Document Title

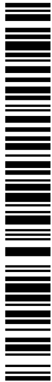
1st amendment of

Summary of the ecotoxicological studies for Fluopyram

Data Requirement(s)

Regulation (EC) No 1107/2009 & Regulation (EU) No 283/2013
Document MCA
Section 8: Ecotoxicological studies – Part 1

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



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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
2021-03-15	Original MCA as submitted by applicant	M-766284-01-1
2021-07-05	Addition of reliability assessments for aquatic organisms and update of aquatic macrophyte endpoint. All additions by the applicant have been highlighted in yellow colour	M-766284-02-1

[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

Fluopyram was included in Annex I to Council Directive 91/414/EEC in 2013 (Regulation (EU) No. 802/2013, Entry into Force on August 22, 2013). This Supplementary Dossier contains only data which were not submitted at the time of the Annex I inclusion of Fluopyram 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.

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.

The document MCA Section 8 summarizes all ecotoxicological data and classification proposal, which are relevant for the approval of Fluopyram and the proposed intended uses, including the representative uses, under Regulation (EC) No 1107/2009 in accordance with the requirements laid down in the Commission Regulation (EU) No 283/2013 and under Classification Regulation (EC) No 1272/2008.

The ecotoxicology MCA was split into two parts, of which this document represents Part 1.

This Part 1 comprises of the sections CA 8 to CA 8.8, while CA 8.9 is presented in Part 2.

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CA 8.1 Effects on birds and other terrestrial vertebrates

CA 8.1.1 Effects on Birds

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

Table 8.1.1- 1: Ecotoxicological endpoints – toxicity studies with birds exposed to fluopyram

Test substance	Test design	Test species	Endpoint		Reference
Fluopyram tech.	Acute oral toxicity	Bobwhite quail (*Colinus virginianus*)	LD_{50}	> 2000 mg a.s./kg bw	[redacted] (2014) M-263049-04-1 KCA 8.1.1.1/01
	Acute oral toxicity	Zebra finch (*Taeniopygia guttata*)	LD_{50}	> 2000 mg a.s./kg bw	[redacted] (2008) M-307871-02-1 KCA 8.1.1.1/02
			LD_{50}	= 3036 mg a.s./kg bw [A]	Extrapolated acc. to chapter 2.1.2 of EFSA Journal 2009; 7(12):1438
	Acute oral toxicity	Chicken (*Gallus domesticus*)	LD_{50}	> 5000 mg a.s./kg bw	[redacted] (2011) M-446344-01-1 KCA 8.1.1.1/03
	Dietary toxicity (short-term)	Bobwhite quail (*Colinus virginianus*)	LC_{50} LDD_{50}	> 5000 mg a.s./kg feed > 1845.4 mg a.s./kg bw/d	[redacted] (2007) M-264902-02-1 KCA 8.1.1.2/01
	Dietary toxicity (short-term)	Mallard duck (*Anas platyrhynchos*)	LC_{50} LDD_{50}	> 5000 mg a.s./kg feed > 1643 mg a.s./kg bw/d	[redacted] (2005) M-262710-01-1 KCA 8.1.1.2/02
	20-weeks feeding chronic, reproduction	Bobwhite quail (*Colinus virginianus*)	NOEC NOED	< 250 mg a.s./kg feed < 23 mg a.s./kg bw/d	[redacted] (2008) M-299245-02-1 KCA 8.1.1.3/01
	22-weeks feeding chronic, reproduction	Bobwhite quail (*Colinus virginianus*)	NOAEC NOAED NOEC NOED	80 mg a.s./kg feed 7.2 mg a.s./kg bw/d 50 mg a.s./kg feed 4.5 mg a.s./kg bw/d	[redacted]. (2008) M-298723-01-1 KCA 8.1.1.3/02
	19-weeks feeding chronic, reproduction	Mallard duck (*Anas platyrhynchos*)	NOEC NOED NOEC NOED	500 mg a.s./kg feed 40 mg a.s./kg bw/d 200 mg a.s./kg kg feed 18 mg a.s./kg bw/d	[redacted] (2008) M-299277-01-1 KCA 8.1.1.3/03 DAR
	Chronic, reproduction: EC_{10} calculation	Bobwhite quail (*Colinus virginianus*) – both chronic	Lowest EC_{10}	7.8 mg a.s./kg bw/d (14-day survivors per eggs set)	[redacted] (2019) M-667209-01-1 KCA 8.1.1.3/04

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Test substance	Test design	Test species	Endpoint		Reference
		studies combined			
	Chronic, reproduction: EC_{10} calculation	Mallard duck (*Anas platyrhynchos*)	EC_{10}	78.6 mg a.s./kg bw/d (eggs laid per hen)	[redacted] (2019) M-667211-01-1 KCA 8.1.1.3/05

a.s. = active substance
A Factor 1.518 for 10 birds/dose level with a single mortality (study result: 12 individuals and 1 mortality)

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CA 8.1.1.1 Acute oral toxicity to birds

Active substance fluopyram

Data Point:	KCA 8.1.1.1/01
Report Author:	
Report Year:	2011
Report Title:	Acute oral toxicity for bobwhite quail (Colinus virginianus) with AE C656948 techn. a.s.
Report No:	BAR/LD 074
Document No:	M-263049-04-1
Guideline(s) followed in study:	Commission Directive 96/46EC of 16 July 1996 amending Council Directive 91/414EEC Concerning the Placing of Plant Protection Compounds on the Market Equivalent to US EPA OPPTS Guideline No. 850.2000
Deviations from current test guideline:	Current Guideline: OECD 223 (2016) Deviations: This study was conducted before the current guideline was adopted in 2016. Instead of one limit dose three dose levels were used. The birds were housed with 2 individuals per cage and not individually as recommended in OECD guideline 223. As neither regurgitation nor any signs of intoxication were observed for any of the birds following dosing this deviation was not considered relevant. The space available for each bird in the pen was about 475 cm2, and thus below the 1000 cm2 recommended in the guideline. Birds were held at a temperature of 12.8 to 26.5 °C, below the recommended range of 15-27 °C. Weighing of birds was not performed on day 3 as recommended by the guideline. These deviations are not expected to have an impact on the study results. All validity criteria were met.
Previous evaluation:	yes, evaluated and accepted in DAR (2011)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

Fluopyram technical was administered orally to 10 adult bobwhite quails (5 males and 5 females) at dose levels of 0, 500, 1000, and 2000 mg a.s./kg bw. Birds were held at a temperature of 12.8 to 26.5 °C with a relative humidity of 20.8 to 57.8 % and approximately 14 hours light per day. The 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.

After application the birds of the highest treatment group (2000 mg a.s./kg body weight) refused food consumption almost completely which caused severe body weight loss and mortality in four cases (3 males, 1 female) combined with extreme emaciation. In the second week the survivors started to eat again and recovered until test termination. Reduced food consumption to a less severe degree occurred also in the two lower treatment groups. One bird of each of these groups died with signs of emaciation, while all the other recovered completely. No pathological changes were found at necropsy of the survivors.

The study fulfilled all validity criteria of OECD 223 guideline.

The acute oral LD_{50} for bobwhite quail was > 2000 mg a.s./kg bw (extrapolated with probit analysis to 3119 mg a.s./kg bw) and the NOAED was < 500 mg a.s./kg bw.

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I. MATERIAL AND METHODS

Test item: fluopyram, specification No.: 102000012455; Batch No.: 08528/0002; Sample identification: TOX 06970-00; purity: 94.5 % w/w.

Test design: Bobwhite quail (estimated ca. 25 weeks old) were orally dosed with fluopyram at 0, 500, 1000, and 2000 mg a.s./kg bw (adjusted for purity). The test substance was placed in capsules based on treatment level and body weight and administered to the birds. Control birds received empty gelatine capsules. Each dosage group comprised 10 birds which were assigned to 5 cages. One cage contained one male and one female bird. Each cage had floor space that measured approximately 38 × 25 cm with a ceiling of 24 cm.

Birds were acclimatized for approximately 15 days prior to being randomized into test groups. At the start of acclimation, the quail were well developed and similar to birds from wild population. When determining body weight on day -1, one bird at 500 mg a.s./kw bw level was found in a physically weak condition, probably due to difficulties in acclimation. It was removed and replaced by a bird from the control group. The control group consisted therefore at the time of application only of 9 birds. Only birds that appeared healthy were used for the study. Water and feed were provided ad libitum during acclimation and during the test, except during periods of fasting prior to testing. Birds were starved for 16 hours prior to oral administration. Birds were held at a temperature of 12.8 to 26.5 °C with a relative humidity of 20.8 to 57.8 %. The photoperiod was 8 hours of light per day during acclimation and throughout the test.

Observations on mortality and signs of intoxication were made continuously during the first hour and hourly up to 8 hours on the day of dosing and at least once work-daily throughout the 14 days observation period. Body weights were recorded prior to test initiation (day -1), on day 7 and day 14 after test start. Food consumption was determined by pen for each dosage group and control group for days 0-3, 3-7 and 7-14.

Statistics: The Probit-Test (Toxcalc version 1.0) was used to calculate the LD_{50}.

Dates of experimental work: February 28th to March 29th, 2005.

II. RESULTS AND DISCUSSION

Validity criteria:

Table 8.1.1.1- 1: Validity criteria (according to OECD 223, adopted 26 July 2016)

Validity criteria	Required	Obtained
Control mortality	≤ 10 %	0 %

Mortality and clinical observations:

After application the birds of the highest treatment group (2000 mg a.s./kg body weight) refused food consumption almost completely which caused severe body weight loss and mortality of 4 birds (3 males, 1 female) combined with extreme emaciation. In the second week the survivors started to eat again and recovered until test termination. Reduced food consumption to a less severe degree occurred also in the two lower treatment groups. One bird of each of these groups died with signs of emaciation, while all the other recovered completely.

At test start in the lowest treatment group (500 mg a.s./kg bw), 7 of the 10 birds showed symptoms including diarrhoea, soft excrement or ptosis. By day 6 all symptoms had disappeared though on day 8 one male bird died (cage 19) after soft excrement occurred the previous day. This bird has been emaciated.

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The second highest treatment group (1000 mg a.s./kg bw) group showed more symptoms which occurred for a longer time period. The symptoms displayed include diarrhoea, soft excrement, apathy, ptosis, fluffed feathers and one mortality. One mortality occurred on day 13 of the study (cage 15). On day 14 of the study symptoms ceased except in one case (diarrhoea).

The highest treatment group (2000 mg a.s./kg bw) showed the highest number of symptoms, including diarrhoea, soft excrement, apathy, ptosis, fluffed feathers, red excrements, reduced vigilance and four fatalities. On day 9, one bird died (cage 9) being severely emaciated, another one was sacrificed for that reason. On day 10 (cage 8) and on day 12 (cage 7) one further bird died prematurely, both extremely emaciated. By day 14 all symptoms ceased in the remaining 6 birds. Death had occurred to the other 4 birds.

Table 8.1.1.1- 2: Summary of mortalities and clinical symptoms

Treatment level [mg a.s./kg bw]	Overall mortality (females and males)	Number dosed	Clinical symptoms (type)
Control	0	9 [A]	diarrhoea
500	1	10	soft excrement, diarrhoea, ptosis
1000	1	10	diarrhoea, soft excrement, fluffed feathers, ptosis, apathy, red coloured excrement, reduced vigilance, excretion of uric acid
2000	4	10	diarrhoea, apathy, fluffed feathers, ptosis, reduced vigilance, red coloured excrement, soft excrement

[A] One day prior to study initiation (at initial weighing on day -1), one bird from the 500 mg a.s./kg bw level was replaced by a bird from the control group, resulting in 9 control birds

Body weight and feed consumption:

Table 8.1.1.1- 3: Mean body weight of surviving birds

Treatment level [mg a.s./kg bw]	Mean body weight ± S.D. [g]					
	Females			Males		
	Day -1	Day 7	Day 14	Day -1	Day 7	Day 14
Control	199.8 ± 21.3	198.2 ± 17.6	201.4 ± 20.5	202.8 ± 31.6	205.0 ± 25.8	208.3 ± 20.8
500	189.8 ± 6.6	187.0 ± 13.2	192.8 ± 6.0	197.0 ± 21.6	195.8 ± 24.2	204.5 ± 18.1
1000	203.2 ± 17.9	190.2 ± 15.1	184.4 ± 10.1	209.6 ± 14.4	206.2 ± 16.8	213.5 ± 16.9
2000	199.6 ± 6.3	143.4 ± 17.7	166.0 ± 16.6	198.8 ± 11.7	137.8 ± 30.9	182.0 ± 8.5

S.D.: Standard deviation

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Table 8.1.1.1- 4: Mean food consumption

Treatment level [mg a.s./kg bw]	Mean food consumption [g/bird/d]		
	Day 0 - 3	**Day 3 - 7**	**Day 7 - 14**
Control	12.4	13.2	11.9
500	8.8	11.4	12.5
1000	3.4	4.6	11.0
2000	3.8	1.2	9.3

Gross pathology:

No severe changes were found with the survivors, while the prematurely dead birds showed apparent signs of emaciation (e.g. of weight loss or reduction of organs).

Biological findings:

Table 8.1.1.1- 5: Acute oral toxicity to Bobwhite Quail

Test substance	Fluopyram
Test object	Bobwhite Quail (male, female)
LD_{50} [mg a.s./kg bw]	> 2000 extrapolated to 3119 [A]
No observed adverse effect dose (NOAED) [mg a.s./kg bw]	< 500

[A] Calculated by probit methods

III. CONCLUSION

Based on this study the LD_{50} value for bobwhite quail exposed to fluopyram was determined to be > 2000 mg a.s./kg bw (extrapolated with probit analysis to 3119 mg a.s./kg bw).

The NOAED was < 500 mg a.s./kg bw.

Assessment and conclusion by applicant:

The study and its data are considered as acceptable and reliable for use in risk assessment.

The endpoint is:

LD_{50} > 2000 mg a.s./kg bw

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Data Point:	KCA 8.1.1.1/02
Report Author:	[redacted]
Report Year:	2008
Report Title:	AE C656948 - Acute oral toxicity test (LD50) with the zebra finch (Taeniopygia guttata) following OECD draft guideline 223
Report No:	EBGMP117-1
Document No:	M-307871-02-1
Guideline(s) followed in study:	OECD Draft Guideline 223 U.S. EPA OPPTS 850.2100
Deviations from current test guideline:	Current Guideline: OECD 223 (2016) Deviations: The photoperiod was 10 hours light, above the 8 hours light as recommended. No information on medication reported for 14 days prior to test start. Weighing of birds was not performed on day 3 as recommended by the guideline. These deviations are not expected to have an impact on the study results. All validity criteria were met.
Previous evaluation:	yes, evaluated and accepted in Addendum 3 to the DAR (2011)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive summary

Fluopyram technical was administered orally to 6 Zebra Finches (3 male and 3 female) at the limit dose of 2000 mg a.s./kg bw. Birds were held at a temperature of 15-25 °C with a humidity of 45-70 % and 10 hours light per day. Birds were observed for 14 days for mortality and sublethal symptoms. This study was conducted as two consecutive 14-day limit tests. Since one death was observed in the first limit test, and no signs of toxicity were observed in the other birds, six more birds were dosed at the limit dose in a second test. Body weight and average feed consumption were measured for the treatment and the control group. Gross necropsies were performed on all mortalities and on all survivors at test termination. For both limit tests, observations were made for mortality, morbidity and symptoms of intoxication daily until test termination (day 14).

The study fulfils all validity criteria of OECD 223 guideline.

There were no mortalities in the control group and in the 2000 mg a.s./kg bw treatment group in the second limit test. A single mortality was observed in the 2000 mg a.s./kg bw treatment group in the first limit test.

Based on this study the acute oral LD_{50} for zebra finch exposed to fluopyram as a single oral dose was determined to be > 2000 mg a.s./kg bw.

I. Material and Methods

Test item: fluopyram; specification No.: 102000012455; Batch No.: 08528/002; TOX No.: 0793200; purity: 94.7 % w/w.

Test design: Adult Zebra finches (*Taeniopygia guttata*) were orally dosed with fluopyram at the limit dose of 2000 mg a.s./kg bw. Adults zebra finches (*Taeniopygia guttata*) weighing 9.5 - 13.9 g at test initiation (first limit test) and 10.2 - 15.0 g at the initiation of the second limit test were used as test organisms. Test birds were housed indoors by dosage groups in batteries of pens containing one randomly assigned bird. There were 6 birds per treatment which comprised 3 male and 3 female. Each pen had floor space that measured approximately 33 × 48 cm with a ceiling of 31 cm.

This study was conducted as two consecutive 14-day limit tests. Since one death was observed in the treatment group of the first limit test, and no signs of toxicity were observed in the other birds, six more

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birds were dosed at the limit in a second test. Control birds were sham dosed with the same carrier used with the test substance. The test substance was dispersed in corn oil and orally intubated directly into the proventriculus of each bird.

All test birds were acclimated to the study room and test caging prior to test initiation. All zebra finches appeared to be in good health at initiation of the test. The birds were fasted for at least 12 hours prior to dosing at experimental start. Birds were maintained at 15-25 °C and a relative humidity of 45-70 %. The photoperiod was 10 hours of light per day during acclimation and throughout the test. The birds were exposed to an average light intensity of 467 lux (43.3 footcandles).

For both limit tests, following dosing, birds were observed for regurgitation and toxic effects continuously for the first two hours. Following the initial observation period, birds were observed for mortality, morbidity and symptoms of intoxication at 4 additional intervals during the first 24 hours post-dosing (day 0). Observations were conducted at least once daily thereafter until test termination (day 14). Feed consumption was measured in each cage for days 0-7 and 7- 14. Any feed added or replaced during these intervals was also measured. Body weights were measured immediately prior to dosing, at day 7 post-dosing and at test termination (day 14), and upon death if prior to termination.

Gross necropsies were performed on all mortalities and on all survivors at test termination. The gross necropsies included examination of the general physical condition, digestive tract, liver, kidneys, lungs, gall bladder, breast muscles, heart, and spleen.

Dates of experimental work: April 28th to June 5th, 2008.

II. RESULTS AND DISCUSSION

Validity criteria:

Table 8.1.1.1- 6: Validity criteria (according to OECD 223, adopted 26 July 2016)

Validity criteria	Required	Obtained
Control mortality	≤ 10 %	0 %

Clinical observations:

Observations of piloerection, ataxia, lethargy, wing droop, and incoordination were observed in all birds in the 2000 mg a.s./kg body weight group. The single fatality also displayed wing droop, laboured breathing, and lack of responsiveness.

The remaining birds recovered within approximately 29 hours post-dosing during the first limit test. No abnormal physical conditions were noted during the post-mortem examinations conducted at the termination of the first limit test.

Observation of piloerection and incoordination were also observed in the second limit test. All birds in the second limit test recovered within approximately 9 hours post-dosing. No abnormal physical conditions were noted during the post-mortem examinations conducted at the termination of the second limit test.

Mortality and behaviour:

One mortality was noted on day 0 in the single treatment group of the first limit test. This bird died approximately 20 hours post-dosing. No mortalities occurred during the second limit test in the control group, or 2000 mg a.s./kg treatment group.

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Table 8.1.1.1- 7: Summary of mortalities and clinical symptoms

Treatment level [mg a.s./kg bw]	Overall mortality (females and males)	Number dosed	Clinical symptoms (type)
First limit test			
Control	0	6	-
2000	1	6	piloerection, ataxia, lethargy, wing droop, and incoordination, wing droop, laboured breathing, and lack of responsiveness [A]
Second limit test			
Control	0	6	-
2000	0	6	Piloerection and incoordination [B]

[A] All birds recovered within approximately 29 hours post-dosing
[B] All birds recovered within approximately 9 hours post-dosing.

Body weight:

Table 8.1.1.1- 8: Summary of mean body weight

Zebra finch	Mean body weight [g] [A]					
Treatment level [mg a.s./kg bw]	Females			Males		
	Day 0	Day 7	Day 14	Day 0	Day 7	Day 14
First limit test						
Control	12.8	14.1	13.7	11.8	12.7	13.7
2000	11.1	11.7	11.3	12.8	13.6	13.0
Second limit test						
Control	11.3	11.3	11.7	13.6	14.3	13.8
2000	13.4	14.4	14.7	13.1	13.6	13.8

[A] Mean body weights not given in study report. Calculations were performed based on individual body weights of females and males on each assessment day.

Table 8.1.1.1- 9: Mean feed consumption per day

Zebra finch	Mean feed consumption [g/bird/d]			
Treatment level [mg a.s./kg bw]	First limit test		Second limit test	
	Week 1	Week 2	Week 1	Week 2
Control	3.5	3.1	3.1	3.1
2000	2.8	2.9	4.0	3.7

Biological findings:

Table 8.1.1.1- 10: Acute oral toxicity to Zebra Finch

Test substance	Fluopyram
Test object	Zebra Finch (male, female)
LD_{50} [mg a.s./kg bw]	> 2000

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III. CONCLUSION

The acute oral LD_{50} value for zebra finch exposed to fluopyram as a single oral dose was determined to be > 2000 mg a.s./kg bw.

Assessment and conclusion by applicant:

The study and its data are considered as acceptable and reliable for use in risk assessment.

The endpoint is:

LD_{50} > 2000 mg a.s./kg bw. Based on one mortality among 12 dosed birds, the LD_{50} can be extrapolated to 3036 mg a.s./kg bw.

****

Data Point:	KCA 8.1.1.1/03
Report Author:	[redacted]
Report Year:	2011
Report Title:	Acute oral toxicity (MLD) of fluopyram technical to chicken
Report No:	R/11846/ATC/11
Document No:	M-446344-01-1
Guideline(s) followed in study:	not applicable
Deviations from current test guideline:	Current Guideline: OECD 223 (2016) Deviations: A limit dose with 3 birds plus 3 birds for the control were used instead of minimum of 5 organism recommended. No information on random allocation of birds nor on space available for each bird are given in the report. No information on medication prior to test start nor temperature was given in the report. The photoperiod was 12 hours light, above the 10 hours light as recommended in the guideline for other species than quail and mallard. These deviations are not expected to have an impact on the study results. All validity criteria were met.
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

Fluopyram technical was administered orally to 3 chickens (*Gallus domesticus*) at the limit dose of 5000 mg a.s./kg bw. Birds were held at ambient temperature with adequate ventilation and illumination and 12 hours light per day. Birds were observed for 21 days for mortality and sublethal symptoms. Body weight and average feed consumption were measured for the treatment and the control group. Gross necropsies were performed on all survivors at test termination.

The study fulfils all validity criteria of OECD 223 guideline.

No mortality and no clinical signs were observed for the birds in the limit dose of 5000 mg a.s./kg bw and in the control group throughout 21 days observation period.

The acute oral LD_{50} value for chicken exposed to fluopyram as a single oral dose was determined to be > 5000 mg/kg bw.

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I. MATERIAL AND METHODS

Test item: Fluopyram, specification No.: 102000017196; Batch No: AE C656948-01-05; TOX No.: 9244-00; purity: 98.2 %.

Test design: The chickens (*Gallus domesticus*, 9 to 10 weeks old) were single orally dosed with fluopyram (with 0.2 % Tween80) at the limit dose of 5000 mg a.s./kg bw. The control group was administered vehicle alone. Chickens weighing 1531-1762 g were used as test organisms. Chickens were housed individually with 3 chickens per treatment. The administration was done by oral gavage to each bird in a single dose, using a suitably graduated syringe and a stainless steel intubation needle.

All test birds were acclimated for approximately one week prior to test initiation and were starved overnight prior to oral administration. Water and feed were provided *ad libitum* during acclimation and during the test, except during periods of fasting prior to testing. Birds were maintained at ambient temperature with adequate ventilation. The photoperiod was 12 hours of light per day during acclimation and throughout the test.

Dosing was followed by a subsequent observation period of 21 days. Observations on mortality were made at various intervals for 6 hours on day of dosing and then twice a day for 21 days. Body weights were recorded prior to dosing (day 0), on the day of dosing (day1, fasting body weight), on study day 3, 7, 14 and day 21. Food consumption was determined for each dosage group and control group for days 1-3, 3-7, 7-14 and 14-21. At the end of the study all surviving birds were sacrificed and subjected to a complete necropsy.

Dates of experimental work: June 23rd to July 30th, 2011

II. RESULTS AND DISCUSSION

Validity criteria

Table 8.1.1.1- 11: Validity criteria (according to OECD 223, adopted 26 July 2016)

Validity criteria	Required	Obtained
Control mortality	≤ 10 %	0 %

Observations:

Mortality and behaviour:

No mortality and no clinical signs were observed in the control and the single treatment group (5000 mg a.s./kg bw) throughout 21 days observation period.

Table 8.1.1.1- 12: Summary of mortalities and signs of intoxication

Treatment level [mg a.s./kg bw]	Overall mortality	Number dosed	Observed effects
Control	0	3	-
5000	0	3	-

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Body weight development:

Body weight gain was not affected due to treatment and was found to be comparable with that of the control group.

Table 8.1.1.1- 13: Body weight and % body weight gain

Treatment level [mg a.s./kg bw]	Mean bean body weight ± SD [g]					Mean body weight gain [%]			
	Day 0	Day 3	Day 7	Day 14	Day 21	Day 0-3	Day 0-7	Day 0- 14	Day 0-21
Control	1705.00 ± 49.39	1726.33 ± 48.23	1781.00 ± 46.94	1857.33 ± 47.44	2014.67 ± 54.93	1.25	4.46	8.94	18.17
5000	1579.33 ± 50.64	1596.33 ± 50.21	1646.00 ± 49.57	1718.67 ± 51.60	1863.00 ± 57.56	1.08	4.23	8.83	17.96

SD: Standard deviation
Day 0: 1 day prior to dosing

Food consumption:

No effect on food intake was observed in the treated birds at 5000 mg/kg bw as compared to that of vehicle control group birds.

Table 8.1.1.1- 14: Food consumption per day

Treatment level [mg a.s./kg bw]		Food consumption [g/bird/day]					Mean Food consumption [g/bird/day]
		Day 0	Day 1-3	Day 3-7	Day 7-14	Day 14-21	
Control	Average	153.3	158.7	166.8	179.6	202.9	172.25
5000	Average	151.7	155.3	163.3	176.9	198.5	169.13
	% of control	99	98	98	98	98	98

Pathological findings:

No gross pathological changes were encountered in any of the birds sacrificed at the termination of the observation period.

Biological findings:

Table 8.1.1.1- 15: Acute oral toxicity to birds

Test substance	Fluopyram
Test object	Chicken
LD_{50} [mg a.s./kg bw]	> 5000

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III. CONCLUSION

The acute oral LD_{50} value for chicken exposed to fluopyram as a single oral dose was determined to be > 5000 mg a.s./kg bw.

Assessment and conclusion by applicant:

The study is not conducted under GLP but at a certified testing facility, and its data are reported transparently. Therefore the study is considered as acceptable and reliable for use in risk assessment. The endpoint is:

LD_{50} > 5000 mg a.s./kg bw

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CA 8.1.1.2 Short-term dietary toxicity to birds

Active substance fluopyram

Data Point:	KCA 8.1.1.2/01
Report Author:	[redacted]
Report Year:	2007
Report Title:	AE C656948 (tech. a.s.) - 5-day-dietary LC50 for bobwhite quail (Colinus virginianus)
Report No:	BAR/LC 023
Document No:	M-264902-02-1
Guideline(s) followed in study:	U.S.EPA Ecological Effects Test Guidelines, OPPTS 850.2200 Avian Dietary Toxicity Test (April 1996); OECD guideline 205 for testing of chemicals "Avian Dietary Toxicity Test" (April 1984)
Deviations from current test guideline:	Current Guideline: OECD 205 (1984) Deviations: The temperature of the brooding compartment was in the range 31.8-39.2 °C for the chicks at age 0-7 days and in the range 27.9-34.2 °C for the chicks at age 8-14 days, slightly differing from the recommended 35-38 °C and 30-32 °C, respectively. At the age of the chicks >14 days, the temperature was 28.0-32.5 °C, slightly higher than the recommended 25-28 °C. The relative humidity was not reported. These deviations are not expected to have an impact on the study results. All validity criteria were met.
Previous evaluation:	yes, evaluated and accepted in DAR (2011)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive summary

Young Northern Bobwhite chicks (10 days old) were exposed for 5 days to nominal dietary concentrations of 0 (control), 279, 562, 1120, 2338 and 4785 mg fluopyram technical/kg feed. There were 10 chicks (unknown sex) per test concentration and 20 (unknown sex) for the control. Birds were held at a temperature of 27.9 to 39.2 °C and 12 hours light per day. Observations for mortality and signs of intoxication were made at least once daily. Body weights were recorded on day 0 at test initiation, on day 5 and at test termination on day 8. Feed consumption for each level was recorded daily during the exposure, and recovery periods. Gross necropsies were carried out on all prematurely deaths and on all survivors. Samples were collected to verify the test concentrations administered and to confirm the stability and homogeneity of the test substance in the diets.

The study fulfilled all validity criteria of the of OECD 205 guideline.

No mortality and abnormal behaviours occurred. At the end of the exposure period (day 5) the body weight in the highest test concentration (5000 mg a.s./kg feed) was significantly reduced. At test termination on day 8, there was a small significant reduction of the body weight detected in the highest test concentration (5000 mg a.s./kg feed) compared to the control. No clear relationship between treatment and food consumption was found. 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 fluopyram to 12-day old Northern Bobwhite chicks was determined to be > 5000 mg a.s./kg feed, corresponding with an LDD_{50} of > 1845.4 mg a.s./kg bw/d.

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I. MATERIAL AND METHODS

Test item: Fluopyram, specification No.: 102000012455; Batch No.: 08528/0002; Sample code: PBF-2005-0058-TOX-07153; purity: 95.0 %

Test design: Young Bobwhite (*Colinus virginianus*) chicks (10 days old) were exposed to fluopyram for 5 days to nominal dietary concentrations (techn. a.s.) of 0 (control), 313, 625, 1250, 2500 and 5000 mg a.s./kg feed. There were 10 chicks (unknown sex) per test concentration and 20 (unknown sex) for the control which were housed with 10 chicks per cage. Control birds received basal (untreated) feed. Each cage had floor space that measured approximately 100 × 55 cm with a ceiling of 25 cm.

Birds were acclimatized for approximately 7 days prior to the exposure. At the start of acclimation, the chicks were well developed and similar to birds from wild population. Only birds that appeared healthy were used for the study. Water and feed were provided ad libitum during acclimation and during the test. Room temperature was between 27.9 to 39.2 °C during the acclimation and test periods. The photoperiod was maintained at 12 hours of light per day during acclimation and throughout the test.

Observations for mortality and on signs of intoxication were made daily during acclimatization, twice on the first exposure day, continued at least once daily throughout the following study days until terminal sacrifice. Body weights were recorded on day 0 at study initiation, on day 5 and at termination of the test on day 8. Feed consumption was determined by cage for each treatment and control group for days 0-5 and 5-8. Gross necropsies were carried out on all prematurely deaths and on all survivors.

Statistics: For statistical evaluation of possible treatment related effects, the data for testing endpoints were processed as unpaired comparisons of each treatment level with untreated control. Initially the data were analysed on variances homogeneous distribution (Bartlett's test, $p < 0.05$). In case of homogeneous variances, subsequent analyses were conducted using parametric techniques (Bonferroni t-test adjustment); otherwise the Bonferroni Welch t-test adjustment for inhomogeneous variances was used. All described statistical procedures were carried out by using ToxRat Professional (Version 2.09).

Analytics: Samples of the test diets were collected at test start 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 three samples which have been randomly chosen out of the food batch concentrations at 100 and 5000 mg a.s./kg feed (prepared on March 17th, 2005). Samples for stability analysis were taken from the batch concentrations at 100 and 5000 mg a.s./kg feed (prepared on April 14th, 2005) and stored at test conditions for 1 day and deep frozen (-18 °C) for 14 days.

Dates of experimental work: August 16th to August 31st, 2005

II. RESULTS AND DISCUSSION

Validity criteria:

Table 8.1.1.2- 1: Validity criteria (according to OECD 205, adopted 04 April 1984)

Validity Criteria	Required	Obtained
Control mortality	≤ 10 %	0 %
Test concentration maintained over the 5 day exposure period	≥ 80 % of nominal	89 - 96 % of nominal
Treatment related effects in the lowest treatment level	No effects should occur	Fulfilled

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Analytical results:

Samples collected at test start to verify test substance concentrations for the 313, 625, 1250 and 5000 mg a.s./kg feed diets had measured concentrations of 279, 562, 1121, 2338 and 4785 mg a.s./kg feed, respectively. These values represented 89, 90, 90, 94 and 96 % of nominal concentrations, respectively.

No residues of fluopyram were found in the control samples.

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 SANTE/2020/12830, Rev.1.

Table 8.1.1.2- 2: Analysis of diet test

Nominal dietary concentration [mg a.s./kg feed]	Measured dietary concentration Day 0 [mg a.s./kg feed]	% of nominal
313	279	89
625	562	90
1250	1121	90
2500	2338	94
5000	4785	96

Diet samples were collected from the 100 and 5000 ppm a.s. test concentrations and were analysed to evaluate the homogeneity of the test substance in the diet. Measured concentrations for the two test concentrations were 99 mg a.s./kg feed and 5047 mg a.s./kg feed, respectively.

Analysis of diet samples collected from feeders after being held at study conditions for 24 hours revealed 96 and 98 % of the nominal values for the batch concentrations at 100 and 5000 mg a.s./kg feed. Analysis of diet samples collected from feeders after being held in a deep freezer (-18 °C) for 14 days resulted in 95 % and 99 % of the nominal values for the batch concentrations at 100 and 5000 mg a.s./kg feed.

Observations:

Mortality and clinical observations:

No mortality and abnormal behaviours occurred.

Table 8.1.1.2- 3: Summary of mortalities and clinical symptoms

Treatment level [mg a.s./kg feed]	Overall mortality (females and males)			Number dosed	Signs of intoxication (type)
	Day -3 – Day -1 (Pre-exposure)	Day 0 – Day 4 (Exposure)	Day 5 – Day 7 (Post exposure)		
Control	0	0	0	20	-
313	0	0	0	10	-
625	0	0	0	10	-
1250	0	0	0	10	-
2500	0	0	0	10	-
5000	0	0	0	10	-

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Body weight and feed consumption:

At the end of the exposure period the body weight in the highest test concentration (5000 mg a.s./kg feed) was significantly reduced. At test termination there was a small significant reduction of the body weight detected in the highest test concentration (5000 mg a.s./kg feed) compared to the control.

Table 8.1.1.2- 4: Quail mean body weight and change of body weight

Nominal treatment concentration [mg a.s./kg feed]	Mean bodyweight ± S.D. [g]			Change of body weight [%]	
	Day 0	Day 5	Day 8	Exposure Period 5 days	Post-Exposure Period 3 days
Control	24.0 ± 1.9	35.7 ± 2.6	42.8 ± 53.3	+ 48.9	+ 19.9
313	23.1 ± 0.8	34.5 ± 1.7	41.7 ± 2.1	+ 49.4	+ 20.9
625	23.6 ± 1.7	34.2 ± 2.4	42.6 ± 2.7	+ 45.2	+ 24.4
1250	24.3 ± 1.6	34.3 ± 2.5	42.0 ± 3.3	+ 41.3	+ 22.4
2500	24.2 ± 1.9	32.8 ± 4.7	39.7 ± 6.2	+ 35.7	+ 20.9
5000	24.1 ± 1.5	27.6 ± 3.1 *	36.0 ± 4.0 *	+ 14.7	+ 30.4

S.D.: Standard deviation
* Statistically different from the control group at $p < 0.05$

No clear relationship for the food consumption was found. At the highest test concentration (5000 mg a.s./kg feed) the mean food consumption was on some days remarkably higher than that of the control, during exposure period as well as during post treatment, but this high food consumption was not consistent over the whole test period. The other test groups consumed a little bit less than the control until day 6. On day 7 the control consumed more food than the other groups.

Table 8.1.1.2- 5: Quail mean feed consumption

Treatment [mg a.s./kg feed]	Mean food consumption [g/bird/day]	
	Exposure Period 5 days	Post-Exposure Period 3 days
Control	7.1	11.8
313	6.9	9.5
625	5.0	6.9
1250	6.0	7.0
2500	6.6	7.6
5000	10.0	15.8

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Table 8.1.1.2- 6: Adult bird feed consumption: Daily dietary dose

Treatment [mg a.s./kg feed]	Mean body weight Exposure Period [g bw]	Mean food consumption Exposure Period [g feed/ bird/ day]	Mean food consumption/ mean body weight [A] [kg feed/ kg bw/ day]	Daily dietary dose [B] [mg a.s./kg bw/day]
Control	29.8	7.1	0.238	0
313	28.8	6.9	0.240	66.7
625	28.9	5.0	0.173	96.9
1250	29.3	6.0	0.205	230.4
2500	28.5	6.6	0.232	538.0
5000	25.8	10.0	0.388	1845.4

bw: Body weight
[A] Values not presented in study report. Calculated on the basis of results for food consumption and body weight (bw) given in study report
[B] Based on measured concentration in test diets at test start.

Gross pathology:

No signs of necropsy abnormality were found.

Biological findings:

Table 8.1.1.2- 7: Short-term dietary toxicity to birds

Test substance	Fluopyram	
Test object	Bobwhite Quail chicks (10 days)	
Exposure	dietary	
	[mg a.s./kg feed]	**[mg a.s./kg bw/d]**
LC_{50} / LD_{50}	> 5000 (nom)	> 1845.4 (im)
Lowest observed effect level (LOEL)	5000 (nom)	1845.4 (im)
No observed effect level (NOEL)	2500 (nom)	538.0 (im)

im: Initially measured concentration in test diets
nom: Nominal concentration in test diets

III. CONCLUSION

The subacute dietary LC_{50} of fluopyram to 10-day old Northern Bobwhite chicks was determined to be > 5000 mg a.s./kg feed, corresponding with an LDD_{50} of > 1845.4 mg a.s./kg bw/d.

Based on all parameters measured, the NOEL was 2500 mg a.s./kg feed.

Assessment and conclusion by applicant:

The study and its data are considered as acceptable and reliable for use in risk assessment.

The endpoints are:

LC_{50} > 5000 mg a.s./kg feed

LDD_{50} > 1845.4 mg a.s./kg bw/d

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Data Point:	KCA 8.1.1.2/02
Report Author:	[redacted]
Report Year:	2005
Report Title:	AE C656948 techn. a.s. : 5-day-dietary LC50 mallard duck (Anas platyrhynchos)
Report No:	BAR/LC 020
Document No:	M-262710-01-1
Guideline(s) followed in study:	U.S.EPA Ecological Effects Test Guidelines, OPPTS 850.2200 Avian Dietary Toxicity Test (April 1996); U.S. EPA OPPTS Guideline No. 850.2200 OECD guideline 205 for testing of chemicals "Avian Dietary Toxicity Test" (April 1984)
Deviations from current test guideline:	Current guideline: OECD 205 (1984) Deviations: At the age of the chicks >14 days, the temperature was 26.2 - 29.2 °C, slightly higher than the recommended 22 - 28°C. The relative humidity was not reported. These deviations are not expected to have an impact on the study results. All validity criteria were met.
Previous evaluation:	yes, evaluated and accepted in DAR (2011)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive summary

Mallard duck chicks (12 day old) were exposed for 5 days to nominal dietary concentrations of 0 (control), 313, 625, 1250, 2500 and 5000 mg a.s./kg feed. There were 10 hatchlings per test concentration and 20 for the control. Birds were held at a temperature of 26.2 to 35 °C and 16 hours light per day. Observations for mortality and signs of intoxication were made at least once daily. Body weights were recorded on day 0 at study initiation, on day 5 and test termination on day 8. Feed consumption for each level was recorded daily during the exposure and recovery period. Gross necropsies were carried out on all prematurely deaths and on all survivors. Samples were collected to verify the test concentrations adminstered and to confirm the stability and homogeneity of the test substance in the diets.

The study fulfilled all validity criteria of the OECD 205 guideline.

In the control group and in the 1250 mg a.s./kg bw group one bird each died prematurely, showing severe signs of emaciation. The death was not considered treatment related. There were no effects on food consumption and body weight development in the test groups compared to the control group. The frequent occurrence of dark red pancreas at necropsy at 5000 mg a.s./kg feed was 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.
The subacute dietary LC_{50} of fluopyram technical in Mallards was determined to be > 5000 mg a.s./kg feed, corresponding with an LDD_{50} of > 1643 mg a.s./kg bw/d.

I. Material and Methods

Test item: Fluopyram technical, specification No.: 102000012455; Batch No.: 08528/0002; Sample code: PBF-2005-0046-TOX-06970; purity: 94.7 %

Test design: Young mallard ducks (*Anas platyrhynchos*) chicks (12 day old) were exposed to fluopyram for 5 days to nominal dietary concentrations (techn. a.s.) of 0 (control), 313, 625, 1250, 2500 and 5000 mg a.s./kg feed. There were 10 chicks (unknown sex) per test concentration and 20 (unknown sex)

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for the control, which were housed with 10 chicks per cage. Control birds received basal (untreated) feed. Each cage had floor space that measured approximately 100 × 70 cm with a ceiling of 40 cm.

