/02
Report Author:	[redacted]
Report Year:	2004
Report Title:	Effects of BAM (2.6-dichlorobenzamide, AE C653711) on non-target terrestrial plants: seedling emergence and seedling growth test (Tier 2) Test item: BAM (2.6-dichlorobenzamide) AE C653711, substance pure Code: AE F653711 00 1B96 0001
Report No:	M-225892-01-2
Document No:	M-225892-01-2
Guideline(s) followed in study:	OECD: 208A (draft, 2000)
Deviations from current test guideline:	Method: Deviations from current guideline SANCO/3029/99 rev.4: No details regarding the linearity is reported. Nevertheless, data on accuracy and precision are presented to confirm the method validation. The accuracy of the method was proved by analysing a reference solution with a similar concentration. Recoveries were within the acceptable range of 70–110% and the RSD values were below 20%. The analytical method can be regarded as fit for purpose.; Study: Current Guideline: OECD 208 (2006) For oilseed rape the seedling emergence was 65% instead ≥ 70 %. No information on humidity and light intensity was reported. The plant density was 5 seeds per pot and not 2. These deviations are not expected to have impacted the study results. The temperature ranged from 14 to 39°C. This is outside the proposed range of 22°C ± 10 °C. The humidity ranged from 12 – 103%. This is outside the range of 70% ± 25%. The light intensity was not reported. Planting density was higher than recommended by the current guideline (more plants per pot, smaller pots).
Previous evaluation:	yes, evaluated and accepted DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The purpose of this specific study is to evaluate the effect of M-01 (AE C653711) on the seedling emergence and seedling growth of eight plant species representing 6 different plant families. Seeds of eight plant species were sown into soil in which various concentrations were incorporated. Eight pots per treatment group with 5 seeds per pot were used for testing.

Plants were grown and set under greenhouse conditions. Assessments for the respective plants were made 7, 14 and 21 days after emergence of 50% seeds in the control treatments. Assessments were made for plant survival, visual phytotoxicity, plant growth stage and shoot dry weight.

The validity criteria of the study were fulfilled for all species except for oilseed rape. For this species, emergence in the control was 65% only, failing to reach 70%. It should be noted that the emergence in all treatment rates of oilseed rape was between 87.5 and 97.5%. The analysis of M-01 (AE C653711) in the initial test item stock solution revealed a measured concentration of 95.5% of nominal.

There were no visible symptoms of phytotoxicity from any treatment concentration in any of the species tested apart from some minor growth stage delays. The maximum rate of 12.1 µg/kg did not result in adverse effects on emergence, survival or biomass of any of the 8 species tested in this study.

I. MATERIAL AND METHODS:

Test item: The study was conducted using M-01 (AE C653711); Code: AE C653711 00 1B96 0001, Batch No.: 08018ET, purity: 96.2%, appearance: beige powder.

Test species: Seeds of eight plant species, i.e. corn (*Zea mays*), cucumber (*Cucumis sativa*), oats (*Avena sativa*), oilseed rape (*Brassica napus*), onion (*Allium cepa*), pea (*Pisum sativum*), soybean (*Glycine max*) and sugar beet (*Beta vulgaris*) were sown into soil in which various concentrations of the fluopicolide metabolite M-01 (AE C653711) were incorporated.

Seeds used on the study had not been treated with pesticides or repellents prior to test initiation. Eight pots per treatment group with 5 seeds per pot were used for testing. Standard soil (silty loam) sieved to 2 mm with an organic carbon content of 1.19% and a pH value of 7.4 was used for testing. The test item was dissolved in methanol and added to soil and thoroughly mixed to provide the maximum concentration of 12.1 µg/kg. An aliquot of this soil (dry weight) was then subjected to serial dilution to provide the 5 other test concentrations. Details of the range of application rates are summarized in the following table:

Application rates during the study

Test item rates in µg/kg soil		**0.011**	**0.045**	**0.18**	**0.75**	**3.0**	**12.1**
Species							
ZEAMA	*Zea mays*	X	X	X	X	X	X
CUMSA	*Cucumis sativus*	X	X	X	X	X	X
AVESA	*Avena sativa*	X	X	X	X	X	X
BRSNN	*Brassica napus*	X	X	X	X	X	X
ALLCE	*Allium cepa*	X	X	X	X	X	X
PIBSX	*Pisum sativum*	X	X	X	X	X	X
GLXMA	*Glycine max*	X	X	X	X	X	X
BEAVX	*Beta vulgaris*	X	X	X	X	X	X

Plants were grown and set under glasshouse conditions with a temperature of 23°C ± 5°C and a photoperiod of approximately 16 hours light and 8 hours dark.
Daily checks were made to identify the date when 50% of the seedlings emerge in the controls for each species. Number of plants emerged were thencounted after 7, 14 and 21 days. Visual phytotoxicity ratings were made 7, 14 and 21 days after emergence of 50% of seeds in the control treatments. Growth stages as the final assessment were reported according to BBCH-Monograph. Number of plants that died after application were recorded at the end of the assessment period. The dry weight was determined at the final assessment.
Statistical analysis of data was performed to obtain NOEC/LOEC values and EC_{50}, where possible, using the software ToxRat Professional 2.08.

II. RESULTS AND DISCUSSION:

Validity criteria:
The germination rate of the seeds used in this study was ≥ 70% for all species except for oilseed rape. For this species, emergence in the control was 65% only, failing to reach 70%. It should be noted that the emergence in all treatment rates of oilseed rape was between 87.5 and 97.5%.
The validity criterion of at least 90% survival of the plants during the study period was achieved for the untreated controls for all species tested. No visible phytotoxic effects were observed in the control.

Analytical findings:
The analysis of M-01 (AE C653711) in the initial test item stock solution revealed a measured concentration of 95.5% of nominal.

Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4 with minor acceptable exceptions only.

Biological findings:
The EC_{50} values for emergence and survival for the final assessment are expressed in µg/kg soil and presented in the following table. In this table are also presented the results of the final assessments for shoot dry weight, i.e. the EC_{50} values, the no observed effect rate (NOEC) and the lowest effect rate (LOEC).

Effects of the test item on emergence, survival and shoot dry weight

	Emergence	Survival	Shoot dry weight		
Species	**EC_{50}**	**EC_{50}**	**EC_{50}**	**NOEC**	**LOEC**
Zea mays	> 12.1	> 12.1	> 12.1	> 12.1	> 12.1
Cucumis sativa	> 12.1	> 12.1	> 12.1	> 12.1	> 12.1
Avena sativa	> 12.1	> 12.1	> 12.1	> 12.1	> 12.1
Brassica napus	> 12.1	> 12.1	> 12.1	> 12.1	> 12.1
Allium cepa	> 12.1	> 12.1	> 12.1	> 12.1	> 12.1
Pisum sativum	> 12.1	> 12.1	> 12.1	> 12.1	> 12.1
Glycine max	> 12.1	> 12.1	> 12.1	> 12.1	> 12.1
Beta vulgaris	> 12.1	> 12.1	> 12.1	> 12.1	> 12.1

There were no visible symptoms of phytotoxicity from any treatment concentration in any of the species tested apart from some minor growth stage delays.

Growth stages of the non-target terrestrial plant species at the application rate at the final assessment

Growth stage (BBCH) Min-Max at application rates (in µg/kg soil) at the final assessment							
Species	**Control**	**0.011**	**0.045**	**0.18**	**0.75**	**3.0**	**12.1**
Zea mays	16	16	15-16	15-16	16	16	15-16
Cucumis sativa	13-14	12-14	13-14	13-14	13-14	13-14	10-14
Avena sativa	14-21	14-21	14-21	14	14	14-15	14-22
Brassica napus	14-16	15-16	14-16	14-16	14-16	14-16	14-16
Allium cepa	11-13	12-13	11-13	11-13	11-13	11-13	11-13
Pisum sativum	16-18	16-18	16-18	16-18	16-18	10-18	14-18
Glycine max	14-16	14-15	12-16	14-15	14-15	14-15	12-15
Beta vulgaris	14-16	16	14-16	14-16	16	10-16	16

III. CONCLUSION:

In a Tier 2 seedling emergence and growth test the metabolite M-01 (AE C653711) was tested under greenhouse conditions for effects on the survival, visual phytotoxicity, growth and shoot dry weight of 8 non-target terrestrial plant species. The seeds of the test species were sown into soil in which various concentrations of the test item were incorporated. The maximum rate of 12.1 µg/kg did not result in adverse effects on emergence, survival or biomass of any of the 8 species in this study.

Assessment and conclusion by applicant:

In this Tier 2 seedling emergence and growth study, no adverse effects > 50% on survival, visual phytotoxicity, growth and shoot dry weight of eight non-target terrestrial plant species after incorporation in soil of the metabolite M-01 (AE C653711) were detected. The study is considered reliable.