Birds were acclimatized for approximately 7 days prior to being randomized into test groups. At the start of acclimation, the chicks were well developed and similar to birds from wild population. Only birds that appeared healthy were used for the study. Water and feed were provided ad libitum during acclimation and during the test. Room temperature was between 26.2 to 35 °C during the acclimation and test periods. The photoperiod was maintained at 16 hours of light per day during acclimation and throughout the test.

Observations for mortality and on signs of intoxication were made daily during acclimatization, twice on the first exposure day, continued at least once daily throughout the following study days until terminal sacrifice. Body weights were recorded on day 0 at study initiation, on day 5 and at termination of the test on day 8. Feed consumption was determined by cage for each treatment and control group for the exposure and the post-exposure period. Gross necropsies were carried out on all prematurely deaths and on all survivors.

Statistics: For statistical evaluation of possible treatment related effects, the data for testing endpoints were processed as unpaired comparisons of each treatment level with untreated control. Initially the data were analysed on variances homogeneous distribution (Bartlett's test, $p < 0.05$). In case of homogeneous variances, subsequent analyses were conducted using parametric techniques (Bonferroni t-test adjustment); otherwise the Bonferroni Welch t-test adjustment for inhomogeneous variances was used. All described statistical procedures were carried out by using ToxRat Professional (Version 2.09).

Analytics: Samples of the test diets were collected at test start 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 three samples which have been randomly chosen out of the food batch concentrations at 100 and 5000 mg a.s./kg feed (prepared on March 17th, 2005). Samples for stability analysis were taken from the batch concentrations at 100 and 5000 mg a.s./kg feed (prepared on March 17th, 2005) and stored at test conditions (35 °C) for 1 day and deep frozen (-18 °C) for 14 days.

Dates of experimental work: May 12th to May 28th, 2005

II. RESULTS AND DISCUSSION

Validity criteria:

Table 8.1.1.2- 8: Validity criteria (according to OECD 205, adopted 04 April 1984)

Validity Criteria	Required	Obtained
Control mortality	≤ 10 %	5 % (1 out of 20)
Test concentration maintained over the 5 day exposure period	≥ 80 % of nominal	90 - 93 % of nominal
Treatment related effects in the lowest treatment level	No effects should occur	Fulfilled

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Analytical results:

Samples collected at test start to verify test substance concentrations for the 313, 625, 1250 and 5000 mg a.s./kg feed diets had measured concentrations of 280.9, 580.2, 1119.1, 2307.1 and 4604.5 mg a.s./kg feed, respectively. These values represented 90, 93, 90, 92 and 92 % of nominal concentrations, respectively.

No residues of fluopyram were found in the control samples.

Table 8.1.1.2- 9: Analysis of diet test

Nominal dietary concentration [mg a.s./kg feed]	Measured dietary concentration Day 0 [mg a.s./kg feed]	% of nominal
313	280	90
625	580	93
1250	1119	90
2500	2307	92
5000	4604	92

Diet samples were collected from the 100 and 5000 mg a.s./kg feed test concentrations and were analysed to evaluate the homogeneity of the test substance in the diet. Measured concentrations for the two test concentrations were 99 ppm and 5047 mg a.s./kg feed, respectively.

Analysis of diet samples collected from feeders after being held at study conditions (35 °C) for 24 hours revealed 96 and 98 % of the nominal values for the batch concentrations at 100 and 5000 mg a.s./kg feed. Analysis of diet samples collected from feeders after being held in a deep freezer (-18 °C) for 14 days resulted in 95 % and 99 % of the nominal values for the batch concentrations at 100 and 5000 mg a.s./kg feed.

Observations:

Mortality and clinical observations:

In the control group and in the 1250 mg a.s./kg bw group one bird each died prematurely, showing severe signs of emaciation. The death was not considered treatment related.

Table 8.1.1.2- 10: Summary of mortalities and clinical symptoms

Nominal treatment concentration [mg a.s./kg feed]	Overall mortality (females and males)		Number dosed	Signs of Intoxication (type)
	Day 0 - 4 (Exposure)	Day 5 - 8 (Post exposure)		
Control	0	1	20	-
313	0	0	10	-
625	0	0	10	-
1250	1	0	10	-
2500	0	0	10	-
5000	0	0	10	-

Body weight and feed consumption:

For body weight development, no difference between exposure and control groups were found.

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Table 8.1.1.2- 11: Mallard mean body weight and change of body weight

Nominal treatment concentration [mg a.s./kg feed]	Mean bodyweight ± S.D. [g]			Change of body weight [%]	
	Day 0	Day 5	Day 8	Exposure Period 5 days	Post-Exposure Period 3 days
Control	147.1 ± 15.7	236.4 ± 41.7	309.9 ± 67.7	+ 60	+ 31
313	157.3 ± 13.7	275.9 ± 18.1	350.4 ± 24.8	+ 64	+ 35
625	151.0 ± 19.5	243.7 ± 34.5	322.3 ± 45.9	+ 61	+ 32
1250	143.7 ± 9.5	242.6 ± 22.2	333.7 ± 38.0	+ 68	+ 37
2500	157.0 ± 15.1	253.5 ± 27.1	334.5 ± 33.3	+ 61	+ 32
5000	153.0 ± 15.4	241.8 ± 28.8	329.0 ± 36.0	+ 58	+ 36

S.D.: Standard deviation

No influence on food consumption was observed in any treatment group.

Table 8.1.1.2- 12: Mallard mean feed consumption data

Treatment [mg a.s./kg feed]	Mean food consumption [g/bird/day]	
	Exposure Period 5 days	Post-Exposure Period 3 days
Control	72.9	96.1
313	77.7	96.4
625	73.3	97.3
1250	68.0	104.8
2500	76.2	95.4
5000	70.4	89.2

Table 8.1.1.2- 13: Adult bird feed consumption: Daily dietary dose

Treatment [mg a.s./kg feed]	Mean body weight Exposure Period [g bw]	Mean food consumption Exposure Period [g feed/ bird/ day]	Mean food consumption/ mean body weight [A] [kg feed/ kg bw/ day]	Daily dietary dose [B] [mg a.s./kg bw/day]
Control	191.7	72.9	0.380	0
313	207.6	77.7	0.374	105.2
625	197.4	73.3	0.371	215.6
1250	193.1	68.0	0.352	394.1
2500	205.3	76.2	0.371	856.8
5000	197.4	70.4	0.357	1642.7

bw: Body weight

[A] Values not presented in study report. Calculated on the basis of results for food consumption and body weight (bw) given in study report

[B] Based on measured concentration in test diets at test start.

Gross pathology:

Necropsy of the two prematurely dead birds showed strong emaciation (one dead bird in control on day 7 and one dead birds in 1250 mg a.s./kg feed on day 2).

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One of the birds showed additionally a whitish coloured pancreas (1250 mg a.s./kg feed), the other one showed a dark red pancreas and yellow coloured thickened heart bag (control).

In the control group 2 of 19 survivors showed strong emaciation and various other changes at necropsy (reduced liver, increased spleen, yellow coloured thickened pericardium, a dark red pancreas, signs of visceral gout at the gall bladder, yellowish plaques at the chest).

For the test concentration of 1250 mg a.s./kg feed, 2 of 9 survivors showed reddish coloured pancreas.

For the test concentrations at 313, 625 and 2500 mg a.s./kg feed, there were no findings.

At the highest test concentration (5000 mg a.s./kg feed), 7 of 10 birds showed reddish coloured pancreas. One of them showed additionally a haematoma at the chest.

Biological findings:

Table 8.1.1.2- 14: 5-day-dietary toxicity to birds

Test substance	Fluopyram technical	
Test object	Mallard Duck chicks (12 days old)	
Exposure	dietary	
	[mg a.s./kg feed]	**[mg a.s./kg bw/d]**
LC_{50} / LD_{50}	> 5000 (nom)	> 1643 (nom)
Lowest observed effect level (LOEL)	5000 (nom)	> 1643 (nom)
No observed effect level (NOEL)	2500 (nom)	856.8 (nom)

nom: Nominal concentration in test diets

III. CONCLUSION

The subacute dietary LC_{50} of fluopyram technical in Mallards was determined to be > 5000 mg a.s./kg feed, corresponding with an LDD_{50} of > 1643 mg a.s./kg bw/d.

Based on all parameters measured, the NOEL was 2500 mg a.s./kg feed.

Assessment and conclusion by applicant:

The study and its data are considered as acceptable and reliable for use in risk assessment.

The endpoints are:

LC_{50} > 5000 mg a.s./kg feed

LDD_{50} > 1643 mg a.s./kg bw/d

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CA 8.1.1.3 Sub-chronic and reproductive toxicity to birds

Active substance fluopyram

Data Point:	KCA 8.1.1.3/01
Report Author:	[redacted]
Report Year:	2008
Report Title:	Effect of AE C656948 technical on reproduction of the northern bobwhite quail
Report No:	EBGMP004-1
Document No:	M-299245-02-1
Guideline(s) followed in study:	OECD 206; FIFRA Guideline 71-4; USEPA OPPTS 850.2300
Deviations from current test guideline:	Current Guideline: OECD 206 (1984) Deviations: The birds were 16 weeks old at experimental start, below the minimum age of 20 weeks recommended by the guideline. The floor area per pair was 0.1568 m², below the 0.25 m2 recommended. The temperature during egg storage was 12 °C, lower than the 15-16 °C recommended. The temperature during incubation (37.3 °C) was slightly lower than 37.5 °C recommended by the guideline. The temperature during hatching was not reported. The hatchlings were kept at a temperature range of 32-38 °C during the first and second week, lower than the minimum 35 °C recommended for the first week and above the maximum 32 °C recommended for the second week. The humidity during egg storage was 87.7 %, higher than the recommended 55-75 %. The humidity during hatching was 65.7 %, lower than the recommended 70-75 %. These deviations are not expected to have impacted the study results. All validity criteria were fulfilled
Previous evaluation:	yes, evaluated and accepted in DAR (2011)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

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Executive summary

The purpose of this study was to evaluate the effects of dietary exposure to fluopyram on adult health and reproduction of bobwhite quail. Adult bobwhite quail (*Colinus virginianus*) were exposed to fluopyram over approximately 23 weeks to nominal dietary concentrations of 0 (control), 250, 500 and 1000 mg a.s./kg feed. Bobwhite quail were 16 weeks old at experimental start. Eggs were collected from the parental birds for ~9 weeks during the exposure phase after 14 weeks of a pre-egg laying exposure period (including 8 weeks period of pre-photostimulation). There were 18 pairs of birds at each treatment level with one reproductive pair of birds (i.e. one male and one female) per cage. Due to significant effects upon reproduction at all treatment levels, the recovery potential was examined at the two highest treatment groups (500 and 1000 mg a.s./kg feed) during the final 4 weeks of egg production by providing clean feed to half of the replicates in the 500 and 1000 mg/kg treatments.

Birds were observed for mortality, abnormal behaviour and signs of toxicity; adult body weight and feed consumption were measured; gross pathology was conducted; reproductive parameters, as well as hatchling health, growth and survival, were examined.

The study fulfilled all validity criteria of the OECD 206 guideline.

The mean measured concentrations of fluopyram in week 1, 5, 10, 15, and 20 were determined as 237, 530 and 1082 mg a.s./kg feed representing percent nominal values of 95, 106 and 108 %, respectively.

Clinical observations of adult birds exhibited no treatment related signs of toxicity with the exception of feather loss and minor injuries as a result of cage wear. Adult mortality for the study included one control female bird, two females in the treatment group of 250 mg a.s./kg feed, one female in the treatment group of 500 mg a.s./kg feed and two females in the highest treatment group (1000 mg a.s./kg feed). The observed mortalities were considered incidental and not related to a dose-response effect. Female body weight gain was significantly reduced at the lowest treatment group (250 mg a.s./kg feed) during the first 5-week egg laying period compared to the control. The significant increase in the treatment group of 1000 mg a.s./kg feed corresponded to a very low egg production and slightly increased food consumption for this treatment throughout the study. For food consumption up to week 19 there were no statistically significant differences at any treatment level compared to the control. Although no statistics were performed for the treated and untreated replicates regarding food consumption, an increase in food consumption was apparent for the 500 and 1000 mg/kg untreated replicates for the four weeks of recovery.

Significant adverse effects were observed for most reproductive endpoints at all treatment levels as compared to the control. Effects included reductions in the numbers of eggs laid, increases in the numbers of cracked and defective eggs, increases in embryonic mortality, reductions in eggshell strength and thickness, and reductions in the numbers of hatchlings and 14-day old survivors. In addition, the body weights of hatchlings and 14-day old survivors were inhibited 14-26 % at the two highest treatment groups (500 and 1000 mg a.s./kg feed) compared to the controls. Improvements were observed for most reproductive endpoints during the 4-week recovery period (relative to untreated birds at these levels). For the two highest treatment groups (500 and 1000 mg a.s./kg feed), increases were observed in the numbers of eggs laid, eggs set, viable embryos, live 18-day embryos, hatchlings, and 14-day old survivors, and a decrease was observed in the number of cracked eggs. However, a decrease in the number of defective eggs and increases in hatchling and 14-day old survivor body weights was only clearly observed at the 500 mg a.s./kg feed level during the recovery period. Egg shell strength and thickness improved notably at both levels, where values obtained during recovery approached control levels.

Based on reproduction endpoints, the NOEC for bobwhite quail exposed to fluopyram was < 250 mg a.s./kg feed which corresponds to a NOED of < 23 mg a.s./kg bw/day.

I. MATERIAL AND METHODS

Test item: fluopyram, specification No.: 102000012455; Batch No.: 08528/0002; Tox No.: 06970-01; purity: 94.7 %.

Test design: Adult bobwhite quail (*Colinus virginianus*) were exposed to fluopyram for approximately 23 weeks to nominal dietary concentrations of 0 (control), 250, 500 and 1000 mg a.s./kg feed (adjusted for purity). Test diets were prepared by mixing fluopyram into a premix that was used for preparation of the final diet every seven to ten days. Control diet and each of the three treated diets were presented to the birds each week.

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 SANTE/2020/12830, Rev.1

Bobwhite quail were 16 weeks old at experimental start. There were 18 pairs of birds at each treatment level resulting in 72 pairs for the total study. One reproductive pair of birds (i.e. one male & one female) was housed per cage. Eggs were collected from the parental birds for ~9 weeks during the exposure phase after 14 weeks of a pre-egg laying exposure period (including an 8 weeks period of pre-photostimulation). During the first 5 weeks of the egg-laying period, when all replicates from all levels were fed treated diet, egg Lots A to F were produced. For the final 4 weeks of the egg-laying period (egg Lots G to J), one-half of the replicates from the two highest treatment groups (500 and 1000 mg a.s./kg feed) were offered untreated diet to assess their recovery potential. The remainder of replicates

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as well as all birds from the lowest treatment group (250 mg a.s./kg feed) were maintained on treated diet for the full duration of the study.

The test birds were acclimated to the test facility and study cages for approximately 2 weeks prior to experimental start. During the acclimation, all birds were observed daily. Birds exhibiting abnormal behaviour or debilitating physical injuries were not used for the test.

Adult birds were housed indoors in cages. The adult quail cages measured approximately 56 × 28× 27 cm (length × width × height). Cages were constructed of stainless-steel wire grid and stainless-steel sheeting. Each cage was equipped with both feed and watering nipple. The birds were fed basal diet (Teklad Game Bird Ration feed) and tap water ad libitum during the acclimation and testing period. During the acclimation period and the first 8 weeks of the test (short day length phase) the birds were held under a photoperiod of 7 hours of light and 17 hours dark per day. The photoperiod was then increased to 17 hours of light and 7 hours dark per day for the remainder of the study.

During the study, all adult birds were observed daily for signs of toxicity or abnormal behaviour. Additionally, all offspring were observed daily from hatching until 14 days of age. A record was maintained for all clinical observations and mortalities. Adult body weights were measured at the experimental start (week 1), in week 3, 5, 7 and 9, and at adult termination (week 23). No adult body weights were taken during the egg production phase. Adult feed consumption was measured weekly by cage throughout the study.

Gross pathology was conducted for all birds that died or were euthanized during the course of the study. At the end of the exposure period, all surviving birds of the control and highest dose level were necropsied. Additionally, at least 4 males and 4 females from each of the remaining dose levels were necropsied. Reproductive parameters, as well as hatchling health, growth and survival, were examined. In addition, the effects of adult exposure to fluopyram on the number of eggs laid, fertility, embryo viability, hatchability, offspring survival, and eggshell quality/thickness were evaluated.
Eggs were collected approximately twice daily and placed in an egg cooler (12 °C) until incubation. All eggs laid in a weekly interval were considered as one lot. At the end of the weekly interval, all eggs were removed from the egg cooler and candled to detect cracks. Cracked eggs were recorded and discarded. All eggs laid on one day every other week during the egg laying period were collected for eggshell strength and thickness measurements (Lot's B, D, F, H and J). Eggs that were not cracked or used for eggshell strength and thickness measurements were transferred in an incubator. The transfer of the eggs to the incubator was referred to as "egg set". The eggs were set in the incubator at 37.3 °C. Eggs were candled again on day 11 of incubation to determine embryo viability (fertility) and on day 18 to determine embryo survival. Non-fertile and nonviable eggs were removed, recorded, and discarded. On day 21 of incubation, eggs were placed into a NatureForm Hatcher (37.17 °C, reported in raw data) and allowed to hatch. All hatchlings and unhatched eggs were removed from the hatcher on day 24 and 25 of incubation. The unhatched eggs per parental cage were observed for embryo attempts to hatch (pipping), recorded and discarded on day 25. The body weights of surviving hatchlings were recorded.

Afterwards, hatchlings were housed in brooding cages and kept on untreated diet until 14 days of age when they were weighed again and sacrificed. Thermostats in the brooding compartment of each cage were set to maintain a temperature of approximately 32-38 ºC over the course of the 14-day post hatchling phase at a photoperiod of 14 hours light and 10 hours dark.

Statistics: Data were assessed for normality and homogeneity of variances using a Chi-Square Test and Bartlett's Test, respectively. If the data passed these assumptions, treatment groups were compared to the control group using an analysis of variance (ANOVA) followed by Bonferroni and/or Dunnett's tests. For data that was not homogeneous, a Kruskal Wallis Rank Test was performed. Statistical analyses were not performed on the recovery portion of the study (egg Lots G to J) due to the study design and the nature of the data for the untreated replicates.

Analyses were conducted using ToxStat® statistical software (Version 3.4) at a 95 % confidence level.

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Each of the following parameters was analysed statistically: adult body weight, adult feed consumption, eggs laid, eggs cracked of eggs laid, number of eggs for eggs set, viable embryo, live 18th day embryo per hen, percent number of eggs not cracked of eggs laid per hen, percent number of eggs set of eggs laid per hen, percent viable embryos of eggs set per hen, percent live embryos of viable embryos per hen, number hatched, 14-day survivors of hatchlings, percent hatched of eggs laid per hen, percent hatched of eggs set per hen, percent hatched of live embryos per hen, percent 14-day survivors of eggs set per hen, percent 14-day survivors of number hatched per hen, hatchling body weight per hen, 14-day survivor body weight per hen, egg shell strength and egg shell thickness.

Dates of experimental work: October 19th, 2006 to April 24th, 2007

II. RESULTS AND DISCUSSION

Validity criteria:

Table 8.1.1.3- 1: Validity criteria (according to OECD 206, 1984)

Validity Criteria	Required	Obtained
Adult mortality in control	≤ 10 %	2.8 % (one dead female bird in the controls out of 18 pairs of birds)
Mean number of 14-day old survivors in the controls	≥ 12 per hen (during a 10-week production phase)	39 per hen (during the 9-week production phase)
Eggshell thickness in control	≥ 0.19 mm	0.192 mm (Egg Lots B, D, F) 0.197 mm (Egg Lots H, J)
Concentration of the test item in the feed	≥ 80 % of the nominal concentrations	83.2 - 112 % of the nominal concentrations

Analytical results for dietary concentration:

The measured concentrations of fluopyram in week 1, 5, 10, 15 and 20 ranged between 83.2 and 112 % of nominal concentrations (see table below). The mean measured concentrations of fluopyram were determined as 237, 530, and 1082 mg a.s./kg feed representing 95, 106 and 108 % of nominal concentrations, respectively. Analysis of diet samples collected from feeders after being held at ambient temperature for 10 days (147 % and 116 % of the day 0 values for the 250 and 1000 mg a.s./kg feed a.s. test concentrations) confirmed appropriate maintenance of the treatment concentrations.

No residues of fluopyram were detected in the control diets above the limit of quantification (LOQ: 9.61 mg a.s./kg feed).

A summary of the dietary concentrations is included in the following table.

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Table 8.1.1.3- 2: Feed analysis summary of fluopyram

Nominal dietary concentration [mg a.s./kg feed]	Week 1	Week 5	Week 10	Week 15	Week 20	Mean measured dietary concentration [mg a.s./kg feed]
	Measured dietary concentration [mg a.s./kg feed]					
250	208	231	268	249	230	237
500	542	489	564	535	521	530
1000	1117	1175	997	1012	1108	1082
	% of nominal [A]					Mean % nominal
250	83.2	92.4	107.2	99.6	92.0	95
500	108.4	97.8	112.8	107	104.2	106
1000	111.7	117.5	99.7	101.2	110.8	108

[A] Not given in report. Calculations based on measured dietary concentration on each sampling day.

Observations:

Parental Toxicity

Adult bird mortality:
Six incidental adult mortalities occurred during the test: one in the control group, two in the treatment group of 250 mg a.s./kg feed, one in the treatment group of 500 mg a.s./kg feed and two in the highest treatment group (1000 mg a.s./kg feed). All mortalities were related to female birds. The mortality in the control group was female in Pen 14, which was found dead on 10th February 2007 (Week 16).

The first mortality in the 250 mg a.s./kg test group was female in Pen 406, which was found dead on 23rd December 2006 (Week 10). The second mortality was female in Pen 401, which was found dead on 16th March 2007 (Week 22).

The mortality in the 500 mg a.s./kg test group was the female in Pen 517, which was found dead on 26th January 2007 (Week 15).

The first mortality in the 1000 mg a.s./kg test group was the female in Pen 608, which was found dead on 19th January 2007 (Week 14). The second mortality was female in Pen 612, which was found dead on 1st March 2007 (Week 20).

The observed mortalities were considered incidental and not related to a dose-response effect. No other mortalities occurred during the course of the study.

Minor occurrences of feather loss were observed in the control and all treatment levels starting on week 12 and occurring until adult termination. Additionally, several birds in the treatment group of 250 and 500 mg a.s./kg feed exhibited injuries and/or lacerations. These occurrences were considered a result of normal cage wear and aggression as exhibited in laboratory cage reared birds. There were no significant clinical symptoms or compound related effects observed during the study.

Adult bird observations:

Clinical observations of adult birds exhibited no treatment related signs of toxicity. Feather loss and minor injuries were observed as a result of cage wear.

Adult bird necropsy:

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Adults exposed to fluopyram in the diet showed no indication of treatment effects at necropsy. Feather loss and skin abrasions were noted on several birds for the control and treatment groups as was also observed during the in-life phase of the study. A high percentage of the females had maturing follicles in the control and treatment groups. No lesions or growths were observed on the testes or ovaries for any of the birds.

Adult bird body weight:

There was a statistically significant decrease in female body weight gain at test termination in the lowest treatment group (250 mg a.s./kg feed) when compared to the control group. The significant increase in the treatment group of 1000 mg a.s./kg feed corresponded to a very low egg production and increased food consumption for this treatment throughout the study.

Moreover, the analysis of the US EPA (DAR 2011) detected a significant adverse effect on adult female weight at the lowest treatment level of 250 mg a.s./kg feed and an increase of female weight at 1000 mg/kg feed.

Table 8.1.1.3- 3: Adult Quail mean body weights and weight gains

Nominal dietary concentration [mg a.s./kg feed]	Males			Females		
	Mean weight ± S.D. [g]		Mean weight gain [g]	Mean weight ± S.D. [g]		Mean weight gain [g]
	Start	End	Difference	Start	End	Difference
Control	192 ± 8.7	219 ± 17.1	27	189 ± 7.9	239 ± 24.7	50
250	194 ± 11.7	219 ± 10.8	25	189 ± 8.0	221 (*) ± 24.8	32*
500	195 ± 11.7	219 ± 16.2	24	189 ± 7.9	229 ± 29.4	40
1000	196 ± 11.1	220 ± 20.2	24	189 ± 8.0	249 (*) ± 11.0	60 *

S.D.: Standard deviation
* Significant different from the control ($p < 0.01$)
(*) Significantly different from the control ($p < 0.05$) in the additional statistical evaluation of US EPA – DAR 2011

Adult bird feed consumption:

Since a recovery period was incorporated into the final 4 weeks of the study, food consumption was calculated only to week 19.

There were no statistically significant differences for food consumption (up to week 19) at any treatment level compared to the control.

Table 8.1.1.3- 4: Adult bird feed consumption: Daily dietary dose

Test interval	Nominal dietary concentration [mg a.s./kg feed]	Mean body weight [kg bw]	Mean food consumption ± S.D. [g feed/ bird/ day]	Daily dietary dose [mg a.s./kg bw/day]
Weeks 1 - 19	Control	0.2098	18.5 ± 2.4	0.0
	250	0.2055	19.5 ± 2.6	23
	500	0.2080	19.7 ± 2.9	50
	1000	0.2135	20.5 ± 2.9	104

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Reproduction Toxicity

Significant adverse effects were observed for most reproductive endpoints at all treatment levels as compared to the control.

The tables below present the reproductive endpoints totals for the first 5 weeks of the reproductive phase (Egg Lot's A-F) and reproductive endpoint totals for the final 4 weeks of the reproductive phase (Egg Lot's G-J), respectively.

Table 8.1.1.3- 5: Reproductive endpoint totals - Egg Lots A to F (first 5 weeks of egg laying period)

Reproductive parameter	Nominal dietary concentration [mg a.s./kg feed]			
	Control	250	500	1000
No. of eggs laid (per group) [A]	524	425 *	276 *	260 *
No. of eggs laid / hen [A]	29.11	23.61	15.33 *	14.44 *
No. of eggs cracked / hen [A]	0.33	1.50 *	1.33 *	0.89
No. of eggs defective	3	62 *	54 *	40 *
No. of eggs set / hen [A]	26.44	16.72 *	9.61 *	10.11 *
No. of viable embryos / hen [A]	25.11	13.61 *	5.17 *	5.72 *
No. of live embryos / hen [A]	25.06	10.44 *	3.67 *	4.00 *
No. of hatchlings / hen [A]	23.94	8.83 *	2.28 *	0.39 *
No. of 14-day survivors / hen [A]	23.72	8.28 *	1.39 *	0.06 *
Eggshell mean thickness [mm] [A]	0.19	0.17 *	0.17 *	0.17 *
Eggshell strength [kg] [A]	0.86	0.62 *	0.52 *	0.57 *
Hatchling mean body weight [g] [B]	6.5	6.3	5.6	5.4
14-d survivor mean body weight [g] [B]	33. 5	30.4	24.7	28.2 [C]

* Significantly different from control group.
[A] Data and statistical analysis from US EPA – DAR 2011.
[B] Data from study report. Not statistically analysed.
[C] n = 1.

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Table 8.1.1.3- 6: Reproductive endpoint total – Egg Lots G to J (final 4 weeks of egg-laying period) No statistical analysis was performed on the recovery portion of the study due to the study design and the nature of the data for the untreated replicates.

Reproductive parameter	Nominal dietary concentration [mg a.s./kg feed]					
	Control	250	500		1000	
		Treated	Untreated	Treated	Untreated	Treated
No. of eggs laid (per group)	338	206	120	90	135	81
No. of eggs laid /hen	19	11	13	10	15	9
No. of eggs cracked	7	24	13	15	10	21
No. of eggs defective	2	24	5	18	11	6
No. of eggs set	310	142	92	49	107	49
No. of viable embryos	299	106	84	25	96	25
No. of live embryos	299	83	81	10	95	19
No. of hatchlings	277	69	74	4	88	0
No. of 14-day survivors	276	68	74	4	88	0
Eggshell mean thickness [mm]	0.197	0.174	0.194	0.176	0.193	0.186
Eggshell strength [kg]	0.85	0.63	0.85	0.46	0.83	0.83
Hatchling mean body weight [g]	6.9	6.3	6.8	6.5	6.5	NA
14-d survivor mean body weight [g]	35.3	34.7	36.7	32.2	36.3	NA

NA Not applicable (n = 0)

Table 8.1.1.3- 7: Reproductive performance (normalized as percentage) – Egg Lots A to F (first 5 weeks of egg laying period)

Reproductive parameter	Nominal dietary concentration [mg a.s./kg feed]			
	Control	250	500	1000
% eggs not cracked of eggs laid [B]	99.0	93.8 *	93.5 *	92.2 *
% eggs cracked of eggs laid [A]	1	6	9	6
% of eggs set of eggs laid [B]	91.43	69.71 *	63.14 *	70.09 *
% of viable embryos of eggs set [B]	94.70	79.34 *	54.47 *	53.60 *
% of live embryos of viable embryos [B]	99.87	70.36 *	69.20 *	58.80 *
% of hatchlings of eggs laid [B]	83.18	33.73 *	17.26 *	3.85 *
% of hatchlings of eggs set [B]	91.17	45.57 *	27.11 *	5.02 *
% of hatchlings of live embryos [B]	96.44	77.97 *	59.86 *	17.69 *
% of 14-day survivors of eggs set [B]	90.46	40.20 *	17.73 *	0.44 *
% of 14-day survivors of hatchlings [B]	99.26	88.20 *	46.70 *	12.50 *

* Significantly different from control group.
[A] Data from US EPA – DAR 2011 – not statistically analysed.
[B] Data and statistical analyses from US EPA – DAR 2011.

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Table 8.1.1.3- 8: Reproductive performance (normalized as percentage) – Egg Lots G to J (final 4 weeks of egg-laying period)
No statistical analysis was performed on the recovery portion of the study due to the study design and the nature of the data for the untreated replicates.

Reproductive parameter	Nominal dietary concentration [mg a.s./kg feed]					
		250	500		1000	
	Control	Treated	Untreated	Treated	Untreated	Treated
% eggs cracked of eggs laid	2	12	11	17	7	26
% eggs defective of eggs laid	1	12	4	20	8	7
% of eggs set of eggs laid	92	69	77	54	79	60
% of viable embryos of eggs set	96	75	91	51	90	51
% of live embryos of viable embryos	100	78	96	40	99	76
% of hatchlings of live embryos	93	83	91	40	93	0
% of 14-day survivors of hatchlings	99	99	100	100	100	0

Table 8.1.1.3- 9: Reproductive performance in % of control – Egg Lots A to F (first 5 weeks of egg laying period)

Reproductive parameter	Nominal dietary concentration [mg a.s./kg feed]		
	250	500	1000
	% of control [A]		
No. of eggs laid (per group)	81	53	50
No. of eggs laid / hen	81	53	50
No. of eggs cracked / hen	455	403	270
No. of eggs defective / hen	2067	1800	1333
No. of eggs set / hen	63	36	38
No. of viable embryos / hen	54	21	23
No. of live embryos / hen	42	15	16
No. of hatchlings / hen	37	10	2
No. of 14-day survivors / hen	35	6	0
Eggshell mean thickness [mm]	89	89	89
Eggshell strength [kg]	72	60	49
Hatchling mean body weight [g]	97	86	83
14-d survivor mean body weight [g]	91	74	84 [B]

[A] Not given in report. Calculations based on data for reproductive endpoint totals.
[B] n = 1

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Table 8.1.1.3- 10: Reproductive performance in % of control - Egg Lots G to J (final 4 weeks of egg-laying period)

Reproductive parameter	Nominal dietary concentration [mg a.s./kg feed]				
	250	500		1000	
	Treated	Untreated	Treated	Untreated	Treated
	% of control [A]				
No. of eggs laid (per group)	61	36	27	40	24
No. of eggs cracked / hen	343	186	214	143	300
No. of eggs defective / hen	1200	250	900	550	300
No. of eggs set	46	30	16	35	16
No. of viable embryos	35	28	8	32	8
No. of live embryos	28	27	3	32	6
No. of hatchlings	25	27	1	32	0
No. of 14-day survivors	25	27	1	32	0
Eggshell mean thickness [mm]	88	98	89	98	94
Eggshell strength [kg]	74	100	54	97	98
Hatchling mean body weight [g]	91	99	94	94	NA
14-d survivor mean body weight [g]	98	104	91	103	NA

[A] Not given in report. Calculations based on data for reproductive endpoint totals.
NA Not applicable.

<u>Recovery conclusion</u>
The recovery portion of the study with untreated replicates for the treatment groups of 500 and 1000 mg a.s./kg feed exhibited positive results for most reproductive and hatchling endpoints over a 4-week period. The most dramatic recovery occurred in the untreated replicates for eggshell strength and thickness in which the replicate means were approaching the control values. In addition, the recovery effects for the number of offspring hatched and 14-day survivors in the untreated replicates ranged from 62 to 64 % as opposed to 0 to 4 % for the treated replicates.

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Table 8.1.1.3- 11: Recovery Potential for Untreated Replicates

Reproductive Parameter	Original Nominal Dietary Concentration (mg/kg feed)		
	500	**1000**	**Observation in recovery groups**
Eggs Laid	Yes	Yes	The number of eggs laid increased in both treatments.
Eggs Cracked	Yes	Yes	The number of cracked eggs was reduced in both treatments.
Eggs Defective	Yes	No	The number of defective eggs was reduced at 500 mg/kg feed.
Eggs Set	Yes	Yes	The number of eggs set increased in both treatments.
Viable Embryos	Yes	Yes	The number of viable embryos increased in both treatments.
Live Embryos	Yes	Yes	The number of live embryos increased in both treatments.
Number Hatched	Yes	Yes	The number of hatchlings increased in both treatments.
14-Day Survivors	Yes	Yes	The number of 14-Day survivors increased in both treatments.
Hatchling Body Wgt.	Yes	No	Hatchling body weight increased in both treatments.
Survivor Body Wgt.	Yes	No	Survivor body weight increased at 500 mg/kg.

Wgt.: weight

Biological findings:

Table 8.1.1.3- 12: Subchronic and reproduction toxicity to Bobwhite quail

Test substance	Fluopyram a.s.
Test object	Bobwhite quail
NOEC for parental toxicity [mg a.s./kg feed]	< 250 nom (< 237 measured)
NOEC for reproduction [mg a.s./kg feed]	< 250 nom (< 237 measured)
LOEC for parental toxicity [mg a.s./kg feed]	250 nom (237 measured)
LOEC for reproduction [mg a.s./kg feed]	250 nom (237 measured)
NOED for reproduction [mg a.s./kg bw/day]	< 23 measured
LOED for reproduction [mg a.s./kg bw/day]	23 measured

III. CONCLUSION

The NOEC of parental toxicity was < 250 mg a.s./kg feed (< 23 mg a.s./kg bw/day).

Based on reproduction endpoints, the NOEC for bobwhite quail exposed to fluopyram was < 250 mg a.s./kg feed which corresponds to a NOED of < 23 mg a.s./kg bw/day.

Assessment and conclusion by applicant:

The study and its data are considered as acceptable and reliable for use in risk assessment.

The endpoints are:

NOEC < 250 mg a.s./kg feed

NOED < 23 mg a.s./kg bw/day

Recovery potential from reproductive effects was demonstrated after cessation of treatment.

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Data Point:	KCA 8.1.1.3/02
Report Author:	[redacted]
Report Year:	2008
Report Title:	AE C656948: A reproduction study with the Northern bobwhite
Report No:	149-213
Document No:	M-298723-01-1
Guideline(s) followed in study:	U.S. Environmental Protection Agency Series 850 – Ecological Effects Test Guidelines OPPTS Number 850.2300 FIFRA Subdivision E Section 71-4 OECD Guideline 206
Deviations from current test guideline:	Current Guideline: OECD 206 (1984) Deviations: The floor area per pair was 0.1275 m², below the 0.25 m² recommended by the guideline. The temperature during egg storage was 14.3 ± 0.1 °C, lower than the 15-16 °C recommended. The temperature during incubation (37.3 °C) and hatching phase (37.2 °C) was slightly lower than 37.5 °C recommended by the guideline. The hatchlings were kept at a temperature of 38 °C during their second week, higher than the 30-32 °C recommended. The humidity during egg storage was 86 ± 5 %, higher than the recommended 55-75 %. The humidity during hatching was 77 %, higher than the recommended 70-75 %. The humidity for the hatchlings was not reported. These deviations are not expected to have impacted the study results. All validity criteria were fulfilled.
Previous evaluation:	yes, evaluated and accepted in DAR (2011)
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 evaluate the effects of dietary exposure to fluopyram on health and reproduction of the bobwhite quail. Adult bobwhite quail (*Colinus virginianus*) were exposed to fluopyram over approximately 22 weeks to nominal dietary concentrations of 0 (control), 10, 50, 80 and 180 mg a.s./kg feed. Bobwhite quail were 20 weeks old at experimental start. Eggs were collected from the parental birds for 11 weeks during the exposure phase after 7 weeks of a pre-photo stimulation period and 4 weeks of a pre-egg laying exposure period. There were 16 pairs of birds at each treatment level with one reproductive pair of birds (i.e. one male and one female) per cage. Birds were observed for mortality, abnormal behaviour and signs of toxicity; adult body weight and feed consumption were measured; gross pathology was conducted; reproductive parameters, as well as hatchling health, growth and survival, were examined.

The study fulfilled all validity criteria of the OECD 206 guideline.

The mean measured concentrations of fluopyram in week 2, 3, 4, 12, 16 and 20 were determined as 9.69, 46.7, 75.7 and 175 mg a.s./kg feed representing percent nominal values of 97, 94, 95 and 97 %, respectively.

Clinical observations of adult birds exhibited no treatment related signs of toxicity. Adult mortality for the study included one control bird, two in the treatment group of 10 mg a.s./kg feed and two in the highest treatment group (180 mg a.s./kg feed). There were no treatment–related mortalities, overt signs of toxicity or treatment-related effects upon body weight or feed consumption at any of the concentrations tested. Additionally, there were no treatment-related effects reported upon any of the reproductive parameters measured at the treatment groups of 10, 50 and 80 mg a.s./kg feed. However, at the highest treatment group (180 mg a.s./kg feed) there were treatment-related reductions reported in offspring survival, hatchling body weight and 14-day old survivor body weight.

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The No Observed Adverse Effect Concentration (NOAEC) for parental toxicity of bobwhite quail exposed to fluopyram was 180 mg a.s./kg feed, corresponding to the mean dietary dose of 16.3 mg a.s./kg bw/day (NOAED). The NOAEC for reproduction endpoints was the nominal dietary concentration of 80 mg a.s./kg feed or the mean dietary dose of 7.2 mg a.s./kg bw/day (NOAED).

An additional statistical analysis by the US EPA concluded a NOEC of 50 mg a.s./kg feed which corresponds to a NOED of 4.5 mg a.s./kg bw/day. The original RMS questioned the biological relevance of the findings at 80 mg a.s./kg feed (difference ~8% of control weight for 14-d survivor weight) and suggested to use the mean dietary dose of 7.2 mg a.s./kg bw/day as higher tier reproductive risk assessment endpoint at the population level.

I. MATERIAL AND METHODS

Test item: fluopyram, specification No.: 102000012455; Batch No.: 08528/0002; purity: 94.7 %.

Test design: Adult bobwhite quail (*Colinus virginianus*) were exposed to fluopyram for approximately 22 weeks to nominal dietary concentrations of 0 (control), 10, 50, 80 and 180 mg a.s./kg feed (adjusted for purity). Test diets were prepared by mixing fluopyram into a premix that was used for weekly preparation of the final diet. Control diet and each of the three treated diets were prepared weekly and presented to the birds on Tuesday of each week.

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 SANTE/2020/12830, Rev.1.

Bobwhite quail were 20 weeks old at experimental start. There were 16 pairs of birds at each treatment level resulting in 80 pairs for the total study. One reproductive pair of birds (i.e. one male & one female) was housed per cage. Eggs were collected from the parental birds for 11 weeks during the exposure phase after 7 weeks of a pre-photostimulation period and 4 weeks of a pre-egg laying exposure period.

The test birds were acclimated to the test facility and study cages for approximately 6 weeks prior to experimental start. During the acclimation, all birds were observed daily. Birds exhibiting abnormal behaviour or debilitating physical injuries were not used for the test.

Adult birds were housed indoors in cages. The adult quail cages measured approximately 25× 51 cm (length × width) with a height ranging from 20 to 26 cm due to sloping floors. Cages were constructed of galvanized wire mesh and galvanized sheeting. Each cage was equipped with both feed and watering troughs. The birds were fed basal diet (containing ≥27 % protein, 2.5 % crude fat, <3.8 % crude fibre, 1.0 % calcium, 0.6 % limestone) and tap water ad libitum during the acclimation and testing period. For the first 7 weeks of the test, the birds were held under a photoperiod of 8 hours of light and 16 hours dark per day. The photoperiod was increased to 17 hours of light and 7 hours dark per day at the beginning of week 8 to induce egg laying until adult sacrifice.

During the study, all adult birds were observed daily for signs of toxicity or abnormal behaviour. Additionally, all offspring were observed daily from hatching until 14 days of age. A record was maintained for all clinical observations and mortalities. Adult body weights were measured at the experimental start, in week 2, 4, 7 and 8, and at adult termination. No adult body weights were taken during the egg production phase. Adult feed consumption was measured weekly by cage throughout the study.

Gross pathology was conducted for all birds that died or were euthanized during the course of the study. At the end of the exposure period, all surviving adult birds were euthanized and necropsied. Reproductive parameters, as well as hatchling health, growth and survival, were examined. In addition, the effects of adult exposure to fluopyram on the number of eggs laid, fertility, embryo viability, hatchability, offspring survival, and eggshell quality/thickness were evaluated.

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Eggs were collected daily and placed in a cold room (14.3 ± 0.1 °C) until incubation. All eggs laid in a weekly interval were considered as one lot. At the end of the weekly interval, all eggs were removed from the cold room, counted and eggs for eggshell quality measurements were selected by indiscriminate draw. The remaining eggs were candled to detect eggshell cracks or abnormal eggs. Cracked or abnormal eggs were recorded and discarded. Eggs that were not cracked or used for eggshell strength and thickness measurements were transferred in an incubator after fumigation with formaldehyde gas for 2 hours. The transfer of the eggs to the incubator was referred to as "egg set". The eggs were set in the incubator at 37.3 ± 0 °C. Eggs were candled again on day 11 and 12 of incubation to determine embryo viability (fertility) and on day 21 to determine embryo survival. On day 21 of incubation, eggs were placed into a hatcher (37.3 ± 0 °C) and allowed to hatch. All hatchlings, unhatched eggs, and eggshells were removed from the hatcher on day 25 or 26 of incubation. The body weights of surviving hatchlings were recorded and the group body weight by pen was determined.

Afterwards, hatchlings were housed in brooding pens and kept on untreated diet until 14 days of age when they were weighed again and sacrificed. Thermostats in the brooding compartment of each cage were set to maintain a temperature of approximately 38 ºC over the course of the 14-day post hatchling phase at a photoperiod of 16 hours light and 8 hours dark.

Statistics: In the original report, an analysis of variance (ANOVA) was performed to determine statistically significant differences between groups. Dunnett's multiple comparison procedure was used to compare the four treatment means with the control group mean and to assess the statistical significance of the observed differences. Sample units were the individual cages within each experimental group, except adult body weights where the sample unit was the individual bird. Percentage data were examined using Dunnett's method following arcsine square root transformation for reproductive parameters. Each of the following parameters was analysed statistically: adult body weight, adult feed consumption, eggs laid of maximum laid per hen, eggs cracked of eggs laid per hen, viable embryos of set per hen, live three-week embryos of viable embryos per hen, number hatched of live three-week embryos, 14-day survivors of hatchlings, hatchlings of eggs set, percent 14-day survivors of eggs set per hen, number of hatchlings of maximum set per hen, number of 14-day old survivors of maximum set, egg shell strength, average egg shell thickness, offspring's body weight, 14-d survivor body weight.

In the review of the study for the original Annex 1 listing of fluopyram, additional statistical analysis was conducted using "chicks.sas" (Ver. 3; March 2002), a SAS program provided by EFED/OPP/USEPA. Data for all endpoints were examined graphically using box plots to determine if they exhibited a dose-dependent response, which was ultimately used to select the multiple comparison test to detect LOAEC and NOAEC. Data for each endpoint were tested to determine if their distributions were normal and if their variances were homogeneous using Shapiro-Wilk's and Levene's tests, respectively. Data that satisfied these assumptions were subjected to Dunnett's and William's tests and data that did not satisfy these assumptions were subjected to the non-parametric MannWhitney-U (with a Bonferroni adjustment) and Jonckheere's tests. Data for dead birds were excluded from the analyses.

In this summary, the calculations and statistical results from the additional statistical analysis are presented (taken from M-522897-01-1) because the results of this additional statistical analysis were also included in the original DAR of fluopyram.

Dates of experimental work: July 8th, 2007 to February 12th, 2008

II. RESULTS AND DISCUSSION

Validity criteria:

Table 8.1.1.3- 13: Validity criteria (according to OECD 206, 1984)

Validity Criteria	Required	Obtained
Adult mortality in control	≤ 10 %	2.8 % (one dead male bird in the controls out of 18 pairs of birds)
Mean number of 14-day old survivors in the controls	≥ 12 per hen	24 per hen
Eggshell thickness in control	≥ 0.19 mm	0.225 mm
Concentration of the test item in the feed	≥ 80 % of the nominal concentrations	89.5 - 106 % of the nominal concentrations

Analytical results for dietary concentration:

The measured concentrations of fluopyram in week 2, 3, 4, 12, 16 and 20 ranged between 89.5 and 106 % of nominal concentrations (see table below). The mean measured concentrations of fluopyram were determined as 9.69, 46.7, 75.7 and 175 mg a.s./kg feed representing percent nominal values of 97, 94, 95 and 97 %, respectively. Analysis of diet samples collected from feeders after being held at ambient temperature for seven days (89 %, 90 %, 90 % and 92 % of the day 0 values for the 10, 50, 80 and 180 mg a.s./kg feed a.s. test concentrations) confirmed appropriate maintenance of the treatment concentrations.

No residues of fluopyram were detected in the control diets above the lowest analytical standard (2.50 mg a.s./kg feed).

A summary of the dietary concentrations is included in the following table.

Table 8.1.1.3- 14: Feed analysis summary of fluopyram

Nominal dietary concentration [mg a.s./kg feed]	Week 2	Week 3	Week 4	Week 8	Week 12	Week 16	Week 20	Mean measured dietary concentration [mg a.s./kg feed]
	Measured dietary concentration [A] [mg a.s./kg feed]							
10	10.6	9.70	9.20	9.28	9.69	9.57	9.83	9.69
50	48.4	46.7	46.1	44.8	47.000	46.9	47.4	46.7
80	80.3	76.0	73.8	72.0	73.9	74.5	79.3	75.7
180	178	172	172	168	179	175	183	175
	% of nominal [A]							Mean % nominal
10	106	97.0	92.0	92.5	97.0	96.0	98.5	97
50	96.5	93.5	92.5	89.5	94.0	93.5	95.0	94
80	101	95.0	92.0	90.0	92.5	93	99.5	95
180	99.0	95.5	95.5	93.0	99.0	97.0	102	97

[A] Calculations based on 2 replicates on each sampling week.

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Observations:

Parental Toxicity

Adult bird mortality:

Five incidental adult mortalities occurred during the test: one in the control group, two in the treatment group of 10 mg a.s./kg feed and two in the highest treatment group (180 mg a.s./kg feed). No adult mortalities occurred in the treatment group of 50 and 80 mg a.s./kg feed.

The mortality in the control group was the male in Pen 208, which was found dead on Day 3 of Week 18. Prior to death, the male was observed with a head lesion. At necropsy the bird was emaciated, with an extensive head and neck lesion. Abnormalities were observed during necropsy in several organs (including lung, liver, testis and intestines). Necropsy of the male's mate was unremarkable.
The first mortality in the 10 mg a.s./kg feed test group was the male in Pen 230, which was found dead on day 3 of week 11. Prior to death, the male was observed with a head lesion. At necropsy the bird was thin, with a severe head and neck lesion and a lesion on the inside of the left leg. Abnormalities were observed during necropsy in several organs (including heart, spleen, kidneys and testes). Necropsy of the male's mate was unremarkable.

The second mortality in the 10 mg a.s./kg feed test group was the female in Pen 224, which was found dead on day 0 of week 12. Prior to death, the female was observed with a head lesion. At necropsy, the bird was thin with abnormalities in several organs (including spleen and kidneys). Additionally, it was noted that the ovary was developing. Necropsy of the female's mate showed that while the bird had plumage that was phenotypically male-like at test initiation, the bird was a female with a regressed ovary. Since the birds in Pen 224 were both females, data from this pen were excluded from analysis.

The first mortality in the 180 mg a.s./kg feed test group was the male in Pen 265, which was found dead on day 5 of week 9. Prior to death, the bird was observed with foot lesions. At necropsy the bird was externally unremarkable. Internally, there was a cervical fracture of the vertebrae and the spleen was small and pale. Necropsy of the male's mate was unremarkable.

The second mortality in the 180 mg a.s./kg feed test group was the female in Pen 274, which was found dead on day 3 of week 19. Prior to death, the female was observed with a head lesion, a lesion on the left wing, and a ruffled appearance. At necropsy the bird was slightly thin, with an extensive head and neck lesion, feather loss and bruising on the rump. Abnormalities were observed during necropsy in several organs (including of the neck muscle at the base of the skull, the spleen and kidneys were pale, the cecal contents were pasty, and there were yolk remnants in the abdominal cavity. Additionally, the ovary was regressing. Necropsy of the female's mate showed small testes but was otherwise unremarkable.

No other mortalities occurred during the course of the study. Due to the nature of the lesions observed at necropsy, none of the mortalities that occurred were considered to be related to treatment.

Adult bird observations:

No overt signs of toxicity were observed at any of the concentrations tested. Incidental clinical observations noted during the test included those that normally are associated with injuries and penwear. Such signs included lesions on the head, neck, back, around the vent, or feet, a fractured wing, leg, or toe, ventral head curl, feather loss, a pen worn appearance, unilateral wing droop, and a swollen sinus. Clinical signs observed included a loss of coordination (ataxia), a ruffled appearance, a thin appearance, lower limb weakness, reduced reaction to external stimuli, and lameness, but they were typically associated with the incidental injuries. Except for incidental findings, all birds were normal in appearance and behaviour throughout the study.

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Adult bird necropsy:

Adults exposed to fluopyram in the diet showed no indication of treatment effects at necropsy. The birds that survived to the termination of the adult exposure phase had no compound-related gross lesions.

Adult bird body weight:

There were no apparent treatment-related effects upon adult body weight at any treatment level. No statistically significant differences were observed in the treatment groups of 10, 80 and 180 mg a.s./kg feed compared the control group at any of the body weight intervals. Additionally, mean female bodyweight in the treatment group of 50 mg a.s./kg feed was not significantly different from the control mean at any weight interval. However, when compared to the control group, there was a slight decrease in male body weight in the treatment group of 50 mg a.s./kg feed at test termination that was statistically significant ($p < 0.05$).

The difference observed in male body weight in the 50 mg a.s./kg feed treatment group at test termination was influenced, primarily, by two birds. The males in cages 237 and 248 exhibited approximately 10 % reductions in body weight from week 8 until adult termination. The male in cage 237 was noted with a small, pale spleen at necropsy and the male in cage 248 had sustained incidental injuries during the last six weeks of the exposure period that may have contributed to the weight loss. When the terminal body weight data for the males in cage 237 and 248 were excluded, the mean weight (206 ± 8 g) was comparable to the control group and the difference observed was no longer statistically significant. Since the decrease in mean body weight was small, influenced by two pens, and not concentration responsive, it was considered unrelated to treatment.

Table 8.1.1.3- 15: Adult Quail mean body weights and weight gains

Nominal dietary concentration [mg a.s./kg feed]	Males			Females		
	Mean weight ± S.D. [g]		Mean weight gain ± S.D. [g]	Mean weight ± S.D. [g]		Mean weight gain ± S.D. [g]
	Start	End	Difference	Start	End	Difference
Control	192 ± 14	215 ± 22	23 ± 15	189 ± 14	222 ± 22	32 ± 27
10	191 ± 12	210 ± 14	18 ± 13	191 ± 10	232 ± 25	42 ± 21
50	189 ± 10	201 * ± 15	12 ± 10	188 ± 13	236 ± 23	47 ± 16
80	188 ± 10	208 ± 12	20 ± 8	187 ± 9	229 ± 22	41 ± 22
180	189 ± 10	205 ± 9	16 ± 7	186 ± 10	237 ± 13	49 ± 10

S.D.: Standard deviation
* Significant different from the control ($p<0.01$) only before excluding outliers; after exclusion 206 ± 8 g (n.s.)

Adult bird feed consumption:

There were no apparent treatment-related effects upon feed consumption at any treatment level. No statistically significant differences were observed in the treatment groups of 10 and 50 mg a.s./kg feed compared the control group at any of the feed consumption intervals. There were slight differences from the control in the treatment group of 80 mg a.s./kg feed during week 5, and in the 180 mg a.s./kg feed during weeks 6, 7, and 21, that were statistically significant ($p < 0.05$). However, those differences were slight increases in feed consumption, transient and not consistent over time. Therefore, the differences were not considered to be related to treatment.

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The achieved dose was calculated in the report for the pre-egg laying phase (weeks 1-11), for the egg-laying phase (weeks 12-22), and for the total test duration (weeks 1-22). For use in this summary the overall dose over the total test duration is considered relevant.

Table 8.1.1.3- 16: Adult bird feed consumption: Daily dietary dose

Test interval	Nominal dietary concentration [mg a.s./kg feed]	Mean body weight [kg bw]	Mean food consumption ± S.D. [g feed/ bird/ day]	Daily dietary dose [mg a.s./kg bw/day]
Weeks 1 - 22	Control	202	17	0.0
	10	203	18	0.9
	50	201	18	4.5
	80	200	18	7.2
	180	201	18	16.3

Reproduction Toxicity

In the highest treatment level (180 mg a.s./kg feed) there was a treatment-related reduction in offspring survival. The number of 14-day old survivors as a percentage of hatchlings was significantly different ($p < 0.01$). Furthermore, the weight of the hatchlings and of the 14-d old survivors was significantly lower than in the controls ($p < 0.01$).

In an additional evaluation with the William´s test during the original review of the study for Annex 1 inclusion, the slight reduction in 14-d survivor weight at 80 mg a.s./kg feed was also detected as statistically significant.

The tables below present the reproductive endpoints. Data and decimal places are presented according to Appendix C of the DAR 2011 by US EPA.

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Table 8.1.1.3- 17: Reproductive endpoint totals

Reproductive parameter	Nominal dietary concentration [mg a.s./kg feed]				
	Control	10	50	80	180
No. of replicates	15	14	16	16	14
No. of eggs laid (per group)	496	540	662	710	495
No. of eggs laid / hen	33.07	38.57	41.38	44.38	35.36
No. of eggs laid / hen / day	0.31	0.36	0.39	0.42	0.33
No. of eggs cracked / hen	0.33	0.14	0.38	0.88	0.14
No. of eggs set / hen	29.20	34.36	37.06	38.31	31.36
No. of viable embryos / hen	26.93	32.43	30.69	33.38	26.93
No. of live embryos / hen	26.67	32.36	30.50	33.50	26.57
No. of hatchlings / hen	25.40	31.29	30.13	30.81	24.50
No. of 14-day survivors / hen	24.33	26.93	28.94	28.19	19.86
Eggshell mean thickness [mm]	0.22	0.23	0.23	0.22	0.23
Eggshell mean strength [kg]	10.86	11.62	11.22	10.33	10.25
Hatchling mean body weight [g]	5.64	5.64	5.67	5.56	5.08 *
14-d survivor mean body weight [g]	28.57	27.36	28.20	26.19 (*)	24.38 *

* Significantly different from the control ($p<0.01$) in the original statistical evaluation
(*) Significantly different from the control ($p<0.05$) in the additional statistical evaluation of US EPA – DAR 2011

Table 8.1.1.3- 18: Reproductive performance (normalized as percentage)

Reproductive parameter (means)	Nominal dietary concentration [mg a.s./kg feed]				
	Control	10	50	80	180
% eggs not cracked of eggs laid	99.20	99.57	99.36	98.12	99.68
% of eggs set of eggs laid	84.66	87.54	88.14	85.98	88.06
% of viable embryos of eggs set	92.37	91.29	75.93	91.93	90.91
% of live embryos of viable embryos	95.49	99.77	99.62	98.80	98.96
% of hatchlings of eggs laid	71.90	77.81	66.80	72.41	72.88
% of hatchlings of eggs set	84.51	88.07	74.87	84.56	82.61
% of hatchlings of live embryos	96.14	96.91	97.99	93.38	91.55
% of 14-day survivors of eggs set	80.67	76.56	71.82	75.70	63.20 **
% of 14-day survivors of hatchlings	95.79	87.59 *	94.60	89.96	75.36 **

* Significantly different from the control ($p<0.05$)
** Significantly different from the control ($p<0.01$)

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Table 8.1.1.3- 19: Reproductive performance in % of control

Reproductive parameter	Nominal dietary concentration [mg a.s./kg feed]			
	10	50	80	180
	% of control [A]			
No. of eggs laid (per group)	109	133	143	100
No. of eggs laid / hen	118	124	133	106
No. of eggs laid / hen / day	116	126	135	106
No. of eggs cracked / hen	43	113	263	43
No. of eggs set / hen	118	127	131	107
No. of viable embryos / hen	120	114	126	100
No. of live embryos / hen	121	114	126	100
No. of hatchlings / hen	123	119	121	96
No. of 14-day survivors / hen	111	119	116	82
Eggshell mean thickness	100	101	99	101
Eggshell mean strength	107	103	95	94
Hatchling mean body weight	100	101	99	90
14-d survivor mean body weight	96	99	92	85

[A] Not given in report. Calculations based on data for reproductive endpoint totals.

Biological findings:

Table 8.1.1.3- 20: Subchronic and reproduction toxicity to Bobwhite quail

Test substance	**Fluopyram a.s.**
Test object	**Bobwhite quail**
NOAEC for parental toxicity [mg a.s./kg feed]	180 nom (175 measured)
NOEC for reproduction [mg a.s./kg feed]	50 nom (46.7 measured)
NOAEC for reproduction [mg a.s./kg feed]	80 nom (75.7 measured)
NOEAD for parental toxicity [mg a.s./kg bw/day]	16.3 (measured)
NOED for reproduction [mg a.s./kg bw/day]	4.5 (measured)
NOAED for reproduction [mg a.s./kg bw/day]	7.2 (measured)

III. CONCLUSION

The NOAEC of parental toxicity was 180 mg a.s./kg feed (corresponding to a NOAED of 16.3 mg a.s./kg bw/day).

Based on treatment-related reductions in offspring survival, hatchling body weight and 14-day old survivor body weight in the highest treatment level of 180 mg a.s./kg feed, the NOAEC for reproductive toxicity was set in the original report at 80 mg a.s./kg feed which corresponds to a NOAED of 7.2 mg a.s./kg bw/day. The additional statistical analysis by the US EPA in the DAR concluded a NOEC of 50 mg a.s./kg feed which corresponds to a NOED of 4.5 mg a.s./kg bw/day.

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Assessment and conclusion by applicant:

The study and its data are considered as acceptable and reliable for use in risk assessment.

The endpoints are:

NOAEC = 80 mg a.s./kg feed

NOAED = 7.2 mg a.s./kg bw/day

The additional statistical analysis by the US EPA in the DAR concluded the following endpoints:

NOEC = 50 mg a.s./kg feed

NOED = 4.5 mg a.s./kg bw/day

In the opinion of the applicant, the biological relevance of the effect on 14-day survivor body weight at 80 mg a.s./kg feed (which is behind the NOEC determined by US EPA at 50 mg a.s./kg feed) is questionable. This was also the assessment of the original RMS who proposed to use the NOAEC of 80 mg a.s./kg feed (equivalent to 7.2 mg a.s./kg bw/d) as higher tier endpoint in population level risk assessments for fluopyram.

The difference at 80 mg a.s./kg feed on 14-day survivor body weight is only 8.3% (i.e., within the relative standard deviation of the control of 10.8% for that endpoint) and not statistically significant in a pair-wise comparison to the control.

Furthermore, this difference was obtained under constant exposure over 22 weeks of exposure, whilst fluopyram rapidly dissipates from foliage or insects under realistic field conditions (DT_{50} ~ 5 days, see MCA section 8.9).

Finally, rapid reversibility of effects on chick weight was demonstrated at treatment levels as high as 500 and 1000 mg a.s./kg feed in study M-299245-02-1 (KCA 8.1.1.3/01).

Thus, the minor effect on chick body weight after 22 weeks of exposure to 80 mg a.s./kg feed is unlikely to occur under field conditions with only short-term exposure of wild birds.

Therefore, it is proposed to maintain the option to employ the NOAEC of 80 mg a.s./kg feed (equivalent to 7.2 mg a.s./kg bw/d) as higher tier endpoint in population level risk assessments for fluopyram.

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Data Point:	KCA 8.1.1.3/03
Report Author:	[redacted]
Report Year:	2008
Report Title:	Effect of AE C656948 technical on reproduction of the mallard duck (Anas platyrhynchos)
Report No:	EBGMP041
Document No:	M-299277-01-1
Guideline(s) followed in study:	OECD 206; FIFRA Guideline 71-4; USEPA OPPTS 850.2300
Deviations from current test guideline:	Current Guideline: OECD 206 (1984) Deviations: The birds were 16 weeks old at experimental start, below the minimum age of 9 months recommended by the guideline. However, the younger birds ensured that the birds have not been through a reproductive cycle and that egg production does not begin too soon after exposure. The floor area per pair was 0.4819 m², below the 1.0 m² recommended by the guideline. However, this cage size was considered successful for both husbandry and reproduction in mallards. The temperature during egg storage was 11.3 °C, lower than the 14-16 °C recommended. The temperature during incubation (37.3 °C) and hatching phase (37.2 °C) was slightly lower than 37.5 °C recommended by the guideline. The hatchlings were kept at a temperature of 32-38 °C during the 14-day post hatchling phase, higher than the 32-35 °C and 28-32 °C recommended for the first and second week, respectively. The humidity during egg storage was 95.8 %, higher than the recommended 60-85 %. The humidity during incubation and hatching was 55.1 and 69.6 %, respectively, lower than the recommended 60-75 and 75-85 %. The humidity for the hatchlings during the 14-day post hatchling phase was 55.7 %, lower than the recommended 60-85 %. There were 68 % viable embryos of eggs set, lower than the recommended 85-98 %. These deviations are not expected to have impacted the study results. All validity criteria were fulfilled.
Previous evaluation:	Yes, evaluated and accepted in DAR (2011)
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 evaluate the effects of dietary exposure to fluopyram on health and reproductive capacity of birds, represented by mallard ducks. Adult mallard ducks (*Anas platyrhynchos*) were exposed to fluopyram over approximately 19 weeks to nominal dietary concentrations of 0 (control), 100, 200 and 500 mg a.s./kg feed. Mallard ducks were 16 weeks old at experimental start. Eggs were collected from the parental birds for 8 weeks (after 8 weeks of a pre-photostimulation period followed by 3 weeks of photostimulation before the egg-laying period).

There were 15 pairs of birds at each treatment level with one reproductive pair of birds (i.e. one male and one female) per cage. Birds were observed for mortality, abnormal behaviour and signs of toxicity; adult body weight and feed consumption were measured; gross pathology was conducted; reproductive parameters, as well as hatchling health, growth and survival, were examined.

The study fulfilled all validity criteria of OECD 206 guideline.

The mean measured amounts of fluopyram tech. in week 1, 5, 10, 15, and 19 were determined as 0, 100, 183, and 428 mg/kg feed representing percent nominal values of 100, 92 and 86 %, respectively.

There were no treatment–related mortalities, overt signs of toxicity or treatment-related effects upon body weight or feed consumption with the exception of feather loss and minor injuries as a result of cage wear. There were no compound related symptoms of toxicity in the adults and offspring.

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Additionally, there were no statistically significant adverse effects for any reproductive endpoint in the study.

The No Observed Effect Concentration (NOEC) for both, parental toxicity and reproduction endpoints, of mallard ducks exposed fluopyram tech. was 500 mg a.s./kg feed, corresponding to 40 mg a.s./kg bw/day (NOED). The Lowest Observed Effect Concentration (LOEC) for both, parental toxicity and reproduction, was reported as > 500 mg a.s./kg food, corresponding to > 40 mg a.s./kg bw/day (LOED). The additional statistical analysis by the US EPA in the DAR concluded a NOEC of 200 mg a.s./kg feed which corresponds to a NOED of 18 mg a.s./kg bw/day.

I. Material and methods

Test item: fluopyram, specification No.: 102000012455; Batch No.: 08528/0002; Tox No. 07932-00; purity: 94.7 % w/w.

Test design: Adult mallard ducks (*Anas platyrhynchos*) were exposed to fluopyram for approximately 19 weeks to nominal dietary concentrations of 0 (control), 100, 200 and 500 mg a.s./kg feed (adjusted for purity). Test diets were prepared by mixing fluopyram into a premix that was used for weekly preparation of the final diet. Control diet and each of the treated diets were prepared weekly and presented to the birds each week.

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 SANTE/2020/12830, Rev.1.

Mallard ducks were 16 weeks old at experimental start. There were 15 pairs of birds at each treatment level resulting in 60 pairs for the total study. One reproductive pair of birds (i.e. one male & one female) was housed per cage. Eggs were collected from the parental birds for 8 weeks (after 8 weeks of a pre-photostimulation period followed by 3 weeks of photostimulation before the egg-laying period).

The test birds were acclimated to the test facility and study cages for approximately 2 weeks prior to experimental start. During the acclimation, all birds were observed daily. Birds exhibiting abnormal behaviour or debilitating physical injuries were not used for the test.

Adult birds were housed indoors in cages. The adult quail cages measured approximately 79 × 61 × 53 cm (length × width × height). Cages were constructed of stainless-steel wire grid and stainless-steel sheeting. Cage floors were constructed of plastic-coated steel wire and slopped to accommodate egg collection. Each cage was equipped with both feed and watering troughs. The birds were fed basal diet (Teklad Game Bird Ration) and tap water ad libitum during the acclimation and testing period. The photoperiod through the acclimation period (2 weeks) and the first 8 weeks of the test the birds were held under a photoperiod of 7 hours of light and 17 hours dark per day. The photoperiod was then increased to 17 hours of light and 7 hours dark per day for the remainder of the study.

During the study, all adult birds were observed daily for signs of toxicity or abnormal behaviour. Additionally, all offspring were observed daily from hatching until 14 days of age. A record was maintained for all clinical observations and mortalities. Adult body weights were measured the at experimental start, in week 3, 5, 7 and 9, and at adult termination. No adult body weights were taken during the egg production phase. Adult feed consumption was measured weekly by cage throughout the study.

Gross pathology was conducted for all birds that died or were euthanized during the course of the study. At the end of the exposure period, all surviving birds of the control and highest dose level were euthanized and necropsied. Additionally, at least five males and five females from each of the remaining dose levels were necropsied. Reproductive parameters, as well as hatchling health, growth and survival were examined. In addition, the effects of adult exposure to fluopyram on the number of eggs laid, fertility, embryo viability, hatchability, offspring survival, and eggshell quality/thickness were evaluated.

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Eggs were collected twice daily and placed in a cold room (11.3 °C) until incubation. All eggs laid in a weekly interval were considered as one lot. At the end of the weekly interval, all eggs were removed from the cold room and candled to detect cracks. Cracked or abnormal eggs were recorded and discarded. Eggs that were not cracked or used for eggshell strength and thickness measurements were transferred in an incubator. The transfer of the eggs to the incubator was referred to as "egg set". The first eggs were set in the incubator at 37.3 ± 0 °C. Eggs were candled again on day 14 of incubation to determine embryo viability (fertility) and on day 21 to determine embryo survival. On day 23 of incubation, eggs were placed into a hatcher (37.2 °C) and allowed to hatch. Hatchlings were removed from the hatching compartments on days 27 and 28 of incubation. The unhatched eggs per parental cage were observed for embryo attempts to hatch (pipping), recorded and discarded on day 28. The body weights of surviving hatchlings were recorded.

Afterwards, hatchlings were housed in brooding pens and kept on untreated diet until 14 days of age when they were weighed again and sacrificed. Thermostats in the brooding compartment of each cage were set to maintain a temperature gradient of approximately 32 to 38 ºC over the course of the 14-day post hatchling phase at a photoperiod of 14 hours light and 10 hours dark.

Statistics: Data from treatment groups were compared to controls using the Shapiro-Wilk's test for normality and Levene's test of equal variance to determine if dose groups had unequal variances. If assumption of normality ($p \leq 0.01$) and homogeneity of variance ($p \leq 0.05$) were met, then parametric analyses were conducted using analysis of variance (ANOVA) followed by Dunnett's test or William's test. If variances were unequal, then the non-parametric analyses were conducted using the Jonckheere or Mann-Whitney procedures.

Statistical analyses were performed using SAS statistical software. The statistical significance was set at $\alpha = 0.05$ (95 % confidence level). Each of the following parameters was analysed statistically: adult body weight, adult feed consumption, eggs laid per hen, eggs cracked of eggs laid per hen, eggs not cracked of eggs laid per hen, viable embryos of set per hen, live three-week embryos of viable embryos per hen, number hatched of live four-week embryos, 14-day survivors of hatchlings, hatchlings of eggs set, percent 14-day survivors of eggs set per hen, number of hatchlings set per hen, number of 14-day old survivors of set, egg shell strength, average egg shell thickness, offspring's body weight.

In the review of the study for the original Annex I listing of fluopyram, an additional statistical was conducted using "chicks.sas" (Ver. 3; March 2002), a SAS program provided by EFED/OPP/USEPA. Data for all endpoints were examined graphically using box plots to determine if they exhibited a dose-dependent response, which was ultimately used to select the multiple comparison test to detect LOAEC and NOAEC. Data for each endpoint were tested to determine if their distributions were normal and if their variances were homogeneous using Shapiro-Wilk's and Levene's tests, respectively. Data that satisfied these assumptions were subjected to Dunnett's and William's tests and data that did not satisfy these assumptions were subjected to the non-parametric Mann-Whitney-U (with a Bonferroni adjustment) and Jonckheere's tests. Data for dead birds were excluded from the analyses.

Dates of experimental work: May 8th, 2007 to October 30th, 2007

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II. RESULTS AND DISCUSSION

Validity criteria:

Table 8.1.1.3- 21: Validity criteria (according to OECD 206, 1984)

Validity Criteria	Required	Obtained
Adult mortality in control	≤ 10 %	0 %
Mean number of 14-day old survivors in the controls	≥ 14 per hen	27.7 per hen
Eggshell thickness in control	≥ 0.34 mm	0.330 mm [A]
Concentration of the test item in the feed	≥ 80 % of the nominal concentrations	86 - 100 % of the nominal concentrations

[A] Considered fulfilled as the 0.330 mm eggshell thickness value was within the normal range of historical control data.

Analytical results for dietary concentration:

The measured concentrations of fluopyram in week 1, 5, 10, 15 and 19 ranged between 80 and 115 % of nominal concentrations (see table below). The mean measured concentrations of fluopyram were determined as 100, 183 and 428 mg a.s./kg feed representing 100, 92, and 86 % of nominal, respectively. These values correspond to daily dietary dose levels of 0, 10, 18, and 40 mg a.s./kg bw/day, respectively. Analysis of diet samples collected from feeders after being held at ambient temperature for 9 days (82 and 90 % of the nominal concentrations for the 100 and 500 mg a.s./kg feed as test concentrations) confirmed appropriate maintenance of the treatment concentrations.

No residues of fluopyram were detected in the control diets above 9.61 mg a.s./kg feed.

A summary of the dietary concentrations is included in the following table.

Table 8.1.1.3- 22: Feed analysis summary of fluopyram

Nominal dietary concentration [mg a.s./kg feed]	Week 1	Week 5	Week 10	Week 15	Week 19	Mean measured dietary concentration [mg a.s./kg feed]
	Measured dietary concentration [mg a.s./kg feed]					
100	92.2	115	99.6	111	83	100 ± 13.3
200	173	205	195	171	171	183 ± 15.9
500	420	469	401	423	426	428 ± 25.0
	% of nominal					Mean % nominal
100	92	115	100	111	83	100
200	86	102	98	85	86	92
500	84	94	80	85	85	86

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Observations:

Parental Toxicity

Adult bird mortality:

No bird mortality occurred, and no birds were sacrificed during the study. There was no significant difference in adult mortality as compared to the control for any treatment level.

Adult bird observations:

No overt signs of intoxication were observed during the study in any adult test group. Minor occurrences of feather loss and minor injuries which were associated with normal laboratory cage wear were observed in the control and several treatment levels. There were no significant clinical symptoms or compound related effects observed during the study.

Adult bird necropsy:

Adults exposed to fluopyram in the diet showed no indication of treatment effects at necropsy. Feather loss was noted on a few birds due to normal cage wear for laboratory reared mallards. No dose-response relationship existed for these few and infrequent observations. The birds that survived to the termination of the adult exposure phase had no compound-related gross lesions.

Adult bird body weight:

Male adult mallard showed a small decrease in mean body weight while the females showed an increase. However, there were no statistically significant differences at any treatment level compared to the control regarding male or female adult body weight change from study initiation to termination.

Table 8.1.1.3- 23: Adult Mallard mean body weights and weight gains

Nominal dietary concentration [mg a.s./kg feed]	Males			Females		
	Mean weight ± S.D. [g]		Mean weight gain [g]	Mean weight ± S.D. [g]		Mean weight gain [g]
	Start	End	Difference	Start	End	Difference
Control	1132 ± 103	1115 ± 92	- 17.5	963 ± 68	1202 ± 101	239.0
100	1136 ± 61	1122 ± 101	- 14.7	959 ± 71	1211 ± 74	251.7
200	1113 ± 109	1083 ± 88	- 29.6	956 ± 63	1250 ± 107	293.7
500	1143 ± 66	1103 ± 86	- 39.6	958 ± 67	1200 ± 92	242.7

S.D.: Standard deviation

Adult bird feed consumption:

There were no statistically significant differences at any treatment level as compared to the control for the food consumption endpoint.

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Table 8.1.1.3- 24: Adult bird feed consumption: Daily dietary dose

Test interval	Nominal dietary concentration [mg a.s./kg feed]	Mean body weight [kg bw]	Mean food consumption ± S.D. [g feed/ bird/ day]	Daily dietary dose [mg a.s./kg bw/day]
Weeks 1 - 19	Control	1.103 [A]	103.7 ± 17.9	0
	100	1.107	104.9 ± 13.9	10
	200	1.100	109.2 ± 19.0	18
	500	1.101	101.6 ± 15.5	40

S.D.: Standard deviation
A Not given in report. Calculations based on the body weight of male and female mallard ducks at test start and at test termination.

Reproduction Toxicity

There were no statistically significant differences at any treatment level as compared to the control for any of the egg endpoints.

The table below presents the reproductive endpoints totals.

Table 8.1.1.3- 25: Reproductive endpoint totals

Reproductive parameter	Nominal dietary concentration [mg a.s./kg feed]			
	Control	**100**	**200**	**500**
No. of replicates	15	15	15	15
No. of eggs laid (per group)	780	810	766	745
No. of eggs laid / hen	52.0	54.0	51.1	49.7
No. of eggs laid / hen / day	0.93 [A]	0.96 [A]	0.91 [A]	0.93 [A]
No. of eggs cracked / hen	0.07	0.07	0.33	0.13
No. of eggs set / hen	47.3	49.1	45.8	45.1
No. of viable 14-day embryos / hen	32.2	34.7	41.1	38.8
No. of 3-week live embryos / hen	32.1	34.1	40.7	38.7
No. of hatchlings / hen	27.9	27.9	28.3	30.0
No. of 14-day survivors / hen	27.7	27.9	28.0	30.0
Eggshell mean thickness [mm]	0.330	0.331	0.329	0.327
Eggshell mean strength [kg]	2.682	2.640	2.676	2.717
Hatchling mean body weight [g]	36.0	36.9	36.5	35.2
14-d survivor mean body weight [g]	254.7	257.9	254.0	245.6 (*)

A Not given in report. Calculations based on an egg laying period of 56 days.
(*) Significantly different from the control ($p<0.05$) in the additional statistical evaluation of US EPA – DAR 2011

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Table 8.1.1.3- 26: Reproductive performance (normalized as percentage)

Reproductive parameter	Nominal dietary concentration [mg a.s./kg feed]			
	Control	100	200	500
% of eggs not cracked of eggs laid	100	100	99	100
% of eggs set of eggs laid	90	91	90	91
% of viable 14-day embryos of eggs set	68	72	89	86
% of live 3-week embryos of viable embryos	100	98	98	100
% of hatchlings of eggs laid	52	51	53	60
% of hatchlings of eggs set	58	56	59	65
% of hatchlings of live embryos	82	83	68	77
% of 14-day survivors of eggs set	57	56	59	65
% of 14-day survivors of hatchlings	99	100	99	100

Table 8.1.1.3- 27: Reproductive performance in % of control

Reproductive parameter	Nominal dietary concentration [mg a.s./kg feed]		
	100	200	500
	% of control [A]		
No. of eggs laid (per group)	104	98	96
No. of eggs laid / hen	104	98	96
No. of eggs laid / hen / day	104 [B]	98 [B]	96 [B]
No. of eggs cracked / hen	100	471	186
No. of eggs set / hen	104	97	95
No. of viable embryos / hen	108	128	120
No. of live embryos / hen	106	127	121
No. of hatchlings / hen	100	101	108
No. of 14-day survivors / hen	101	101	108
Eggshell mean thickness	100	100	99
Eggshell mean strength	98	100	101
Hatchling mean body weight	103	101	98
14-d survivor mean body weight	101	100	96

[A] Not given in report. Calculations based on data for reproductive endpoint totals.
[B] Not given in report. Calculations based on an egg laying period of 56 days.

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Biological findings:

Table 8.1.1.3- 28: Subchronic and reproduction toxicity to Mallard

Test substance	Fluopyram a.s.
Test object	Mallard duck
NOEC for parental toxicity [mg a.s./kg feed]	500 nom (428 measured)
NOEC for reproduction [mg a.s./kg feed]	500 nom (428 measured)
LOEC for parental toxicity [mg a.s./kg feed]	> 500 nom (428 measured)
LOEC for reproduction [mg a.s./kg feed]	> 500 nom (428 measured)
NOED for parental toxicity [mg a.s./kg bw/day]	40 (measured)
NOED for reproduction [mg a.s./kg bw/day]	40 (measured)
LOED for parental toxicity [mg a.s./kg bw/day]	> 40 (measured)
LOED for reproduction [mg a.s./kg bw/day]	> 40 (measured)

III. CONCLUSION

The NOEC for both, parental toxicity and reproduction, was reported as 500 mg a.s./kg feed, corresponding to 40 mg a.s./kg feed, measured (NOED).

The LOEC for both, parental toxicity and reproduction, was reported as > 500 mg a.s./kg feed, corresponding to > 40 mg a.s./kg feed, measured (LOED).

The additional statistical analysis by the US EPA in the DAR concluded a NOEC of 200 mg a.s./kg feed which corresponds to a NOED of 18 mg a.s./kg bw/day.

Assessment and conclusion by applicant:

The study and its data are considered as acceptable and reliable for use in risk assessment.

The endpoints are:

NOEC = 500 mg a.s./kg feed

NOED = 40 mg a.s./kg bw/day

The additional statistical analysis by the US EPA in the DAR concluded the following endpoints:

NOEC = 200 mg a.s./kg feed

NOED = 18 mg a.s./kg bw/day

****

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Data Point:	KCA 8.1.1.3/04
Report Author:	[redacted]
Report Year:	2019
Report Title:	Calculation of EC10 for fluopyram for reproduction endpoints in bobwhite quail
Report No:	M-667209-01-1
Document No:	M-667209-01-1
Guideline(s) followed in study:	None
Deviations from current test guideline:	Current Guideline: 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

In the present study effect concentrations (EC_{10}) were calculated from combined data of two reproduction studies in bobwhite quail exposed to fluopyram (studies by M-299245-02-1 and by M-298723-01-1).

Calculations of EC_{10} were conducted using ToxRat version 3.3.0. Calculations were performed both with summary data and individual replicate data. Effect concentrations are reported for those reproduction endpoints, for which a significant dose response was calculated.

The resulting EC_{10} values were then evaluated regarding their reliability, based on the reliability criterion NW (normalised width) proposed by EFSA (2015). A threshold of NW ≤ 1.0 was applied to identify reliable fits, corresponding to the EFSA categories "excellent" (NW < 0.2), "good" (NW < 0.5) and "fair" (NW < 1.0). Fits with NW ≥ 1.0 ("poor" or "bad") were not considered reliable enough to determine valid EC_{10} values.

When more than one fit resulted in NW < 1.0, then the fit with the lowest NW was considered as the most reliable, and selected for the EC_{10} value concluded below. Where the same NW is obtained in reliable fits, the lowest EC_{10} is selected.

When none of the fits for an assessment endpoint provided for NW < 1, then no reliable EC_{10} values can be generated and the NOAEL was selected as the relevant endpoint.

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Table 8.1.1.3- 29: EC_{10} of all reproduction endpoints with a significant dose response from two combined reproduction studies in bobwhite quail exposed to fluopyram.