CA 8.7 Effects on other terrestrial organisms (flora and fauna)

Data Point:	KCA 8.7/01
Report Author:	[redacted]
Report Year:	2001
Report Title:	Laboratory screening trials to determine insecticidal activity Code: AE C638206
Report No:	C014350
Document No:	M-206449-01-1
Guideline(s) followed in study:	--
Deviations from current test guideline:	Not applicable, as no guideline is available
Previous evaluation:	yes, evaluated and accepted DAR (2005)
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

In the screening assessment the test item was used at dose rates of 1 and 100 ppm to determine whether it exhibits insecticidal activity on relevant entomological target species. Therefore, it was used against seven different insect species (*Spodoptera exigua*, *Heliothis virescens*, *Aphis fabae*, *Nilaparvata lugens*, *Diabrotica undecimpunctata*, *Meloidogyne incognita*, *Tetranychus urticae*). The test models that were used represent the standard characterization screen of the insecticide research department of Aventis CropScience in Frankfurt. All treated species were held in a climate chamber (25°C, 40-60% RH). Assessment was done 6 days after application in percentage of efficacy in comparison to the untreated control. The activity of the test compound is expressed as the concentration (ppm) required to cause 90% effect (LC_{90}). The fungicide AE C638206 tech. showed no efficacy on the seven treated entomological species. As the test methods have been designed to optimize the chances of detecting new insecticidal lead compounds, it is concluded that AE C638206 tech. has no potential for causing insecticidal effects at concentrations below approximately 100 ppm.

I. MATERIALS AND METHODS

Test item: AE C638206 tech., name: Fluopicolide; content of a.s. analysed: > 95%. Material was dissolved in a solvent mixture containing 0.2 mL DMSO + 0.3 mL Acetone + 1.4 mL Methanol and then diluted in deionized water in order to obtain a dose rate of 100 ppm.

Test design: In the screening assessment the test item was used to determine whether it exhibits insecticidal activity on relevant entomological target species. Therefore, it was used against seven different insect species. The test models that were used represent the standard characterization screen of the insecticide research department of Aventis CropScience in Frankfurt. The screening methods are described in the following paragraphs:

-The test compound solution is carefully rinsed over 10 second instar larvae of *Spodoptera exigua* and dropped onto an artificial diet.

- *Heliothis virescens* eggs on filter paper are dipped into the test compound solution for 5 seconds and carefully placed in a Petri dish with the eggs upwards. Additional compound solution is dropped onto diet.

- Seeds of field beans (*Vicia faba*) infested by *Aphis fabae* are dripped into the test compound solution for 5 seconds.

- Rice plants (*Oryza sativa*) are dipped into the test compound solution for 5 seconds. Immediately after the plants are dipped, they are laid into Petri dishes. After drying, the rice plants are infested with approx. 20 planthopper larvae (*Nilaparvata lugens*).

-The test compound solution is dropped onto eggs of *Diabrotica undecimpunctata* and on a sprouted maize seed.

-The test compound solution is pipetted into a nematode (*Meloidogyne incognita*) suspension (dilution factor 1:100). If immobility > 80% occurs after 6 d, the compound solution with the pre-treated nematodes is drenched into soil.

-Seeds of French beans (*Phaseolus vulgaris*) infested with *Tetranychus urticae* are dipped into the test compound solution for 5 seconds.

-Roots of intact seedlings of field beans (*Vicia faba*) are inserted into glass bottles. The test compound solution is pippeted into the bottles (dilution factor 1:10). Afterwards, the plants are infested with *Aphis fabae*.

All treated species were held in a climate chamber (25°C, 40-60% RH). Assessment was done 6 days after application in percentage of efficacy in comparison to the untreated control. The activity of the test compound is expressed as the concentration (ppm) required to cause 90% effect (LC_{90}).

II. RESULTS AND DISCUSSION

Characterization of screening results and calculated LC_{90}

Species	Treated stage	% effect in test	LC_{90} (ppm)
Spodoptera exigua	Larvae	0 at 100 ppm	> 100
Heliothis virescens	Eggs	0 at 100 ppm	> 100
Aphis fabae – contact trial	Mixed population	0 at 100 ppm	> 100
Nilaparvata lugens	Larvae	0 at 100 ppm	> 100
Diabrotica undecimpunctata	Eggs	0 at 100 ppm	> 100
Meloidogyne incognita	Larvae	0 at 1 ppm	> 1
Tetranychus urticae	Mixed population	0 at 100 ppm	> 100
Aphis fabae – systemic trial	Mixed population	0 at 10 ppm	> 10

No insecticidal activity at the tested rates on the treated target species was observed.

III. CONCLUSION

The fungicide AE C638206 tech. showed no efficacy on the eight treated entomological species. As the test methods have been designed to optimize the chances of detecting new insecticidal lead compounds, it is concluded that AE C638206 tech. has no potential for causing insecticidal effects at concentrations below approximately 100 ppm.

Assessment and conclusion by applicant:

The study is considered reliable. The information it provides is supplemental as it is not relevant for use in risk assessments.

Data Point:	KCA 8.7/02
Report Author:	[redacted]
Report Year:	2001
Report Title:	Glasshouse screening trials to determine herbicidal activity AE C638206
Report No:	C013880
Document No:	M-205444-01-1
Guideline(s) followed in study:	--
Deviations from current test guideline:	Not applicable, as no guideline is available for NTTP screening studies
Previous evaluation:	yes, evaluated and accepted DAR (2005)
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The purpose of this screening study was to determine whether the fungicide fluopicolide formulated in a typical screening formulation (fluopicolide WP20) exhibits herbicidal activity. Therefore, 27 plant species were selected to cover important grass and broad-leaved weeds and include globally important arable crop. Two screening stages were performed, and 8 plant species tested at screening Stage I and 24 plant species at screening stage II. For both screening stages, plant seeds were sown in a sandy loam soil in 4 cm diameter peat pots.
The spray solution of 1000 g a.s./ha was applied at a spray volume of 800 L/ha in the first stage. In the second stage the spray solutions of 20, 80, 320 and 1280 g a.s./ha were applied at a spray volume of 600 L/ha. The pots were maintained under glasshouse conditions.
The treated plants were monitored daily for symptoms of herbicidal injury in comparison with untreated control plants. The treated plants showed no herbicidal effect at the Screening Stage 1 and 2.

I. MATERIAL AND METHODS:

Test item: Fluopicolide WP20: Fluopicolide was supplied as a > 95% pure technical grade sample. This material was formulated as a 20% wettable powder (standard WP20 screening formulation).

Test species: 27 species selected to cover important grass and broad-leaved weeds and include globally important arable crops (EPPO code): *Zea mays* (ZEAMA), *Tricitium aestivium* (TRZAS), *Glycine max* (GLXMA), *Beta vulgaris* (BEAVA), *Oryza sativa* (ORYSA), *Abutilon theophrasti* (ABUTH), *Agropyron repens* (AGRRE), *Alopecurus myosuroides* (ALOMY), *Amaranthus retroflexus* (AMARE), *Avena fatua* (AVEFA), *Avena sativa* (AVESA), *Chenopodium album* (CHEAL), *Cyperus esculentus* (CYPES), *Cyperus iria* (CYPIR), *Digitaria sanguinalis* (DIGSA), *Echinochloa crus-galli* (ECHCG), *Galium aparine* (GALAP), *Lolium multiforum* (LOLMU). *Matricaria inodora* (MATIN), *Pharbits purpurea* (PHBPU), *Fallopia convolvulus* (POLCO), *Sesbania exaltata* (SEBEX), *Setaria viridis* (SETVI), *Sorghum halepense* (SORHA), *Stellaria media* (STEME), *Sinapis alba* (SINAL), *Veronica persica* (VERPE).

Two screening stages were performed, and 8 plant species tested at screening Stage I and 24 plant species at screening stage II. For both screening stages, plant seeds were sown in a sandy loam soil in 4 cm diameter peat pots. In order to investigate the soil and foliar activity of fluopicolide, the pots were either treated before the seedlings emerged from the soil (pre-emergence) or after the seedlings had produced the first 2-4 leaves (post-emergence). The pots were placed in a glasshouse at 22°C day and 18°C night.

The Fluopicolide WP 20 screening formulation was diluted in deionized water (containing 0.2% of the wetting agent Agrotin 390) in order to obtain dose rates of 20, 80, 320, 1000 and 1280 g a.s./ha. The spray solution of 1000 g a.s./ha was applied at a spray volume of 800 L/ha in the first stage. In the second stage the spray solutions of 20, 80, 320 and 1280 g a.s./ha were applied at a spray volume of 600 L/ha. After application the pots were returned to the glasshouse.
The treated plants were monitored daily for symptoms of herbicidal injury in comparison with untreated control plants. The plants treated post-emergence were formally scored for injury 7 and 14 days after treatment. The pre-emergence plants were assessed 21 days after treatment. The assessments were made on a scale of 0 (= no effect) to 100 (= complete kill) for visual effect.

II. Results and Discussion:

Biological findings:
The 8 plant species treated pre- or post-emergence at a dose rate of 1000 g a.s./ha showed no herbicidal effect at stage I. At Stage II also no herbicidal effect was observed in the 24 plant species treated pre- or post-emergence with dose rates of 20, 80, 320 and 1280 g technical sample/ha.

III. Conclusions:

In a screening test Fluopicolide WP20 was tested under greenhouse conditions for herbicidal effects on 8 species at stage I (test rate = 1000 g a.s./ha) and 24 species at stage II (test rates = 20, 80, 320 and 1280 g a.s./ha) to cover important grass and broad-leaved weeds and including important global arable crops. No herbicidal effects were observed at both stages at the given rates.

Assessment and conclusion by applicant:

No herbicidal effects on 25 weed and crop species were observed in this screening test for the active substance fluopicolide formulated as a WP20 screening formulation up to a test rate of 1000 g a.s./ha. The study is considered reliable.