Endpoint	EC_{10} ind. Data	EC_{10} sum. Data	Selected endpoint [mg a.s./kg bw]
Eggs laid per hen	n.r. (NW>1)	n.r. (NW>1)	16.3 (NOAEL)
Eggs cracked per eggs laid	n.c.	n.r. (NW>1)	16.3 (NOAEL)
Eggshell strength	n.r. (NW>1)	n.r. (NW>1)	16.3 (NOAEL)
Eggshell thickness	19.2 (NW 0.9)	n.r. (NW>1)	16.3 (NOAEL)
Viable embryos per eggs set	n.c.	n.r.(NW>1)	16.3 (NOAEL)
Live 3-wk embryos per viable embryos	n.c.	n r.(NW>1)	16.3 (NOAEL)
Hatchlings per hen	5.8 (NW 0.9)	15.1 (NW 0.4)	15.1
Hatchlings per eggs set	9.8 (NW 0.7)	n.r. (NW>1)	9.8
Hatchlings per viable embryos	n.c.	n.r. (NW>1)	16.3 (NOAEL)
Hatchlings per live 3-week embryos	n.c.	17.9 (NW 0.6)	17.9
14-day survivors per hen	5.7 (NW 0.9)	14.4 (NW 0.2)	14.4
14-day survivors per eggs set	7.8 (NW 0.8)	n.r. (NW>1)	7.8
14-day survivors per hatchlings	n.c.	n.r. (NW>1)	7.2 (NOAEL)
Initial hatchling bodyweight[3]	n.r. (NW>1)	n.r. (NW>1)	7.2 (NOAEL)
14-day survivor bodyweight[3]	9.2 (NW 0.9)	n.r. (NW>1)	9.2

n.r.: Not reliable
n.c.: Not computable (ToxRat reported the following reason: No statistically significant dose/response was found (p(F)>0.05; i.e. slope of the relationship is not significantly different from zero)

I. MATERIAL AND METHODS

Data used for analysis:

The endpoints and their standard deviations were normalised and given as percent of control. The control used for normalisation was always from the same study as the normalised value. All normalised data, expressed as percent of control, were then combined to one data set for the statistical analysis. Values such as viable eggs of eggs set were calculated only for those animals, for which data were available, i.e. for animals which actually laid eggs.

Where necessary the raw data was re-evaluated to ensure that correct data was used for analysis.

Since ToxRat (the software which was used for the ECx analysis) is not able to correctly combine studies (i.e. it is not possible to relate results of different treatment groups to their respective control groups; ToxRat first combined the controls and then relates effects of treatment groups to the pooled control), the available data could not be analysed as quantal data.

Hence, the following quantal data was normalised by its respective control group: Eggs not cracked per eggs laid, viable embryos per eggs set, live 3-week embryos per viable embryos, hatchlings per eggs set, hatchlings per viable embryos, hatchlings per live 3-week embryos, 14-day survivors per egg set and 14-day survivors per hatchlings.

Overall, the following data were analysed as continuous data: Eggs laid per hen, eggshell thickness, eggshell strength, eggs not cracked per eggs laid, viable embryos per eggs set, live 3-week embryos per viable embryos, hatchlings per hen, hatchlings per eggs set, hatchlings per viable embryos, hatchlings, per live 3-week embryos, 14-day survivors per hen, 14-day survivors per egg set, 14-day survivors per hatchlings, initial hatchling bodyweight, 14-day survivor bodyweight.

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The endpoint “Eggs cracked per eggs laid” could not be directly analysed, since ToxRat can only calculate a decrease for continuous data (a decrease in eggs cracked would be a positive effect). Therefore, this endpoint was analysed as “Eggs not cracked per eggs laid”, the approach nowadays employed in statistical evaluation of bird reproduction studies.

An EC_x analysis for the endpoint “Eggs cracked per hen” was considered not to be needed, since this endpoint is covered by the endpoint “Eggs not cracked per eggs laid”.

Table 8.1.1.3- 30: Overview of the normalised and combined data used for the analysis of each reproduction endpoint.

Endpoint	Dose [mg/kg bw/day]	Mean	SD	N
Eggs laid per hen [% of control]	0[1]	100.0	50.5	18
	0[2]	100.0	63.7	15
	0.9[2]	116.6	44.3	14
	4.5[2]	125.1	59.4	16
	7.2[2]	134.2	43.3	16
	16.3[2]	106.9	60.0	14
	23[1]	80.9	36.7	18
	50[1]	52.7	32.8	18
	104[1]	49.6	37.6	18
Eggs cracked per hen [% of control]	0[1]	100.0	145.5	18
	0[2]	100.0	185.2	15
	0.9[2]	42.9	108.9	14
	4.5[2]	112.5	215.6	16
	7.2[2]	262.5	535.3	16
	16.3[2]	42.9	160.4	14
	23[1]	466.7	401.5	18
	50[1]	400.0	436.6	18
	104[1]	266.7	369.4	18
Eggs cracked per eggs laid [% of control]	0[1]	100.4	1.2	14.0
	0[2]	100.2	1.2	16.0
	0.9[2]	98.9	3.7	16.0
	4.5[2]	100.5	1.2	14.0
	7.2[2]	93.2	7.1	17.0
	16.3[2]	94.4	6.6	18.0
	23[1]	93.2	13.1	16.0
	50[1]	100.4	1.2	14.0
	104[1]	100.2	1.2	16.0
Eggshell thickness [% of control]	0[1]	100.0	5.7	16
	0[2]	100.0	7.3	14
	0.9[2]	100.5	3.0	14
	4.5[2]	100.9	6.0	16

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Endpoint	Dose [mg/kg bw/day]	Mean	SD	N
	7.2[2]	98.9	6.8	16
	16.3[2]	100.8	5.1	13
	23[1]	89.3	10.5	15
	50[1]	88.6	13.0	13
	104[1]	90.3	10.8	10
Viable embryos per eggs set [% of control]	0[1]	100.0	10.8	18
	0[2]	100.0	16.4	14
	0.9[2]	98.8	17.8	14
	4.5[2]	82.2	39.3	16
	7.2[2]	99.5	19.4	16
	16.3[2]	98.4	28.5	14
	23[1]	83.8	18.3	17
	50[1]	57.5	33.4	17
	104[1]	56.6	34.7	15
Live 3-week embryos per viable embryos [% of control]	0[1]	100.0	0.6	18
	0[2]	100.0	13.9	14
	0.9[2]	104.5	0.9	14
	4.5[2]	104.3	0.8	15
	7.2[2]	103.5	1.8	16
	16.3[2]	103.6	2.6	13
	23[1]	67.5	31.9	17
	50[1]	69.3	23.4	15
	104[1]	58.9	33.9	13
Hatchlings per hen [% of control]	0[1]	100.0	50.9	18
	0[2]	100.0	70.2	15
	0.9[2]	123.2	55.2	14
	4.5[2]	118.6	75.2	16
	7.2[2]	121.3	34.9	16
	16.3[2]	96.5	70.6	14
	23[1]	36.4	37.0	18
	50[1]	9.5	10.1	18
	104[1]	1.6	3.2	18
Hatchlings per eggs set [% of control]	0[1]	100.0	13.0	18
	0[2]	100.0	21.2	14
	0.9[2]	104.2	18.3	14
	4.5[2]	94.5	36.8	15
	7.2[2]	100.1	21.8	16
	16.3[2]	105.3	16.3	13
	23[1]	46.8	32.4	17

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Endpoint	Dose [mg/kg bw/day]	Mean	SD	N
	50[1]	29.7	31.0	17
	104[1]	5.5	10.9	15
Hatchlings per viable embryos [% of control]	0[1]	100.0	7.5	18
	0[2]	100.0	13.6	14
	0.9[2]	105.5	4.2	14
	4.5[2]	106.5	5.8	15
	7.2[2]	100.6	10.6	16
	16.3[2]	99.1	14.8	13
	23[1]	56.1	35.7	17
	50[1]	46.7	32.7	15
	104[1]	9.4	16.3	13
Hatchlings per live 3-week embryos [% of control]	0[1]	100.0	7.5	18
	0[2]	100.0	3.9	14
	0.9[2]	100.8	3.5	14
	4.5[2]	101.9	5.4	15
	7.2[2]	97.1	10.4	16
	16.3[2]	95.2	13.1	13
	23[1]	79.4	32.5	16
	50[1]	62.1	36.1	15
	104[1]	18.3	32.6	12
14-day survivors per hen [% of control]	0[1]	100.0	50.9	18
	0[2]	100.0	71.3	15
	0.9[2]	110.7	48.8	14
	4.5[2]	118.9	76.0	16
	7.2[2]	115.8	38.8	16
	16.3[2]	81.6	67.5	14
	23[1]	34.9	36.0	18
	50[1]	5.9	9.2	18
	104[1]	0.2	1.0	18
14-day survivors per eggs set [% of control]	0[1]	100.0	12.9	18
	0[2]	100.0	21.3	14
	0.9[2]	94.9	16.9	14
	4.5[2]	95.0	37.6	15
	7.2[2]	93.8	25.4	16
	16.3[2]	84.4	30.2	13
	23[1]	44.4	31.3	17
	50[1]	19.6	31.5	17
	104[1]	0.5	1.9	15
	0[1]	100.0	2.0	18

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Endpoint	Dose [mg/kg bw/day]	Mean	SD	N
14-day survivors per hatchling [% of control]	0[2]	100.0	6.4	14
	0.9[2]	91.4	9.4	14
	4.5[2]	98.8	8.6	15
	7.2[2]	93.9	15.1	16
	16.3[2]	78.7	22.8	13
	23[1]	95.2	10.6	14
	50[1]	47.1	44.1	13
	104[1]	12.6	25.2	4
Initial hatchling bodyweight [% of control]	0[1]	100.0	6.8	18
	0[2]	100.0	9.6	14
	0.9[2]	99.0	9.2	14
	4.5[2]	99.6	7.4	15
	7.2[2]	97.7	7.2	16
	16.3[2]	89.8	5.9	13
	23[1]	97.1	8.7	16
	50[1]	88.1	6.5	13
	104[1]	85.9	6.7	4
14-day survivor bodyweight [% of control]	0[1]	100.0	6.1	18
	0[2]	100.0	11.2	14
	0.9[2]	95.2	7.5	14
	4.5[2]	98.5	8.2	15
	7.2[2]	91.7	10.3	16
	16.3[2]	84.5	17.3	13
	23[1]	84.7	11.6	16
	50[1]	70.8	11.8	9
	104[1]	68.1	-	1

[1] Data from Stoughton and Lam, 2008
[2] Data from Frey et al., 2008

Statistical evaluation:

According to Commission Regulation (EU) No 283/2013 dated 1 March 2013, chapter 8.1.1.3. "The EC_{10} and EC_{20} shall be reported. Where they cannot be estimated, an explanation shall be provided together with the NOEC expressed in mg substance/kg bw/day".

EC_{10} concentrations were calculated using ToxRat version 3.3.0 with the default regression for all available functions (logit, probit, Weibull) using both, individual and summary data. For quantal data, this was not possible, because ToxRat provides no functionality to analyse summary data for quantal

To characterize the reliability of EC_x values, the 'normalised width' or NW, which is the ratio of the confidence interval of the ECx 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 (see table below).

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Table 8.1.1.3- 31: Classification of ´normalised width' (NW) according to EFSA (2015)

NW	Rating	Application in this evaluation
< 0.2	Excellent	Accepted as reliable
< 0.5	Good	
< 1	Fair	
< 2	Poor	Considered as not reliable (not accepted in this evaluation)
> 2	Bad	

When more than one dose-response function could significantly be fitted, the EC_{10} value with the lowest normalised width (NW) was selected for the respective endpoint. When the same NW was obtained, the fit with the lower EC_{10} is selected.

II. RESULTS AND DISCUSSION

EC_{10} values calculated using ToxRat 3.3.0 and their normalised width (NW) are summarised in the table below for all endpoints. The most reliable EC_{10} (smallest NW) was selected. If no reliable EC_{10} (NW < 1) could be fitted, the NOAEL was selected.

Table 8.1.1.3- 32: Eggs laid per hen

	Individual data			Summary data			Comment
	Logit	Probit	Weibull	Logit	Probit	Weibull	
EC_{10}	2.3	2.5	1.9	14.8	15.4	8.5	Measured data indicate that 10% effect reached at > 16.3 mg/kg bw/d (3.6% difference to control)
CL_L	0.1	0.2	0.1	1.8	3.2	3.1	
CL_U	6.3	6.4	5.6	26.8	26.3	14.8	
Width	6.2	6.2	5.5	25	23.1	11.7	
NW	2.7	2.5	2.9	1.7	1.5	1.4	
Conclusion	No reliable EC_{10}, retain endpoint specific NOAEL = 16.3 mg/kg bw/d						

Table 8.1.1.3- 33: Eggs cracked per eggs laid

	Individual data			Summary data			Comment
	Logit	Probit	Weibull	Logit	Probit	Weibull	
EC_{10}	Not computable			n.c.	n.d.	124.8	Measured data indicate that 10% effect may be reached only above the top test level (104 mg/kg bw/d)
CL_L						40.9	
CL_U						6725.0	
Width						6684.1	
NW						53.6	
Conclusion	No reliable EC_{10}, retain endpoint specific NOAEL = 16.3 mg/kg bw/d						

n.c.: Not computable (ToxRat reported the following reason: No statistically significant dose/response was found (p(F)>0.05; i.e. 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

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Table 8.1.1.3- 34: Eggshell strength

	Individual data			Summary data			Comment
	Logit	Probit	Weibull	Logit	Probit	Weibull	
EC_{10}	6.5	7.1	6.0	12.3	12.6	12.0	Measured data indicate that 10% effect reached at ≈ 16.3mg/kg bw/d
CL_L	0.7	1.0	0.5	n.d.	0.0	n.d.	
CL_U	13.7	14.1	13.3	29.6	28.4	30.6	
Width	13	13.1	12.8	n.d.	28.4	n.d.	
NW	2.0	1.8	2.1	n.d.	2.3	n.d.	
Conclusion	No reliable EC_{10}, retain endpoint specific NOAEL = 16.3 mg/kg bw/d						

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

Table 8.1.1.3- 35: Eggshell thickness

	Individual data			Summary data			Comment
	Logit	Probit	Weibull	Logit	Probit	Weibull	
EC_{10}	53.3	40.7	**19.2**	n.c.	60.4	30.1	Measured data indicate that 10% effect reached at ≈23 mg/kg bw/d
CL_L	33.0	25.7	12.7		22.4	11.2	
CL_U	101.4	80.8	30.8		1844.9	227.5	
Width	68.4	55.1	18.1		1822.5	216.3	
NW	1.3	1.4	0.9		30.2	7.2	
Conclusion	Reliable EC_{10} = 19.2 mg/kg bw/d						

n.c.: not computable (ToxRat reported the following reason: No statistically significant dose/response was found (p(F)>0.05; i.e. slope of the relationship is not significantly different from zero)

Table 8.1.1.3- 36: Viable embryos per eggs set

	Individual data			Summary data			Comment
	Logit	Probit	Weibull	Logit	Probit	Weibull	
EC_{10}	Not computable			12.2	13.0	5.2	Measured data indicate that 10% effect reached at > 16.3 mg/kg bw/d (1.6% difference to control)
CL_L				n.d.	n.d.	0.11	
CL_U				28.9	29.4	13.8	
Width				n.d.	n.d.	13.7	
NW				n.d.	n.d.	2.6	
Conclusion	No reliable EC_{10}, retain endpoint specific NOAEL = 16.3 mg/kg bw/d						

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

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Table 8.1.1.3- 37: Live 3-wk embryos per viable embryos

	Individual data			Summary data			Comment
	Logit	Probit	Weibull	Logit	Probit	Weibull	
EC_{10}	Not computable			13.2	13.6	7.8	Measured data indicate that 10% effect reached at > 16.3 mg/kg bw/d
CL_L				n.d.	n.d.	1.3	
CL_U				31.5	30.6	16.5	
Width				n.d.	n.d.	15.3	
NW				n.d.	n.d.	1.9	
Conclusion	No reliable EC_{10}, retain endpoint specific NOAEL = 16.3 mg/kg bw/d						

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

Table 8.1.1.3- 38: Hatchlings per hen

	Individual data			Summary data			Comment
	Logit	Probit	Weibull	Logit	Probit	Weibull	
EC_{10}	5.7	5.8	3.8	15.5	**15.1**	11.4	Measured data indicate that 10% effect reached at ≈ 15 mg/kg bw/d (13% difference at 16.3 mg/kg bw/d)
CL_L	2.6	2.9	1.5	11.7	11.5	2.6	
CL_U	8.3	8.3	6.2	17.4	16.9	15.5	
Width	5.7	5.4	4.7	5.7	5.4	12.9	
NW	1.0	0.9	1.2	0.4	0.4	1.1	
Conclusion	Reliable EC_{10} = 15.1 mg/kg bw/d						

Table 8.1.1.3- 39: Hatchlings per eggs set

	Individual data			Summary data			Comment
	Logit	Probit	Weibull	Logit	Probit	Weibull	
EC_{10}	9.3	**9.8**	7.0	12.0	12.5	10.2	Measured data indicate that 10% effect reached at >16.3 mg/kg bw/d (actual numbers up to 16.3 mg/kg bw/d are > control)
CL_L	5.4	5.9	3.6	0.1	0.4	0.0	
CL_U	12.6	13.0	10.2	19.6	20.1	19.5	
Width	7.2	7.1	6.6	19.5	19.7	19.5	
NW	0.8	0.7	0.9	1.6	1.6	1.9	
Conclusion	Statistically reliable EC_{10} = 9.8 mg/kg bw/d but not well matching data						

Table 8.1.1.3- 40: Hatchlings per viable embryos

	Individual data			Summary data			Comment
	Logit	Probit	Weibull	Logit	Probit	Weibull	
EC_{10}	Not computable			12.9	13.4	11.5	Measured data indicate that 10% effect reached at >16.3 mg/kg bw/d (actual numbers up to 16.3 mg/kg bw/d are > control)
CL_L				0.7	1.6	0.4	
CL_U				22.4	22.4	22.0	
Width				21.7	20.8	21.6	
NW				1.7	1.6	1.9	
Conclusion	No reliable EC_{10}, retain endpoint specific NOAEL = 16.3 mg/kg bw/d						

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Table 8.1.1.3- 41: Hatchlings per live 3-week embryos

	Individual data			Summary data			Comment
	Logit	Probit	Weibull	Logit	Probit	Weibull	
EC_{10}	Not computable			19.5	19.6	**17.9**	Measured data indicate that 10% effect reached at >16.3 mg/kg bw/d (actual difference at 16.3 mg/kg bw/d < 5%)
CL_L				11.5	11.8	12.0	
CL_U				26.1	25.9	23.3	
Width				14.6	14.1	11.3	
NW				0.7	0.7	0.6	
Conclusion	Reliable EC_{10} = 17.9 mg/kg bw/d						

Table 8.1.1.3- 42: 14-day survivors per hen

	Individual data			Summary data			Comment
	Logit	Probit	Weibull	Logit	Probit	Weibull	
EC_{10}	5.8	5.7	4.2	**14.4**	14.7	13.1	Measured data indicate that 10% effect reached between 7.2 mg/kg bw/d and 16.3 mg/kg bw/d (actual numbers up to 7.2 mg/kg bw/d are > control)
CL_L	2.9	3.1	1.8	12.8	13.1	10.4	
CL_U	8.2	8.0	6.5	15.6	15.8	14.9	
Width	5.3	4.9	4.7	2.8	2.7	4.5	
NW	0.9	0.9	1.1	0.2	0.2	0.3	
Conclusion	Reliable EC_{10} = 14.4 mg/kg bw/d						

Table 8.1.1.3- 43: 14-day survivors per eggs set

	Individual data			Summary data			Comment
	Logit	Probit	Weibull	Logit	Probit	Weibull	
EC_{10}	7.8	**7.8**	5.6	10.5	10.7	8.1	Measured data indicate that 10% effect reached at > 7.2 mg/kg bw/d
CL_L	4.4	4.6	2.8	3.5	3.7	1.9	
CL_U	10.7	10.6	8.3	14.7	15.0	13.0	
Width	6.3	6.0	5.5	11.2	11.3	11.1	
NW	0.8	0.8	1.0	1.0	1.1	1.4	
Conclusion	Reliable EC_{10} = 7.8 mg/kg bw/d						

Table 8.1.1.3- 44: 14-day survivors per hatchlings

	Individual data			Summary data			Comment
	Logit	Probit	Weibull	Logit	Probit	Weibull	
EC_{10}	Not computable			18.7	18.9	15.5	Measured data indicate that 10% effect reached at > 7.2 mg/kg bw/d (actual difference at 7.2 mg/kg bw/d < 10%)
CL_L				3.5	4.4	3.3	
CL_U				28.9	28.9	25.5	
Width				25.4	24.5	22.2	
NW				1.4	1.3	1.4	
Conclusion	No reliable EC_{10}, retain endpoint specific NOAEL = 7.2 mg/kg bw/d						

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Table 8.1.1.3- 45: Initial hatchling bodyweight

	Individual data			Summary data			Comment
	Logit	**Probit**	**Weibull**	**Logit**	**Probit**	**Weibull**	
EC_{10}	12.1	12.5	4.1	14.9	14.8	6.2	Measured data indicate that 10% effect reached at > 7.2 mg/kg bw/d (actual numbers at 7.2 mg/kg bw/d are > control)
CL_L	3.8	4.5	1.6	2.0	2.4	1.1	
CL_U	19.6	19.7	7.1	26.0	25.5	12.5	
Width	15.8	15.2	5.5	24	23.1	11.4	
NW	1.3	1.2	1.3	1.6	1.6	1.8	
Conclusion	No reliable EC_{10}, retain endpoint specific NOAEL = 7.2 mg/kg bw/d						

Table 8.1.1.3- 46: 14-day survivor bodyweight

	Individual data			Summary data			Comment
	Logit	**Probit**	**Weibull**	**Logit**	**Probit**	**Weibull**	
EC_{10}	9.3	**9.2**	9.1	9.3	9.6	9.1	Measured data indicate that 10% effect reached at > 7.2 mg/kg bw/d (actual difference at 7.2 mg/kg bw/d < 10%)
CL_L	4.7	4.9	4.6	2.6	3.2	2.2	
CL_U	13.4	13.1	13.3	16.6	16.2	16.8	
Width	8.7	8.2	8.7	14	13	14.6	
NW	0.9	0.9	1.0	1.5	1.4	1.6	
Conclusion	Reliable EC_{10} = 9.2 mg/kg bw/d						

Assessment and conclusion by applicant:

Overall, a large proportion of EC_{10}-calculation attempts failed because of a non-significant dose response, or because the regression cannot be considered reliable in view of the unacceptable normalized width of the confidence interval. It is also important to assess the match or mismatch with the actually observed percent of effects at the predicted EC_{10}.

The lowest reliable EC_{10} was 7.8 mg/kg bw/d (14-day survivors per eggs set), with normalised width of 0.8. This value may be used in the avian reproductive risk assessment.

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Data Point:	KCA 8.1.1.3/05
Report Author:	[redacted]
Report Year:	2019
Report Title:	Calculation of EC10 for fluopyram for reproduction endpoints in mallard
Report No:	19020-BAY
Document No:	M-667211-01-1
Guideline(s) followed in study:	None
Deviations from current test guideline:	Current 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}) were calculated from data of a reproduction study in mallard exposed to fluopyram (M-299277-01-1).

Calculations of EC_{10} were conducted using ToxRat version 3.3.0. Calculations were performed both with summary data and individual replicate data. Effect concentrations are reported for those reproduction endpoints, for which a significant dose response was calculated.

The resulting EC_{10} values were then evaluated regarding their reliability, based on the reliability criterion NW (normalised width) proposed by EFSA (2015). A threshold of NW ≤ 1.0 was applied to identify reliable fits, corresponding to the EFSA categories “excellent” (NW < 0.2), “good” (NW < 0.5) and “fair” (NW < 1.0). Fits with NW ≥ 1.0 (“poor” or “bad”) were not considered reliable enough to determine valid EC_{10} values.

When more than one fit resulted in NW < 1.0, then the fit with the lowest NW was considered as the most reliable, and selected for the EC_{10} value concluded below. Where the same NW is obtained in reliable fits, the lowest EC_{10} is selected.

When none of the fits for an assessment endpoint provided for NW < 1, then no reliable EC_{10} values can be generated and the NOAEL was selected as the relevant endpoint.

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Table 8.1.1.3- 47: EC_{10} for reproduction endpoints from Christ & Lam (2008) exposed to fluopyram

Endpoint	EC_{10} ind. Data	EC_{10} sum. Data	Selected endpoint [mg a.s./kg bw]
Eggs laid per hen	n.c.	78.6 (NW 0.2)	78.6
Eggs cracked per eggs laid	n.c.	-[1]	>40 (No effect)
Eggshell strength	n.c.	n.c.	>40 (No effect)
Eggshell thickness	n.c.	n.d.	>40 (No effect)
Viable embryos per eggs set	n.c.	-[1]	>40 (No effect)
Live 3-wk embryos per viable embryos	n.c.	-[1]	>40 (No effect)
Hatchlings per hen	n.c.	n.c.	>40 (No effect)
Hatchlings per eggs set	n.c.	-[1]	>40 (No effect)
Hatchlings per viable embryos	n.c.	-[1]	>40 (No effect)
Hatchlings per live 3-week embryos	n.c.	-[1]	>40 (No effect)
14-day survivors per hen	n.c.	n.c.	>40 (No effect)
14-day survivors per hatchlings	n.c.	-[1]	>40 (No effect)
Initial hatchling bodyweight	n.d.	n.c.	>40 (No effect)
14-day survivor bodyweight	n.d.	n.r. (NW>1)	>40 (No effect)

[1] For quantal data no evaluation with summary data is possible.
n.r.: Not reliable
n.c.: Not computable (ToxRat reported the following reason: No statistically significant dose/response was found (p(F)>0.05; i.e. 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.

I. MATERIAL AND METHODS

Data used for analysis:

The following data were analysed as continuous data: Eggs laid per hen, eggshell thickness, eggshell strength, hatchlings per hen, 14-day survivors per hen, initial hatchling bodyweight, 14-day survivor bodyweight.

The following endpoints were analysed as quantal data: Eggs cracked per eggs laid, viable embryos per eggs set, live 3-week embryos per viable embryos, hatchlings per eggs set, hatchlings per viable embryos, hatchlings per live 3-week embryos, 14-day survivors per egg set, 14-day survivors per hatchlings.

Table 8.1.1.3- 48: Overview of the data used for the analysis of each reproduction endpoint

Endpoint	Dose [mg/kg bw/day]	Mean	SD	N
Eggs laid per hen [N]	0	52.0	13.7	15
	10	54.0	12.5	15
	18	51.1	10.3	15
	40	49.7	9.6	15
Eggs cracked per hen [N]	0	0.1	0.3	15
	10	0.1	0.3	15

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Endpoint	Dose [mg/kg bw/day]	Mean	SD	N
	18	0.3	0.6	15
	40	0.1	0.4	15
Eggs cracked per eggs laid [%]	0	0.1	0.7	15
	10	0.1	0.5	15
	18	0.7	1.3	11
	40	0.3	0.7	15
Eggshell thickness [mm]	0	0.33	0.02	15
	10	0.33	0.02	15
	18	0.33	0.02	15
	40	0.33	0.02	15
Eggshell strength [kg]	0	2.7	0.3	15
	10	2.6	0.2	15
	18	2.7	0.3	15
	40	2.7	0.2	15
Viable embryos per eggs set [%]	0	68.3	42.6	15
	10	71.9	35.1	15
	18	88.8	16.7	11
	40	85.9	25.6	15
Live 3-week embryos per viable embryos [%]	0	99.5	1.3	12
	10	98.4	2.9	14
	18	98.1	4.6	11
	40	99.7	0.8	15
Hatchlings per hen [N]	0	27.9	21.9	15
	10	27.9	16.7	15
	18	28.3	15.6	15
	40	30.0	14.7	15
Hatchlings per eggs set [%]	0	57.7	38.3	15
	10	56.3	28.2	15
	18	59.1	26.9	15
	40	65.5	27.8	15
Hatchlings per viable embryos [%]	0	81.7	19.1	12
	10	82.0	17.6	14
	18	66.6	27.0	11
	40	76.9	21.6	15
Hatchlings per live 3-week embryos [%]	0	82.1	19.4	12
	10	83.4	17.6	14
	18	67.7	27.2	11
	40	77.1	21.7	15
14-day survivors per	0	27.7	21.9	15

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Endpoint	Dose [mg/kg bw/day]	Mean	SD	N
hen [N]	10	27.9	16.7	15
	18	28.0	15.6	15
	40	30.0	14.7	15
14-day survivors per eggs set [%]	0	57.4	38.6	15
	10	56.2	28.3	15
	18	55.4	26.0	11
	40	65.5	27.8	15
14-day survivors per hatchling [%]	0	99.5	1.3	12
	10	99.7	1.1	14
	18	99.0	1.9	11
	40	100.0	0.0	15
Initial hatchling bodyweight [g]	0	36.0	2.1	12
	10	36.9	2.5	14
	18	36.5	1.8	15
	40	35.2	2.4	15
14-day survivor bodyweight [g]	0	254.7	10.8	12
	10	257.9	15.4	14
	18	254.0	13.9	15
	40	245.6	9.9	15

Statistical evaluation:

According to Commission Regulation (EU) No 283/2013 dated 1 March 2013, chapter 8.1.1.3. "The EC_{10} and EC_{20} shall be reported. Where they cannot be estimated, an explanation shall be provided together with the NOEC expressed in mg substance/kg bw/day".

Since in recent regulatory guidelines on EC_x and BMD_x calculation, it is generally preferred to use individual data for the regression instead of summarised data (see e.g. EFSA, 2017 for BMD_x calculations and OECD, 2006 and OECD 2014 for EC_x calculations), individual data is used for the statistical analysis, whenever possible.

EC_{10} concentrations were calculated using ToxRat version 3.3.0 with the default regression for all available functions (logit, probit, Weibull). When for continuous data no significant dose response was found, but an effect of 10 % or more was found in the highest dose group, then the regression was also calculated using summarised data for all available dose response functions (logit, probit and Weibull). For quantal data, this was not possible, because ToxRat provides no functionality to analyse replicates for quantal data.

To characterize the reliability of EC_x values, the 'normalised width' or NW, which is the ratio of the confidence interval of the ECx and the median ECx, was also calculated. The use of NW was recently proposed by EFSA (2015). The smaller the NW, the better the reliability (see table below).

Table 8.1.1.3- 49: Classification of ´normalised width' (NW) according to EFSA (2015)

NW	Rating	Application in this evaluation

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< 0.2	Excellent	Accepted as reliable
< 0.5	Good	
< 1	Fair	
< 2	Poor	Considered as not reliable (not accepted in this evaluation)
> 2	Bad	

When more than one dose-response function could significantly be fitted, the EC_{10} value with the lowest normalised width NW was selected for the respective endpoint. When the same NW was obtained, the fit with the lower EC_{10} is selected.

II. Results and Discussion

EC_{10} values calculated using ToxRat 3.3.0 and their normalised width (NW) are summarised in the table below for all endpoints. The most reliable EC_{10} (smallest NW) was selected. If no reliable EC_{10} (NW < 1) could be fitted, the NOAEL was selected.

Table 8.1.1.3- 50: Eggs laid per hen

	Individual data			Summary data			Comment
	Logit	Probit	Weibull	Logit	Probit	Weibull	
EC_{10}	n.c.				n.d.	78.6	The highest tested dose (40 mg/kw bw/d) resulted in an effect of 4.5%. The visual fit of the dose-response is poor.
CL_L					n.d.	71.2	
CL_U					n.d.	88.1	
Width					n.d.	16.9	
NW					n.d.	0.2	
Conclusion	Reliable EC_{10} = 78.6 mg/kg bw/d						

n.c.: Not computable (ToxRat reported the following reason: No statistically significant dose/response was found (p(F)>0.05; i.e. 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

Table 8.1.1.3- 51: 14-day survivor bodyweight

	Individual data			Summary data			Comment
	Logit	Probit	Weibull	Logit	Probit	Weibull	
EC_{10}	n.c.	n.d.	n.d.	55.8	n.c.		The highest tested dose (40 mg/kg bw/d) resulted in an effect of 3.6 %.
CL_L		n.d.	n.d.	48.5			
CL_U		n.d.	n.d.	115.6			
Width		n.d.	n.d.	67.1			
NW		n.d.	n.d.	1.2			
Conclusion	No reliable EC_{10}, retain endpoint specific NOAEL = >40 mg/kg bw/d						

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Assessment and conclusion by applicant:

Overall, a large proportion of EC_{10}-calculation attempts failed because of a non-significant dose response, or because the regression cannot be considered reliable in view of the unacceptable normalized width of the confidence interval. It is also important to assess the match or mismatch with the actually observed percent of effects at the predicted EC_{10}.

The only reliable EC_{10} was 78.6 mg/kg bw/d (eggs laid per hen), with a normalised width (NW 0.2). This value may be used 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 fluopyram are reported in the toxicology section.

The evaluation of these studies for selection of the wild mammal reproductive risk assessment endpoint according to EFSA 2009 and EFSA 2015 is presented in section MCA 8.1.2.2.

Table 8.1.2- 1: Endpoints used in the mammalian risk assessment

Test substance	Test design	Test species	Endpoint		Reference
Fluopyram	Acute oral	Rat	LD_{50}	> 2000 mg a.s./kg bw	[redacted]) M-259398-01-1 KCA 5.2.1/01
	Two-generation study	Rat	NOAEL	= 14.5 mg a.s./kg bw/d	[redacted] (2008) M-299334-01-1 KCA 5.6.1/02

CA 8.1.2.1 Acute oral toxicity to mammals

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

Test substance	Test design	Test species	Endpoint		Reference
Fluopyram	Acute oral	Rat	LD_{50}	> 2000 mg a.s./kg bw	[redacted] (2005) M-259398-01-1 KCA 5.2.1/01

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CA 8.1.2.2 Long-term and reproduction toxicity to mammals

Wild mammal reproductive risk assessment for fluopyram

The wild mammal reproductive risk assessment profile of fluopyram is of low concern. This section reviews the current regulatory agreed value (14.5 mg/kg bw/d males / 17.2 mg/kg bw/d females) with consideration of the updated EFSA guidance (EFSA 2015, Apx A) including a review of the available laboratory toxicology data. Further explanations about the data available and its ecotoxicological relevance for this endpoint are provided below. The updated methodology found no evidence that the current value which is based on the lowest relevant NOAEL (220 ppm in the rat multigeneration reproduction study) should be changed. This value is recommended for use in the relevant risk assessments.

This section comprises the following elements to document the evaluation for selecting the wild mammal reproductive risk assessment endpoint according to EFSA 2009 and EFSA 2015:

A) Concept, data, rationale and conclusions

B) Plot with $NOAEL_{ETX}$ and $LOAEL_{ETX}$ proposed for wild mammals

C) Short summaries of the evaluated studies with justification for the proposed $NOAEL_{ETX}$

D) Table with the proposed $NOAEL_{ETX}$ arranged according to EFSA 2015

A) Concept, data, rationales and conclusions

EFSA 2015 has revised the EFSA 2009 guidance on a tiered wild mammal chronic risk assessment endpoint selection process, and specified the range of studies to be considered: now, a number of repeated dose studies in rodents (28-d, 90-d) shall be considered additional to the reproduction and developmental toxicity studies. Ecologically relevant endpoint selection shall be facilitated by arranging the NOAELs on selected parameters from these studies in a tabled format, organized along a scheme of wild mammal reproduction phases as well as a graphical format of selected NOAELs and LOAELs.

Therefore, the following toxicological studies with fluopyram are reviewed below, with the objective to present both a brief overview on all findings, and a proposal for which of these findings should be selected for the tabled overview on the wild mammals reproduction phases:

Studies to check according to EFSA 2015:

- 28-d oral toxicity study in rat (non-GLP rangefinder) M-085510-01-1
- 28-d oral toxicity study in mice (non-GLP-rangefinder) M-088486-01-1
- 90-day oral toxicity study in rat (OECD 408) M-250946-01-1
- 90-day oral toxicity study in mice (OECD 408) M-251136-01-1
- Multigeneration study in rat (OECD 416) M-299334-01-1
- Developmental study in rat (OECD 414) M-299438-01-2
- Developmental study in rabbit (OECD 414) M-279773-01-1

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Additionally, a range of additional repeat dose studies in rodents are checked for whether they provide additional findings of relevance for the tabled overview:

Studies checked additionally for potentially relevant specific findings:

- 90-d oral neurotoxicity study in rat (OECD 424) M-299110-01-1
- One generation reproduction study in rat (pilot) M-299533-01-1
- Chronic toxicity and carcinogenicity study in rat (OECD 453) M-298339-01-1
- Chronic toxicity and carcinogenicity study in mice (OECD 453) M-295688-01-1

Generally, the studies were evaluated based on their summary in the DAR (2011). Where necessary, the study reports were consulted in order to retrieve additional information. Detailed summaries of the studies cited can also be found in the accompanying version of the active substance dossier MCA Section 05, Toxicological and metabolism studies (2021) which also includes more details about non-target mode of action studies including an endocrine disruption assessment.

Relevance of endpoints and effects for the wild mammal risk assessment was assessed based on the protection goals in EFSA 2009, i.e. the absence of visible mortality and of effects on populations. Effects were judged relevant if they impact survival, development to maturity or reproductive success. These comprise mortality, clinical signs, effects on bodyweight, developmental milestones, offspring production and offspring quality. Effects on organ weights, clinical chemistry or histopathological findings are only considered as potential evidence for the interpretation of a toxicity mode of action or time course of relevant apical effects.

Overall, the wild mammal reproductive risk assessment profile of fluopyram indicates low concern. Reproductive success across two exposed generations is not affected up to 1200 ppm (highest dose tested), even considering some effects on bodyweight (below 10% or 20% for effects on bodyweight gain). There were no effects on locomotor performance. Mortality and severe clinical signs appear at following repeated exposure at high dose levels – in mice mortality was observed after 17 days at 5000 ppm and in male rats an increase in mortality was first observed after 32 weeks of treatment at 750 ppm.

The available toxicological studies with fluopyram show that increasing exposure duration leads to increased level of incidence and severity of effects on target organs and that the key toxicological findings are largely reversible following up to 90 days of exposure.

From the toxicological perspective, the key mode of action is an induction of detoxification processes which are visible in metabolic enzyme inductions, centrilobular hypertrophy and increased liver weight. Kidney weight is also increased. Secondary to the increased metabolic activity, thyroid hormone levels are affected inducing compensatory increase of thyroid activity and weight. The liver toxicity markers and the correlated thyroid hormone level changes and thyroid gland changes seen after 90 days of exposure in the rat were reversible within the 28 day recovery phase, indicating recovery of the liver and thyroid once the exposure to fluopyram is terminated. Taking into account the rapid dissipation of fluopyram on food items for wild mammals (DT_{50} on foliage ca. 5 days), realistic exposure duration in the field will not exceed 2-3 weeks after an application. Thus, findings after life-time exposure, such as in the rodent chronic / carcinogenicity studies, are considered irrelevant for wild mammal endpoint selection.

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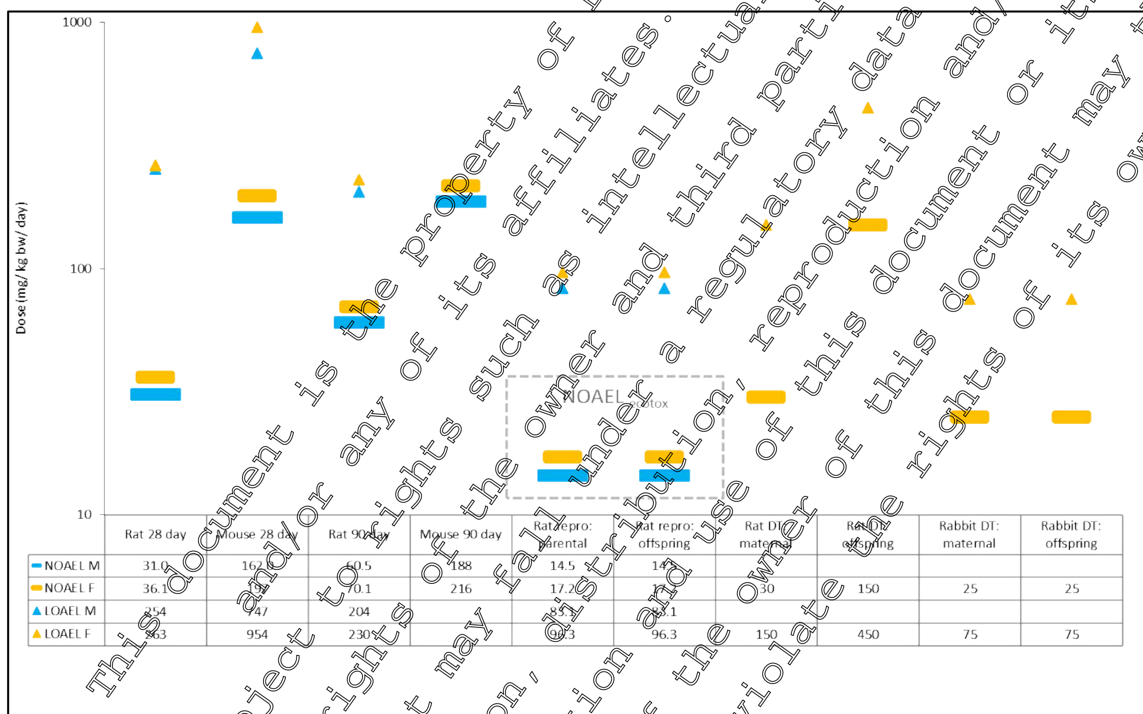
According to these principles, the review of the fluopyram studies for the wild mammal reproductive risk assessment endpoint allows to conclude that the previously selected NOAEL of 220 ppm (14.5 mg/kg bw/d) from the 2-generation rat reproduction study is still considered relevant.

This endpoint is based on slight body weight, liver and kidney changes at 1200 ppm (82.8 mg/kg bw/d, highest dose tested) with no effects on mortality, clinical signs, developmental milestones (independent of bodyweight effect), offspring production or offspring quality and as such is highly protective for wild mammal population level risk assessment.

B) Plot with NOAEL$_{ETX}$ (bars) and LOAEL$_{ETX}$ (triangles) proposed for wild mammals



	Rat 28 day	Mouse 28 day	Rat 90 day	Mouse 90 day	Rat repro: parental	Rat repro: offspring	Rat DT: maternal	Rat DT: offspring	Rabbit DT: maternal	Rabbit DT: offspring
NOAEL M	31.0	162.0	60.5	188	14.5	14.5				
NOAEL F	36.1	197	70.1	216	17.2	17.2	30	150	25	25
LOAEL M	254	747	204		83.1	83.1				
LOAEL F	263	954	230		96.3	96.3	150	450	75	75

C) Short summaries of the evaluated studies with justification for the proposed NOAEL$_{ETX}$

28-d oral toxicity study in rat (non-GLP rangefinder) (M-085510-01-1, 2004; DAR page 58)
Fluopyram was administered in the diet for 28 days to Wistar rats at 0, 50, 400 and 3200 ppm (equating to 4.0, 31.0 and 254 mg/kg/d in males and 4.6, 36.1 and 263 mg/kg/d in females).

Mortality or clinical signs: no mortality or clinical signs in any group.

Bodyweight gain and food consumption: Bodyweight gain was reduced at 3200 ppm in both sexes in week 1 (13% in males, 25% in females); over 4 weeks bodyweight gain was comparable in males and reduced by 14% in females. Food consumption of females was reduced ~10% in females at 3200 ppm.

Hematology and clinical chemistry: various findings at 3200 ppm.
Organ weights: Thyroid gland and kidney weights were increased in males at 400 and 3200 ppm. Hepatic enzyme induction was observed at 400 and 3200 ppm.

Increased activity of CAR/PXR enzymes was measured by PROD and BROD at 400 and 3200 ppm.

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Risk assessment endpoints 28-d rat:
Human health assessment in DAR: NOEL = 50 ppm ($NOAEL_{TOX}$ not derived)
Wild mammal assessment proposal: $NOAEL_{ETX}$ 400 ppm based on bodyweight gain and food consumption at 3200 ppm. Effects on liver enzymes, thyroid and kidney weights can be disregarded since rapid reversibility was shown in 90-d rat study. Potentially population-relevant apical long-term effects on survival and reproduction are not expected from such transient effects following in-field-relevant short peak exposure scenarios (DT50 in foliage ca. 5 days).

28-d oral toxicity study in mice (non-GLP rangefinder) (M-088486-01-1, 2004; DAR page 78)
Fluopyram was administered in the diet for 28 days to C57BL/6J mice at 0, 150, 1000 and 5000 ppm (equating to 24.7, 162 and 747 mg/kg/d in males and 31.1, 197 and 954 mg/kg/d in females).

Mortality or clinical signs: All males and 3/5 females at 5000 ppm were sacrificed between study Days 17 and 27, with clinical signs including reduced motor activity, hunched posture, piloerection, wasted appearance, coldness to touch, labored respiration and distended abdomen. No mortalities or clinical signs occurred in the other dose groups.

Bodyweight gain and food consumption: unaffected at 150 and 1000 ppm, apart from a slight and transient decrease in males at 1000 ppm that was not statistically significant.

Organ weights and histopathology: In the decedent animals at 5000 ppm treatment-related effects were seen in the adrenal glands, liver, lungs, spleen, thymus and thyroid gland. Liver weights were increased at 150 and 1000 ppm. Effects at 150 ppm were limited to hypertrophy of hepatocytes.

Risk assessment endpoints 28-d mice:
Human health assessment in DAR: NOEL < 150 ppm (NOAEL not derived)
Wild mammal assessment proposal: $NOAEL_{ETX}$ 1000 ppm based on mortality at 5000 ppm. Slight, transient and non-significant effects on bodyweight gain at 1000 ppm are not considered relevant.

90-day oral toxicity study in rat (OECD 408) (M-250946-01-1, 2005; DAR from page 63)
Fluopyram was administered in the diet for 90 days to Wistar rats at 0, 50, 200, 1000, and 3200 ppm (equating approximately to 0, 3.06, 12.5, 60.5 and 204 mg/kg/day in males and 0, 3.63, 14.6, 70.1, and 230 mg/kg/day in females). An additional 10 males and 10 females fed either 0 or 3200 ppm of test diet for 90 days were afterwards maintained for 28 days on non-treated diet to examine the reversibility of any effects seen during the exposure phase (recovery group).

Mortality or clinical signs: There were no treatment-related effects in any group.

Bodyweight and food consumption: At 3200 ppm, mean bodyweight was decreased by between 4 and 6% in males and 4 and 8% in females throughout the course of the study, compared to controls. The effect on bodyweight was primarily due to an initial decrease in mean bodyweight gain per day during the first week of treatment in males and females (-26 and -29%, respectively, $p<0.01$), compared to controls. From study week 2 and throughout the remainder of the exposure phase of the study, mean bodyweight gain per day was essentially comparable to the controls in both sexes. However, the effect on mean bodyweight was still observed even after the subsequent 28 days of non-exposure. The magnitude of the difference in the mean bodyweight of the recovery group at the end of the recovery phase (90d + 28d: M: -7.4%, F: -6.2%) was similar to that at day 1 of the recovery phase (90d + 0d: M: -7.3%, F: -7.2%).

Thus, the functional growth rate recovered rapidly and reached control level even during exposure at 3200 ppm, but the initial bodyweight decrease during the first week of treatment could not be compensated during the recovery phase, probably because by then growth had largely plateaued in these adults (about 5% bodyweight gain over 4 weeks).

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Food consumption was statistically significantly reduced at several measurement intervals at 3200 ppm.

No statistically significant effect on bodyweight or food consumption occurred at 50, 200 or 1000 ppm.

Hematology: Several parameters were statistically significant at 3200 ppm but were generally reversible during the recovery phase (e.g. recovery of y-glutamyltransferase and ALP as markers for liver toxicity).

Urine analysis: The presence of casts in the urine is to be seen in connection to the hyaline droplet nephropathy observed at histopathology examinations. Lower incidence and severity at the end of the recovery phase suggest reversibility.

Hormone analysis: A dose dependent increase of T3, T4 and TSH was observed in both sexes at most time points and for most parameters at all dose levels when compared to controls, however, without being significant or of relevant magnitude at the lower two dose levels of 200 and 50 ppm. All changes observed at the end of the dosing phase were considered to be reversible during the 1-month recovery period.

Organ weights: Liver and kidney weights were statistically significantly higher at 3200 and 1000 ppm, when compared to controls. A tendency towards higher liver and kidney weights was also noted at 200 ppm (not statistically significant). There was a tendency towards higher thyroid weights at 3200 ppm.

After the recovery phase, the liver to bodyweight ratio at 3200 ppm was still statistically significantly higher than in controls. Mean kidney weights in the high dose male group were still statistically significantly higher than in controls. However, the magnitude of variation compared to control was clearly lower than at the end of the dosing phase, indicating reversibility.

The thyroid weights of females at 3200 ppm remained increased after the recovery phase, but the microscopic changes in the thyroid were reversible and therefore this finding is considered to be non-adverse.

Gross and histopathology: Enlarged and dark liver and/or prominent lobulation of the liver were observed at 3200 and 1000 ppm. These findings corroborate the centrilobular hypertrophy noted at the microscopic examination. In the thyroid gland, a higher incidence of minimal to slight diffuse hypertrophy of follicular cells was seen at 3200 and 1000 ppm in both sexes compared to controls and internal historical control data.

After the recovery phase, liver and thyroid gland microscopic changes noted after 90 days of treatment were found to be reversible.

In the kidney, hyaline droplet nephropathy and hyaline casts were higher at 3200 and 1000 ppm in males, in comparison with controls. Hyaline droplet nephropathy was also slightly higher at 200 ppm in males.

After the recovery phase, basophilic tubules, hyaline droplets in proximal tubules, granular casts in the medulla and hyaline casts were persistent in the kidney of some males in the high dose group but with a lower severity rating, demonstrating partial recovery and thus principal reversibility of the findings.

Neurotoxicity: no treatment related effects were observed on locomotor activity, sensory reactivity and grip strength up to 1000 ppm, with slightly reduced grip strength of females considered secondary to bodyweight effects at 3200 ppm.

Conclusion: Effects after 90 days of exposure were found to be reversible in the majority of cases. Even where full recovery was not observed after 28 days on untreated diet, the magnitude of effects, the severity rating and/or the incidence were generally lower than after 90 days of exposure indicating reversibility and ongoing recovery. Female thyroid weight remained increased after 28 days on untreated diet, however, as the changes in thyroid hormones reflecting thyroid activity stimulation and the microscopic changes were reversible, the higher thyroid weights were considered non-adverse.

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Following a decrease of bodyweight after the first week of treatment in the high dose groups, bodyweight evolution was similar to control animals in both sexes; however this initial differential in absolute bodyweight remained throughout the rest of the exposure period and after 28 days on untreated diet. After 90 days, the growth phase of the rats had largely plateaued, so that only very little compensatory growth could narrow the gap in absolute bodyweight between control and the 3200 ppm recovery group.

Risk assessment endpoints 90-d rat: **Human health assessment in DAR: NOAEL 200** **Wild mammal assessment proposal: NOAEL$_{ETX}$ 1000 ppm** based on bodyweight effects at 3200 ppm. Reversible effects on hormones or organs are not considered to induce population-relevant apical long-term effects in field relevant short peak exposure scenarios (DT50 in foliage ca. 5 days).

90-day oral toxicity study in mice (OECD 408) (M-251136-01-1, 2005; DAR from page 83)
Fluopyram was administered in the diet for 90 days to C57BL/6J mice at 0, 30, 150 and 1000 ppm (equating to 0, 5.4, 26.6 and 188 mg/kg/d in males and 0, 6.8, 32.0 and 216 mg/kg/d in females).

Mortalities and clinical signs: There were no treatment-related mortalities or clinical signs in any dose group. **Bodyweight** was unaffected by treatment in any dose group. **Food consumption** was affected by treatment at 1000 ppm in males.

Blood analysis showed various changes down to 30 ppm.

Organ weights: Liver weight increases with associated histological changes were observed at 150 and 1000 ppm. Adrenal gland weights were increased at 1000 ppm.

Histopathology detected changes in the liver at 150 ppm and above, and in adrenal glands at 1000 ppm.

Risk assessment endpoints 90-d mice: **Human health assessment in DAR: NOEL 30 ppm (NOAEL 150 ppm)** **Wild mammal assessment proposal: NOAEL$_{ETX}$ 1000 ppm** based on the absence of mortality, clinical signs or effects on bodyweight up to the highest dose level tested.

Multigeneration study in rat (OECD 416) (M-299334-01-1, 2008; DAR from page 175)
Fluopyram was administered to Han Wistar rats over two generations in the diet at 0, 40, 220 and 1200 ppm (0, 20, 110 and 600 ppm during lactation), equating to average doses as presented below:

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Phase	Sex	Generation	Achieved dose [mg/kg bw/d]		
			40 ppm	220 ppm	1200 ppm
Premating	Males	P-gen	2.7	15.1	83.1
Premating	Males	F1-gen	2.6	13.9	82.4
Premating	Males	combined	2.7	14.5	82.8
Premating	Females	P-gen	3.2	17.6	96.3
Premating	Females	F1-gen	3.1	16.8	95.6
Premating	Females	combined	3.2	17.2	96.0
Gestation	Females	P-gen	3.0	15.5	90.3
Gestation	Females	F1-gen	2.8	14.4	95.9
Gestation	Females	combined	2.9	15.0	93.1
Lactation	Females	P-gen	3.1	15.9	92.5
Lactation	Females	F1-gen	3.3	18.1	103.2
Lactation	Females	combined	3.2	17.0	97.9

Mortality and clinical signs: There were no test substance-related mortalities or clinical observations observed during the course of this study at any dietary dose level tested in either generation

Bodyweight and food consumption: For males and females there were no treatment related effects on food consumption at any dose level. The males did not exhibit any test substance-related effects on bodyweight at any dietary dose level tested. The females of the 1200 ppm dose group exhibited slight declines in bodyweight (BW) or bodyweight gain (BWG) during different phases of the test:

Pre-mating P-generation: final BW lower by 4.4%, BWG week 0-10 lower by 20% (both not stat. sign.)

Pre-mating F1-gen: final BW lower by 3.0% (ns), BWG week 0-10 lower by 10.8% (sign.)

Gestation P-gen: BW lower by ≤ 6.3% d0, d6, d13 (all sign); final BW lower 4.0% (ns), BWG d0-20 +1.1%

Gestation F1-gen: BW not affected, BWG d0-20 above control

Lactation P-gen: BW lower by 4.7% d0 (sig.), afterwards not sign. BWG d0-21 above control

Lactation F1-gen: and BWG unaffected

Reproductive function: There were no test substance-related effects observed on the estrous cycle number or length in either generation at any dietary level tested. There were no test substance-related effects observed on any sperm parameter evaluated at any dietary level tested for either generation.

Reproductive performance: Overall reproductive performance was not affected for any parameter (e.g., mating, fertility or gestation indices, days to insemination, gestation length, or the median number of implants) in either generation at any dietary level tested.

Clinical Chemistry and Hematology: Test substance-related changes were limited to the highest dose level of 1200 ppm.

Organ weights: liver and kidney weights were increased at 1200 ppm, and spleen weight was reduced at 220 and 1200 ppm.

Pathology: treatment related changes at 1200 ppm (kidney, liver).

Offspring viability and clinical signs: no effects at any dose level tested.

Pup weight: bodyweight gain over days 0-21 of lactation was slightly reduced in the F1 pups (n.s.) and the F2 pups (sign).

Sexual maturation: A slight delay in preputial separation in the F1-males of the 1200 ppm dose group (mean 42.5 days vs. 41.0 days in the control) was observed. Although statistically significant, the number of days to passing was well within this laboratory's historical control range (42.4 – 43.8) and is considered to be secondary to the slight decline in male bodyweight gain. There were no findings on preputial separation in any other dietary dose level tested. There was no effect observed on vaginal patency or anogenital distance at any dietary dose level tested.

Pup organ weights: not affected in the F1 pups. Findings in F2-pups that were statistically significantly different from controls including spleen and thymus (reduced) and brain (increased).

Pup pathology: no treatment related findings at any treatment level.

Risk assessment endpoints rat 2-gen repro:
Human health assessment in DAR: parental NOAEL 220 ppm, reproductive NOAEL 1200 ppm, offspring NOAEL 220 ppm **Wild mammal assessment proposal:** **parental and offspring $NOAEL_{ETX}$ 220 ppm (14.5 mg/kg bw/d)** based on minor effects on maternal bodyweight and pup bodyweight at 1200 ppm with no effects on survival or reproductive parameters at any dose level. **Reproductive NOAEL ≥ 1200 ppm (93.1 mg/kg bw/d).**

Developmental study in rat (OECD 414) (M-299438-01-2, 2008; DAR from page 191)
Fluopyram was administered daily by gavage to pregnant female Sprague-Dawley rats from GD 6 to 20 at 0, 30, 150 and 450 mg/kg bw/d.

Maternal mortality and clinical signs: There were no test substance-related mortalities or clinical observations observed during the course of this study at any treatment level.

Maternal bodyweight and food consumption:

At 450 mg/kg bw/day, maternal bodyweight gain over gestation days 6-21 was 16 % compared with the controls (stat. sign.). At 150 mg/kg bw/d, maternal bodyweight gain over GD 6-21 was 6% lower than in the controls (not sign.). At 30 mg/kg bw/day, mean maternal corrected bodyweight change was similar to the controls.

Food consumption was statistically significantly lower than controls at various intervals until GD 14 at 450 and 150 mg/kg bw/d, but thereafter comparable to controls. At 30 mg/kg bw/day, mean maternal food consumption was 10 % lower than the controls between GD 6 to 8 (sign.) and thereafter similar to the controls.

Pregnancy rate: no effect (pregnancy rates were 96 % in all treated group and in the controls)

Maternal organ weights: maternal liver weights were increased at 450 and 150 mg/kg bw/day,

Maternal necropsies and microscopic findings: At autopsy of the dams, enlarged liver was observed in 4/23 females at 450 mg/kg bw/day compared with 0/23 cases in the control group. Histopathological changes were observed in the liver at 450 and 150 mg/kg bw/day and consisted of diffuse centrilobular hepatocellular hypertrophy.

Litter data: At 450 mg/kg bw/day, mean fetal bodyweight was 5 % (sign) lower for both the combined and separate sexes. Other litter parameters including number of live fetuses, early or late resorptions and dead fetuses, were unaffected by treatment.

Fetal necropsy findings

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External observations: There were no malformations or treatment-related increase in variations observed at the external fetal observation.

Visceral and skeletal observations: a slightly increased incidence of two visceral and two skeletal minor variations at 450 mg/kg bw/d was considered treatment-related.

Risk assessment endpoints rat developmental toxicity study:
Human health assessment in DAR: maternal NOAEL 30 mg/kg bw/d, fetal NOAEL 150 mg/kg bw/d

Wild mammal assessment proposal: Maternal and fetal $NOAEL_{ETX}$ 150 mg/kg bw/d (only minor effects on maternal bodyweight gain, not stat. sign.), based on more pronounced effects on maternal bodyweight and minor fetal bodyweight effects at 450 mg/kg bw/d.

Developmental study in rabbit (OECD 414) (M-279773-01-1, 2006; DAR from page 198)

Fluopyram was administered daily by gavage to pregnant female New Zealand White rabbits from GD 6 to 28 at 0, 10, 25 and 75 mg/kg bw/d.

Maternal mortality and clinical signs: There were no test substance-related mortalities or clinical observations observed during the course of this study at any treatment level.

Maternal bodyweight and food consumption:

At 75 mg/kg bw/day, mean bodyweight gain was reduced at various intervals. Mean bodyweight change at 25 and 10 mg/kg bw/day was comparable with the controls.

Food consumption at 75 mg/kg bw/d was reduced by between 22 to 34 % (sign) for all intervals between GD 14 to 26. Food consumption at 25 and 10 mg/kg bw/day was similar to the controls.

Pregnancy rate: no effect (pregnancy rates were 96 % in all treated group and in the controls), no abortions.

Maternal organ weights and necropsy: no treatment related findings.

Litter data: At 75 mg/kg bw/day, mean fetal bodyweight was 11 % lower than the controls (sign). Other litter parameters, including number of live fetuses, early or late resorptions, fetal death status and percentage of male fetuses were unaffected by treatment at all dose levels tested.

Fetal necropsy findings

External observations: No treatment-related malformations.

Visceral and skeletal observations: No treatment-related malformations.

Risk assessment endpoints rabbit developmental toxicity study
Human health assessment in DAR: maternal NOAEL 25 mg/kg bw/d, fetal NOAEL 25 mg/kg bw/d
Wild mammal assessment proposal: Maternal and fetal $NOAEL_{ETX}$ 25 mg/kg bw/d based on effects on maternal bodyweight and fetal bodyweight at 75 mg/kg bw/d.

Studies checked additionally for potentially relevant specific findings

90-d oral neurotoxicity study in rat (OECD 424) (M-299110-01-1, 2008; DAR p. 293, brief overview only)

Fluopyram was administered in the diet for 13 weeks to Wistar rats at nominal concentrations of 0, 100, 500 and 2500 ppm. Mean achieved doses were 6.69, 33.2 and 164.2 mg/kg/day for males and 8.05, 41.2 and 197.1 mg/kg/day for females.

Mortality and clinical signs: no treatment-related effects at any dose level throughout the study

Bodyweight and food consumption: at 2500 ppm, bodyweight gain was slightly decreased in males (10%, not sign.) and females (26%, sign.). Food consumption was reduced at 2500 ppm in males and females, and in females at 500 ppm, over several measurement intervals.

Neurotoxicity: No evidence of neurotoxicity was observed at any treatment-level.

Motor and locomotor activity: no treatment-related differences to control at any treatment level

Ophthalmology: no treatment-related findings

Hematology: minor changes without consistent dose response

Clinical chemistry: Cholesterol (males and females) and triglyceride (females only) levels were increased in high dose animals

Gross pathology: no treatment related findings

Organ weights: treatment-related increase of liver and kidney weights at 2500 ppm.

Micropathology: no treatment-related changes in the neural and non-neural tissues in the brain

females) was considered the NOAEL for this endpoint.

Risk assessment endpoints from the 90-day neurotoxicity study in rats:
Human health assessment in DAR: NOAEL = 2500 ppm for neurotoxicity, 500 ppm for systemic toxicity
Wild mammal assessment proposal: $NOAEL_{ETX}$ = 2500 ppm. The study can be considered relevant for wild mammals, as it adds information on (the lack of) neurotoxicity, and also because the rats at the highest dose level of 2500 ppm did not present any deficiencies in motor and locomotor performance, confirming that the moderate effects on bodyweight at this treatment level are unlikely to translate into behavioral deficits in wild mammals that could result in declines of population survival or reproduction. Therefore, the slight bodyweight differences resulting from fluopyram exposure in the toxicological studies could be considered as non-relevant for the wild mammal risk assessment.

One generation reproduction study in rat (pilot) (M-299533-01-1, 2008; DAR page 176 (brief overview only)

Fluopyram was administered in the diet to Wistar rats at 0, 30, 150, 750 and 1500 ppm. Diets were administered from beginning of the study until sacrifice, except during the lactation period (Days 0-21) where the concentration in the female diet was adjusted down by 50%.

Phase	Sex	Achieved dose [mg/kg bw/d]			
		30 ppm	150 ppm	750 ppm	1500 ppm
Premating	Males	2.0	10.2	49.6	102.1
Premating	Females	2.3	11.6	57.7	118.2
Gestation	Females	2.2	10.8	55.5	117.3
Lactation	Females	2.4	11.9	60.7	113.5

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Mortality and clinical signs: There were no test substance-related mortalities or clinical observations observed during the course of this study at any dietary level.

Bodyweight and food consumption: The males did not exhibit any test substance-related effects on bodyweight or food consumption at any dietary level tested.

Females (premating): Slight declines in bodyweight gain at 1500 ppm (10.6%). Declines in food consumption of the females at 750 and 1500 ppm during the first week of premating are considered to be due to initial palatability of the compound. By the second week of premating the food consumption values for females were comparable to controls.

Females (gestation and lactation): no effects on body weight, bodyweight gain or food consumption at any dietary level tested.

Reproductive function: Estrous cycling or sperm analysis were not performed in this one generation study.

Reproductive performance: not affected for any parameter (e.g., mating, fertility or gestation indices, days to insemination, gestation length, or the median number of implants) at any treatment level.

Clinical Chemistry and Hematology: Test substance-related changes were limited to the highest dose level of 1500 ppm.

Organ weights: liver and kidney weights were increased at 750 and 1500 ppm

Gross pathology: no test substance-related observations in the adults at any treatment level.

Micropathology: not performed in this one-generation study.

Offspring viability and clinical signs: no effects at any test level.

Pup weight: no effects at any test level.

Sexual maturation: not evaluated in this one-generation study.

Pup organ weights: not affected at any test level.

Pup gross pathology: no test substance-related observations in the pups at any treatment level.

Pup micropathology: not performed in this one-generation study.

Risk assessment endpoints rat 1-gen repro:
Human health assessment in DAR: not assigned. The conclusions in the report are:
Parental systemic NOAEL 150 ppm (increased organ weights)
Reproductive NOAEL ≥ 1,500 ppm (no reproductive findings observed in the highest dose tested)
Offspring NOAEL ≥ 1500 ppm (no effects observed on the offspring)
Wild mammal assessment proposal: the endpoints of this study are not used for wild mammals TER_{LT} calculations which are based on the studies required by EFSA 2015. However, the lack of effects on the pup weights in this one-generation study confirms the findings of the 2-generation rat reproduction study, that significant effects on pup weights were limited to the second generation, which is of limited relevance for realistic wild mammal exposure scenarios.

Chronic toxicity and carcinogenicity study in rat (OECD 453) (M-298339-01-1, 2008; DAR from page 135)

Fluopyram was administered in the diet to Wistar rats at 0, 30, 150 and 750/375 ppm (males) and 0, 30, 150 and 1500 ppm to females. Mean achieved doses over two years were 0, 1.20, 6.0 and 29 mg/kg bw/d in males and 0, 1.68, 8.6 and 89 mg/kg bw/d in females.

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Mortality or clinical signs: Following week 32, increased mortality was observed in the male high dose group (750 ppm, average dose over weeks 1-32: 38.4 mg/kg bw/d).

Achieved dose of males over weeks 1-32 [mg/kg bw/d]		
weeks	**150 ppm**	**750 ppm**
1-4	11.89	59.6
5-8	8.72	43.9
9-12	7.51	38.0
13-16	6.89	35.4
17-20	6.78	34.7
21-24	6.67	33.0
25-28	6.39	31.3
29-32	6.17	31.0
mean over weeks 1-32	**7.63**	**38.4**

In total, 11/70 males were found dead or were sacrificed prematurely for humane reasons in the high dose group compared to 6/70 in the control group. The main clinical signs in these early decedent males included signs associated with morbidity (limited use of hindlimbs, reduced motor activity, general pallor, wasted appearance). These findings were considered to be treatment-related but no clear factor contributing to the death of these animals could be established at the microscopic examination. In week 85, the high treatment level was reduced from 750 to 375 ppm for the males. No effect on mortality was noted in females. In the female high dose group (1500 ppm), a higher incidence of hair loss and wasted appearance was noted, in comparison to the controls. No treatment-related clinical signs were noted at the mid and low dose levels in either sex.

Bodyweight gain and food consumption: In the male high dose group (750/375 ppm), bodyweight or bodyweight gain were essentially comparable to the controls throughout the study.

In the female high dose group (1500 ppm), mean bodyweight or bodyweight gain were essentially comparable to the controls throughout the first three months of treatment. Thereafter, differences in bodyweight or bodyweight gain were observed at various time points.

At the mid and low dose levels (150 and 30 ppm), bodyweight or bodyweight gain parameters were unaffected by the treatment in both sexes over the two years of the study.

Apart from minor and transient differences, food consumption was essentially comparable to controls in all treatment groups.

Ophthalmologic examination: In the female high dose group, pale retinal fundus was observed in 4/67 animals, compared to no case in the controls. No treatment-related ophthalmological findings were noted at any dose level tested in males or at the mid and low dose levels in females at the end of the first year of treatment. At the end of the second year of treatment, various ophthalmological findings were observed in males at the top and mid dose, and in females at the top dose.

Hematology: various findings in males and females in the high dose group.

Clinical chemistry: various findings in females in the high dose group

Urine analysis: abnormal color of urine of high dose females, transient increase of incidence and severity of cellular casts in the high and mid dose males.

Organ weights: in males and females of the high dose groups increased weights of liver, kidney and thyroid (only females) after 12 months. After 24 months, liver weight was also increased in low and mid dose males.

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Gross pathology: treatment-related findings were noted in the liver and kidney. In the female high dose group, a higher incidence of enlarged liver, dark liver, white foci or red foci on the liver was observed among the unscheduled deaths compared to the controls.

Microscopic pathology: after 12 months there was no evidence of a treatment-related effect on the incidence of neoplastic findings. A number of non-neoplastic findings was reported in the liver, kidneys and thyroids of high dose females and high and mid dose males.

At the end of the carcinogenicity phase (after 24 months of exposure), a higher incidence of tumors in the liver (carcinoma and adenoma) was noted in the female high dose group only, in comparison to the controls. These findings were associated with non-neoplastic/preneoplastic changes and were seen at a dose causing marked hepatocellular toxicity. There was no evidence of a treatment-related increased incidence of tumors of any type in any other organ. A number of non-neoplastic findings was reported in the liver, kidneys and thyroids and other organs, with typically higher incidences and severity in the top dose level in females and the high and mid dose in males.

Risk assessment endpoints from the chronic toxicity and carcinogenicity study in rats:
Human health assessment in DAR: NOAEL = 30 ppm
Wild mammal assessment proposal: the outcome of this study can be disregarded for wild mammals, due to the excessively long and environmentally irrelevant duration of treatment before onset of potentially relevant effects.
The most critical finding in this study is mortality of males after 32 weeks of exposure to 750 ppm. However, 32 weeks of exposure is not considered realistic for wild mammals, and the spacing factor between the no-mortality level 150 ppm (7.63 mg/kg bw/d over weeks 1-32) and 750 ppm (38.4 mg/kg bw/d over weeks 1-32) is large. No mortality occurred after 10 weeks of exposure at 82.8 mg/kg bw in the rat reproduction study or even after 13 weeks at 204 mg/kg bw/d in the rat 90-d study. Taking into account the short DT_{50} of fluopyram in the feed of wild mammals (ca 5 days in foliage), exposure over 32 weeks or more is clearly unrealistic even for repeated application scenarios. Thus, the mortality in the chronic study can be considered as irrelevant and covered by the assessment of the 90-day and repro studies, as required by EFSA 2015.
The second potentially relevant finding is liver tumors in females at 1500 ppm. However, these tumors did not lead to increased mortality, and are the consequence of long-term induction of increased liver activity, as also seen in all other studies.
Under realistic field conditions, exposure of wild mammals will be much shorter, so that liver tumor induction is unlikely (no tumors observed in the rat 90-day studies, the rat repro study or after 1 year in this study).

Chronic toxicity and carcinogenicity study in mice (OECD 453) (M-295688-01-1, 2007; DAR from page 160)
Fluopyram was administered in the diet to C57BL/6J mice at 0, 30, 150 and 750 ppm. Mean achieved doses over 78 weeks were 0, 4.2, 20.9 and 105 mg/kg bw/d in males and 0, 5.3, 26.8 and 129 mg/kg bw/d in females.

Mortality and clinical signs: There were no treatment-related mortalities or clinical signs at any dose level in either sex during the course of the study.

Bodyweight and food consumption: mean bodyweight and bodyweight gain at 750 ppm were unaffected until week 30 in males and week 14 in females, afterwards slightly reduced at various measurements. Bodyweight parameter were unaffected in either sex at the low and the mid dose over the entire study duration.

Hematology: Slightly higher mean platelet counts were noted at 750 ppm in males.

Clinical chemistry: not conducted in this study

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Organ weights: increased weights of liver and reduced weight of kidneys after 12 months in males and females of the high dose groups. After 24 months, liver weight was also increased in low and mid dose males and females, and kidney weight was reduced in males and females of the high dose group. Heart and adrenal gland weights were increased in the top dose.

Gross pathology: After the 12-month chronic phase, enlarged livers were observed at 750 ppm.

After the 18-month carcinogenicity phase, enlarged liver and/or dark liver was found in some males and females at 750 and 150 ppm. These findings were correlated with relevant histopathological findings.

Furthermore, an enlarged thyroid gland was found in one high dose male and two high dose females as compared to no such effect at all other dose levels in both sexes.

Microscopic pathology: After the 12-month chronic phase, follicular cell hyperplasia was noted in the thyroid of 2/10 and 2/9 males at 750 and 150 ppm, respectively.

After the 18-month carcinogenicity phase, treatment related non-neoplastic findings were reported in the liver, kidney and thyroid gland in males and females at 750 and 150 ppm. In the thyroid gland, a higher incidence of follicular cell adenoma was noted in males at 750 ppm.

Risk assessment endpoints from the chronic toxicity and carcinogenicity study in mice:
Human health assessment in DAR: NOAEL = 30 ppm
Wild mammal assessment proposal: the outcome of this study can be disregarded for wild mammals, due to the excessively long and environmentally irrelevant duration of treatment before onset of potentially relevant effects.
For wild mammals, there are no potentially critical findings in this study that would not be covered by the assessment of the 90-day and repro studies, as required by EFSA 2015. There was no treatment related mortality or clinical signs. Effects on bodyweight were mild and occurred only after long-term exposure. Under realistic field conditions, exposure of wild mammals will be much shorter. The thyroid adenomas did not result in mortality or developmental deficiencies, and reversibility of thyroid hormone stimulation was seen the rat 90-day study during the recovery phase. Under realistic field conditions, exposure of wild mammals will be much shorter so that thyroid tumor induction is unlikely (no thyroid induction observed in the mouse 90-day study).

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D) Table with the proposed $NOAEL_{ETX}$ arranged according to EFSA 2015

Effects to check	**Studies to check**	**Relevant endpoint observations**	**$NOAEL_{ETX}$ proposal**	**Comment**
1) Effects on bodyweight change, behavioural effects and systemic toxicity[2]	28-d oral toxicity study in rat M-085510-01-1	Survival: LOAEL > 3200 ppm Clinical signs: LOAEL > 3200 ppm **Bodyweight: LOAEL 3200 ppm** Feed consumption: LOAEL 3200 ppm Clinical chemistry: LOAEL 3200 ppm Organ weights and Histopathology (liver, thyroid, kidney): LOAEL 400 ppm	400 ppm (31.0/36.1 mg/kg bw/d))	
	28-d oral toxicity study in mice M-088486-01-1	**Survival: LOAEL 5000 ppm** **Clinical signs: LOAEL 5000 ppm** **Bodyweight: LOAEL 5000 ppm** Food consumption: not affected in survivors Organ weight and Histopathology: LOAEL 150 ppm	1000 ppm (162/197 mg/kg bw/d)	
	90-day oral toxicity study in rat (OECD 408) M-250946-01-1	Survival: LOAEL > 3200 ppm **Clinical signs and FOB: LOAEL = 3200 ppm** **Bodyweight: LOAEL = 3200 ppm** Feed consumption: LOAEL = 3200 ppm Clinical chemistry: LOAEL = 1000 ppm Histopathology (liver, thyroid, kidney): LOAEL 1000 ppm Organ weights: LOAEL 1000 ppm	1000 ppm (60.5/70.1 mg/kg bw/d)	Most effects seen at 3200 ppm decreased during 4-week post-exposure phase (indicating reversibility)

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Effects to check	Studies to check	Relevant endpoint observations	NOAEL$_{ETX}$ proposal	Comment
	90-day oral toxicity study in mice (OECD 408) M-251136-01-1	Survival: LOAEL > 1000 ppm Bodyweight: LOAEL > 1000 ppm Feed consumption: LOAEL = 3200 ppm Clinical chemistry: LOAEL = 1000 ppm Histopathology (liver, adrenal glands): LOAEL 1000 ppm Organ weights: LOAEL 1000 ppm	≥1000 ppm (188/216 mg/kg bw/d)	
	Multigeneration study in rat (OECD 416) M-299334-01-1	Survival: LOAEL > 1200 ppm Clinical signs: LOAEL > 1200 ppm **Bodyweight: LOAEL = 1200 ppm (F)** Feed consumption: LOAEL > 1200 ppm Clinical chemistry: LOAEL = 1200 ppm Histopathology LOAEL = 1200 ppm Mating index: LOAEL > 1200 ppm Fertility index: LOAEL > 1200 ppm	220 ppm (14.5/17.2 mg/kg bw/d)	
	Developmental study in rat (OECD 414) M-299438-01-2	Survival: LOAEL > 450 mg/kg bw/d Clinical signs: LOAEL > 450 mg/kg bw/d **Bodyweight: LOAEL = 150 mg/kg bw/d** Feed consumption: LOAEL = 150 mg/kg bw/d Organ weight (liver): LOAEL = 150 mg/kg bw/d Histopathology LOAEL = 150 mg/kg bw/d	150 mg/kg bw/d	Bodyweight gain at 150 mg/kg only lower by 6% and not stat. sign.

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Effects to check	Studies to check	Relevant endpoint observations	NOAEL_{ETX} proposal	Comment
	Developmental study in rabbit (OECD 414) M-279773-01-1	Survival: LOAEL > 75 mg/kg bw/d Clinical signs: LOAEL > 75 mg/kg bw/d **Bodyweight: LOAEL = 75 mg/kg bw/d** Feed consumption: LOAEL = 75 mg/kg bw/d	25 mg/kg bw/d	
2) Indices of gestation, litter size, pup and litter weight[3]	Multigeneration study in rat (OECD 416) M-299334-01-1	Mating index: LOAEL > 1200 ppm Fertility index: LOAEL > 1200 ppm Gestation index: LOAEL > 1200 ppm Litter size: LOAEL > 1200 ppm Pup birth weight: LOAEL > 1200 ppm	≥1200 ppm (82.8/93.1 mg/kg bw/d)	Use dose of females during gestation
	Developmental study in rat (OECD 414) M-299438-01-2	Litter size: LOAEL > 450 mg/kg bw/d **Fetal weight: LOAEL 450 mg/kg bw/d**	150 mg/kg bw/d	
	Developmental study in rabbit (OECD 414) M-279773-01-1	Litter size: LOAEL > 75 mg/kg bw/d **Fetal weight: LOAEL 25 mg/kg bw/d**	25 mg/kg bw/d	
3) Indices of viability, pre- and post-implantation loss	Multigeneration study in rat (OECD 416) M-299334-01-1	Livebirth index: LOAEL > 1200 ppm Viability index: LOAEL > 1200 ppm Lactation index: LOAEL > 1200 ppm	≥1200 ppm (82.8/93.1 mg/kg bw/d)	Use dose of females during gestation
	Developmental study in rat (OECD 414) M-299438-01-2	pre- and post-implantation loss: LOAEL > 450 mg/kg bw/d	≥450 mg/kg bw/d	
	Developmental study in rabbit (OECD 414) M-279773-01-1	pre- and post-implantation loss: LOAEL > 75 mg/kg bw/d	≥75 mg/kg bw/d	
4) Embryo/ foetal toxicity including teratological effects	Multigeneration study in rat (OECD 416) M-299334-01-1	No particular assessments of fetal toxicity but no effects on number of normal pups: LOAEL > 1200 ppm	≥1200 ppm (82.8/93.1 mg/kg bw/d)	Use dose of females during gestation

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Effects to check	Studies to check	Relevant endpoint observations	NOAEL$_{ETX}$ proposal	Comment
	Developmental study in rat (OECD 414) M-299438-01-2	External observations: LOAEL > 450 mg/kg bw/d Visceral observations: LOAEL = 450 mg/kg bw/d Skeletal observations: LOAEL = 450 mg/kg bw/d	150 mg/kg bw/d	Minor variations (2 visceral, 2 skeletal) could also be considered irrelevant for wild mammals
	Developmental study in rabbit (OECD 414) M-279773-01-1	**External observations: LOAEL = 75 mg/kg bw/d (increased number of small fetuses)** Visceral observations: LOAEL > 75 mg/kg bw/d Skeletal observations: LOAEL > 75 mg/kg bw/d	25 mg/kg bw/d	
5) Number aborting and number delivering early	Multigeneration study in rat (OECD 416) M-299334-01-1	Number aborting or early deliveries: LOAEL > 1200 ppm	≥1200 ppm (82.8/93.1 mg/kg bw/d)	Use dose of females during gestation
	Developmental study in rat (OECD 414) M-299438-01-2	Number aborting or early deliveries: LOAEL > 450 mg/kg bw	≥450	
	Developmental study in rabbit (OECD 414) M-279773-01-1	Number aborting or early deliveries: LOAEL > 75 mg/kg bw	≥75 mg/kg bw/d	
6) Systemic toxicity and effects on adult bodyweight	Multigeneration study in rat (OECD 416) M-299334-01-1	Survival: LOAEL > 1200 ppm Clinical signs: LOAEL > 1200 ppm **Bodyweight: LOAEL = 1200 ppm (F only)** Feed consumption: LOAEL > 1200 ppm Clinical chemistry: LOAEL = 1200 ppm Histopathology LOAEL = 1200 ppm Mating index: LOAEL > 1200 ppm Fertility index: LOAEL > 1200 ppm	220 ppm (14.5/17.2 mg/kg bw/d)	Large spacing (~5x) between LOAEL and NOAEL

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Effects to check	Studies to check	Relevant endpoint observations	NOAEL$_{ETX}$ proposal	Comment
	Developmental study in rat (OECD 414) M-299438-01-2	Survival: LOAEL > 450 mg/kg bw/d Clinical signs: LOAEL > 450 mg/kg bw/d **Bodyweight: LOAEL = 450 mg/kg bw/d** Feed consumption: LOAEL = 150 mg/kg bw/d Organ weight (liver): LOAEL = 150 mg/kg bw/d Histopathology LOAEL = 150 mg/kg bw/d	150 mg/kg bw/d	
	Developmental study in rabbit (OECD 414) M-279773-01-1	Survival: LOAEL > 75 mg/kg bw/d Clinical signs: LOAEL > 75 mg/kg bw/d **Bodyweight: LOAEL = 75 mg/kg bw/d** Feed consumption: LOAEL = 75 mg/kg bw/d	25 mg/kg bw/d	
7) Indices of post-natal growth, indices of lactation and data on physical landmarks	Multigeneration study in rat (OECD 416) M-299334-01-1	Lactation index: LOAEL > 1200 ppm **Post-natal growth: LOAEL = 1200 ppm** **Physical landmarks: LOAEL = 1200 ppm**	220 ppm (14.5/17.2 mg/kg bw/d)	
	Developmental studies (OECD 414)	Not relevant (no post-natal data)		
8) Survival and general toxicity up to sexual maturity	Multigeneration study in rat (OECD 416) M-299334-01-1	Survival: LOAEL > 1200 ppm Clinical signs: LOAEL > 1200 ppm **Bodyweight: LOAEL = 1200 ppm (F only)** Feed consumption: LOAEL > 1200 ppm Clinical chemistry: LOAEL = 1200 ppm Histopathology LOAEL = 1200 ppm.	220 ppm (14.5/17.2 mg/kg bw/d)	
	Developmental studies (OECD 414)	Not relevant (no post-natal data)		

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Effects to check	Studies to check	Relevant endpoint observations	NOAEL$_{ETX}$ proposal	Comment
Conclusions:				
The wild mammal reproductive risk assessment profile of fluopyram is of low concern. Mortality and clinical signs appear only at very high exposure. Moderate effects on bodyweight are below 10% (20% for effects on bodyweight gain) and do not impact on locomotor performance. Reproductive success is not affected even at very high doses.		Overall lowest relevant NOAEL (220 ppm in the rat multigeneration reproduction study)	**14.5 mg/kg bw/d males** **17.2 mg/kg bw/d females** (premating P and F1)	

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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. As the log P_{OW} of the active substance Fluopyram is above the trigger, an evaluation of secondary poisoning is conducted. For the evaluation please refer to the MCP Section 10, Point 10.1.1 and 10.1.2 for the representative formulations.