Data Point:	KCA 8.7/03
Report Author:	[redacted]
Report Year:	2003
Report Title:	AE C638206 technical ingredient: Determination of effects on growth of pure cultures of a soil fungus, Mucor circinelloides (Zygomycetes), on nutrient medium
Report No:	LKC-SF-01/03
Document No:	M-235058-01-1
Guideline(s) followed in study:	--
Deviations from current test guideline:	Not applicable, as no agreed guideline is available
Previous evaluation:	yes, evaluated and accepted DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The study investigated the influence of the test item on growth of pure cultures of a soil fungus, *Mucor circinelloides* (Zygomycetes), on malt agar mixed with sterile, silty sand soil. The soil, which served as a carrier for the test substance and a source of micro-nutrients for the fungus, was treated with the test item to receive concentrations of 0.3, 1.5, 3.0, 7.5, 15.0 and 30.0 mg a.s./kg dry weight soil. Controls contained malt agar and untreated, sterile soil. Colonies were grown in the dark at 20 ± 2 °C. Three replicate dishes were prepared per test concentration. The percent of inhibition was determined after 3 days. Significant differences in growth between treated and control samples were detected only in the highest test treatment (30 mg a.s./kg dry weight soil). The effects were less than 25 %. Therefore, the NOEC was ≥ 30.0 mg a.s./kg dry weight soil and the EC_{50} was > 30.0 mg a.s./kg dry weight soil.

I. Materials and Methods

Test item: AE C638206 tech., name: Fluopicolide; batch/FL no.: OP2050046; content of a.s. analysed: 96.1 %; development code of a.s.: AE C638206; development no.: 3000315241.

Test design: Small pieces of agar (4 mm in diameter) containing mycelium from an actively growing *Mucor circinelloides* culture were used to inoculate the centers of petri dishes containing nutrient medium prepared by mixing malt agar with sterile, AE C638206-treated, silty sand soil (0.6 % org. C, pH 5.3).

The soil, which served as a carrier for the test substance, and a source of micro-nutrients for the fungus, was treated with the test item to receive concentrations of 0.3, 1.5, 3.0, 7.5, 15.0 and 30.0 mg a.s./kg dry weight soil. Controls contained malt agar and untreated, sterile soil. Colonies were grown in the dark at 20 ± 2 °C. Three replicate dishes were prepared per test concentration. Percent inhibition of growth was determined after 3 days by comparison of mean diameters (± std. deviation) of treated colonies to mean diameters of the untreated controls.

II. RESULTS AND DISCUSSION

Effect of fluopicolide on growth of pure cultures of *Mucor circinelloides* on nutrient medium

Concentration tested (mg a.s./kg dry wt soil)	Mean Colony diameters ± standard deviation (cm) from 3 replicates	Inhibition of growth in % of untreated control
0	7.3 ± 0.1	0
0.3	7.1 ± 0.1	3
1.5	7.1 ± 0.3	3
3.0	7.3 ± 0.1	0
7.5	7.1 ± 0.2	3
15.0	7.0 ± 0.1	4
30.0	6.8 ± 0.0	7

Significant differences in growth between treated and control samples were detected only in the highest test treatment (30 mg a.s./kg dry weight soil). The effects were less than 25 %. Therefore, the NOEC was ≥ 30.0 mg a.s./ kg dry weight soil and the EC_{50} was > 30.0 mg a.s./ kg dry weight soil.

Validity criteria:

Internationally agreed and accepted guidelines for determining the influence of test items on pure cultures of soil fungi could not be found in the literature. Therefore, validity criteria were not defined.

III. CONCLUSION

The endpoints are:

NOEC ≥ 30.0 mg a.s./kg dry weight soil

EC_{50} > 30.0 mg a.s./kg dry weight soil

Assessment and conclusion by applicant:

The study is considered reliable. However as this study is not a data requirement it will not be further considered in the risk assessment.

Data Point:	KCA 8.7/04
Report Author:	[redacted]
Report Year:	2003
Report Title:	AE C638206 technical ingredient: Determination of effects on growth of pure cultures of a soil fungus, Phytophthora nicotianae (Oomycetes), on nutrient medium
Report No:	LKC-SF-03/03
Document No:	M-235061-01-1
Guideline(s) followed in study:	--
Deviations from current test guideline:	Not applicable, as no agreed guideline is available
Previous evaluation:	yes, evaluated and accepted DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The study investigated the influence of the test item on growth of pure cultures of a soil fungus, *Phytophthora nicotianae* (Oomycetes), vegetable juice agar with sterile, AE C638206 tech. – treated, silty sand soil. The soil, which served as a carrier for the test substance, and a source of micro-nutrients for the fungus, was treated with the test item to receive concentrations of 0.3, 1.5, 3.0, 7.5, 15.0 and 30.0 mg a.s./kg dry weight soil. Colonies were grown in the dark at 20 ± 2 °C. Three replicate dishes were prepared per test concentration. Percent inhibition of growth was determined after 6 days. Significant differences in growth between treated and control samples were detected in the test concentrations with 1.5, 3.0, 7.5, 15 and 30 mg a.s./kg dry weight soil. At these rates the differences were > 25%. Therefore, the NOEC is 0.3 mg a.s./kg dry weight soil. The EC_{50} is 1.2 mg a.s./kg dry weight soil.

I. Materials and Methods

Test item: AE C638206 tech., name: Fluopicolide; batch/FL no.: OP2050046; content of a.s. analysed: 96.1 %; development code of a.s.: AE C638206; development no.: 3000315241.

Test design: Small pieces of agar (4 mm in diameter) containing mycelium from an actively growing *Phytophthora nicotianae* culture were used to inoculate the centers of petri dishes containing nutrient medium prepared by mixing vegetable juice agar with sterile, AE C638206 tech. – treated, silty sand soil (0.6% org. C, pH 5.3). The soil, which served as a carrier for the test substance, and a source of micro-nutrients for the fungus, was treated with enough AE C638206 tech. to give concentrations of 0.3, 1.5, 3.0, 7.5, 15.0 and 30 mg a.s./kg dry weight soil. Controls contained vegetable juice agar and untreated, sterile soil. Colonies were grown in the dark at 20 ± 2°C. Three replicate dishes were prepared per test concentration. Percent inhibition of growth was determined after 6 days by comparison of mean diameters (± std. deviation) of treated colonies to mean diameters of the untreated controls.

II. RESULTS AND DISCUSSION

Effect of fluopicolide on growth of pure cultures of *Phytophthora nicotianae* on nutrient medium

Concentration tested (mg a.s./kg dry wt soil)	Mean colony diameters ± standard deviation (cm) from 3 replicates	Inhibition of growth in % of untreated control
0	5.6 ± 0.2	0
0.3	6.0 ± 0.1	0
1.5	2.2 ± 0.3	61
3.0	1.5 ± 0.2	73
7.5	1.3 ± 0.2	77
15.0	1.0 ± 0.4	82
30.0	0.6 ± 0.2	89

Significant differences in growth between treated and control samples were detected in the test concentrations with 1.5, 3.0, 7.5, 15 and 30 mg a.s./kg dry weight soil. At these rates the differences were > 25%. Therefore, the NOEC is 0.3 mg a.s./kg dry weight soil. The EC_{50} is 1.2 mg a.s./kg dry weight soil.

Validity criteria:

Internationally agreed and accepted guidelines for determining the influence of test items on pure cultures of soil fungi could not be found in the literature. Therefore, validity criteria were not defined.

III. CONCLUSION

The endpoints are:

NOEC = 0.3 mg a.s./kg dry weight soil

EC_{50} = 1.2 mg a.s./kg dry weight soil

Assessment and conclusion by applicant:

The study is considered reliable. However, as this study is not a data requirement it will not be further considered in the risk assessment.

Data Point:	KCA 8.7/05
Report Author:	[redacted]
Report Year:	2003
Report Title:	AE C638206 technical ingredient: Determination of effects on growth of pure cultures of a soil fungus, Cladorrhinum foesundissimum (Deuteromycetes), on nutrient medium
Report No:	LKC-SF-07/03
Document No:	M-235063-01-1
Guideline(s) followed in study:	--
Deviations from current test guideline:	Not applicable, as no agreed guideline is available
Previous evaluation:	yes, evaluated and accepted DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The study investigated the influence of the test item on growth of pure cultures of a soil fungus, *Cladorrhinum foesundissimum* (Deuteromycetes), on malt agar mixed with sterile, silty sand soil. The soil, which served as a carrier for the test substance, and a source of micro-nutrients for the fungus, was treated with the test item to receive concentrations of 0.3, 1.5, 3.0, 7.5, 15.0 and 30.0 mg a.s./kg dry weight soil. Colonies were grown in the dark at 20 ± 2 °C. Three replicate dishes were prepared per test concentration. Percent inhibition of growth was determined after 7 days. No significant differences in growth between treated and control samples were detected. The effects were less than 25 %. Therefore, the NOEC was ≥ 30.0 mg a.s./kg dry weight soil and the EC_{50} was > 30.0 mg a.s./kg dry weight soil.

I. Materials and Methods

Test item: AE C638206 tech., name: Fluopicolide; batch/FL no.: OB2050046; content of a.s. analysed: 96.1 %; development code of a.s.: AE C638206; development no.: 3000315241.