Data Point:	KCA 8.1.3/01
Report Author:	
Report Year:	2009
Report Title:	Fluopyram: A study on the bioaccumulation by the earthworm Eisenia fetida
Report No:	08P1RD
Document No:	M-349703-01-1
Guideline(s) followed in study:	Proposal for a new Guideline for OECD Guidelines for the Testing of Chemicals - Bioaccumulation: Soil Test using terrestrial Oligochaetes. Second Draft (May 2007), ECT Oekotoxikologie GmbH, Flörsheim/Main, Germany
Deviations from current test guideline:	Current Guideline: OECD 317 (2010) Deviation: none. All validity criteria were met.
Previous evaluation:	yes, evaluated and accepted in DAR (2011)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The bioconcentration potential of fluopyram to earthworm *Eisenia fetida* was determined in a laboratory test. *Eisenia fetida* (clitellate adults) were exposed to fluopyram [non labelled and ^{14}C-labelled], for 21 days at a nominal concentration of 1.5 mg a.s./kg artificial soil dry weight (dw) (uptake phase). After 21 days of exposure, the adult worms were transferred into untreated artificial soil and kept for a further 21 days (elimination phase). During both phases samples were taken at different sampling points (8 samplings during uptake phase, 9 samplings during elimination phase dates for treated soil and 3 samplings for the solvent control). The bioaccumulation factor of the test item in the worms was assessed after 21 days of uptake (exposure). The Non-eliminated Residues (NER) was assessed after 21 days of elimination.

The study fulfilled all validity criteria of the OECD guideline 317 (2010).

Fluopyram was taken up by the worms rapidly. A steady state was reached. The bioaccumulation factor (BAF_k) was: 1.10 kg soil/kg worm as calculated on a wet weight (ww) basis and 3.13 kg soil/kg worm as normalised for dry weight (dw). Based on the lipid content of the worms and the total organic carbon content (OC) of the artificial soil a biota soil accumulation factor (BSAF) was calculated: 1.27 kg OC/kg lipid as calculated on a dry weight (dw) basis. The test item was lost rapidly from the test organisms after transfer to control soil. The non-eliminated residues (NER_{21d}) at the end of the elimination phase were: 3.0 % of body residues at steady state based on the kinetic model used and 5.2 % of body residues at steady state based on measured body residues.

I. MATERIALS AND METHODS

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Test item (non-labelled): Fluopyram, Batch No.: Mix-Batch:08528/0002; purity: 94.7 %.

Test item (^{14}C-labelled): [phenyl-UL-^{14}C] Fluopyram; purity: > 99 %.

Test organisms: Earthworms, *Eisenia fetida* (Lumbricidae, Earthworms). Only adult worms (with clitellum) with a wet weight between 250 and 600 mg were used. The worms were approx. 14 months old. The animals were taken from a synchronised culture. The age of individuals did not differ by more than 4 weeks. The worms selected for the test were acclimatised in artificial soil under test conditions three days before starting the test.

Test design: *Eisenia fetida* (clitellate adults) were exposed to fluopyram for 21 days at a nominal concentration of 1.5 mg a.s./kg artificial soil dry weight (dw) (consisted of 1.44 mg a.s./kg soil (dw) (non-labelled fluopyram) and 0.06 mg a.s./kg soil (dw) (^{14}C-labelled fluopyram) (uptake phase). The test item was applied once at the beginning of the test. Additionally, a solvent control (acetone) with untreated artificial soil was included. After 21 days of exposure, the adult worms were transferred into untreated artificial soil and kept for a further 21 days (elimination phase).

During the uptake and elimination phase samples were taken at different sampling points (uptake phase: 8 sampling dates; elimination phase: 9 sampling dates for treated soil and 3 sampling dates for the solvent control). For each sampling date three replicate of the treatment level and 4 replicate samples of the solvent controls were used (kinetics). Each test container (kinetics) contained one earthworm. At the end of the uptake phase (day 21), additional test containers of the treatment and the solvent control (6 replicates for the treatment, 4 replicates for the solvent control) were used for determination of parent compound (analytics). Each of these test containers contained 10 earthworms.

Treated worm and soil samples were taken on days 0, 1, 2, 4, 7, 9, 14, 17 and 21 of the uptake phase to determine the total radioactive residues (TRR). During elimination phase worms and soil samples were taken at day 0.25, 1, 2, 4, 7, 9, 14, 17 and 21 of the elimination phase to determine the total radioactive residues (TRR). Additionally, worm and soil samples from the solvent control were taken at day 21 of the elimination phase. The bioaccumulation factor of the test item in the worms was assessed after 21 days of uptake (exposure). The Non-eliminated Residues (NER) was assessed after 21 days of elimination. The lipid content was measured in samples of acclimatised worms on day 0.
Test conditions: The adult worms were fed with 20 g food (finely ground cow manure) per kg soil dry weight once before start of the uptake and elimination phase. The artificial soil was prepared according to the guideline No. 207 with the following constituents (percentage distribution on dry weight basis): 73 - 74 % quartz sand, 5 % Sphagnum peat, 20 % Kaolinite clay, ca. 1% Calcium carbonate ($CaCO_3$). The pH value was adjusted to 6 ± 0.5 using calcium carbonate. The moisture content was adjusted 40 – 60 % of WHC_{max} using deionised water.

Climatic conditions: The temperature was in the range 18 - 22 °C with a photoperiod of 16 hours light and a light intensity of 400 - 800 lux.

Chemical analysis:

Soil samples were analysed by LSC and HPLC. The identity of [phenyl-UL-^{14}C]-Fluopyram was confirmed by LC-MS/MS (please refer for analytical determination to report by M-345856-01-1, KCA 8.1.3/03).

Earthworm samples were analysed by HPLC. The identification of metabolites was performed by HPLC co-chromatography with radiolabelled reference items.

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 SANTE/2020/12830, Rev.1.

Dates of work: October 16th to December 16th, 2008

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II. RESULTS AND DISCUSSION

Biological results:

Table 8.1.3- 1: Worm dry weight at start of the test (day 0 of uptake phase) measured in purged worms after acclimatisation in artificial soil (n = 4; mean value)

Sampling date	Mean worm dry weight [% of wet weight]	Mean water content of worms [% of wet weight]
Day 0 (uptake phase, gut-purged)	24.7	75.3

Table 8.1.3- 2: Lipid content in worms at day 0 (uptake phase) measured in purged worms after acclimatisation in artificial soil

Sampling date	Mena lipid content [% of worm wet weight ± SD]	Mean lipid content [% of worm dry weight]
Day 0 (uptake phase)	2.38 ± 0.10	9.65 ± 0.39

Table 8.1.3- 3: Total organic carbon content (TOC) at day 0 (uptake phase) in artificial control soil

Sampling date	Total organic carbon content [Mean % of soil dry weight ± S.D.]
Day 0 (uptake phase)	3.31 ± 0.09

S.D.: Standard deviation

Bioaccumulation:

Uptake Phase:

At the end of the uptake phase a steady state was reached. No statistically significant (ANOVA, p ≤ 0.05) difference between the three latest sampling points (days 14, 17 and 21) was determined with respect to the concentration measured in worms (C_W) and the accumulation factors (AF).

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Table 8.1.3- 4: Summary of steady state concentrations and accumulation factors

Parameter	Calculated values using regression model	Values measured on day 21 of uptake
C_a at steady state [dpm/g wet weight]	10390	10530
C_a at steady state [dpm/g dry weight]	38778	39302
BAF [ww/ww]; kg soil/ kg worm	1.10	1.13
BAF [dw/dw]; kg soil/kg worm	3.13	3.25
BAF_k (ww/ww; modelled; kg soil/ kg worm)	1.10	--
k_e (ww/ww; modelled, [1/ d])	23.98	--
k_s (ww/ww; modelled, in kg soil/ kg of worm/ d)	26.26	--

C_a: Concentration in worm tissue;
BAF: Bioaccumulation factor;
BAF_k: Kinetic bioaccumulation factor;
k_s: Uptake rate constant;
ww: wet weight;
dw: dry weight

Based on the lipid content of the worms and the total organic carbon content (OC) of the artificial soil a biota soil accumulation factor (BSAF) was calculated to be:

1.27 kg OC/kg lipid as calculated on a dry weight (dw) basis.

Elimination phase:

The calculated elimination rate constants k_a and k_e were 2.0 and 0.06 d^{-1}, respectively.
The Non-Eliminated Residues (NER-21 days) at the end of elimination phase were:

- 3.0 % of the body residues at steady state based on the kinetic model used.
- 5.2 % of body residues at steady state based on measured body residues.

Total radioactive residues in earthworm:

The TRR (Total Radioactive Residue) in the earthworms was calculated from the radioactivity in the extracts and the remaining solids and amounted to 1.399 mg/kg.

Approximately 99 % of the TRR was extractable using a mixture of acetonitrile/water (8/2, v/v). The major compound in the extract was fluopyram and amounted to 1.251 mg/kg (89.4 % of TRR). A minor compound fluopyram-7-hydroxy, was detected in amounts of 0.132 mg/kg (9.4 % of TRR).

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Table 8.1.3- 5: Summary of characterisation and identification of radioactive residues in earthworms exposed to soil treated with [phenyl-UL-^{14}C]-fluopyram [A]

Report name	Earthworms (TRR = 1.399 mg/kg)	
	[% of the TRR]	[mg/kg]
Fluopyram	89.4	1.251
Fluopyram-7-hydroxy	9.4	0.132
Total identified	**98.9**	**1.383**
Total extractable (combined extracts)	**98.9**	**1.383**
Total bound residues (PES)	**1.1**	**0.016**

[A] Please refer to analytical report M-343213-01-1, KCA 8.1.3/02

Residues in soil:

The identity of [phenyl-UL-^{14}C]-fluopyram was confirmed by LC-MS/MS. Besides the test item only one minor product was detected with about max. 4 area %. The product was not identified but is likely the 7-hydroxy metabolite of fluopyram based on retention time.

Table 8.1.3- 6: Summary of residues in soil samples treated with [phenyl-UL-^{14}C]-fluopyram [A]

Sample Code	Total Fluopyram/soil sample		
	Moist soil [µg/sample]	Moist soil [µg a.s./kg soil]	Dry soil [µg a.s./kg soil]
"00118"	n.d.	n.d.	n.d.
"00119"	n.d.	n.d.	n.d.
"00120"	53.5	**991.0**	**1288**
"00121"	53.3	**965.5**	**1255**
"00122"	56.8	**993.2**	**1291**
"00123"	55.1	**968.9**	**1260**
"00130"	n.d.	n.d.	n.d.

n.d.: not detected (< 0.4 % of applied radioactivity)
[A] Please refer to analytical report M-345856-01-1, KCA 8.1.3/03

The recovery was in the range of 86.3 – 88.2 % of the applied radioactivity. The difference to the applied amount is due to calculation of application to dry soil and analysis of wet soil. The back-calculation was done based on weight differences between wet and dry soil given from the supplier of the samples.

Validity criteria:

The validity criteria of the OECD guideline 317 (2010) were fulfilled.

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Table 8.1.3- 7: Validity criteria

Validity criteria acc. to OECD 317 (2010)	Required	Obtained
Mortality of the adult test animals during uptake and elimination phase.	≤ 10 %	1.7 %
Weight reduction of the adult test animals during uptake and elimination phase compared to the initial fresh weight (at start of each phase).	≤ 20 %	None (weight growth of 41 – 72 % during uptake phase)

III. CONCLUSION

All validity criteria of the OECD guideline 317 (2010) were met.

The test item was taken up by the worms rapidly. A steady state was reached.

The bioaccumulation factor (BAFk) was 1.10 kg soil/kg worm as calculated on a wet weight (ww) basis and 3.13 kg soil/kg worm as normalised for dry weight (dw).

Based on the lipid content of the worms and the total organic carbon content (OC) of the artificial soil a biota soil accumulation factor (BSAF) was calculated: 1.27 kg OC/kg lipid as calculated on a dry weight (dw) basis.

The test item was lost rapidly from the test organisms after transfer to control soil. The Non-Eliminated Residues (NER) at the end of the elimination phase were: 3.0 % of body residues at steady state based on the model used and 5.2 % of body residues at steady state based on measured body residues.

Assessment and conclusion by applicant:

The study and its data are considered as acceptable and reliable for use in risk assessment.

The endpoints are:

Bioaccumulation factor (BAFk) = 1.10 kg soil/kg worm (based on wet weight)

Bioaccumulation factor (BAFk) = 3.13 kg soil/kg worm (based on dry weight)

Biota soil accumulation factor (BSAF) = 1.27 kg organic carbon content/kg lipid (based on dry weight)

The additional evaluation by the RMS in the DAR (2011) concluded the following endpoints:

The Reviewer agrees with the author that steady state was reached within the test duration. The calculated BAFk of 1.10 kg soil/kg worm as calculated on a wet (ww) basis 3.13 kg soil/kg worm as nominalised for dry weight (dw) are acceptable. According to the Guidance Document for Risk assessment for Birds and Mammals (EFSA Journal 2009; 7 (12): 1438) for the risk assessment the bioconcentration factor for earthworm should be defined as concentration in earthworm related to fresh weight to concentration in soil related to dry weight ($PEC_{worm\ fresh\ weight}$/ $C_{soil\ dry\ weight}$). For reasons of consistency the RMS calculated from the experimental data a BCF or rather BAF according to this definition. Considering the measured mean ratio of 1.3 between dry weight and wet weight in the experimental treatment in a simple calculation procedure a value of 0.85 based on concentration in worm (wet weight) and concentration in soil (dry weight) can be derived.

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Data Point:	KCA 8.1.3/02
Report Author:	[redacted]
Report Year:	2009
Report Title:	Fluopyram: A study on the bioaccumulation by the earthworm - Metabolism investigations
Report No:	MEF-09/113
Document No:	M-343213-01-1
Guideline(s) followed in study:	OECD Guideline for the Testing of Chemicals, Second Draft Version (since May 2007) - Bioaccumulation: Soil Test using terrestrial Oligochaetes
Deviations from current test guideline:	Current Guideline: not applicable
Previous evaluation:	yes, evaluated and accepted in DAR (2011)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Summary

In a preceding in life study (Fluopyram: A Study on the Bioaccumulation by the Earthworm *Eisenia fetida*, study no. 08P1RD), adult earthworms were exposed to soil spiked with [phenyl-UL-^{14}C]AE C656948 (Fluopyram).

In the current study, the combined earthworm sample (ID 0126 to 0129) was extracted with acetonitrile/water (8/2; v/v) and pure acetonitrile. The TRR (Total Radioactive Residue) in the earthworm sample was calculated by summation of the radioactivity in the extracts and in the remaining solids (PES – post extraction solids). The TRR amounted to 1.399 mg/kg. Approximately 99 % of the TRR was extractable.

After further purification of the combined extract, parent compound and metabolites were quantified by HPLC. The identification of metabolites was performed by HPLC co-chromatography with radiolabeled reference items. The majority of the residue in earthworms was parent compound (1.251 mg/kg, 89.4 % of the TRR). The metabolite AE C656948-7-hydroxy was detected in amounts of 0.132 mg/kg (9.4 % of the TRR).

A summary of characterization and identification of radioactive residues in earthworms is presented below.

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Table 8.1.3- 8: Summary of characterisation and identification of radioactive residues in earthworms exposed to soil treated with [phenyl-UL-^{14}C]-fluopyram

Report name	Earthworms (TRR = 1.399 mg/kg)	
	[% of the TRR]	[mg/kg]
Fluopyram	89.4	1.251
Fluopyram-7-hydroxy	9.4	0.132
Total identified	**98.9**	**1.383**
Total extractable (combined extracts)	**98.9**	**1.383**
Total bound residues (PES)	**1.1**	**0.016**

Assessment and conclusion by applicant:

The study and its data are considered as acceptable and reliable for use in risk assessment.

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Data Point:	KCA 8.1.3/03
Report Author:	[redacted]
Report Year:	2009
Report Title:	[Phenyl-UL-14C]AE C656948 (fluopyram): Determination of residues in soil samples from ETX study ECT 08P1RD
Report No:	MEF-09/153
Document No:	M-345856-01-1
Guideline(s) followed in study:	not specified
Deviations from current test guideline:	Current Guideline: not applicable
Previous evaluation:	yes, evaluated and accepted in DAR (2011)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Summary

The concentration of [phenyl-UL-^{14}C]AE C656948 (Fluopyram) was determined in moist soil samples from study: Fluopyram: A Study on the Bioaccumulation by the earthworm *Eisenia fetida*. The study was carried out in compliance with valid GLP regulations. The soil samples (50.6 g – 57.2 g) were extracted with acetonitrile /water 3-times under ambient temperature and additionally once under reflux conditions. Extracts were analyzed by LSC and HPLC. The identity of [phenyl-UL-^{14}C]AE C656948 (Fluopyram) was confirmed by LC-MS/MS.

Table 8.1.3- 9: Summary of residues in soil samples treated with [phenyl-UL-^{14}C]-fluopyram

Sample Code	Total Fluopyram/soil sample		
	Moist soil [µg/sample]	Moist soil [µg a.s./kg soil]	Dry soil [µg a.s./kg soil]
"00118"	n.d.	n.d.	n.d.
"00119"	n.d.	n.d.	n.d.
"00120"	53.5	**991.0**	**1288**
"00121"	53.3	**965.5**	**1255**
"00122"	56.8	**993.2**	**1291**
"00123"	55.1	**968.9**	**1260**
"00130"	n.d.	n.d.	n.d.

n.d.: not detected (< 0.4 % of applied radioactivity)

Assessment and conclusion by applicant:

The study and its data are considered as acceptable and reliable for use in risk assessment.

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Data Point:	KCA 8.1.3/04
Report Author:	[redacted]
Report Year:	2011
Report Title:	Fluopyram - Evaluation of OECD joint review dossier - WNL 6656 - Fluopyram - OECD-Joint Review/ EU-Wirkstoffpruefung zur Aufnahme von Wirkstoffen in Anhang I der Richtlinie 91/414/EWG
Report No:	M-409909-01-1
Document No:	M-409909-01-1
Guideline(s) followed in study:	not specified
Deviations from current test guideline:	Current Guideline: not applicable
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	Yes

In the previous submission this statement was evaluated. However, it is no longer of relevance for the active substance renewal process.

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

The risk to birds and mammals is assessed in the respective representative formulation Document MCP, Section 10.1.

Amphibians and Reptiles

There are no data requirements or agreed specific protection goals for amphibians and reptiles under 1107/2009. No standardized guidelines for studies with terrestrial amphibian life stages and reptiles are available. Therefore, information on effects of fluopyram on reptiles or terrestrial stages of amphibians is not available.

CA 8.1.5 Endocrine disrupting properties

The potential for endocrine disrupting properties in birds and other non-target terrestrial vertebrates is assessed based on a weight of evidence (WoE) using information gathered from studies across different vertebrate species, following the recommendations of the ECHA-EFSA Guidance for the identification of endocrine disruptors under Regulations (EC) No 1107/2009 (ECHA-EFSA, 2018).

This analysis is captured in two formats: Appendix I of EFSA's administrative guidance on submission of dossiers (EFSA, 2019) and Appendix E an Excel file, completed in line with the template for reporting the available information relevant for ED assessment (Appendix E.1 to the Guidance) and submitted as a supplement to this document.

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For birds, the EU ED guidance reports that only a limited number of standardised *in vivo* methods are available, and that little information can be gained from those guidelines concerning potential ED-related effects (ECHA-EFSA, 2018). This guidance sets out the parameters investigated according to the OECD TG 206 (OECD 1984; Level 4 of the OECD CF) and the OCSPP 890.2100 (US EPA, 2015; Level 5 of the OECD CF) together with their relevance for identifying a substance with a potential for endocrine disruption according to the EATS modalities. It concludes that the main investigated parameters in the OECD TG 206 study are only '*sensitive to, but not diagnostic of, EATS*' (OCSPP 890.2100 data are not available for this active substance). It is also reported that based on the OECD GD 150 (OECD, 2018), these parameters are not assignable to a specific modality. Therefore, these avian *in vivo* studies are considered as complementary information and not sufficient to conclude on whether an active substance meets the ED criteria in non-target organisms.

For other non-target terrestrial vertebrates, the potential for endocrine disrupting properties assessment is based on a weight of evidence assessment of all the available relevant data, which includes endocrine activity and adversity assays using *in vitro* and *in vivo* test models, primarily in mammalian systems. For specific study details please refer to the separate sections in this dossier:

CA 5.8.3 Endocrine disrupting properties

CA 8.1.1.3 Sub-chronic and reproductive toxicity to birds

CA 8.1.2.2 Long-term and reproduction toxicity to mammals

CA 8.2 Effects on aquatic organisms *then* CA 8.2.3 Endocrine disrupting properties

This section provides a summary of the conclusions provided in Appendix I which in-turn is based on data collated in the Appendix E.

Appendix I - Assessment of the endocrine disrupting properties of the active substance fluopyram in accordance with Commission Regulation (EU) 2018/605

Data Point:	KCA 8.1.5/01
Report Author:	[redacted]
Report Year:	2021
Report Title:	Appendix I - Assessment of the endocrine disrupting properties of the active substance fluopyram in accordance with Commission Regulation (EU) 2018/605
Report No:	M-764022-01-1
Document No:	M-764022-01-1
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

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Table 8.1.5- 1: Outline of studies available for ecotoxicology assessment of potential endocrine disrupting properties of fluopyram in birds and other non-target terrestrial vertebrates

Type of toxicity	Study type	Reference
Endocrine activity	Specific studies are not performed for birds and other non-target terrestrial vertebrates. For relevant terrestrial vertebrate information please refer to: – Appendix I Section 2.1.2.1: Table 4: WoE for T-mediated endocrine activity. – Appendix I Section 2.1.2.1: Table 10: WoE for EAS-mediated endocrine activity.	M-764022-01-1 KCA 8.1.5/01
Endocrine mediated adversity	Specific studies are not performed for birds and other non-target terrestrial vertebrates. For relevant terrestrial vertebrate information please refer to: – Appendix I Section 2.1.2.1: Table 3: WoE for T-mediated endocrine adversity. – Appendix I Section 2.1.2.1: Table 9: WoE for EAS-mediated endocrine adversity.	M-764022-01-1 KCA 8.1.5/01
Complementary information	Avian Reproduction Test according to OECD TG 206. For further details about these studies, please refer to the relevant Appendix E and MCA Section 8.1.1.3.	
	20-weeks feeding chronic, reproduction — Bobwhite quail (*Colinus virginianus*)	M-299245-02-1 KCA 8.1.1.3/01
	22-weeks feeding chronic, reproduction — Bobwhite quail (*Colinus virginianus*)	M-298723-01-1 KCA 8.1.1.3/02
	19-weeks feeding chronic, reproduction — Mallard duck (*Anas platyrhynchos*)	M-299277-01-1 KCA 8.1.1.3/03

Modality summaries (based on Appendix I Section 3 (M-764022-01-1, KCA 8.1.5/01)

Conclusion on the assessment of Thyroid (T)-modality in birds and other non-target terrestrial vertebrates

For birds as non-target organisms, OECD level 4 studies conducted according to OECD TGs 206 (Avian Reproduction Test) are available to potentially investigate T-mediated adversity. However, the range of effects observed in these avian studies are considered as not assignable to a specific modality and only potentially sensitive to, but not diagnostic of a T-mediated mode of action (ECHA-EFSA ED Guidance, 2018). For further details about these studies, please refer to the relevant Appendix E and the accompanying MCA Section 8.1.1.3.

Based on this information, fluopyram does not meet the ED criteria for the T-modality in birds as non-target organisms.

For mammals as non-target organisms, T-mediated adversity and endocrine activity have been sufficiently investigated. In summary, fluopyram caused microscopic changes in the thyroid and in thyroid hormones in the rat and mouse. However, the Mode of Action (MoA) analysis provided sufficient evidence to demonstrate the most plausible MoA was a secondary effect on the thyroid via enhanced hepatic clearance of thyroid hormones. In addition, a direct MoA could be excluded. As the effects on the thyroid are secondary to the effects of fluopyram on the liver, it can be concluded that fluopyram shows no adversity with regard to the T modality. For further details about these studies,

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please refer to Appendix I Section 2 ED assessment for humans and in particular Section 2.1. ED assessment for T-modality Tables 2 and 3.

Based on the weight of the evidence, it can be concluded that fluopyram does not meet the ED criteria for the T-modality in mammals as non-target organisms.

Conclusion on the assessment of Estrogen, Androgen and Steroidogenic (EAS) -modalities in birds and other non-target terrestrial vertebrates

For birds as non-target organisms, OECD level-4 studies conducted according to OECD TGs 206 (Avian Reproduction Test) are available to potentially investigate EAS-mediated adversity. However, the range of effects observed in these avian studies are considered as not assignable to a specific modality and only potentially sensitive to, but not diagnostic of an EAS-mediated mode of action (ECHA-EFSA ED Guidance, 2018). For further details about these studies, please refer to the relevant Appendix E and the accompanying MCA Section 8.1.1.3.

Based on this information, fluopyram does not meet the ED criteria for the EAS-modalities in birds as non-target organisms.

For mammals as non-target organisms, EAS-mediated adversity and endocrine activity have been sufficiently investigated. There were no morphological or functional changes observed in any of the organs sensitive to the E, A, or S modalities, and no adverse effects were observed in any of these organs in any study conducted with fluopyram. No EAS-mediated endocrine activity or adversity was observed either *in vivo* or *in vitro* in any of the mechanistic studies conducted using fluopyram. For further details about these studies, please refer to Section 2. ED assessment for humans and in particular Section 2.2. ED assessment for EAS-modalities.

Based on the weight of the evidence, it can be concluded that fluopyram does not meet the ED criteria for the EAS-modalities in mammals as non-target organisms.

<u>Assessment and conclusion by applicant:</u> It is concluded that fluopyram does not meet the ED criteria for birds and other non-target terrestrial vertebrates according to Commission Regulation (EU) 2018/605.

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CA 8.2 Effects on aquatic organisms

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

Test substance	Test species	Time scale/ Study type	Endpoint	Reference
Fluopyram tech.	Fish, acute *Oncorhynchus mykiss*	96 h / static	LC_{50} > 1.89 mg a.s./L (nom)	[redacted] (2008) M-277770-02-1 KCA 8.2.1/01
	Fish, acute *Lepomis macrochirus*	96 h / static	LC_{50} > 5.68 mg a.s./L (nom)	[redacted] (2008) M-278441-02-1 KCA 8.2.1/02
	Fish, acute *Pimephales promelas*	96 h / static	LC_{50} > 4.95 mg a.s./L (mm) [A]	[redacted] (2008) M-298918-01-1 KCA 8.2.1/03
	Fish, acute *Cyprinus carpio*	96 h / static	LC_{50} = 30.5 mg a.s./L (mm) [B]	[redacted] (2006) M-280108-01-1 KCA 8.2.1/04
	Fish, acute *Cyprinodon variegatus*	96 h / static	LC_{50} > 0.98 mg a.s./L (mm)	[redacted] (2006) M-279167-01-1 KCA 8.2.1/05
	Fish, chronic (ELS) *Pimephales promelas*	33 d / flow-through	NOEC = 0.135 mg a.s./L (mm) **EC_{10} = 0.162 mg a.s./L (mm)**	[redacted] (2006) M-279440-01-1 KCA 8.2.2.1/01 **Recalculation by [redacted] (2020) M-758375-01-1 KCA 8.2.2.1/02**
	Fish, BCF flow-through *Lepomis macrochirus*	28 d exposure + 14 d depuration / flow-through	BCF (whole fish, wet weight) = 18 BCF (whole fish, normalized to 5 % lipid content) = 16	[redacted] (2008) M-298506-01-1 KCA 8.2.2.3/01
	Invertebrate, acute *Daphnia magna*	48 h / static	EC_{50} > 20 mg a.s./L (nom)	[redacted] (2006) M-278709-01-1 KCA 8.2.4.1/01
	Sediment dweller; sub-chronic *Leptocheirus plumulosus* (spiked sediment)	10 d / static	LC_{50} > 100 mg a.s./kg (mm) NOEC = 100 mg a.s./kg (mm)	[redacted] (2008) M-297751-01-1 KCA 8.2.4.2/01
	Invertebrate, acute *Crassostrea virginica*	96 h / flow-through	EC_{50} > 0.44 mg a.s./L (mm) (shell deposition and mortality)	[redacted] (2006) M-282691-01-1 KCA 8.2.4.2/02
	Invertebrate, acute *Americamysis bahia*	96 h / flow-through	LC_{50} > 0.50 mg a.s./L (mm)	[redacted] (2007) M-282839-01-2 KCA 8.2.4.2/03

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Test substance	Test species	Time scale/ Study type	Endpoint	Reference
	Invertebrate, chronic *Daphnia magna*	21 d / static-renewal	NOEC = 1.25 mg a.s./L (nom) **EC_{10}: not determined** [C]	(2008) M-282102-02-1 KCA 8.2.5.1/01 **Recalculation by (2020)** M-758376-01-1 **KCA 8.2.5.1/02**
	Sediment dweller, chronic (54 d, Life cycle) *Chironomus tentans* (spiked sediment)	54 d / static-renewal	NOEC = 26 mg a.s./kg (mm) **EC_{10}: not determined** [C]	(2008) M-298809-01-1 KCA 8.2.5.3/01 **Recalculation by (2020)** M-758550-01-1 **KCA 8.2.5.3/02**
	Sediment dweller, chronic (28 d, Life Cycle) *Chironomus riparius* (spiked water)	28 d / static	NOEC = 1.39 mg a.s./L (nom) EC_{10} = 0.50 mg a.s./L (nom) EC_{15} = 1.57 mg a.s./L (nom) EC_{50} > 32 mg a.s./L (nom) [C]	(2008) M-298266-01-1 KCA 8.2.5.4/01
	Sediment dweller, chronic *Leptocheirus plumulosus* (spiked sediment)	28 d / Static-renewal	NOEC = 38 mg a.s./kg (mm) LC_{50} > 94 mg a.s./kg (mm)	(2008) M-298810-02-1 KCA 8.2.5.4/02
	Green algae *Pseudokirchneriella subcapitata* [D] (currently known as *Raphidocelis subcapitata*)	0 - 72 h / static	E_rC_{50} = 8.9 mg a.s./L (mm) E_rC_{10} = 7.1 mg a.s./L (mm) E_bC_{50} = 3.97 mg a.s./L (mm) **E_yC_{50} = 4.26 mg a.s./L (nom)**	(2007) M-286541-01-1 KCA 8.2.6.1/01 **Recalculation by (2020)** M-757659-01-1 **KCA 8.2.6.1/03**
	Freshwater diatom *Navicula pelliculosa*	0 - 72 h / static	E_rC_{50} = 9.08 mg a.s./L (mm) E_rC_{10} = 5.23 mg a.s./L (mm) E_bC_{50} = 5.62 mg a.s./L (mm) **E_yC_{50} = 5.64 mg a.s./L (mm)**	(2007) M-289899-01-1 KCA 8.2.6.2/01 **Recalculation by (2020)** M-757699-01-1 **KCA 8.2.6.2/04**
	Marine diatom *Skeletonema costatum*	0 - 72 h / static	E_rC_{50} > 1.13 mg a.s./L (mm) E_rC_{10} > 1.13 mg a.s./L (mm) E_bC_{50} > 1.13 mg a.s./L (mm) **E_yC_{50} > 1.13 mg a.s./L (mm)**	(2007) M-287289-01-1 KCA 8.2.6.2/03 **Recalculation by (2020)** M-757680-01-1 **KCA 8.2.6.1/06**

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Test substance	Test species	Time scale/ Study type	Endpoint	Reference
	Aquatic macrophyte, *Lemna gibba*	7 d / static	E_rC_{50} = 2.51 mg a.s./L (nom) E_rC_{10} = 1.58 mg a.s./L (nom) E_yC_{50} = 2.12 mg a.s./L (nom)	(2021) M-283647-02-1 KCA 8.2.7/01
	Amphibia, *Xenopus laevis*	48 h / static	LC_{50} > 3.0 mg a.s./L (nom)	(2010) M-395416-01-1 KCA 8.2.8/01
Fluopyram-7-hydroxy	Invertebrate, acute *Daphnia magna*	0 - 48 h / static	EC_{50} > 88.7 mg p.m./L (nom)	(2020) M-759029-01-1 KCA 8.2.4.1/02
	Green algae *Pseudokirchneriella subcapitata* (currently known as *Raphidocelis subcapitata*)	0 -72 h / static	E_rC_{50} = 20.9 mg p m./L (nom) E_rC_{10} = 20.2 mg p m./L (nom) E_yC_{50} = 13.0 mg p m./L (nom)	(2020) M-758708-01-1 KCA 8.2.6.1/05
		0 -96 h / static	E_rC_{50} = 21.1 mg p m./L (nom) E_rC_{10} = 20.4 mg p m./L (nom) E_yC_{50} = 13.7 mg p m./L (nom) E_bC_{50} = 12.6 mg p m./L (nom)	
	Aquatic macrophyte *Lemna gibba*	7 d / static	E_rC_{50} = 9.2 mg p m./L (mm) E_rC_{10} = 5.2 mg p m./L (mm) E_yC_{50} = 7.1 mg p m./L (mm)	(2020) M-759030-01-1 KCA 8.2.7/02
Trifluoro-acetic acid (TFA)	Fish, acute *Brachydanio rerio*	96 h / static	LC_{50} > 1200 mg p m./L (nom Na-TFA) > 1008 mg p m./L (nom TFA) [E]	(1992) M-247889-01-1 KCA 8.2.1/06
	Invertebrate, acute *Daphnia magna*	0 - 48 h / static	EC_{50} > 1200 mg p m./L (nom Na-TFA) > 1008 mg p m./L (nom TFA) [E]	(1992) M-247890-01-1 KCA 8.2.4.1/03
	Invertebrate, chronic *Daphnia magna*	21 d / Semi-static	NOEC ≥ 30 mg p m./L (nom Na-TFA) ≥ 25.2 mg p.m./L (nom TFA) [E] EC_{10} not determined [C]	(2010) M-615126-01-1 KCA 8.2.5.1/03
	Green algae *Pseudokirchneriella subcapitata* [D] (currently known as *Raphidocelis subcapitata*)	0 -72 h / static	E_rC_{50} > 1.2 mg p.m./L (nom Na-TFA) > 1.01 mg p.m./L (nom TFA) [E] E_rC_{10} > 1.2 mg p m./L (nom Na-TFA) > 1.01 mg p.m./L (nom TFA) [E] E_bC_{50} > 1.2 mg p m./L (nom Na-TFA) > 1.01 mg p.m./L (nom TFA) [E] E_yC_{50} > 1.2 mg p m./L (nom Na-TFA) > 1.01 mg p.m./L (nom TFA) [E]	(1993) M-247818-02-1 KCA 8.2.6.1/06 Re-evaluation by (2021) M-762268-02-1 KCA 8.2.6.1/07

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Test substance	Test species	Time scale/ Study type	Endpoint	Reference
	Green algae *Pseudokirchneriella subcapitata* D (currently known as *Raphidocelis subcapitata*)	0 -72 h / static	E_rC_{50} = 160 mg p m./L (nom Na-TFA) = 134.4 mg p.m./L (nom TFA) E E_rC_{10} = 2.239 mg p m./L (nom Na-TFA) = 1.881 mg p.m./L (nom TFA) E E_bC_{50} > 4.8 mg p m./L (nom Na-TFA) > 4.03 mg p.m./L (nom TFA) E E_yC_{50} = 4.190 mg p m./L (nom Na-TFA) = 3.52 mg p.m./L (nom TFA) E	(1992) M-247820-01-1 KCA 8.2.6.1/08 Re-evaluation by (2021) M-762208-02-1 KCA 8.2.6.1/09
	Green algae *Pseudokirchneriella subcapitata* D (currently known as *Raphidocelis subcapitata*)	0 -72 h / Static	E_rC_{50} = 237.07 mg p.m./L (nom) = 241.95 mg p.m./L (mm) E_rC_{10} = 5.59 mg p.m./L (nom) = 5.80 mg p.m./L (mm) E_bC_{50} = 26.866 mg p.m./L (mm) E_yC_{50} = 18.956 mg p.m./L (mm)	(2017) M-615180-01-1 KCA 8.2.6.1/12 Re-evaluation by (2021) M-762267-01-1 KCA 8.2.6.1/13
	Aquatic macrophyte *Lemna gibba*	7 d / static	E_rC_{50} = 1100 mg p.m./L (nom Na-TFA) = 924 mg p m./L (nom TFA) E E_rC_{50} = >2016 mg p.m./L (nom TFA) E NOE_rC = 252 mg p m./L (nom TFA) E E_rC_{10} = 308 mg p m./L (nom TFA) E	et al. (1993) M-247900-01-1 KCA 8.2.7/03 Endpoint recalculation by (2021) M-768038-01-1 KCA 8.2.7/06

a.s. = active substance, pm = pure metabolite, prod. = product
mm = mean measured; nom = nominal
Studies written in grey type are referring to studies in the corresponding Baseline-dossier, whereas studies in black type are studies of the Supplemental dossier

A Practical limit of water solubility
B In all test levels precipitations were observable so the LC_{50} is clearly above the water solubility of the test item.
C Not determined due to mathematical reasons
D Formerly known as *Selenastrum capricornutum*
E As the study was conducted with sodium trifluoroacetate which is the sodium salt of trifluoroacetic acid, the endpoint was converted to Trifluoroacetic acid with factor 0.84.

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CA 8.2.1 Acute toxicity to fish

Active substance fluopyram

Data Point:	KCA 8.2.1/01
Report Author:	■■■■■■
Report Year:	2008
Report Title:	Acute toxicity of AE C656948 (tech.) to fish (Oncorhynchus mykiss) under static conditions
Report No:	EBGMP017
Document No:	M-277770-02-1
Guideline(s) followed in study:	EPA-FIFRA § 72-1/SEP EPA-540/9-85-006 (1982/1985 ; OPPTS 850.1075 (Public Draft, 1996) ; Directive 92/69/EEC, C.1 (1992) ; OECD No. 203 (rev. 1992)
Deviations from current test guideline:	Current Guideline: 203 (2019) Deviations: The fish length at test start was 5.9 ± 0.6 cm and thus higher than the maximum 6 cm recommended in OECD 203. This deviation was not expected to have impacted the study results. All validity criteria were met.
Previous evaluation:	yes, evaluated and accepted in DAR (2011)
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 fish were exposed to fluopyram in groups of 30 (two replicates of 15 fish) to an aqueous solution of the test material at the single nominal concentrations of 1.89 mg a.s./L (corrected for purity) for a period of 96 hours. Additionally, a control and solvent control were included. Observations of mortality and other signs of toxicity were made approximately 4, 24, 48, 72 and 96 hours after test initiation.

Concentrations of fluopyram were verified by HPLC-UV on days 0, 2 and 4 for the single concentration and the controls. Measured concentrations were in the 93-102 % range of nominal concentrations and no residues were found in the control and solvent control samples above 0.1029 mg a.s./L which was used as the lowest standard concentration during the study. Biological results are based on nominal concentrations of fluopyram.

The study fulfils all validity criteria of OECD 203 guideline.

There were no behavioural abnormalities or mortalities of the fish in the controls and in the single test concentration.

The endpoints based on nominal concentrations were: LC_{50} - 96 hours: > 1.89 mg a.s./L and NOEC: ≥ 1.89 mg a.s./L.

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I. MATERIALS AND METHODS

Test material	Fluopyram (AE C656948) Specification No.: 102000018148-01 Batch No: EFIM000511 Purity: 94.7 % w/w
Guideline(s) adaptation	None specified
Test species	Rainbow trout (*Oncorhynchus mykiss*)
Acclimation	At least 14 days to test conditions. Health during acclimation: less than 3 % mortality in the 48-hour acclimation period before testing, all unsuitable fish (e.g. injured, deformed, etc.) were eliminated prior to the assignment of test groups.
Organism age/size	Mean length: 59 ± 6 mm at test start Mean body weight: 2.1 ± 6 g at test start
Test solutions	Nominal concentration: 1.89 mg a.s./L (corrected for purity) (corresponding to 2.00 mg test item/L) Corresponding mean measured concentration: 1.80 mg a.s./L Control: water Solvent control: dimethylformamide (0.1 mL/L) Evidence of undissolved material: No test material was observed and the test medium appeared clear.
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: 15
Exposure	Static Total exposure duration: 96 hours
Test Vessel Loading	0.79 g fish/L test medium
Feeding during test	None
Test conditions	Temperature: 12.0 – 12.6 °C Photoperiod: 16 hours light, 8 hours dark Light intensity: not reported pH: 6.5 - 7.0 Water hardness: 40 - 60 mg $CaCO_3$/L Dissolved oxygen: 64 – 104 % of saturation (aeration was added on study day 1 over night and on day 3 for 5 hours to maintain dissolved oxygen concentrations > 60 % of saturation) Conductivity: not reported Alkalinity: not reported
Parameters Measured / Observations	Fish were observed for mortalities and sub-lethal behavioural effects after 4, 24, 48, 72 and 96 hours. Dissolved oxygen, temperature and pH were determined daily in each replicate. Additionally, temperature was measured hourly by a data logger.
Sampling for Chemical analysis	Samples of test solutions were taken at test initiation (0 hour), after 48 hours and at test termination (96 hours) for analysis of test substance. The chemical analyses were performed by using a High-performance liquid chromatograph (HPLC) equipped with an UV-detector.

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Data analysis	Not needed as limit test.

II. Results and Discussion

Table 8.2.1- 1: Validity criteria

Validity criteria	Required	Obtained
Mortality in control during test	≤ 10 %	0 %
Dissolved oxygen saturation	≥ 60 %	64 – 104 %

Analytical results:

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 SANTE/2020/12830, Rev.1.

Recoveries on day 0, 2 and day 4 were between 91 and 102 % of nominal (see table below). Biological results are based on nominal concentrations of fluopyram.

No residues of fluopyram were found in the control and solvent control samples above 0.1029 mg a.s./L which was used as the lowest standard concentration during the study. The limit of quantification was 5000 mg a.s./L and the limit of detection 1700 mg a.s./L.

Table 8.2.1- 2: Analytical results

Nominal conc. [mg a.s./L]		Measured concentration [A] [mg a.s./L]			% of nominal [A]			Mean [A] measured concentration [mg a.s./L]	Mean % of nominal [A]
(not corrected for purity)	(corrected for purity)	Day 0	Day 2	Day 4	Day 0	Day 2	Day 4		
2.0	1.89 [B]	1.92	1.75	1.71	102	93	91	1.80	95

A Not given in report, Calculated based on measured concentrations of 2 replicate samples.
B Considering a purity of 94.7 % of the active substance.

Biological results:

Observations:

No mortalities or sub-lethal findings were observed in the controls and in single the test concentration during the test.

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Table 8.2.1- 3: Mortality

Nominal concentration [mg a.s./L]	Dead fish No. (%)				
	Exposure time				
	4 h	24 h	48 h	72 h	96 h
Control	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Solvent control	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
1.89	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

III. CONCLUSION

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

LC_{50} - 96 hours (95 % C.L):	**> 1.89 mg a.s./L** [A] (n.d.)
NOEC - 96 hours: highest concentration without an effect	≥ 1.89 mg a.s./L

[A] Practical limit of water solubility

Assessment and conclusion by applicant:

The study and its data are considered as acceptable and reliable for use in risk assessment.

The endpoint is:

LC_{50} (96 hours) > 1.89 mg a.s./L.

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Data Point:	KCA 8.2.1/02
Report Author:	██████
Report Year:	2008
Report Title:	Acute toxicity of AE C656948 (tech.) to fish (Lepomis macrochirus) under static conditions
Report No:	EBGMP052
Document No:	M-278441-02-1
Guideline(s) followed in study:	EPA-FIFRA § 72-1/SEP-EPA-540/9-85-006 (1982/1985 ; OPPTS 850.1075 (Public Draft, 1996) ; Directive 92/69/EEC, C.1 (1992) ; OECD No. 203 (rev. 1992)
Deviations from current test guideline:	Current Guideline: 203 (2019) Deviations: The fish length at test start was 4.8 ± 0.3 cm and thus higher than the maximum 3 cm recommended in OECD 203. The deviation was not expected to have impacted the study results. All validity criteria were met.
Previous evaluation:	yes, evaluated and accepted in DAR (2011)
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 fish were exposed to fluopyram in groups of 30 (two replicates of 15 fish per test level) to an aqueous solution of the test item at the single nominal concentration of 5.68 mg a.s./L (corrected for purity) for a period of 96 hours. Additionally, a control and solvent control were included. Observations of mortality and other signs of toxicity were made approximately 4, 24, 48, 72 and 96 hours after test initiation.

Concentrations of fluopyram were verified by HPLC – UV on days 0, 2 and 4 for the single concentration and the controls. Measured concentrations were in the 89 - 97 % range of nominal concentrations and no residues were found in the control and solvent control samples above 0.1029 mg a.s./L which was used as the lowest standard concentration during the study. Biological results are based on nominal concentrations of fluopyram.

The study fulfils all validity criteria of OECD 203 guideline.

There were no behavioural abnormalities or mortalities of the in the controls and in the single test concentration.

The endpoints based on nominal concentrations were: LC_{50} – 96 hours: > 5.68 mg a.s./L and NOEC – 96 hours: ≥ 5.68 mg a.s./L.

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I. MATERIALS AND METHODS

Test material	Fluopyram (AE C656948) Specification No.: 102000012455 Batch No: 08528/0002 Purity: 94.7 % w/w
Guideline(s) adaptation	None specified
Test species	Bluegill sunfish (*Lepomis macrochirus*)

Acclimation	At least 14 days to test conditions. Health during acclimation: less than 3 % mortality was noted before testing, all unsuitable fish (e.g. injured, deformed, etc.) were eliminated prior to the assignment of test groups.
Organism age/size	Mean length: 4.8 ± 0.3 cm at test start Mean body weight: 1.4 ± 0.1 g at test start
Test solutions	Nominal concentration 5.68 mg a.s./L (corrected for purity) (corresponding to 6.00 mg test item/L) Corresponding mean measured concentration: 5.21 mg a.s./L Control: water Solvent control: dimethylformamide (0.1 mL/L) Evidence of undissolved material: Tiny amounts of test item were observed at the surface after 24 hours until test end. In addition, tiny amounts of the test item were lying at the bottom after 24 hours.
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: 15
Exposure	Static Total exposure duration: 96 hours
Test Vessel Loading	0.53 g fish/L test medium
Feeding during test	None
Test conditions	Temperature: 21.5 - 22.5 °C Photoperiod: 16 hours light, 8 hours dark Light intensity: not reported pH: 6.2 - 7.3 Water hardness: 40 - 60 mg $CaCO_3$/L Dissolved oxygen: 62 - 99 % of saturation (aeration was added on study day 1 overnight, on day 2 for 2 hours and on day 3 for 4 hours to maintain dissolved oxygen concentrations > 60 % of saturation) Conductivity: not reported Alkalinity: not reported
Parameters Measured/ Observations	Fish were observed for mortalities and sub-lethal behavioural effects after 4, 24, 48, 72 and 96 hours. Dissolved oxygen, temperature and pH were determined daily in each replicate. Additionally, temperature was measured hourly by a data logger.
Sampling for Chemical analysis	Samples of test solutions were taken at test initiation (0 hour), after 48 hours and at test termination (96 hours) for analysis of test substance. The chemical analyses were performed by using a High performance liquid chromatograph (HPLC) equipped with an UV – detector.
Data analysis	Not needed as limit test.

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II. RESULTS AND DISCUSSION

Table 8.2.1- 4: Validity criteria

Validity criteria	Required	Obtained
Mortality in control during test	≤ 10 %	0 %
Dissolved oxygen saturation	≥ 60 %	62 - 99 %

Analytical results:

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 SANTE/2020/12830, Rev.1.

Recoveries on day 0, 2 and day 4 were between 89 and 97 % of nominal (see table below). Biological results are based on nominal concentrations of fluopyram.

No residues of fluopyram were found in the control and solvent control samples above 0.1029 mg a.s./L which was used as the lowest standard concentration during the study. The limit of quantification was 5000 mg a.s./L and the limit of detection 1700 mg a.s./L.

Table 8.2.1- 5: Analytical results

Nominal concentration [mg a.s./L] [A]		Measured concentration [mg a.s./L]			% of nominal [B]			Mean measured concentration [mg a.s./L]	Mean % of nominal
(not corrected for purity)	(corrected for purity)	Day 0	Day 2	Day 4	Day 0	Day 2	Day 4		
6.00	5.68	5.49	5.08	5.05	97	89	89	5.21	92

[A] Considering a purity of 94.7 % of the active substance.
[B] Not given in report. Calculated based on measured concentrations of 2 replicate samples.

Biological results:

Observations:

No mortalities or sub-lethal findings were observed in the controls and in single the test concentration during the test.

Table 8.2.1- 6: Mortality

Nominal concentration [mg a.s./L]		Dead fish No. (%)				
(not corrected for purity)	(corrected for purity)	Exposure time				
		4 h	24 h	48 h	72 h	96 h
Control		0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Solvent control		0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
6.00	5.68	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

[A] Considering a purity of 94.7 % of the active substance.

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III. CONCLUSION

The study meets the validity criteria according to OECD 203 (2019) and the endpoints based on nominal concentrations were:

LC_{50} – 96 hours (95 % C.I.):	**> 5.68 mg a.s./L** [A] (n.d.)
NOEC – 96 hours: highest concentration without an effect	≥ 5.68 mg a.s./L

[A] Practical limit of water solubility

Assessment and conclusion by applicant:

The study and its data are considered as acceptable and reliable for use in risk assessment.

The endpoint is: LC_{50} (96 hours) > 5.68 mg a.s./L

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Data Point:	KCA 8.2.1/03
Report Author:	[redacted]
Report Year:	2008
Report Title:	Acute toxicity of AE C656948 technical to the fathead minnow (Pimephales promelas) under static conditions
Report No:	EBGMP237
Document No:	M-298918-01-1
Guideline(s) followed in study:	US EPA 72-1; OPPTS Guideline 850.1075; OECD Guideline 203
Deviations from current test guideline:	Current Guideline: 203 (2019) Deviations: The fish length at test start was not reported. The missing information was not expected to have impacted the study results. All validity criteria were met.
Previous evaluation:	Yes, evaluated and accepted in DAR (2011)
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 Fathead minnow (*Pimephales promelas*) in a static system. Juvenile fish were exposed to fluopyram in groups of 10 (one replicate of 10 fish per test level) to an aqueous solution of the test item at nominal concentrations of 0.31, 0.63, 1.25, 2.50 and 5.00 mg a.s./L for a period of 96 hours. Additionally, a control and solvent control were included. Observations of mortality and other signs of toxicity were made approximately 4, 24, 48, 72 and 96 hours after test initiation.

Concentrations of fluopyram were verified by a Liquid Chromatograph / Tandem Mass Spectrometry system (LC/MS/MS) on days 0 and 4 for the concentrations and the controls. Measured concentrations were in the 83 - 109 % range of nominal concentrations and no residues were found in the control and solvent control samples above the limit of quantification (LOQ: 0.030 mg a.s./L). Biological results are based on mean measured concentrations of fluopyram.

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The study fulfils all validity criteria of OECD 203 guideline.

There were no behavioural abnormalities or mortalities of the fish in the controls and in the test concentrations.

The endpoints based on mean measured concentrations were: LC_{50} – 96 hours: > 4.95 mg a.s./L, LOEC – 96 hours: > 4.95 mg a.s./L and NOEC – 96 hours: 4.95 mg a.s./L.

I. MATERIALS AND METHODS

Test material	Fluopyram (AE C656948) Specification No.: 102000012455 Batch No: 08528/0002 Purity: 94.7 % w/w
Guideline(s) adaptation	None specified
Test species	Fathead minnow *(Pimephales promelas)*
Acclimation	At least 14 days to test conditions. Health during acclimation: no mortality in the 48 - hour acclimation period before testing.
Organism age/size	Mean length: 41.5 ± 2.6 mm at test end Mean body weight: 0.62 ± 0.11 g at test end
Test solutions	Nominal concentration: 0.31 – 0.63 – 1.25 – 2.50 – 5.00 mg a.s./L Corresponding mean measured concentration: 0.30 – 0.57 – 1.23 – 2.60 – 4.95 mg a.s./L Control: water Solvent control: dimethylformamide (0.1 mL/L) Evidence of undissolved material: not reported
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	0.21 g fish/L test medium
Feeding during test	None
Test conditions	Temperature: 22.1 - 22.8 °C Photoperiod: 16 hours light, 8 hours dark; with 30 min transition periods Light intensity: 718 - 1008 lux pH: 7.6 - 8.1 Water hardness: 50 - 54 mg $CaCO_3$/L Dissolved oxygen: 84 - 96 % of saturation Conductivity: 170 - 194 µmhos/cm Alkalinity: 42 - 45 mg/L
Parameters Measured / Observations	Fish were observed for mortalities and sub-lethal behavioural effects after 4, 24, 48, 72 and 96 hours. Dissolved oxygen, temperature and pH were determined daily in each replicate.

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	Additionally, temperature was measured hourly by a data logger. Alkalinity, hardness and conductivity were determined on day 0 and day 4.
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. The chemical analysis was performed using a Liquid Chromatograph / Tandem Mass Spectrometry system (LC/MS/MS)
Data analysis	Based on the nature of the data (no mortalities) statistical calculations were not necessary. The NOEC, LOEC and LC_{50}-values were empirically determined.

II. RESULTS AND DISCUSSION

Table 8.2.1- 7: Validity criteria

Validity criteria	Required	Obtained
Mortality in control during test	≤ 10 %	0 %
Dissolved oxygen saturation	≥ 60 %	84 - 96 %

Analytical results:

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 SANTE/2020/12830, Rev.1.

Recoveries on day 0 and day 4 were between 83 and 109 % of nominal (see table below). Biological results are based on nominal concentrations of fluopyram.

No residues of fluopyram were found in the control and solvent control samples above the limit of quantification (0.030 mg a.s./L).

Table 8.2.1- 8: Analytical results

Nominal concentration [mg a.s./L]	Measured concentration [mg a.s./L]		% of nominal [A]		Mean measured concentration [mg a.s./L]	Mean % of nominal
	Day 0	Day 4	Day 0	Day 4		
0.31	0.32	0.28	102	89	0.30	96
0.63	0.62	0.53	98	83	0.57	91
1.25	1.32	1.13	106	90	1.23	98
2.50	2.47	2.74	99	109	2.60	104
5.00	4.99	4.90	100	98	4.95	99

Biological results:

Observations:

No mortalities or sub-lethal findings were observed in the controls and in the test concentrations during the test.

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Table 8.2.1- 9: Mortality

Mean measured concentration [mg a.s./L]	Dead fish No. (%)				
	Exposure time				
	4 h	**24 h**	**48 h**	**72 h**	**96 h**
Control	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Solvent control	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
0.30	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
0.57	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
1.23	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
2.60	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
4.95	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

III. Conclusion

The study meets the validity criteria according to OECD 203 (2019) and the endpoints based on mean measured concentrations were:

LC_{50} – 96 hours (95 % C.I.):	**> 4.95 mg a.s./L** [A] (n.d.)
LOEC – 96 hours: lowest concentration with an effect	≥ 4.95 mg a.s./L
NOEC – 96 hours: highest concentration without an effect	≥ 4.95 mg a.s./L

[A] Practical limit of water solubility

Assessment and conclusion by applicant:

The study and its data are considered as acceptable and reliable for use in risk assessment.

The endpoint is: LC_{50} (96 hours) > 4.95 mg a.s./L

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Data Point:	KCA 8.2.1/04
Report Author:	[redacted]
Report Year:	2006
Report Title:	Acute toxicity of AE C656948 (tech.) to fish (Cyprinus carpio) under static conditions
Report No:	EBGMP020
Document No:	M-280108-01-1
Guideline(s) followed in study:	JMAFF 12 Nousan No. 8147 (2000), EPA-FIFRA § 72-1/SEP-EPA 540/9-85-006 (1982/1985) OPPTS 850.1075 (Public Draft, 1996), Directive 92/69/EEC C.1 (1992) OECD No. 203 (rev.1992)
Deviations from current test guideline:	Current Guideline: 203 (2019) Deviations: The fish length at test start was 5.3 ±0.7 cm and thus higher than the maximum 4 cm recommended in OECD 203. Analytical recoveries were between 20 - 55 % of the nominal concentrations. These deviations were not expected to have impacted the study results. All validity criteria were met.
Previous evaluation:	yes, evaluated and accepted in DAR (2011)
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 fish were exposed to fluopyram in groups of 10 (one replicate of 10 fish per test level) to an aqueous solution of the test item at nominal concentrations of 11.8, 23.7, 47.4, 94.7 and 189 mg a.s./L (corrected for purity) (corresponding to 12.5, 25.0, 50.0, 100 and 200 mg test item/L) for a period of 96 hours. Additionally, a control was included. Observations of mortality and other signs of toxicity were made approximately 4, 24, 48, 72 and 96 hours after test initiation.

Concentrations of fluopyram were verified by HPLC – UV on days 0, 2 and 4 for the concentrations and the control. Measured concentrations were in the 11 - 65 % range of nominal concentrations and no residues were found in the control samples above 0.514 mg a.s./L, which was used as the lowest standard concentration during the study. Biological results are based on mean measured concentrations of fluopyram.

The study fulfils all validity criteria of OECD 203 guideline.

There were behavioural observations on fish caused by the test item in the four highest test concentrations (11.5, 16.2, 24.6 and 37.7 mg a.s./L, mean measured concentrations). At the second highest test concentrations (11.5 mg a.s./L, mean measured concentration) fish showed the following symptoms after 96 hours: remained for unusually long periods on the bottom of the aquarium; were inactive or displayed abnormally low activity; remained for unusually long periods at the water surface; showed laboured respiration. In addition, fish also showed turning dark in coloration laying on their sides or backs in the three highest test concentrations (16.2, 24.6 and 37.7 mg a.s./L, mean measured concentrations).

The endpoints based on mean measured concentrations were: LC_{50} – 96 hours: 30.5 mg a.s./L and NOEC – 96 hours: 6.46 mg a.s./L.

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I. MATERIALS AND METHODS

Test material	Fluopyram (AE C656948) Specification No.: 102000012455 Batch No: 08528/0002 Purity: 94.7 % w/w
Guideline(s) adaptation	None specified
Test species	Common carp (*Cyprinus carpio*)
Acclimation	At least 14 days to test conditions. Health during acclimation: less than 5 % mortality in the 48 – hour acclimation period before testing, all unsuitable fish (e.g. injured, deformed, etc.) were eliminated prior to the assignment of test groups.
Organism age/size	Mean length: 53 ± 7 mm at test start Mean body weight: 1.9 ± 0.8 g at test start
Test solutions	Nominal concentrations: 11.8 – 23.7 – 47.4 – 94.7 – 189 mg a.s./L (corrected for purity) (corresponding to: 12.5 – 25.0 – 50.0 – 100 – 200 mg test item /L) Corresponding mean measured concentrations: 6.46 – 11.5 – 16.2 – 24.6 – 37.7 mg a.s./L Control: water Evidence of undissolved material: In all test concentrations the test item was lying at the bottom during the test. Furthermore, in the two highest test concentrations (24.6 and 37.7 mg a.s./L) during 0 and 48 hours, the test item was observed at the water surface as well an intensive turbidity was caused by the test item. In the two highest test concentrations (24.6 and 37.7 mg a.s./L) during 72 and 96 hours of exposure, the test item was observed at the water surface as well as a homogeneous dispersion in the water and a turbidity was determined.
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	0.48 g fish/L test medium
Feeding during test	None
Test conditions	Temperature: 22.3 - 24.6 °C Photoperiod: 16 hours light, 8 hours dark Light intensity: not reported pH: 6.8 - 7.2 Water hardness: 40 - 60 mg $CaCO_3$/L Dissolved oxygen: 83 - 100 % of saturation) (aeration was added to reach the oxygen saturation point) Conductivity: < 0.2 µS/cm Alkalinity: not reported
Parameters Measured/ Observations	Fish were observed for mortalities and sub-lethal behavioural effects after 4, 24, 48, 72 and 96 hours. Dissolved oxygen, temperature and pH were determined daily in each replicate. Additionally, temperature was measured hourly by a data logger.

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Sampling for Chemical analysis	Samples of test solutions were taken at test initiation (0 hour), after 48 hours and at test termination (96 hours) for analysis of test substance. The chemical analyses were performed by using a High-performance liquid chromatograph (HPLC) equipped with an UV – detector.
Data analysis	The LC_{50} values and the 95 %-confidence intervals were calculated using one of three statistical techniques: moving average, logit analysis or probit analysis. The 96 h- LC_{50} value was estimated by Logit analysis. All calculations were carried out using Microsoft Excel data sheets. All statistical evaluations were done using the commercial program ToxRat Professional (ToxRat® version 2.09).

II. RESULTS AND DISCUSSION

Table 8.2.1- 10: Validity criteria

Validity criteria	Required	Obtained
Mortality in control during test	≤ 10 %	0 %
Dissolved oxygen saturation	≥ 60 %	77 - 100 %

Analytical results:

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 SANTE/2020/12830, Rev.1.

Recoveries on day 0, 2 and day 4 were between 11 and 65 % of nominal (see table below). Biological results are based on mean measured concentrations of fluopyram.

No residues of fluopyram were found in the control samples above 0.514 mg a.s./L, which was used as the lowest standard concentration during the study.

Table 8.2.1- 11: Analytical results

Nominal concentration [mg a.s. /L]		Measured concentration [mg a.s./L]			% of nominal [A]			Mean measured concentration [mg a.s./L]	Mean % of nominal
(not corrected for purity)	(corrected for purity)	Day 0	Day 2	Day 4	Day 0	Day 2	Day 4		
12.5	11.8	4.89	6.86	7.62	41	58	65	6.46	55
25.0	23.7	12.1	10.8	11.5	51	46	49	11.5	48
50.0	47.4	19.6	14.0	14.9	41	30	31	16.2	34
200	94.7	35.7	20.4	17.8	38	22	19	24.6	26
100	189	67.2	24.2	21.6	36	13	11	37.7	20

[A] Not given in report.

Biological results:

Observations:

There were behavioural observations on fish caused by the test item in the four highest test concentrations (11.5, 16.2, 24.6 and 37.7 mg a.s./L, mean measured concentrations). At the second highest test concentrations (11.5 mg a.s./L, mean measured concentration) fish showed the following

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symptoms after 96 hours: remained for unusually long periods on the bottom of the aquarium; were inactive or displayed abnormally low activity; remained for unusually long periods at the water surface; showed laboured respiration. In addition, fish also showed turning dark in coloration laying on their sides or backs in the three highest test concentrations (16.2, 24.6 and 37.7 mg a.s./L, mean measured concentrations).

There were neither any adverse effects nor any mortality in the control group.

Table 8.2.1- 12: Mortality

Mean measured concentration [mg a.s./L]	Dead fish No. (%)				
	Exposure time				
	4 h	24 h	48 h	72 h	96 h
Control	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Solvent control	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
6.46	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
11.5	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
16.2	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
24.6	0 (0)	0 (0)	4 (40)	4 (40)	4 (40)
37.7	0 (0)	2 (20)	7 (70)	8 (80)	8 (80)

III. CONCLUSION

The study meets the validity criteria according to OECD 203 (2019) and the endpoints based on mean measured concentrations were:

LC_{50} – 96 hours (95 % C.I.):	**30.5 mg a.s./L** [A] (21.5 - 69.6 mg a.s./L)
NOEC – 96 hours: highest concentration without an effect (based on sublethal effects)	6.46 mg a.s./L
NOEC – 96 hours: highest concentration without an effect (based on mortality)	16.2 mg a.s./L

[A] In all test levels precipitations were observable so the LC_{50} is clearly above the water solubility of the test item.

Assessment and conclusion by applicant:

The study and its data are considered as acceptable and reliable for use in risk assessment.

The endpoint is: LC_{50} (96 hours) = 30.5 mg a.s./L

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Data Point:	KCA 8.2.1/05
Report Author:	[redacted]
Report Year:	2006
Report Title:	Acute toxicity of AE C656948 technical to the sheepshead minnow (Cyprinodon variegatus) under static conditions
Report No:	EBGMP053
Document No:	M-279167-01-1
Guideline(s) followed in study:	FIFRA 72-3, OPPTS Guideline 850.1075, OECD Guideline 203. The aforementioned 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:	Current Guideline: 203 (2019) Deviations: The fish length at test start was not reported. The missing information was not expected to have impacted the study results. All validity criteria were met.
Previous evaluation:	yes, evaluated and accepted in DAR (2011)
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 Sheepshead minnow (*Cyprinodon variegatus)* in a static system. Juvenile fish were exposed to fluopyram in groups of 10 (one replicate of 10 fish per test level) to an aqueous solution of the test item at nominal concentrations of 0.063, 0.125, 0.25, 0.50 and 1.0 mg a.s./L for a period of 96 hours. Additionally, a control and solvent control were included. Observations of mortality and other signs of toxicity were made approximately 4, 24, 48, 72 and 96 hours after test initiation.

Concentrations of fluopyram were verified by HPLC – UV on day 0 and 4 for the concentrations and the controls. Measured concentrations were in the 96 – 120 % range of nominal concentrations and no residues were found in the control and solvent control samples above the limit of quantification (LOQ: 0.006 mg a.s./L). Biological results are based on mean measured concentrations of fluopyram.

The study fulfils all validity criteria of OECD 203 guideline.

There were no behavioural abnormalities or mortalities of the fish in all test concentrations and in the controls.

The endpoints based on mean measured concentrations were: LC_{50} – 96 hours > 0.98 mg a.s./L, LOEC – 96 hours: > 0.98 mg a.s./L and NOEC – 96 hours: 0.98 mg a.s./L.

I. MATERIALS AND METHODS

Test material	Fluopyram (AE C656948) Specification No.: 102000012455 Batch No: 08528/0002 Purity: 94.7 % w/w
Guideline(s) adaptation	None specified
Test species	Sheepshead minnow *(Cyprinodon variegatus)*
Acclimation	At least 14 days to test conditions. Health during acclimation: no mortality in the 48-hour acclimation period before testing.

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Organism age/size	Mean length: 21.8 ± 3.2 mm at test end Mean body weight: 0.35 ± 0.17 g at test end
Test solutions	Nominal concentration: 0.063 – 0.125 – 0.25 – 0.50 – 1.0 mg a.s./L Corresponding mean measured concentration: 0.072 – 0.138 – 0.25 – 0.51 – 0.98 mg a.s./L Control: water Solvent control: dimethylformamide (0.1 mL/L) Evidence of undissolved material: No precipitates were observed during the exposure period.
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: 21.3 - 22.2 °C Photoperiod: 16 hours light, 8 hours dark; with 30 min transition periods Light intensity: 549 - 667 lux pH: 7.9 - 8.1 Water hardness: not reported Dissolved oxygen: 78 - 91 % of saturation Conductivity: not reported Alkalinity: not reported Salinity: 17 ‰
Parameters Measured / Observations	Fish were observed for mortalities and sub-lethal behavioural effects after 4, 24, 48, 72 and 96 hours. Dissolved oxygen and salinity were determined on day 0, 2 and 4. The pH was measured on day 0 and 4. Temperature was measured hourly by a data logger and daily by manual reading.
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. The chemical analyses were performed by using a High-performance liquid chromatograph (HPLC) equipped with an UV – detector.
Data analysis	Based on the nature of the data (no mortalities) statistical calculations were not necessary. The NOEC, LOEC and LC_{50}-values were empirically determined.

II. RESULTS AND DISCUSSION

Table 8.2.1- 13: Validity criteria

Validity criteria	Required	Obtained
Mortality in control during test	≤ 10 %	0 %
Dissolved oxygen saturation	≥ 60 %	78 - 91 %

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Analytical results:

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 SANTE/2020/12830, Rev.1.

Recoveries on day 0 and day 4 were between 96 and 120 % of nominal (see table below). Biological results are based on mean measured concentrations of fluopyram.

No residues of fluopyram were found in the control and solvent control samples above the limit of quantification (LOQ: 0.006 mg a.s./L).

Table 8.2.1- 14: Analytical results

Nominal concentration [mg a.s./L]	Measured concentration [mg a.s./L]		% of nominal		Mean measured concentration [mg a.s./L]	Mean % of nominal
	Day 0	Day 4	Day 0	Day 4		
0.063	0.075	0.069	120	110	0.072	115
0.125	0.142	0.135	113	108	0.138	111
0.25	0.25	0.25	100	100	0.25	100
0.50	0.51	0.51	101	102	0.51	102
1.00	0.96	0.99	96	99	0.98	98

Biological results:

Observations:

No fish showed any abnormal signs in all test concentrations and the controls.

Table 8.2.1- 15: Mortality

Nominal concentration [mg a.s./L]	Mean measured concentration [mg a.s./L]	Dead fish No. (%)				
		Exposure time				
		4 h	24 h	48 h	72 h	96 h
Control	Control	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Solvent control	Solvent control	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
0.063	0.072	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
0.125	0.138	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
0.25	0.25	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
0.50	0.51	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
1.00	0.98	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

III. CONCLUSION

The study meets the validity criteria according to OECD 203 (2019) and the endpoints based on mean measured concentrations were:

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LC_{50} – 96 hours (95 % C.I.):	**> 0.98 mg a.s./L** [A] (not determined)
LOEC – 96 hours: lowest concentration with an effect	> 0.98 mg a.s./L
NOEC – 96 hours: highest concentration without an effect	0.98 mg a.s./L

[A] Highest level tested.

Assessment and conclusion by applicant:

The study and its data are considered as acceptable and reliable for use in risk assessment.

The endpoint is: LC_{50} (96 hours) > 0.98 mg a.s./L

Metabolite trifluoroacetic acid (TFA)

Data Point:	KCA 8.2.1/06
Report Author:	[redacted]
Report Year:	1992
Report Title:	The acute toxicity of sodium trifluoroacetate to the zebra fish Brachydanio rerio
Report No:	C047202
Document No:	M-247889-01-1
Guideline(s) followed in study:	OECD: 203 (1984)
Deviations from current test guideline:	Current guideline: OECD 203 (2019) Deviation: The fish length at test start was 2.3 to 3.4 cm and thus slightly higher than the maximum 2 cm recommended in OECD 203. No information on the test vessel loading is given. This deviation and the missing information were not expected to have impacted the study results. All validity criteria were met.
Previous evaluation:	yes, evaluated and accepted in flurtamone RAR (2017)
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 *Danio rerio (*formerly *Brachydanio rerio*) in a static system. Juvenile fish were exposed to sodium trifluoroacetate in groups of 20 (two replicates of 10 fish) to an aqueous solution of the test item at the single nominal concentration of 1200 mg p.m./L for a period of 96 hours. Additionally, a water control was included. Observations of mortality and other signs of toxicity were made approximately 4, 24, 48, 72 and 96 hours after test initiation.

Concentrations of sodium trifluoroacetate were verified by ion chromatography on days 0 and 4 for the single concentration and the control. Measured concentrations were in the 100-102 % range of nominal concentrations and no residues were found in the control between 0.04 and 0.9 mg p.m./L. Biological results are based on nominal concentrations of sodium trifluoroacetate.

The study fulfils all validity criteria of OECD 203 guideline.

There were no behavioural abnormalities or mortalities of the fish in the controls and in the single test concentration of 1200 mg p.m./L.

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The endpoints based on nominal concentrations of sodium trifluoroacetate were: LC_{50} – 96 hours: > 1200 mg p.m./L and NOEC - 96 hours: 1200 mg p.m./L.

The converted endpoints based on nominal concentrations of trifluoroacetic acid were: LC_{50} – 96 hours: > 1008 mg p.m./L and NOEC - 96 hours: 1008 mg p.m./L.

I. Materials and Methods

Test material	Sodium trifluoroacetate Batch No: ACA9135AB Purity: 99 % w/w
Guideline(s) adaptation	None specified
Test species	Zebra fish (*Brachydanio rerio*)
Acclimation	Not reported.
Organism age/size	Mean length: range: 2.3 – 3.4 cm at test start Mean body weight: 0.23 g/fish
Test solutions	Nominal concentration: 1200 mg p.m./L Corresponding mean measured concentration: 1210 mg p.m./L Control: ISO-water Evidence of undissolved material: Not reported
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	Not reported
Feeding during test	None
Test conditions	Temperature: 21.0 - 22.8 °C Photoperiod: 16 hours light, 8 hours dark Light intensity: not reported pH: 7.6 - 7.9 Water hardness: ~ 250 mg $CaCO_3$/L Dissolved oxygen: 100 - 104 % of saturation (8.3 – 8.7 mg/L) Conductivity: not reported Alkalinity: not reported
Parameters Measured / Observations	Fish were observed for mortalities and sub-lethal behavioural effects after 4, 24, 48, 72 and 96 hours. Dissolved oxygen, temperature and pH were determined daily in each replicate.
Sampling for Chemical analysis	Duplicate samples of test solutions were taken at test start (0 hour) and at test termination (96 hours). The chemical analyses were performed by means of ion chromatography.
Data analysis	Not needed as limit test.

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II. RESULTS AND DISCUSSION

Table 8.2.1- 16: Validity criteria

Validity criteria	Required	Obtained
Mortality in control during test	≤ 10 %	0 %
Dissolved oxygen saturation	≥ 60 %	100 – 104 %

Analytical results:

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 SANTE/2020/12830, Rev.1.

Recoveries on day 0 and day 4 were between 99 and 102 % of nominal (see table below). Biological results are based on nominal concentrations of sodium trifluoroacetate.

Residues of sodium trifluoroacetate were found in the control samples were reported to be between < 0.02 and 0.9 mg p.m./L. The limit of detection was 0.02 mg p.m./L.

Table 8.2.1- 17: Analytical results

Nominal concentration [mg p.m./L]	Replicate	Measured concentration [A] [mg p m./L]		% of nominal [A]		Mean measured concentration [mg p m./L]	Mean % of nominal [A]
		Day 0	Day 4	Day 0	Day 4		
Control	A	0.04	0.9	-	-	< 1	-
	B	< LOD	< LOD	-	-		
1200	A	1220	1220	102	102	1210	100.8
	B	1200	1190	100	99		

Limit of detection (LOD): 0.02 mg/L

[A] Not given in report. Calculated based on measured concentrations of 2 replicate samples.

Biological results:

Observations:

No mortalities or sub-lethal findings were observed in the control and in single the test concentration during the test.

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Table 8.2.1- 18: Mortality

Nominal concentration [mg p.m./L]	Replicate	Dead fish No. (%)				
		Exposure time				
		3 h	24 h	48 h	72 h	96 h
Control	A	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	B	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
1200	A	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	B	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

III. Conclusion

The study meets the validity criteria and the endpoints based on nominal concentrations of sodium trifluoroacetate were:

LC_{50} - 96 hours (95 % C.I.):	**> 1200 mg p.m./L** [A] (not applicable)
NOEC - 96 hours: highest concentration without an effect	1200 mg p.m./L [A]

[A] Based on the molecular weights, a concentration of 1200 mg sodium trifluoroacetate/L corresponds to 1008 mg trifluoroacetate acid/L. As the study was conducted with sodium trifluoroacetate which is the sodium salt of trifluoroacetic acid, the endpoint was converted to trifluoroacetic acid with factor 0.84.

<u>Assessment and conclusion by applicant:</u>

The study and its data are considered as acceptable and reliable for use in risk assessment.

The endpoint is: LC_{50} (96 hours) > 1200 mg p.m./L (sodium trifluoroacetate) corresponding to > 1008 mg p.m./L (trifluoroacetic acid).

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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:	
Report Year:	2006
Report Title:	Early-life stage toxicity of AE C656948 (tech.) to fish (Pimephales promelas)
Report No:	EBGMP054
Document No:	M-279440-01-1
Guideline(s) followed in study:	EPA-FIFRA § 72-4a/SEP-EPA-560/6-82-002 (1982), ASTM E 1241-92 (1992), OPPTS 850.1400 (1996), OECD No. 210 (1992)
Deviations from current test guideline:	Current Guideline: 210 (2013) Deviations: None. All validity criteria were met.
Previous evaluation:	yes, evaluated and accepted in DAR (2011)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
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. Fluopyram was applied at nominal concentrations of 0.0185, 0.0370, 0.0740, 0.148, 0.296, 0.592 and 1.18 mg a.s./L (corrected for purity). Additionally, a water control and a solvent control were included. The test comprised 4 replicates of 15 eggs after hatching phase 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 fluopyram were verified with HPLC – UV on days -1, 0, 7, 14, 21, 28 and 33 for each concentration and control. Measured concentrations were in the 87 - 103 % range of nominal concentrations and no residues above 0.00514 mg a.s./L were measured in the controls, which was used as the lowest standard concentration during this study. The biological results were based on the mean measured concentrations of 0.0176, 0.0380, 0.0647, 0.135, 0.271, 0.570 and 1.05 mg a.s./L.

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

During the post hatch period between study day 9 and study day 18 observations occurred sporadically in controls and nearly all test concentrations and were not test item-related. From study day 20 on sublethal effects were only observed in test levels with 0.271, 0.570 and 1.05 mg a.s./L (mean measured concentrations).

The endpoints based on mean measured concentrations were: The overall chronic 33-day-NOEC was 0.135 mg a.s./L, based on length and morphological/ behavioural effects, which was the most sensitive parameter. The overall 33-day-LOEC was 0.271 mg a.s./L.

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I. MATERIALS AND METHODS

Test material	Fluopyram (AE C656948) Specification No.: 102000012455 Batch No: 08528/0002 Purity: 94.7 % w/w
Guideline(s) adaptation	None specified.
Test species	Fathead Minnow (*Pimephales promelas*)
Organism age/size at study initiation	Embryos less than 24 h old
Test solutions	Nominal concentrations: 0.0185 – 0.0370 – 0.0740 – 0.148 – 0.296 – 0.592 – 1.18 mg a.s./L (corrected for purity) Corresponding mean measured concentrations: 0.0176 – 0.0380 – 0.0647 – 0.135 – 0.271 – 0.570 – 1.05 mg a.s./L Controls: water Solvent control: dimethylformamide (0.1 mL/L) Evidence of undissolved material: Two days before test start, heavy precipitations were observed in the stock solutions, the mixing vessels and in the test aquaria of the two highest test concentrations (0.592 and 1.18 mg a.s./L). Therefore, these test solutions were removed, and new solutions prepared.
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: 25 eggs at test initiation, thinned to 15 larvae after hatching phase
Exposure	Flow-through Total exposure duration: 33 days (5-day-hatch and 28 d post-hatch)
Test Vessel Loading	0.05 g fish/L test medium per 24 h (at the end of the study)
Feeding during test	Newly hatched larvae were fed live brine shrimp nauplii ad libitum two to four times daily until one day before study termination.
Test conditions	Temperature: 24.7 - 26.0 °C Photoperiod: 16 hours light, 8 hours dark, 30 min transition periods Light intensity: 253 - 304 lux pH: 6.5 – 7.4 Water hardness: 43 - 46 mg/L as $CaCO_3$ (2.4 - 2.6 °dH; calculated based on 1 °dH = 17.8 mg $CaCO_3$/L) Dissolved oxygen: 89 - 97 % of saturation Conductivity: 93 - 163 µmhos/cm Alkalinity: not reported Begin of post-hatch period: day 3 to 5
Parameters Measured / Observations	The temperature was measured in one altering replicate of the control, solvent control and all other test levels on study days 0, 7, 14, 21, 28 and 33. The temperature in one control aquarium was recorded hourly by a data logger. Dissolved oxygen and pH were measured in one altering replicate of the control, solvent control and all other test levels on study days 0, 7, 14, 21, 28 and 33. Total hardness was measured on study days 0 and 33. Conductivity was measured in the dilution water before splitting and was documented hourly by a data logger. Eggs were observed daily for mortality (nonviable eggs removed) and hatching. In the post hatch phase fish were observed daily for abnormal behaviour, abnormal physical

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	changes and mortality (dead organisms were removed). At test end the length, the wet and dry weight for each individual fish was determined.
Sampling for chemical analysis	Samples of test solutions were taken from two alternating replicate test chambers on days -1, 0, 7, 14, 21, 28 and 33 (test termination). The chemical analyses were performed with HLPC – UV
Data analysis	For each analysed parameter the following statistical tests were conducted: – T-test to determine if control and solvent control data can be pooled. Control data were pooled if the t-test criteria were met. – R/s–test procedure in order to test the correspondence with normal distribution – Cochran´s test for homogeneity of variances – Time to hatch and hatching success data were arcsine transformed before analysis using the Williams-t-Test. – Larval Survival data on study day 5 were arcsine transformed before analysis using the Bonferroni-Holm-U-Test. – Survival data on study day 33 were arcsine transformed before analysis using the Williams-t-Test. – Growth data, expressed as dry weight, were analysed without previous data transformation using the Williams-t-Test. – Growth data, expressed as length, were analysed without previous data transformation using the Bonferroni-Holm-U-Test. Statistical analyses were conducted using ToxRat Professional (Version 2.09, ToxRat Solutions GmbH) with a statistical significance based on a 95 % confidence level (α = 0.05).