Test design: Suspensions of spores from *Cladorrhinum foesundissimum* cultures were used to inoculate the cultures of petri dishes containing nutrient medium prepared by mixing malt agar with sterile, AE C638206 tech. – treated, silty sand soil (0.6% org. C, pH 5.3). The soil, which served as a carrier for the test substance and a source of micro-nutrients for the fungus, was treated with enough AE C638206 tech. to give concentrations of 0.3, 1.5, 3.0, 7.5, 15.0 and 30.0 mg a.s./kg dry weight soil. Controls contained malt agar and untreated, sterile soil. Colonies were grown in the dark at 20 ± 2 °C. Three replicate dishes were prepared per test concentration. Percent inhibition of growth was determined after 7 days by comparison of mean diameters (± std. deviation) of treated colonies to mean diameters of the untreated controls.

II. RESULTS AND DISCUSSION

Effect of fluopicolide on growth of pure cultures of *Cladorrhinum foesundissimum* on nutrient medium

Concentration tested (mg a.s./kg dry wt soil)	Mean colony diameters ± standard deviation (cm) from 3 replicates	Inhibition of growth in % of untreated control
0	6.1 ± 0.2	0
0.3	6.1 ± 0.2	0
1.5	6.0 ± 0.1	2
3.0	6.0 ± 0.1	2
7.5	6.3 ± 0.2	0
15.0	6.1 ± 0.3	0
30.0	6.1 ± 0.1	0

No significant differences in growth between treated and control samples were detected. The effects were less than 25 %. Therefore, the NOEC was ≥ 30.0 mg a.s./kg dry weight soil and the EC_{50} was > 30.0 mg a.s./kg dry weight soil.

Validity criteria:

Internationally agreed and accepted guidelines for determining the influence of test items on pure cultures of soil fungi could not be found in the literature. Therefore, validity criteria were not defined.

III. CONCLUSION

The endpoints are:

NOEC ≥ 30.0 mg a.s./kg dry weight soil

EC_{50} > 30.0 mg a.s./kg dry weight soil.

Assessment and conclusion by applicant:

The study is considered reliable. However, as this study is not a data requirement it will not be further considered in the risk assessment.

Data Point:	KCA 8.7/06
Report Author:	[redacted]
Report Year:	2003
Report Title:	AE C638206 technical ingredient: Determination of effects on growth of pure cultures of a soil fungus, Penicillium janthinellum (simplicissimum) (Ascomycetes), on nutrient medium
Report No:	LKC-SF-11/03
Document No:	M-235065-01-1
Guideline(s) followed in study:	--
Deviations from current test guideline:	Not applicable, as no agreed guideline is available
Previous evaluation:	yes, evaluated and accepted DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The study investigated the influence of the test item on growth of pure cultures of a soil fungus, *Penicillium janthinellum* (Ascomycetes), on malt agar mixed with sterile, silty sand soil. The soil, which served as a carrier for the test substance, and a source of micro-nutrients for the fungus, was treated with the test item to receive concentrations of 0.3, 1.5, 3.0, 7.5, 15.0 and 30.0 mg a.s./kg dry weight soil. Colonies were grown in the dark at 20 ± 2 °C. Three replicate dishes were prepared per test concentration. Percent inhibition of growth was determined after 11 days. No significant differences in growth between treated and control samples were detected. The effects were less than 25 %. Therefore, the NOEC was ≥ 30.0 mg a.s./kg dry weight soil and the EC_{50} was > 30.0 mg a.s./kg dry weight soil.

I. Materials and Methods

Test item: AE C638206 tech., name: Fluopicolide; batch/FI no.: OB2050046; content of a.s. analysed: 96.1 %; development code of a.s.: AE C638206; development no.: 3000315241.

Test design: Suspensions of spores from *Penicillium janthinellum* cultures were used to inoculate the cultures of petri dishes containing nutrient medium prepared by mixing malt agar with sterile, AE C638206 tech. – treated, silty sand soil (0.6% org. C, pH 5.3). The soil, which served as a carrier for the test substance and a source of micro-nutrients for the fungus, was treated with enough AE C638206 tech. to give concentrations of 0.3, 1.5, 3.0, 7.5, 15.0 and 30.0 mg a.s./kg dry weight soil. Controls contained malt agar and untreated, sterile soil. Colonies were grown in the dark at 20 ± 2 °C. Three replicate dishes were prepared per test concentration. Percent inhibition of growth was determined after 11 days by comparison of mean diameters (± std. deviation) of treated colonies to mean diameters of the untreated controls.

II. RESULTS AND DISCUSSION

Effect of fluopicolide on growth of pure cultures of *Penicillium janthinellum* on nutrient medium

Concentration tested (mg a.s./kg dry wt soil)	Mean colony diameters ± standard deviation (cm) from 3 replicates	Inhibition of growth in % of untreated control
0	6.0 ± 0.5	0
0.3	5.7 ± 0.6	5
1.5	5.5 ± 04	8
3.0	5.2 ± 0.3	5
7.5	6.4 ± 0.1	13
15.0	6.4 ± 0.2	7
30.0	6.3 ± 0.1	12

No significant differences in growth between treated and control samples were detected. The effects were less than 25 %. Therefore, the NOEC was ≥ 30.0 mg a.s./kg dry weight soil and the EC_{50} was > 30.0 mg a.s./kg dry weight soil.

Validity criteria:

Internationally agreed and accepted guidelines for determining the influence of test items on pure cultures of soil fungi could not be found in the literature. Therefore, validity criteria were not defined.

III. CONCLUSION

The endpoints are:

NOEC ≥ 30.0 mg a.s./kg dry weight soil

EC_{50} > 30.0 mg a.s./kg dry weight soil

Assessment and conclusion by applicant:

The study is considered reliable. However, as this study is not a data requirement it will not be further considered in the risk assessment.

Data Point:	KCA 8.7/07
Report Author:	[redacted]
Report Year:	2003
Report Title:	AE C638206 technical ingredient: Determination of effects on growth of pure culture of a soil fungus, Suillus granulatus (Basidiomycetes), on nutrient medium
Report No:	LKC-SF-05/03
Document No:	M-218467-01-1
Guideline(s) followed in study:	--
Deviations from current test guideline:	Not applicable, as no agreed guideline is available
Previous evaluation:	yes, evaluated and accepted DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The study investigated the influence of the test item on growth of pure cultures of a soil fungus, *Suillus granulatus* (Basidiomycetes), on malt agar mixed with sterile, silty sand soil. The soil, which served as a carrier for the test substance, and a source of micro-nutrients for the fungus, was treated with the test item to receive concentrations of 0.3, 1.5, 3.0, 7.5, 15.0 and 30.0 mg a.s./kg dry weight soil. Controls contained malt agar and untreated, sterile soil. Colonies were grown in the dark at 20 ± 2 °C. Three replicate dishes were prepared per test concentration. The percent of inhibition was determined after 35 days. No significant differences in growth between treated and control samples were detected. The effects were less than 25 %. Therefore, the NOEC was ≥ 30.0 mg a.s./kg dry weight soil and the EC_{50} was > 30.0 mg a.s./kg dry weight soil.

I. Materials and Methods

Test item: AE C638206 tech., name: Fluopicolide; batch/FL no.: 00202050046; content of a.s. analysed: 96.1 %; development code of a.s.: AE C638206; development no.: 3000315241.

Test design: Small pieces of agar (4 mm in diameter) containing mycelium from an actively growing *Suillus granulatus* culture were used to inoculate the centers of petri dishes containing nutrient medium prepared by mixing malt agar with sterile, AE C638206-treated, silty sand soil (0.6 % org. C, pH 5.3).

The soil, which served as a carrier for the test substance, and a source of micro-nutrients for the fungus, was treated with the test item to receive concentrations of 0.3, 1.5, 3.0, 7.5, 15.0 and 30.0 mg a.s./kg dry weight soil. Controls contained malt agar and untreated, sterile soil. Colonies were grown in the dark at 20 ± 2 °C. Three replicate dishes were prepared per test concentration. Percent inhibition of growth was determined after 35 days by comparison of mean diameters (± std. deviation) of treated colonies to mean diameters of the untreated controls.

II. RESULTS AND DISCUSSION

Effect of fluopicolide on growth of pure cultures of *Suillus granulatus* on nutrient medium

Concentration tested (mg a.s./kg dry wt soil)	Mean colony diameters ± standard deviation (cm) from 3 replicates	Inhibition of growth in % of untreated control
0	2.7 ± 0.3	0
0.3	2.4 ± 0.1	11
1.5	2.4 ± 0.1	11
3.0	2.7 ± 0.2	0
7.5	2.4 ± 0.1	11
15.0	2.5 ± 0.1	7
30.0	2.6 ± 0.2	4

No significant differences in growth between treated and control samples were detected. The effects were less than 25 %. Therefore, the NOEC was ≥ 30.0 mg a.s./kg dry weight soil and the EC_{50} was > 30.0 mg a.s./kg dry weight soil.

Validity criteria:

Internationally agreed and accepted guidelines for determining the influence of test items on pure cultures of soil fungi could not be found in the literature. Therefore, validity criteria were not defined.

III. CONCLUSION

The endpoints are:

NOEC ≥ 30.0 mg a.s./kg dry weight soil

EC_{50} > 30.0 mg a.s./kg dry weight soil

Assessment and conclusion by applicant:

The study is considered reliable. However, as this study is not a data requirement it will not be further considered in the risk assessment.

Data Point:	KCA 8.7/08
Report Author:	[redacted]
Report Year:	2003
Report Title:	AE C653711 (AE C653711 00 1B97 0001): Determination of effects on growth of pure cultures of a soil fungus, Mucor circinelloides (Zygomycetes), on nutrient medium
Report No:	LKC-SF-02/03
Document No:	M-235067-01-1
Guideline(s) followed in study:	--
Deviations from current test guideline:	Not applicable, as no agreed guideline is available
Previous evaluation:	yes, evaluated and accepted DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The study investigated the influence of the test item M-01 (AE C653711, 2,6-dichlorobenzamide) on growth of pure cultures of a soil fungus, *Mucor circinelloides* (Zygomycetes), on malt agar mixed with sterile, silty sand soil. The soil, which served as a carrier for the test substance, and a source of micro-nutrients for the fungus, was treated with the test item to receive concentrations of 0.3, 1.5, 3.0, 7.5, 15.0 and 30.0 mg p.m./kg dry weight soil. Controls contained malt agar and untreated, sterile soil. Colonies were grown in the dark at 20 ± 2 °C. Three replicate dishes were prepared per test concentration. The percent of inhibition was determined after 3 days. No significant differences in growth between treated and control samples were detected. The effects were less than 25 %. Therefore, the NOEC was ≥ 30.0 mg p.m./kg dry weight soil and the EC_{50} was > 30.0 mg p.m./kg dry weight soil.

I. MATERIALS AND METHODS

Test item: M-01 (AE C653711; chemical name: 2,6-dichlorobenzamide; batch/FL no.:8808018; content of a.s. analysed: 97.0 %; development code of a.s.: AE C653711 00 1B97 0001; development no.: 3000325964.

Test design: Small pieces of agar (4 mm in diameter) containing mycelium from an actively growing *Mucor circinelloides* culture were used to inoculate the centers of petri dishes containing nutrient medium prepared by mixing malt agar with sterile, AE C653711-treated, silty sand soil (0.6 % org. C, pH 5.3).

The soil, which served as a carrier for the test substance, and a source of micro-nutrients for the fungus, was treated with the test item to receive concentrations of 0.3, 1.5, 3.0, 7.5, 15.0 and 30.0 mg p.m./kg dry weight soil. Controls contained malt agar and untreated, sterile soil. Colonies were grown in the dark at 20 ± 2 °C. Three replicate dishes were prepared per test concentration. Percent inhibition of growth was determined after 3 days by comparison of mean diameters (+ std. deviation) of treated colonies to mean diameters of the untreated controls.

II. RESULTS AND DISCUSSION

Effect of M-01 (AE C653711) on growth of pure cultures of *Mucor circinelloides* on nutrient medium

Concentration tested (mg p.m./kg dry wt soil)	Mean Ccolony diameters + standard deviation (cm) from 3 replicates	Inhibition of growth in % of untreated control
0	7.0 ± 0.2	0
0.3	7.0 ± 0.3	0
1.5	6.9 ± 0.1	1
3.0	6.9 ± 0.1	1
7.5	7.0 ± 0.2	0
15.0	7.0 ± 0.1	0
30.0	6.8 ± 0.2	3

No significant differences in growth between treated and control samples were detected. The effects were less than 25 %. Therefore, the NOEC was ≥ 30.0 mg p.m./kg dry weight soil and the EC_{50} was > 30.0 mg p.m./kg dry weight soil.

Validity criteria:

Internationally agreed and accepted guidelines for determining the influence of test items on pure cultures of soil fungi could not be found in the literature. Therefore, validity criteria were not defined.

III. CONCLUSION

The endpoints are:

NOEC ≥ 30.0 mg p.m./kg dry weight soil

EC_{50} > 30.0 mg p.m./kg dry weight soil.

Assessment and conclusion by applicant:

The study is considered reliable. However as this study is not a data requirement it will not be further considered in the risk assessment.

Data Point:	KCA 8.7/09
Report Author:	[redacted]
Report Year:	2003
Report Title:	AE C643711 (AE C653711 00 1B97 0001): Determination of effects on growth of pure cultures of a soil fungus, Phytophthora nicotianae (Oomycetes), on nutrient medium
Report No:	LKC-SF-04/03
Document No:	M-235068-01-1
Guideline(s) followed in study:	--
Deviations from current test guideline:	Not applicable, as no agreed guideline is available
Previous evaluation:	yes, evaluated and accepted DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The study investigated the influence of the test item M-01 (AE C653711, 2,6-dichlorobenzamide) on growth of pure cultures of a soil fungus, *Phytophthora nicotianae* (Oomycetes), on vegetable juice agar mixed with sterile, silty sand soil. The soil, which served as a carrier for the test substance, and a source of micro-nutrients for the fungus, was treated with the test item to receive concentrations of 0.3, 1.5, 3.0, 7.5, 15.0 and 30.0 mg p.m./kg dry weight soil. Colonies were grown in the dark at 20 ± 2 °C. Three replicate dishes were prepared per test concentration. Percent inhibition of growth was determined after 6 days. No significant differences in growth between treated and control samples were detected. The effects were less than 25 %. Therefore, the NOEC was ≥ 30.0 mg p.m./kg dry weight soil and the EC_{50} was > 30.0 mg p.m./kg dry weight soil.

I. Materials and Methods

Test item: M-01 (AE C653711), chemical name: 2,6-dichlorobenzamide; batch/FL no.:8808018; content of a.s. analysed: 97.0 %; development code of a.s.: AE C653711 00 1B97 0001; development no.: 3000325964.

Test design: Small pieces of agar (4 mm in diameter) containing mycelium from an actively growing *Phytophthora nicotianae* culture were used to inoculate the centers of petri dishes containing nutrient medium prepared by mixing vegetable juice agar with sterile, AE C653711-treated, silty sand soil (0.6 % org. C, pH 5.3).

The soil, which served as a carrier for the test substance, and a source of micro-nutrients for the fungus, was treated with the test item to receive concentrations of 0.3, 1.5, 3.0, 7.5, 15.0 and 30.0 mg p.m./kg dry weight soil. Controls contained vegetable juice agar and untreated, sterile soil. Colonies were grown in the dark at 20 ± 2 °C. Three replicate dishes were prepared per test concentration. Percent inhibition of growth was determined after 6 days by comparison of mean diameters (+ std. deviation) of treated colonies to mean diameters of the untreated controls.

II. RESULTS AND DISCUSSION

Effect of M-01 (AE C653711) on growth of pure cultures of *Phytophthora nicotianae* on nutrient medium

Concentration tested (mg p.m./kg dry wt soil)	Mean colony diameters + standard deviation (cm) from 3 replicates	Inhibition of growth in % of untreated control
0	5.8 ± 0.1	0
0.3	5.6 ± 0.2	3
1.5	5.5 ± 0.1	5
3.0	5.2 ± 0.3	10
7.5	6.4 ± 0.1	0
15.0	6.4 ± 0.2	0
30.0	6.3 ± 0.1	0

No significant differences in growth between treated and control samples were detected. The effects were less than 25 %. Therefore, the NOEC was ≥ 30.0 mg p.m./kg dry weight soil and the EC_{50} was > 30.0 mg p.m./kg dry weight soil.

Validity criteria:

Internationally agreed and accepted guidelines for determining the influence of test items on pure cultures of soil fungi could not be found in the literature. Therefore, validity criteria were not defined.

III. CONCLUSION

The endpoints are:

NOEC ≥ 30.0 mg p.m./kg dry weight soil

EC_{50} > 30.0 mg p.m./kg dry weight soil.

Assessment and conclusion by applicant:

The study is considered reliable. However as this study is not a data requirement it will not be further considered in the risk assessment.

Data Point:	KCA 8.7/10
Report Author:	[redacted]
Report Year:	2003
Report Title:	AE C653711 (AE C653711 00 1B97 0001): Determination of effects on growth of pure cultures of a soil fungus, Cladorrhinum foesundissimum (Deuteromycetes), on nutrient medium
Report No:	LKC-SF-08/03
Document No:	M-235070-01-1
Guideline(s) followed in study:	--
Deviations from current test guideline:	Not applicable, as no agreed guideline is available
Previous evaluation:	yes, evaluated and accepted DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The study investigated the influence of the test item M-01 (AE C653711, 2,6-dichlorobenzamide) on growth of pure cultures of a soil fungus, *Cladorrhinum foesundissimum* (Deuteromycetes), on malt agar mixed with sterile, silty sand soil. The soil, which served as a carrier for the test substance, and a source of micro-nutrients for the fungus, was treated with the test item to receive concentrations of 0.3, 1.5, 3.0, 7.5, 15.0 and 30.0 mg p.m./kg dry weight soil. Colonies were grown in the dark at 20 ± 2 °C. Three replicate dishes were prepared per test concentration. Percent inhibition of growth was determined after 7 days. No significant differences in growth between treated and control samples were detected. The effects were less than 25 %. Therefore, the NOEC was ≥ 30.0 mg p.m./kg dry weight soil and the EC_{50} was > 30.0 mg p.m./kg dry weight soil.

I. Materials and Methods

Test item: M-01 (AE C653711), chemical name: 2,6-dichlorobenzamide; batch/FL no.:8808018; content of a.s. analysed: 97.0 %; development code of a.s.: AE C653711 00 1B97 0001; development no.: 3000325964.

Test design: Suspensions of spores from *Cladorrhinum foesundissimum* cultures were used to inoculate the centers of petri dishes containing nutrient medium prepared by mixing malt agar with sterile, AE C653711-treated, silty sand soil (0.6 % org. C, pH 5.3).

The soil, which served as a carrier for the test substance, and a source of micro-nutrients for the fungus, was treated with the test item to receive concentrations of 0.3, 1.5, 3.0, 7.5, 15.0 and 30.0 mg p.m./kg dry weight soil. Controls contained malt agar and untreated, sterile soil. Colonies were grown in the dark at 20 ± 2 °C. Three replicate dishes were prepared per test concentration. Percent inhibition of growth was determined after 7 days by comparison of mean diameters (+ std. deviation) of treated colonies to mean diameters of the untreated controls.

II. RESULTS AND DISCUSSION

Effect of M-01 (AE C653711) on growth of pure cultures of *Cladorrhinum foesundissimum* on nutrient medium

Concentration tested (mg p.m./kg dry wt soil)	Mean colony diameters + standard deviation (cm) from 3 replicates	Inhibition of growth in % of untreated control
0	6.2 ± 0.2	0
0.3	6.3 ± 0.2	0
1.5	6.2 ± 0.1	0
3.0	6.3 ± 0.3	0
7.5	6.2 ± 0.2	0
15.0	6.2 ± 0.1	0
30.0	6.3 ± 0.1	0

No significant differences in growth between treated and control samples were detected. The effects were less than 25 %. Therefore, the NOEC was ≥ 30.0 mg p.m./kg dry weight soil and the EC_{50} was > 30.0 mg p.m./kg dry weight soil.

Validity criteria:

Internationally agreed and accepted guidelines for determining the influence of test items on pure cultures of soil fungi could not be found in the literature. Therefore, validity criteria were not defined.

III. CONCLUSION

The endpoints are:

NOEC ≥ 30.0 mg p.m./kg dry weight soil

EC_{50} > 30.0 mg p.m./kg dry weight soil.

Assessment and conclusion by applicant:

The study is considered reliable. However, as this study is not a data requirement it will not be further considered in the risk assessment.

Data Point:	KCA 8.7/11
Report Author:	[redacted]
Report Year:	2003
Report Title:	AE C653711 (AE C653711 00 1B97 0001: Determination of effects on growth of pure cultures of a soil fungus, Penicillium janthinellum (simplicissimum) (Ascomycetes), on nutrient medium
Report No:	LKC-SF-12/03
Document No:	M-235072-01-1
Guideline(s) followed in study:	--
Deviations from current test guideline:	Not applicable, as no agreed guideline is available
Previous evaluation:	yes, evaluated and accepted DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The study investigated the influence of the test item M-01 (AE C653711, 2,6-dichlorobenzamide) on growth of pure cultures of a soil fungus, *Penicillium janthinellum* (Ascomycetes), on vegetable juice agar mixed with sterile, silty sand soil. The soil, which served as a carrier for the test substance, and a source of micro-nutrients for the fungus, was treated with the test item to receive concentrations of 0.3, 1.5, 3.0, 7.5, 15.0 and 30.0 mg p.m./kg dry weight soil. Colonies were grown in the dark at 20 ± 2 °C. Three replicate dishes were prepared per test concentration. Percent inhibition of growth was determined after 11 days. No significant differences in growth between treated and control samples were detected. The effects were less than 25 %. Therefore, the NOEC was ≥ 30.0 mg p.m./kg dry weight soil and the EC_{50} was > 30.0 mg p.m./kg dry weight soil.

I. Materials and Methods

Test item: M-01 (AE C653711), chemical name: 2,6-dichlorobenzamide; batch/FL no.:8808018; content of a.s. analysed: 97.0 %; development code of a.s.: AE C653711 00 1B97 0001; development no.: 3000325964.

Test design: Suspensions of spores from *Penicillium janthinellum* cultures were used to inoculate the centers of petri dishes containing nutrient medium prepared by mixing malt agar with sterile, AE C653711-treated, silty sand soil (0.6 % org. C, pH 5.3).

The soil, which served as a carrier for the test substance, and a source of micro-nutrients for the fungus, was treated with the test item to receive concentrations of 0.3, 1.5, 3.0, 7.5, 15.0 and 30.0 mg p.m./kg dry weight soil. Controls contained vegetable juice agar and untreated, sterile soil. Colonies were grown in the dark at 20 ± 2 °C. Three replicate dishes were prepared per test concentration. Percent inhibition of growth was determined after 11 days by comparison of mean diameters (+ std. deviation) of treated colonies to mean diameters of the untreated controls.

II. RESULTS AND DISCUSSION

Effect of M-01 (AE C653711) on growth of pure cultures of *Penicillium janthinellum* on nutrient medium

Concentration tested (mg a.s./kg dry wt Soil)	Mean colony diameters + standard deviation (cm) from 3 replicates	Inhibition of growth in % of untreated control
0	6.3 ± 0.2	0
0.3	6.0 ± 0.1	5
1.5	5.9 ± 0.6	6
3.0	5.8 ± 0.6	8
7.5	6.2 ± 0.4	2
15.0	5.7 ± 0.5	10
30.0	6.0 ± 0.6	5

No significant differences in growth between treated and control samples were detected. The effects were less than 25 %. Therefore, the NOEC was ≥ 30.0 mg p.m./kg dry weight soil and the EC_{50} was > 30.0 mg p.m./kg dry weight soil.

Validity criteria:

Internationally agreed and accepted guidelines for determining the influence of test items on pure cultures of soil fungi could not be found in the literature. Therefore, validity criteria were not defined.

III. CONCLUSION

The endpoints are:

NOEC ≥ 30.0 mg p.m./kg dry weight soil

EC_{50} > 30.0 mg p.m./kg dry weight soil

Assessment and conclusion by applicant:

The study is considered reliable. However, as this study is not a data requirement it will not be further considered in the risk assessment.

Data Point:	KCA 8.7/12
Report Author:	[redacted]
Report Year:	2003
Report Title:	AE C653711 (AE C653711 00 1B97 0001): Determination of effects on growth of pure culture of a soil fungus, Suillus granulatus (Basidiomycetes), on nutrient medium
Report No:	LKC-SF-06/03
Document No:	M-218466-01-1
Guideline(s) followed in study:	--
Deviations from current test guideline:	Not applicable, as no agreed guideline is available
Previous evaluation:	yes, evaluated and accepted DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The study investigated the influence of the test item M-01 (AE C653711, 2,6-dichlorobenzamide) on growth of pure cultures of a soil fungus, *Suillus granulatus* (Basidiomycetes), on malt agar mixed with sterile, silty sand soil. The soil, which served as a carrier for the test substance, and a source of micro-nutrients for the fungus, was treated with the test item to receive concentrations of 0.3, 1.5, 3.0, 7.5, 15.0 and 30.0 mg p.m./kg dry weight soil. Controls contained malt agar and untreated, sterile soil. Colonies were grown in the dark at 20 ± 2 °C. Three replicate dishes were prepared per test concentration. The percent of inhibition was determined after 35 days. No significant differences in growth between treated and control samples were detected. The effects were less than 25 %. Therefore, the NOEC was ≥ 30.0 mg p.m./kg dry weight soil and the EC_{50} was > 30.0 mg p.m./kg dry weight soil.

I. Materials and Methods

Test item: M-01 (AE C653711), chemical name: 2,6-dichlorobenzamide; batch/FL no.:8808018; content of a.s. analysed: 97.0 %; development code of a.s.: AE C653711 00 1B97 0001; development no.: 3000325964.

Test design: Small pieces of agar (4 mm in diameter) containing mycelium from an actively growing *Suillus granulatus* culture were used to inoculate the centers of petri dishes containing nutrient medium prepared by mixing malt agar with sterile, AE C653711-treated, silty sand soil (0.6 % org. C, pH 5.3).

The soil, which served as a carrier for the test substance, and a source of micro-nutrients for the fungus, was treated with the test item to receive concentrations of 0.3, 1.5, 3.0, 7.5, 15.0 and 30.0 mg p.m./kg dry weight soil. Controls contained malt agar and untreated, sterile soil. Colonies were grown in the dark at 20 ± 2 °C. Three replicate dishes were prepared per test concentration. Percent inhibition of growth was determined after 35 days by comparison of mean diameters (+ std. deviation) of treated colonies to mean diameters of the untreated controls.

II. RESULTS AND DISCUSSION

Effect of M-01 (AE C653711) on growth of pure cultures of *Suillus granulatus* on nutrient medium

Concentration tested (mg p.m./kg dry wt soil)	Mean colony diameters + standard deviation (cm) from 3 replicates	Inhibition of growth in % of untreated control
0	2.6 ± 0.1°	0
0.3	2.5 ± 0.2	4
1.5	2.5 ± 0.2	4
3.0	2.5 ± 0.0	4
7.5	2.6 ± 0.1	0
15.0	2.6 ± 0.0	0
30.0	2.5 ± 0.1	4

No significant differences in growth between treated and control samples were detected. The effects were less than 25 %. Therefore, the NOEC was ≥ 30.0 mg p.m./kg dry weight soil and the EC_{50} was > 30.0 mg p.m./kg dry weight soil.

Validity criteria:

Internationally agreed and accepted guidelines for determining the influence of test items on pure cultures of soil fungi could not be found in the literature. Therefore, validity criteria were not defined.

III. CONCLUSION

The endpoints are:

NOEC ≥ 30.0 mg p.m./kg dry weight soil

EC_{50} > 30.0 mg p.m./kg dry weight soil.

Assessment and conclusion by applicant:

The study is considered reliable. However as this study is not a data requirement it will not be further considered in the risk assessment.

Data Point:	KCA 8.7/13
Report Author:	[redacted]
Report Year:	2003
Report Title:	AE C638206 SC 480: Effects on soil litter degradation
Report No:	C038792
Document No:	M-225764-01-1
Guideline(s) followed in study:	--
Deviations from current test guideline:	Method: none Study: Current Guideline: OECD Guidance Document No 56 (2006) No deviations
Previous evaluation:	yes, evaluated and accepted in DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The study investigated the influence of the test item Fluopicolide SC 480 on soil litter degradation. Therefore, six plots in the field Am Hohenseh 410 of Bayer Experimental Parm Höfchen Burscheid, Germany were treated at 24th of March at an application rate of 106.8 g test item/ha corresponding to 42.61 g a.s./ha. Six plots served as untreated control plots. All plots measured 9 × 9 m = 81 m². By careful harrowing the test item was incorporated into the upper 10 cm soil layer to achieve a plateau concentration of 28.4 µg a.s./kg soil dry weight in 0-10 cm soil depth. On the same day untreated summer barley, variety 'Scarlett', was sown onto all plots. On the 7th of April 2003, 40 litter bags (12 cm × 22 cm, mesh size 8 mm) filled with 4 g of dry straw each were buried per plot. Irrigation with 10 mm of water was performed on the 9th of April 2003. Soil samples were taken on the 25th of March 2003 one day after application and incorporation of the plateau concentration. The application of the rate representing the plateau concentration of fluopicolide resulted in soil residues of 185.5 µg fluopicolide/kg dry soil, which is 114.7% of the nominal amount of 28.4 µg/kg dry soil. The degradation of the straw was determined for the time periods of 0 – 29, 0 – 92 and 0 – 184 days by recording the weight of undegraded straw. The results of this study show that at no sampling time (29, 92 and 184 days after introduction of litter-bags into the soil) a statistically significant difference in proportion of straw degradation could be observed between untreated control plots and the plots treated with Fluopicolide SC 480. From the results of this study it can be concluded that residues of Fluopicolide SC 480 in soil even after long-term use (plateau concentration) have no influence on organic matter breakdown after 1, 3 and 6 months.

Objective:

This study was designed to evaluate the influence of Fluopicolide SC 480 on soil litter degradation.

I. MATERIAL AND METHODS

Test item: The study was conducted using Fluopicolide SC 480, Batch-No.: OP220233, development No.: 3000315241, TADS-No.: TADS14510, density: 1.216 g/cm^3, content of fluopicolide: 485 g/L, purity of fluopicolide: 99.3%.
Six plots in the field Am Hohenseh 410 of Bayer Experimental Farm Höfchen Burscheid, Germany were treated at 24th of March at an application rate of 106.8 g test item/ha corresponding to 42.61 g a.s./ha. Six plots served as untreated control plots. All plots measured 9 × 9 m = 81 m^2. By careful harrowing the test item was incorporated into the upper 10 cm soil layer to achieve a plateau concentration of 28.4 µg a.s./kg soil dry weight in 0-10 cm soil depth. On the same day untreated summer barley, variety 'Scarlett', was sown onto all plots. The seed rate was 166 kg/ha. On the 7th of April 2003, 40 litter bags (12 cm × 22 cm, mesh size 8 mm) filled with 4 g of dry straw each were buried per plot. Irrigation with 10 mm of water was performed on the 9th of April 2003. Soil samples were taken on the 25th of March, 2003 one day after application and incorporation of the plateau concentration. The application of the rate representing the plateau concentration of fluopicolide resulted in soil residues of 185.5 µg fluopicolide/kg dry soil, which is 114.7% of the nominal amount of 28.4 µg/kg dry soil. The degradation of the straw was determined for the time periods of 0 – 29, 0 – 92 and 0 – 184 days by recording the weight of undegraded straw. Day 0 was set on April 07, 2003 when litter bags had been buried. Calculating the difference of the weight of straw at the start of the experiment and the remaining weight at sampling time allowed determination of the degree of degradation. Data were statistically analysed by Student-t-Test (two sided, α = 0.05).

Dates of experimental work: March 24, 2003 – October 08, 2003.

II. RESULTS AND DISCUSSION

Validity criteria:

Validity Criteria according to OECD Guidance Document No 56 (2006)	**Obtained**
Degradation of straw after 6 months ≥ 60% in control	Yes
Coefficient of variation ≤ 40% for the data generated within the first 6 months for soil litter degradation of the control	Yes

Means of 6 plots	**Control**	**Fluopicolide SC 480**	**% of Control**
0-29 d*			
g straw degraded	0.77	0.78	**100.7**
% straw degraded	19.34	19.48	
0-92 d*			
g straw degraded	2.17	2.05	**94.5**
% straw degraded	54.14	51.18	
0-184 d*			
g straw degraded	3.72	3.68	**98.9**
% straw degraded	93.03	91.92	

*day 0 was set on April 07, 2003 when litter bags had been buried

The results of this study show that at no sampling time (29, 92 and 184 days after introduction of litter-bags into the soil) a statistically significant difference in proportion of straw degradation could be observed between untreated control plots and the plots treated with Fluopicolide SC 480.

Full details and acceptable validation data to support the analytical method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4 with minor acceptable exceptions only.

III. CONCLUSION

From the results of this study it can be concluded that residues of Fluopicolide SC 480 in soil even after long-term use (plateau concentration) have no influence on organic matter breakdown after 1, 3 and 6 months.

Assessment and conclusion by applicant:

The study is considered reliable. However, as litterbag studies are not required anymore, this study is not further considered in the risk assessment.

Data Point:	KCA 8.7/14
Report Author:	[redacted]
Report Year:	2004
Report Title:	AE C638206-BAM (AE C653711): Effects on soil litter degradation
Report No:	C039658
Document No:	M-227095-01-1
Guideline(s) followed in study:	--
Deviations from current test guideline:	Method: none Study: Current Guideline: OECD Guidance Document No 56 (2006) No deviations
Previous evaluation:	yes, evaluated and accepted in DAR (2008)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The study investigated the influence of the test item M-01 (AE C653711) on soil litter degradation. Therefore, six plots in the field Amhohenseh 410 of Bayer Experimental Farm Höfchen Burscheid, Germany were treated at 24th of March at an application rate of 18.71 g test item/ha corresponding to 18.15 g p.m./ha. Six plots served as untreated control plots. All plots measured 9 × 9 m = 81 m^2. By careful harrowing the test item was incorporated into the upper 10 cm soil layer to achieve a plateau concentration of 12.1 µg a.s./kg soil dry weight in 0-10 cm soil depth. On the same day untreated summer barley, variety 'Scarlett', was sown onto all plots. On the 7th of April 2003, 40 litter bags (12 cm × 22 cm, mesh size 8 mm) filled with 4 g of dry straw each were buried per plot. Irrigation with 10 mm of water was performed on the 9th of April 2003. Soil samples were taken on the 25th of March 2003 one day after application and incorporation of the plateau concentration. The application of the rate representing the plateau concentration of M-01 (AE C653711) resulted in soil residues of 9.73 µg fluopicolide/kg dry soil, which is 80.4% of the nominal amount of 12.1 µg/kg dry soil. The degradation of the straw was determined for the time periods of 0 – 29, 0 – 92 and 0 – 184 days by recording the weight of undegraded straw. The results of this study show that at no sampling time (29, 92 and 184

days after introduction of litter-bags into the soil) a statistically significant difference in proportion of straw degradation could be observed between untreated control plots and the plots treated with M-01 (AE C653711). From the results of this study it can be concluded that residues of M-01 (AE C653711) in soil even after long-term use (plateau concentration) have no influence on organic matter breakdown after 1, 3 and 6 months.

Objective:

This study was designed to evaluate the influence of M-01 (AE C653711) on soil litter degradation.

I. MATERIAL AND METHODS

Test item: The study was conducted using M-01 (AE C653711), Batch-No.: 8808018, development No.: 3000325964, purity: 97.0%.
Six plots in the field Amhohenseh 410 of Bayer Experimental Farm Höfchen Burscheid, Germany were treated at 24th of March at an application rate of 18.71 g test item/ha corresponding to 18.15 g p.m./ha. Six plots served as untreated control plots. All plots measured 9 × 9 m = 81 m^2. By careful harrowing the test item was incorporated into the upper 10 cm soil layer to achieve a plateau concentration of 12.1 µg a.s./kg soil dry weight in 0-10 cm soil depth. On the same day untreated summer barley, variety 'Scarlett', was sown onto all plots. The seed rate was 166 kg/ha. On the 7th of April 2003, 40 litter bags (12 cm × 22 cm, mesh size 8 mm) filled with 4 g of dry straw each were buried per plot. Irrigation with 10 mm of water was performed on the 9th of April 2003. Soil samples were taken on the 25th of March, 2003 one day after application and incorporation of the plateau concentration. The application of the rate representing the plateau concentration of M-01 (AE C653711) resulted in soil residues of 9.73 µg/kg dry soil, which is 80.4% of the nominal amount of 12.1 µg/kg dry soil. The degradation of the straw was determined for the time periods of 0 – 29, 0 – 92 and 0 – 184 days by recording the weight of undegraded straw. Day 0 was set on April 07, 2003 when litter bags had been buried. Calculating the difference of the weight of straw at the start of the experiment and the remaining weight at sampling time allowed determination of the degree of degradation. Data were statistically analysed by Student-t-Test (two sided, $\alpha = 0.05$).

Dates of experimental work: March 24, 2003 – October 24, 2003.

II. RESULTS AND DISCUSSION

Validity criteria:

Validity Criteria according to OECD Guidance Document No 56 (2006)	Obtained
Degradation of straw after 6 months ≥ 60% in control	Yes
Coefficient of variation ≤ 40% for the data generated within the first 6 months for soil litter degradation of the control	Yes

Means of 6 plots	Control	M-01 (AE C653711)	% of Control
0-29 d*			
g straw degraded	0.77	0.78	**101.2**
% straw degraded	19.34	19.58	
0-92 d*			
g straw degraded	2.17	2.06	**95.0**
% straw degraded	54.14	51.43	
0-184 d*			
g straw degraded	3.72	3.71	**99.8**
% straw degraded	93.03	92.81	

*day 0 was set on April 07, 2003 when litter bags had been buried

The results of this study show that at no sampling time (29, 92 and 184 days after introduction of litter-bags into the soil) a statistically significant difference in proportion of straw degradation could be observed between untreated control plots and the plots treated with M-01 (AE C653711).

Full details and acceptable validation data to support the analytical method are presented within document M-CA 4 which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

III. CONCLUSION

From the results of this study it can be concluded that residues of M-01 (AE C653711) in soil even after long-term use (plateau concentration) have no influence on organic matter breakdown after 1, 3 and 6 months.

Assessment and conclusion by applicant:

The study is considered reliable. However, as litterbag studies are not required anymore, this study is not further considered in the risk assessment.

CA 8.8 Effects on biological methods for sewage treatment

Data Point:	KCA 8.8/01
Report Author:	[redacted]
Report Year:	2002
Report Title:	Activated Sludge, Respiration Inhibition Test of AE C638206 Technical
Report No:	B003666
Document No:	M-240681-01-1
Guideline(s) followed in study:	OECD 209 (1993)
Deviations from current test guideline:	Current Guideline: OECD 209 (2010) The test was conducted as a range-finder only with less replicates per concentrations but more concentrations of the test substance. Nitrification respiration was not checked in the test. However, there was no need to differenciate heterotrophic and nitrification respirations in this test since there was no significant inhibition of total respiration. The duration of the test was 30 minutes, whereas the new guideline recommends a duration of 3 hours. The suspended solids concentration was 3.8 g/L instead of 1.5 g/L in the new version of the guideline. These deviations are not considered to invalidate the conclusion of the test since the validity criteria are all met. The combination of the higher concentration of activated sludge and shorter test duration produced a control oxygen uptake high enough to reveal an inhibition by the test item if there were any.
Previous evaluation:	yes, evaluated and accepted in DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Data Point:	KCA 8.8/02
Report Author:	[redacted]
Report Year:	2018
Report Title:	Statement - Certificate of analysis for fluopicolide study on activated sludge respiration inhibition (Heim, 2001; M-240681-01-1)
Report No:	M-634699-01-1
Document No:	M-634699-01-1
Guideline(s) followed in study:	--
Deviations from current test guideline:	Not applicable
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	Yes

Executive Summary

The objective of this study was to evaluate fluopicolide technical for its potential inhibitory effects on microbial respiration in an activated sludge suspension. The respiration rate of an activated sludge inoculum in a synthetic sewage suspension, after aerating for 30 minutes in the presence of fluopicolide technical, was compared to the respiration rate of an activated sludge inoculum in a synthetic sewage suspension to which no test substance was added. A range-finding test was conducted at fluopicolide technical concentrations of 1.4, 3.3, 6.4, 12.0, and 25.4 mg a.s./L in order to provide a range of treatments. A reference substance 3,5-dichlorophenol, a known microbial inhibitor was tested to verify normal sensitivity of the microbial population. The two control flasks containing an activated sludge and synthetic feed mixture with no fluopicolide technical exhibited respiration rates within 15% of each other. Exposure of the activated sludge and synthetic feed mixture to the reference substance resulted in percent inhibition values of 22.8, 57.2, and 85.9% for the 3.2, 10, and 32 mg/L treatments, respectively. The corresponding EC_{50} is 8.1 mg/L. The respiration rate of the abiotic control, 12.7 mg O_2 / L × h indicated that oxygen uptake by fluopicolide technical was 16% of the mean respiration rate of the controls, 78.4 mg O_2 / L × h. Inhibition of the respiration rate was 14.7, 0.3, 31.5, 11.2, and 13.2% for the 1.4, 3.3, 6.4, 12.0, 25.4 mg a.s./L treatments, respectively. Because respiration rate reduction was < 50% in all fluopicolide technical treatments, an EC_{50} value could not be calculated and was estimated to be > 25.4 mg/L, the highest concentration tested. A definitive test was not conducted since the inhibition at the highest concentration tested was < 50%.

I. Material and Methods:

Test item: Fluopicolide technical, Batch code: AE C638206 001C990005; Batch No: 2050190/PP241024/2; purity 97.1% w/w.

The activated sludge was exposed to fluopicolide technical at different concentrations (1.4, 3.3, 6.4, 12.0 and 25.4 mg a.s./L) in a range-finding test. Two control flasks containing an activated sludge and synthetic feed mixture with no substance added were also tested. An abiotic control was dosed with fluopicolide at 25.2 mg a.s./L during the test and was used to measure chemical oxygen uptake. No inoculum was added to this flask. A reference substance 3,5-dichlorophenol, a known microbial inhibitor, was tested to verify normal sensitivity of the microbial population. Reference substance concentrations of 3.2, 10 and 32 mg/L were tested. The respiration rate of each mixture was determined after a 30-minute contact period with permanent aeration.

The temperature of the environmental chamber ranged from 19.2 to 19.8°C during the test. The pH of the 500 mg/L reference substance stock solution and the activated sludge inoculum were measured to be 7.97 and 8.11, respectively, before use in the contact flask solutions. The pH values for the contact flask solution taken after the 30-minute contact ranged from 8.2 to 8.54.

II. RESULTS AND DISCUSSION:

Validity Criteria

The test was performed according to the OECD guideline 209 published in 1984, the corresponding validity criteria are met. The validity criteria of the updated guideline (2010) are the following:

Validity criteria (OECD 209, 2010)	Required	Observed
Oxygen uptake in blank controls	≥ 20 mg O_2 /g suspended solid/h	78.4 mg O_2/L h at 3.8 g suspended solid/L so 20.6 mg O_2 /g suspended solid/h
Coefficient of variation of oxygen uptake in the blank control replicates at the end of the definitive test	≤ 30%	Within a 15% range
EC_{50} for 3.5-DCP for total respiration	within 2 to 25 mg/L	8.1 mg/L

Analytical Findings:

The test item and reference compound concentrations were not confirmed by analytical methods, they were based on nominal concentrations.

Biological Findings:

Percent inhibition values for the test substance treatments in the range-finding test ranged from 0.3 to 31.5% and showed no dependence through the range of concentrations tested (1.5 to 25 mg a.s./L). Based on these results, an EC_{50} value for fluopicolide could not be determined and a definitive test was not performed. The respiration rate of the abiotic control, 12.7 mg O_2 / L × h, indicated that oxygen uptake by fluopicolide was 16% of the mean respiration rate of the controls, 78.4 mg O_2 / L × h.
The EC_{50} for 3.5-DCP is 8.1 mg/L.

Since fluopicolide did not produce significant inhibition of oxygen respiration in the range-finding test, no definitive test was performed.

Respiration rates and percent inhibition values for fluopicolide and 3,5-Dichlorophenol

Treatment [mg a.s./L]	Respiration rate [mg O_2 / L × h]	% Inhibition
Control 1	80.9	-
Control 2	75.8	-
Control, mean	**78.4**	-
1.4 mg a.s./L test item	66.8	14.7
3.3 mg a.s./L test item	78.1	0.3
6.4 mg a.s./L test item	53.7	31.5
12.0 mg a.s./L test item	69.6	11.2
25.4 mg a.s./L test item	68.0	13.2
25.2 mg a.s./L test item (abiotic control)	12.7	-
3.2 mg/L reference item	60.5	22.8
10 mg/L reference item	33.6	57.2
32 mg/L reference item	11.0	85.9

III. CONCLUSION:

Under the conditions of the test, the EC_{50} of fluopicolide is clearly in excess of 25.4 mg a.s./L, the highest tested concentration due to solubility limit.

Assessment and conclusion by applicant:

This test was conducted as a range-finder with concentrations up to the solubility limit. All validity criteria of the latest OECD guideline are met. The results show that fluopicolide does not significantly inhibit the activated sludge respiration.

CA 8.9 Monitoring data

No ecological monitoring studies were conducted. For monitoring of fluopicolide in the environment please refer to the Summary MCA Section 7, Point 7.5.