II. Results and Discussion

Table 8.2.2.1- 1: Validity criteria

Validity criteria	Required by OECD 210 (1992)	Required by OECD 210 (2013)	Obtained
Dissolved oxygen concentration throughout the test	Between 60 % to 100 % saturation	≥ 60 % saturation	89 - 97 % saturation
Water temperature difference between test chambers or between successive days at any time during the test	± 1.5 °C max	± 1.5 °C max	Fulfilled
Analytical measure of the test concentrations	Compulsory	Compulsory	Done
Hatching success of controls	> 66 %	> 70 %	95 % (Control), 87 % (Solvent control)
Post-hatch survival of controls	> 70 %	> 75 %	97 % (Control) 93 % (Solvent control)
Solubilising agent when used	No significant effect on survival nor any other adverse effects	Not required	Fulfilled

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Analytical results:

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 SANTE/2020/12830, Rev.1.

Recoveries on day -1 were between 56 and 126 % of nominal concentrations (see table below). Recoveries over the remaining test period (day 0 to 33) ranged between 79 and 106 % of nominal concentrations (please refer to table below). The biological results of the study are based on the mean measured test concentrations.

No residues of fluopyram were measured in the control and solvent control samples above 0.00514 mg a.s./L, which was used as the lowest standard concentration during this study.

Table 8.2.2.1- 2: Analytical results

Nominal concentration [mg a.s./L]	Mean measured concentration [mg a.s./L]							Mean measured concentration [mg a.s./L]
	Day -1	Day 0	Day 7	Day 14	Day 21	Day 28	Day 33	
0.0185	0.0234	0.0178	0.0162	0.0177	0.0176	0.0182	0.0178	0.0176
0.037	0.045	0.0363	0.0392	0.0385	0.0374	0.0375	0.0392	0.0380
0.074	0.067	0.0589	0.0633	0.0690	0.0664	0.0651	0.0653	0.0647
0.148	0.129	0.129	0.136	0.147	0.128	0.132	0.138	0.135
0.296	0.261	0.267	0.268	0.272	0.266	0.266	0.288	0.271
0.592	0.590	0.619	0.470	0.552	0.580	0.600	0.599	0.570
1.18	0.660	0.957	1.01	1.15	0.996	1.08	1.10	1.05
Nominal concentration [mg a.s./L]	**% of nominal [A]**							**Mean % of nominal**
	Day -1	**Day 0**	**Day 7**	**Day 14**	**Day 21**	**Day 28**	**Day 33**	
0.0185	126	96	88	96	95	98	96	95
0.037	122	98	106	104	101	101	106	103
0.074	91	80	86	93	89	88	88	87
0.148	87	87	92	99	86	89	93	91
0.296	88	90	91	92	90	90	97	91
0.592	100	105	79	93	98	101	101	96
1.18	56	81	86	97	84	92	93	89

A Not given in report. Calculations are based on mean measured concentrations of each sampling day (-1, 0, 7, 14, 21, 28 and 33).

Biological results:

Observations

During the post hatch period between study day 9 and study day 18 the following morphological and behavioural effects were observed: swollen belly, lordosis – an extended spinal column, kyphosis – a flexed spinal column, scoliosis- lateral curvature of the spin, showed weaker coloration, showed loss of equilibrium with lateral deviation from normal orientation, laid inactive on the bottom of the aquarium, laid on their sides or backs, turned in a vertical position or remained for unusually long periods at the water surface. These observations occurred sporadically in controls and nearly all test concentrations and were not test item-related.

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During the post hatch period between study day 20 and test termination the following morphological and behavioural effects were observed: deformed mouth, ventral haematoma, showed laboured respiration, remained for unusually long periods at the water surface, turned dark in colouration, swollen belly, showed loss of equilibrium with lateral deviation from their normal orientation or lordosis – an extended spinal column. From study day 20 on sublethal effects were only observed in three highest test concentrations (0.271, 0.570 and 1.05 mg a.s./L, mean measured concentrations), so these findings occurred dose-related.

Growth

The standard length showed statistically significant differences at the three highest test concentrations (0.271, 0.570 and 1.05 mg a.s./L, mean measured concentrations) compared to the pooled control.

The dry weights showed statistically significant difference between the pooled control and the two highest test concentrations (0.570 and 1.05 mg a.s./L, mean measured concentrations).

Table 8.2.2.1- 3: Length and dry weight on day 33

Mean measured concentration [mg a.s./L]	Mean length (SD) [mm]	Mean wet weight [mg]	Mean dry weight (SD) [mg]
Control	20.9 (1.4)	152.9 (34.9)	35.1 (8.6)
Solvent control	21.0 (1.3)	162.3 (35.9)	36.8 (9.0)
Pooled control	21.0 (1.3)	157.6 (35.4)	36.0 (8.8)
0.0176	21.4 (1.5)	n.a.	39.5 (11.1)
0.0380	21.4 (1.5)	n.a.	41.5 (9.5)
0.0647	21.1 (1.4)	n.a.	38.1 (9.3)
0.135	20.9 (1.5)	n.a.	39.7 (11.2)
0.271	19.9 (2.1) *)	n.a.	36.5 (15.0)
0.570	16.7 (2.7) *)	n.a.	22.1 (13.0) *
1.05	10.8 (n.a.) *)	n.a.	4.1 (n.a.) *

n.a.: Not applicable; wet weight measurements only performed for control fish
SD: Standard deviation
* Statistically significant difference from pooled control ($\alpha = 0.05$) using Williams-t-Test
*) Statistically significant difference from pooled control ($\alpha = 0.05$) using Bonferroni-Holm-U-Test

Time to hatch and hatching success

The day 5-percent hatch ranged from 87 to 95 %.

Egg hatching began on study day 3 and continued until study day 5 (day 0 post hatch). Start and end of hatching showed no significant difference compared to the pooled control data. There were statistically significant differences in time to hatch on study day 4 (study -1 post hatch) at the three highest test concentrations (0.271, 0.570 and 1.05 mg a.s./L, mean measured concentrations) compared to the pooled control. This interim and transient effect was considered not to be biologically relevant and did not change the NOEC.

Hatching success was evaluated on day 0 post hatch. There was no significant difference in hatching success in any test concentration compared to the pooled control data.

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Table 8.2.2.1- 4: Time to hatch and hatching success for Fathead minnow during early life stage toxicity test

Mean measured concentration [mg a.s./L]	Mean (SD) % hatch by study day		
	Day 3	Day 4	Day 5
Control	4 (5.7)	74 (21.0)	95 (6.0)
Solvent control	0 (0.0)	81 (6.0)	87 (8.9)
Pooled controls	2 (2.8)	78 (13.5)	91 (7.4)
0.0176	0 (0.0)	77 (6.0)	89 (5.0)
0.0380	1 (2.0)	74 (7.7)	90 (9.5)
0.0647	1 (2.0)	87 (13.2)	91 (10.5)
0.135	2 (2.3)	84 (14.2)	93 (2.0)
0.271	0 (0.0)	91 (3.8) *	92 (4.6)
0.570	5 (7.6)	95 (5.0) *	95 (5.0)
1.05	2 (2.4)	92 (4.6) *	92 (4.6)

SD Standard Deviation
* Statistically significant difference from pooled controls (α=0.05) using Williams-t-Test.

Larvae survival on day 5 and day 33

Larval survival was analysed before thinning on study day 5. Data analysis showed statistically significant difference in comparison to the pooled control data in the highest test concentration (1.05 mg a.s./L, mean measured concentration).

Fry survival was analysed at test termination on study day 33. Data analysis showed statistically significant difference in comparison to the pooled control data in the two highest test concentrations (0.570 and 1.05 mg a.s./L, mean measured concentrations).

Table 8.2.2.1- 5: Fry survival on day 5 and day 33

Mean measured concentrations [mg a.s./L]	% fry survival	
	Day 5	Day 33
	Mean (SD)	Mean (SD)
Control	100 (0.0)	97 (3.8)
Solvent control	100 (0.0)	93 (5.4)
Pooled controls	100 (0.0)	95 (4.6)
0.0176	100 (0.0)	92 (3.3)
0.0380	100 (0.0)	88 (6.4)
0.0647	100 (0.0)	93 (5.4)
0.135	100 (0.0)	90 (11.5)
0.271	100 (0.0)	87 (10.9)
0.570	100 (0.0)	70 (13.9) *
1.05	95 (2.4) *)	15 (12.6) *

SD Standard deviation
* Statistically significant difference from pooled controls (α = 0.05) using Williams-t-Test.
*) Statistically significant difference from pooled controls (α = 0.05) using Bonferroni-Holm-U-Test.

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III. CONCLUSION

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

Parameter	NOEC: highest concentration without an effect [mg a.s./L]	LOEC: lowest concentration with an effect [mg a.s./L]	EC_{10}: [A] (95 % C.I.) [mg a.s./L]	EC_{20}: [A] (95 % C.I.) [mg a.s./L]
% Hatch (Time to hatch, Day 3 to 5)	≥ 1.05	> 1.05	-	-
Hatching success (Day 5)	≥ 1.05	> 1.05	- [B]	- [B]
Larval survival (Day 5)	0.570	1.05	- [C]	- [C]
Fry survival (Day 33)	0.271	0.570	0.162 (n.d. – 0.377)	0.280 (n.d. – 0.734)
Growth – Dry weight	0.271	0.570	0.379 (0.003 – 0.502)	0.459 (0.025 – 0.572)
Growth - Total length	**0.135**	0.271	0.385 (0.081 – 0.551)	0.564 (0.237 – 0.722)
Morphological and behavioural effects	**0.135**	0.271	-	-

[A] Please refer to recalculation M-758375-01-1

[B] There was less than 10 % effect on the hatching success compared to the pooled controls, so no additional calculations were done.

[C] Due to only 5 % effect within the highest tested concentration compared to the pooled controls, no EC_{10} or EC_{20} calculation was possible.

Reliability assessment (EFSA 2015)

The following table provides reliability indicators for EC_{10} values for *Pimephales promelas.*

Biological endpoints	EC_{10} [mg a.s./L]	95% CI	NW	Relationship $EC_{10}/EC_{20/50}$
Fry survival	0.162	n.d. – 0.377	- [A]	- [A]
Growth – Dry Weight	0.379	0.003 – 0.502	1.317 (poor)	EC_{20}, low < EC_{10} (medium)[B]
Growth – Total Length	0.385	0.081 – 0.551	1.221 (poor)	EC_{20}, low < EC_{10} (medium)[B]

[A] No NW calculation could be completed when the confidence interval could not include a lower bound value.

[B] An EC_{50} was not calculated, therefore the relationship could not be determined completely. The NW score, however, was poor and indicates that the NOEC should be used instead of the EC_{10}.

Assessment and conclusion by applicant:

The study and its data are considered as acceptable and reliable for use in risk assessment.

The endpoint is:

NOEC (based on length and morphological and behavioural effects) = 0.135 mg a.s./L

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Data Point:	KCA 8.2.2.1/02
Report Author:	[redacted]
Report Year:	2020
Report Title:	Re-evaluation of a fish early life stage study performed with Pimephales promelas and AE C656948 (technical) (Nieden, D., 2006; M-279440-01-1)
Report No:	M-758375-01-1
Document No:	M-758375-01-1
Guideline(s) followed in study:	--
Deviations from current test guideline:	Current Guideline: not applicable Deviations: not applicable
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	Yes

Summary

EC_{10} and EC_{20} values were calculated for the fish early life stage study M-279440-01-1 with *Pimephales promelas* to fulfil the data requirements according to regulation EU 283/2013. Additionally, the validity criteria were re-evaluated according to the current guideline OECD 201 (2011).

The recalculations were performed with the software ToxRat Professional (Version 3.2.1) with the mean measured concentration provided in the report.

Endpoints were calculated for post-hatch survival, length, and dry weight, and are presented below. The results are presented as the critical effect and threshold concentration as observed at the end of experimental time; EC: Effective concentration for xx % reduction (95% Confidence limits). Calculations could not be done for hatching success and survival of larvae, as explained below. Results are compared to pooled controls.

Hatching success: there was less than 10 % effect on the hatching success, compared to the pooled controls, so no additional calculations were done.

Survival of larvae until thinning step: there was less than 10 % effect on the survival rate of hatched larvae until the thinning step, compared to the pooled controls. Due to only 5 % effect within the highest tested concentration compared to the pooled controls, no EC_{10} or EC_{20} calculation was possible.

Post-hatch survival: As more than 10 % effect were observed, a re-calculation using a logit-model was performed.

Length: As more than 10 % effect were observed, a re-calculation using a logit-model was performed.

Dry weight: As more than 10 % effect were observed, a re-calculation using a logit-model was performed.

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Table 8.2.2.1- 6: Re-calculated EC_{10} and EC_{20} values based on mean measured concentration

Parameter	Fluopyram (AE C656948) [mg a.s./L]	
	EC_{10} (95 % C.I.)	EC_{20} (95 % C.I.)
Hatching success	Not determined	Not determined
Survival of larvae until thinning step	Not determined	Not determined
Post-hatch survival	0.162 (n.d. – 0.377)	0.280 (n.d. – 0.734)
Length	0.385 (0.081 – 0.551)	0.564 (0.237 – 0.722)
Dry weight	0.379 (0.003 – 0.502)	0.459 (0.025 – 0.572)

C.I.: Confidence interval
n.d.: Not determined

Overall, no recalculated endpoint differs for the NOEC or LOEC compared to the existing report. No recalculated EC_{10} is below the overall NOEC of 0.135 mg a.s./L as already mentioned in the existing report.

Assessment and conclusion by applicant:

The study and its data are considered as acceptable and reliable for use in risk assessment.

The endpoints are:

NOEC = 0.135 mg a.s./L (based on growth and morphological and behavioural effects)

EC_{10} = 0.162 mg a.s./L (based on post-hatch survival)

According to the AGD, EC_{10} are preferred endpoints for risk assessment. As no recalculated EC_{10} endpoint differs for the NOEC or LOEC compared to the existing report, the NOEC based on growth and morphological and behavioural effects is the most sensitive parameter of the study and is proposed to be used in the risk assessment.

CA 8.2.2.2 Fish full life cycle test

Since data from a fish early life stage study (see CA 8.2.2.1) are available and as fluopyram is not considered as an endocrine disruptor (see CA 8.2.3), a fish full life cycle test is not required.

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CA 8.2.2.3 Bioconcentration in fish

Data Point:	KCA 8.2.2.3/01
Report Author:	
Report Year:	2008
Report Title:	[pyridyl-2,6-14C]- fluopyram bioconcentration and biotransformation in fish (Lepomis macrochirus)
Report No:	EBGMP116
Document No:	M-298506-01-1
Guideline(s) followed in study:	OECD 305 (1996), EPA-FIFRA § 72-6 (1982), EPA-FIFRA § 165-4 (1982), OPPTS 850.1730 (1996)
Deviations from current test guideline:	Current Guideline: 305 (2012) Deviations: The particulate matter before test start was not reported. The length measurement was only reported for day 0 and not at sampling time as recommended in OECD 305. The missing information was not expected to have impacted the study results. All validity criteria were met.
Previous evaluation:	yes, evaluated and accepted in Addendum 3 to the DAR (2011)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The bioconcentration potential of fluopyram from the aqueous environment into bluegill sunfish *(Lepomis macrochirus)* was determined in a continuous flow-through exposure system. The bioconcentration part of the study included a 28-day uptake period and a 14-day depuration period. One replicate of 60 juvenile fish were exposed to two treatments at nominal concentrations of 6.0 and 60 µg [pyridyl-2,6-^{14}C]-fluopyram/L dilution water. Additionally, a solvent control of 60 juvenile fish was included. For the biotransformation portion of the study, one group with 30 fish was exposed to a nominal concentration of 60 µg [pyridyl-2,6-^{14}C]-fluopyram/L dilution water. Fish and water samples were collected throughout the uptake and depuration period for radioactivity measurement. The fish were dissected into edible and non-edible tissues.

Samples for analysis of the concentration of the test substance in the water were verified by HPLC and LSC on study days -1, 0, 1, 3, 7, 10, 14, 21, 28, 29, 31, 35, 38 and 42. During the 28-day bioconcentration (uptake) phase (Days 0- 28) concentration of [pyridyl-2,6-^{14}C]-fluopyram in the low (6.0 µg a.s./L) and high treatment (60 µg a.s./L) were in the 94.7-110 % and 91.5-107 % range, respectively. During the biotransformation portion of the study the recoveries ranged between 56.2 and 102 %. During the depuration phase of the study no residues of [pyridyl-2,6-^{14}C]-fluopyram were determined above 0.05 µg a.s./L in the low treatment group (6.0 µg a.s./L) and above 0.6 µg a.s./L in the high treatment group (60 µg a.s./L). The limit of quantification for LSC was 20 dpm. No residues of [pyridyl-2,6-^{14}C]-fluopyram were determined in the samples of the solvent control tank; the measured radioactivity was below 78.6 dpm.

The study fulfils all validity criteria of the OECD 305 guideline.

The average percent lipids over the entire study period ranged from 8 to 11 %, from 5 to 10 %, and from 5 to 11 % in the whole fish samples in the solvent control (aquarium A), in the low treatment (aquarium B), and high treatment (aquarium C), respectively. The overall mean percent lipid content in samples from aquaria A, B, and C on day 0 and 28 was 7.03 %. The kinetic bioconcentration factors based on TRR (BCF_{TRR},) were 47.6 (edible tissue) and 87.9 (whole fish) for the low treatment (6.0 µg [pyridyl-2,6-^{14}C]-fluopyram/L) and 35.9 (edible tissue) and 65.7 (whole fish) for the high treatment (60 µg [pyridyl-2,6-^{14}C]-fluopyram/L).

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The steady-state BCF for parent fluopyram based on whole fish (wet weight) was calculated to be 18 and the steady-state BCF for parent fluopyram normalized to 6 % lipid content was 16.

I. MATERIALS AND METHODS

Test material	Fluopyram Radiolabelled test substance: [pyridyl-2,6-^{14}C]-fluopyram Sample ID: KATH 6022 Radiochemical purity: >99 %	* = position of radiolabel
Guideline(s) adaptation	None specified	
Test species	Bluegill sunfish (*Lepomis macrochirus*)	
Acclimation	Fish were acclimated in culture tanks, at a photoperiod of 16 hours light and 8 hours dark, for at least 14 days prior to initiation of testing. No mortality was noted during the acclimation period.	
Details on test organisms at test initiation:	Bioconcentration part: Mean body wet weight: 8.6 g ± 2.2 (aquaria A, B and C) Mean body length: 7.4 cm ± 0.6 (aquaria A, B and C) Biotransformation part: Mean body wet weight: 22.4 g ± 3.9 (aquarium D) Mean body length: 10.1 cm ± 0.6 (aquarium D)	
Test solutions	Four aquaria (A, B, C, D) were used in the test: Bioconcentration part: Nominal concentrations: 6.0 and 60 µg [pyridyl-2,6-^{14}C]-fluopyram/L (Aquaria B and C) Mean measured concentrations: 5.98 µg a.s./L for 6.0 µg/L treatment group; 59.9 µg a.s./L for 60 µg/L treatment group. Solvent control (aquarium A): 0.1 mL/L dimethylformamide (DMF) (Aquarium A) Biotransformation part: Nominal concentration: 60 µg [pyridyl-2,6-^{14}C]- fluopyram/L (Aquarium D) Mean measured concentrations: 55.1 µg a.s./L 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	Bioconcentration part (aquaria A, B and C): No. of organisms per vessel (concentration/ solvent control): 60 Biotransformation part (aquarium D): No. of organisms per vessel: 30 (15 fish each for 7 and 14 days of exposure)	
Exposure	Test type: flow through (delivery rate of flow-meter: 25 L/h) Route of exposure: aqueous Bioconcentration part (aquarium A, B and C): Total exposure duration (uptake phase): 28 days	

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	Total depuration duration: 14 days Biotransformation part (aquarium D): Total exposure duration: 7 and 14 days exposure
Test Vessel Loading at test initiation:	Biomass loading rate: Bioconcentration part (aquaria A, B and C): 5.2 g fish/L and 0.86 g fish/L/day Biotransformation part (aquarium D): 1.1 g fish/L/day
Test conditions:	Temperature: 22.8 – 23.4 °C (weekly measurements); 23.1 – 23.8 °C (daily measurements in control vessel) Photoperiod: 16 hours light, 8 hours dark Light intensity: not reported pH: 6.6 – 7.3 Water hardness: 40 - 60 mg $CaCO_3$/L (reconstituted water) Dissolved Oxygen: 61 – 99 % of saturation. All test aquaria were aerated during the study. Conductivity: not reported Alkalinity: not reported TOC: <10 mg/L (sum for all test vessels: 46.9 mg carbon/L)
Feeding during test	During the uptake and depuration phase, the fish were fed daily with standard fish-diet at a rate of approximately 1.5- 2 % of mean body weight per day. In the biotransformation part of the study, the fish received 1 % of mean body-weight per day.
Parameters Measured / Observations	Dissolved oxygen, pH and temperature were measured initially and throughout the study in each aquarium once a week. In addition, the daily temperature fluctuation was monitored continuously in the control tank and recorded as hourly mean values. Total organic carbon (TOC) was measured at the beginning of the test and then once a week. Fish were observed initially and daily on working days during the exposure and depuration period for any mortality and/or adverse behaviour. Total lipid content was determined in fish at study initiation and in treated fish sampled on days 0 and 28 of uptake and on study day 42 in the depuration phase.
Sampling for chemical analysis:	Water analysis: - Samples for analysis of the concentration of the test substance in the water by HPLC and LSC were taken on study days -1, 0, 1, 3, 7, 10, 14, 21, 28, 29, 31, 35, 38 and 42. - On each sampling day, three samples of 10 ml water were removed from each aquarium. The concentrations of the test item in test medium were calculated based on liquid scintillation counting (LSC). In additions the measured radioactivity (expressed as disintegrations per minute, dpm) and calculated concentrations of equivalents were determined. - For the determination of metabolites in water, 500 mL samples were taken from the high concentration level of aquarium C (bioconcentration part) and aquarium D (biotransformation part). The samples were stored deep-frozen until analysis. - Stock solutions and water samples were analysed for content and stability in the beginning and the end of the exposure period. Fish analysis: - Fish sampling was done on study days 1, 3, 7, 10, 14, 21, 28, 29, 31, 35, 38 and 42. For biotransformation portion samples were collected on study days 7 and 14 from aquarium D. - Four fish were taken from each tank at each sampling and processed individually. The fish were dissected into edible (Fillet = body, muscle, skin, skeleton) and viscera / nonedible (Viscera = head, fins, internal organs) parts. Afterwards the samples were weighed, incubated in a drying oven for at least 5 days and analysed for the radioactivity (expressed as disintegrations per minute, dpm) in order to determine the TRR (total radioactivity residues). - For lipid content determination 4 additional fish were taken on day 0, 28 and 42.

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	- For the biotransformation part of the study, 15 fish were sampled on day 7 and day 15 from aquarium D. The samples were dissected into edible and viscera parts and then transferred into vials. After determination of the wet weight the samples were investigated for biotransformation of [^{14}C]- fluopyram. Tissues were extracted with acetonitrile/water mixtures. Metabolic profiles of the extracts were measured by reversed phase HPLC with radiodetection. The parent compound was identified in by HPLC co-chromatography. The chemical analysis of radioactivity in fish and water samples was performed by means of liquid scintillation measurements (LSC-measurement). All calculations were performed using Microsoft Excel 2002 and the non-linear kinetic modelling program Origin 6.0 software.

II. Results and Discussion

Table 8.2.2.3- 1: Validity criteria

Validity criteria acc. to OECD 305 (2012)	Required	Obtained
Water temperature variation over the whole test period	±2 °C	22.8 – 23.4 °C
Dissolved oxygen % saturation in all test vessels	> 60 %	61 – 99 %
Concentration of test substance maintained during the uptake phase	± 20 % of mean measured concentration	Fulfilled: Low concentration: 5.39 – 6.62 µg a.s./L (mean: 5.98 µg a.s./L) High concentration: 54.9 - 64.7 µg a.s./L (mean: 59.9 µg a.s./L)
The concentration of the test substance is below its limit of solubility in test water	Test concentration below water solubility of test item in test water	Fulfilled [A]
Mortality or other adverse effects/disease in control and treated fish	< 10 %	0 %

[A] Water solubility of [pyridyl-2,6-^{14}C]- fluopyram: 16.0 mg/L (pH 7)

Analytical results:

- Water analyses:

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 SANTE/2020/12830, Rev.1.

During the 28-day bioconcentration (uptake) phase water concentrations ranged from 5.39 to 6.62 µg/L for the low treatment (6.0 µg/L, aquarium B) and from 54.9 to 64.7 µg/L for the high treatment (60 µg/L, aquarium C). For the biotransformation part of the study the concentrations ranged from 33.7 to 61.7 µg/L (60 µg/L, aquarium D).

In the stock solutions used for all tests [pyridyl-2,6-^{14}C]-fluopyram was stable. The identity of the test item at the end of the exposure phase was confirmed by HPLC co-chromatography with non-radio-labelled AE C656948.

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During the depuration phase of the study no residues of [pyridyl-2,6-^{14}C]-fluopyram were determined above 0.05 µg a.s./L in the low treatment group (6.0 µg a.s./L) and above 0.6 µg a.s./L in the high treatment (60 µg a.s./L). The limit of quantification for LSC was 20 dpm. No residues of [pyridyl-2,6-^{14}C]-fluopyram were determined in the samples of the solvent control tank; the measured radioactivity was below 78.6 dpm.

Table 8.2.2.3- 2: LSC results from the water analyses (expressed as µg/L of [pyridyl-2,6-^{14}C]-fluopyram equivalents)

Study-phase	Study day	Nominal concentration: 6.0 µg a.s./L (Aquarium B)		Nominal concentration: 60 µg a.s./L (Aquarium C)		Nominal concentration: 60 µg a.s./L (Aquarium D)	
		Measured [A] [µg a.s./L]	% of nominal [B]	Measured [A] [µg a.s./L]	% of nominal [B]	Measured [A] [µg a.s./L]	% of nominal [B]
Uptake	0	6.26	104.3	64.1	106.8	57.6	96.0
	1	5.39	89.8	54.9	91.5	55.4	92.3
	3	6.13	102.2	61.4	102.3	60.7	101.2
	7	5.68	94.7	56.7	94.5	33.7	56.2
	8	-	/	-	/	61.7	102.8
	10	5.96	99.3	62.1	103.5	59.8	99.7
	14	5.83	97.2	57.7	96.2	57.1	95.2
	21	6.62	110.3	64.7	107.8	-	/
	28	5.97	99.5	57.8	96.3	-	/
	Mean	5.98	/	59.9	/	55.1	/
Depu-ration	29	0.05	/	0.6	/		
	31	0.02	/	0.1	/		
	35	0	/	0	/		
	38	0	/	0	/		
	42	0	/	0	/		

\- No samples collected.
[A] Calculated concentrations of [pyridyl-2,6-^{14}C]- fluopyram based on results of LSC analyses and specific radioactivity
[B] Not given in the report. Calculated on the basis of the individual measured concentrations at each time point.

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➢ Fish tissue analysis:

Total radioactive residues (TRR):

Total radioactive residues (TRR) fish edible and non-edible tissues are shown in the table below.

Table 8.2.2.3- 3: Total radioactive residues (TRRs) in fish edible and non-edible tissues and calculated values from whole fish (expressed as mg/kg of [pyridyl-2,6-^{14}C]- fluopyram equivalents)

Study day	Total radioactive residues [mg/kg wet weight]					
	Nominal concentration 6 µg a.s./L (Aquarium B)			Nominal concentration: 60 µg a.s./L (Aquarium C)		
	Edible tissue	Whole fish	Viscera tissue	Edible tissue	Whole fish	Viscera tissue
Exposure phase						
1	0.114	0.20	0.34	1.08	2.12	4.00
3	0.100	0.29	0.62	0.87	2.77	6.21
7	0.126	0.39	0.83	1.76	3.77	7.25
10	0.167	0.49	1.01	1.35	3.25	6.60
14	0.230	0.47	0.90	1.69	3.80	7.15
21	0.206	0.42	0.82	1.94	3.52	6.35
28	0.292 #	0.58 #	1.01 #	2.49 #	4.75 #	8.79 #
Depuration phase						
29	0.154	0.25	0.39	1.04	1.90	3.26
31	0.144	0.23	0.37	1.10	1.62	2.50
35	0.165	0.21	0.28	0.84	1.27	1.91
38	0.120	0.16	0.24	0.94	1.37	2.05
42	0.120	0.15	0.19	0.71	0.97	1.31
Mean at steady state [A]	0.292	0.581	1.01	2.49	4.75	8.79

\# Mean of steady state
[A] Based on TRR

Characterisation of metabolites in fish and in test water (Biotransformation part):

The parent compound fluopyram accounted for > 97 % of the radioactivity in the profiles of all water samples after SPE and concentration. In the samples collected during the later exposure phase of fish, the metabolite AE C656948-7-hydroxy was detected with ca. 1 – 2 % of the TRR.

Total radioactive residues (TRR) measured were 0.753 mg/kg in edibles (day 7), 1.533 mg/kg in edibles (day 14), 3.221 mg/kg in viscera (day 7) and 12.597 mg/kg in viscera (day 14).

Between 95 and 97 % of the TRR could be extracted with acetonitrile/water mixtures.

The metabolic profiles for both time points were similar for edibles and viscera, respectively. In edibles the major part of the residue was represented by the parent compound followed by the metabolite AE C656948-7-hydroxy. Samples of viscera exhibited significant higher proportions of conjugates compared to edibles. In viscera, the major compounds were parent compound and AE C656948-7-OH (glucuronic acid conjugate of AE C656948-7-hydroxy).

Minor metabolites detected were AE C656948-8-hydroxy (edibles and viscera), AE C656948-8-OH-GA and AE C656948-pyridyl acetic acid (both in viscera, only).

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Biological results:

Observations:

No mortality of fish was observed throughout the test in all test vessels.

Lipid content in whole fish:

The average percent lipids over the entire study period ranged from 8 to 11 %, from 5 to 10 %, and from 5 to 11 % in the whole fish samples in the solvent control (aquarium A), in the low treatment (aquarium B), and high treatment (aquarium C), respectively (see table below). The overall mean percent lipid content in samples from aquaria A, B, and C on day 0 and 28 was 7.03 %.

Table 8.2.2.3- 4: Lipid content in whole fish samples from the [pyridyl-2,6-^{14}C]- fluopyram - bioconcentration and biotransformation study in bluegill sunfish

Day of study	Sample	% Lipid content (whole fish)			
		Aquarium A (Solvent Control)	6.0 µg a.s./L (Aquarium B)	60 µg a.s./L (Aquarium C)	Mean per sampling day
0	1	3.80	5.73	8.93	6.50
	2	4.83	5.80	6.69	
	3	6.61	6.08	9.84	
	4	6.21	6.32	7.14	
28	1	6.45	5.32	8.05	7.56
	2	11.10	8.70	5.63	
	3	5.68	5.71	9.15	
	4	8.53	9.77	6.68	
42	1	9.18	8.05	9.94	9.92
	2	9.84	5.63	9.85	
	3	12.43	9.15	8.11	
	4	8.46	6.68	10.59	
Overall mean [A] (day 0 + day 28)		7.03 %			

[A] Overall mean from aquaria A, B, and C for day 0 and day 28 used for normalisation of the BCF to 6 % lipid content

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Bioconcentration factors:

Table 8.2.2.3- 5: Bioconcentration factors (BCF) based on TRR for edible, non-edible tissues and whole fish

Day of study	Mean bioconcentration factor (BCF)					
	Edible tissues		Viscera		Whole fish	
	6.0 µg [pyridyl-2,6-^{14}C]-fluopyram/L	60 µg [pyridyl-2,6-^{14}C]-fluopyram/L	6.0 µg [pyridyl-2,6-^{14}C]-fluopyram/L	60 µg [pyridyl-2,6-^{14}C]-fluopyram/L	6.0 µg [pyridyl-2,6-^{14}C]-fluopyram/L	60 µg [pyridyl-2,6-^{14}C]-fluopyram/L
Exposure phase						
1	19.6	18.2	58.4	67.2	34.5	35.6
3	16.8	14.4	105.4	103.2	48.7	46.0
7	21.5	29.7	141.2	122.3	66.2	63.6
10	28.4	22.6	171.1	110.3	82.6	54.3
14	39.1	28.4	153.0	120.2	80.6	63.9
21	34.5	32.2	137.0	105.4	70.5	58.5
28	48.8	41.6	168.2	146.6	97.2	79.2
Depuration phase						
29	25.8	17.3	64.8	54.3	42.2	31.7
31	24.1	18.3	61.2	41.7	38.1	27.0
35	27.6	14.1	47.6	31.9	35.2	22.0
38	20.1	15.7	40.4	34.2	27.3	22.9
42	20.0	11.9	31.9	21.9	24.5	16.1

After 14 days in uncontaminated water 75 % (low treatment, 6.0 µg/L) and 80 % (high treatment, 60 µg/L) of the radioactivity were depurated from whole fish.

Fluopyram accumulated in bluegill sunfish with a total residue bioconcentration factor of about 65.7 to 87.9 for whole fish (sum of radio labelled compounds, fluopyram parent, metabolites and mineralization products) (see table below).

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Table 8.2.2.3- 6: Substance uptake and depuration constants and bioconcentration factors

Parameter (based on TRR)	6.0 µg [pyridyl-2,6-^{14}C]-fluopyram/L			60 µg [pyridyl-2,6-^{14}C]- fluopyram/L		
	Edible tissue	Non-edible tissue	Whole fish	Edible tissue	Non-edible tissue	Whole fish
Kinetic bioconcentration factor (BCF_{TRR})	47.6	156.4	87.9	35.9	121.6	65.7
Time to reach 95 % of steady state [days]	30.6	8.1	14.8	18.4	4.6	7.7
$t_{(1/2)}$ for clearance [days]	7.1	1.9	3.4	4.2	1.1	1.8
Uptake rate constant (k_u) [1/Day]	4.67 (± 0.42)	58.2 (± 2.0)	17.8 (± 1.1)	5.96 (± 0.57)	78.7 (± 3.62)	25.6 (± 1.59)
Depuration rate constant (k_d) [1/Day]	0.098 (± 0.03)	0.37 (± 0.16)	0.20 (± 0.08)	0.17 (± 0.06)	0.65 (± 0.26)	0.39 (± 0.175)

The OriginTM calculated kinetic BCF_{TRR} values for edible parts and whole fish (calculated as the ratio of uptake and depuration rate constant) correspond well with the respective bioconcentration factors (calculated as the ratio of concentration in fish and in water) 48.8 X (edible parts) and 97.2 X (whole fish) for 6.0 µg [pyridyl-2,6-^{14}C]-fluopyram/L and of 41.6 X (edible parts) and 79.2 X (whole fish) for 60 µg [pyridyl-2,6-^{14}C]-fluopyram/L, respectively.

These values correspond to the calculated total residue levels of 0.292 mg/kg edible parts and 0.581 mg/kg whole fish for 6.0 µg [pyridyl-2,6-^{14}C]-fluopyram/L and of 2.49 mg/kg edible parts and 4.75 mg/kg whole fish for 60 µg [pyridyl-2,6-^{14}C]-fluopyram/L, respectively.

Taking into account that in edible parts of the fish 24.7 % of the TRR (sample day 14) were identified as parent compound and in viscera 21.9 % of the TRR (sample day 14) the steady-state-BCF for parent (based on whole fish, wet weight) is 18, the steady-state-BCF for parent (normalised to 6 % lipid content) is 16.

III. CONCLUSION

The average percent lipid content in whole fish samples from the solvent control, from the low (6.0 µg a.s./L) and high treatment level (60 µg a.s./L) at day 0 and 28 was 7.03 %.

The steady-state BCF for parent fluopyram based on whole fish (wet weight) was calculated to be 18 and the steady-state BCF for parent fluopyram normalized to 6 % lipid content was 16.

Assessment and conclusion by applicant:

The study and its data are considered as acceptable and reliable for use in risk assessment.

The endpoints are:

BCF: 18 (whole fish)

BCF_{SS} (normalised to 6 % lipid content): 16

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CA 8.2.3 Endocrine disrupting properties

Table 8.2.3- 1: Outline of studies conducted for ecotoxicology assessment of potential endocrine disrupting properties of fluopyram in aquatic organisms

Type of toxicity	Study type	Reference
In vivo mechanistic assays	*Xenopus* embryonic thyroid assay (XETA) analysis report	████ (2018) M-632157-01-1 KCA 8.2.3/01
	Xenopus embryonic thyroid assay (XETA) analysis report	████ (2020) M-684884-01-1 KCA 8.2.3/02
21-day chronic studies	Fluopyram: Amphibian Metamorphosis Assay for the Detection of Thyroid Active Substances report	████ (2020) M-761814-01-1 KCA 8.2.3/03
	Fluopyram Technical - Short-Term Reproduction Assay with Fathead Minnow (*Pimephales promelas*) report	████ (2020) M-762527-01-1 KCA 8.2.3/04

Study summaries

Data Point:	KCA 8.2.3/01
Report Author:	████
Report Year:	2018
Report Title:	Xenopus embryonic thyroid assay (XETA) analysis report
Report No:	P-2018-0008
Document No:	M-632157-01-1
Guideline(s) followed in study:	Xenopus Embryonic Thyroid Assay (XETA) DRAFT Test Guideline, Version: June 2018
Deviations from current test guideline:	Current Guideline: OECD 248 (2019) Deviation: The test duration was 48 h instead of the recommended 72 h. The temperature was held at 26°C, higher than the recommended 21°C.
Previous evaluation:	No, not previously submitted submitted after the legal deadline
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	No

Executive Summary

The objective of the test is to detect a potential thyroid activity of the test item on *Xenopus laevis* transgenic embryos.

Five concentrations of the test item were prepared in test medium + DMSO in three replicates. A test medium control and a test medium + DMSO control were also prepared. Triiodothyronine (T3) was used as the internal standard in the test. Fluorescence of each embryo in each test condition was measured and analysed.

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Chemical analysis

The mean measured concentrations were within a range to 101.3% to 80.3% (mean percent recovery), except for the lowest test concentration (0.312 µg/L) where the percent of recovery was 79% after the first 24 hours. As this does not represent a major deviation from the 80- 120% recovery range, the results are expressed with respect to nominal concentrations.

Changes in fluorescence

Unspiked mode: no statistically significant variation of fluorescence was induced by the test item at any of the tested concentrations.

Spiked mode: at the highest concentration tested, the test item induced a statistically significant increase in fluorescence. This induction of 9% compared to the T3 3.25 µg/L control group remains below the threshold of 12% which is the minimal induction defining an active concentration in the XETA OECD test guideline.

It can therefore be concluded that the test item fluopyram (technical substance) does not show thyroid activity in the Xenopus Embryonic Thyroid Assay (XETA).

I. MATERIALS AND METHODS

Test material	Fluopyram technical Specification No.: 102000017196 Batch Number: EDTE017337
Guideline(s) adaptation	None
Test species	*Xenopus laevis*
Organism age at study initiation / size at study initiation	Eleutheroembryo Stage 45 according to the Nieuwkoop & Faber development table (Nieuwkoop & Faber 1994).
Number of runs	3 independent runs
Organisms per concentration	60 (20 per run)
Exposure	Duration: 48 hours Medium renewal frequency: at 24 hours
Environmental test conditions	Temperature: 26 °C Illumination: none pH (recorded in the highest test concentration): 7.5
Test medium	Evian® water
Solvent	Dimethylsulfoxide (0.01 %)
Nominal concentrations tested	0.312, 0.625, 1.25, 2.5 and 5 mg a.s./L
Mean measured test concentrations	0.269, 0.550, 1.144, 2.350, 4.704 for unspiked and 0.271, 0.556, 1.129, 2.375, 4.872 for spiked referring to 0.312, 0.625, 1.25, 2.5, and 5, respectively
Mean recovery	101.3% to 80.3%
Parameters Measured / Observations	Macroscopic observation of malformations and survival after 24 and 48 hours. Reading of fluorescence using Leica Macrofluo at test termination (48 hours).

II. RESULTS AND DISCUSSION

<u>Validity criteria:</u>

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According to the OECD TG 248 (2019), for the test to be valid, the following validity criteria should be met for each run and for the pool of the three runs:

Table 8.2.3- 2: Validity criteria

Validity criteria	Required	Obtained			
		Run 1	**Run 2**	**Run 3**	**Pooled data**
Mean fluorescence of the 3.25 µg/L T3 group higher than mean fluorescence of the test medium control group.	≥ 20 %	21 %	32 %	29 %	32.50 %
Statistically significant induction of fluorescence in the T4 control group as compared to the test medium control.	≥ 70 %	141 %	116 %	53.33 %	104 %
Coefficient of variation of fluorescence intensity measured for the test medium control. [A]	≤ 30 %	Not determined			
Mortality in each control group.	≤ 10 %	0 %	0 %	≤ 10 %	≤ 10 %
Initial pH of the exposure solutions for each renewal.	6.5-8.5	7.5	7.5	7.5	7.5
Percentage of malformed organisms in each control group. [A]	≤ 10 %	Not determined			

[A] Not included in original study due to OECD draft test guidance.

The measured pH values of the exposure solutions were found within the acceptable range. An induction lower than the TG threshold of 70% (53.3%) was recorded between the T4 control group and the test medium control in run 3. The study therefore does not fully meet the validity criteria.

Chemical analysis

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 SANTE/2020/12830, Rev.1.

The mean measured concentrations were within a range to 101.3% to 80.3% (mean percent recovery), except for the lowest test concentration (0.312 µg/L) where the percent of recovery was 79% after the first 24 hours. As this does not represent a major deviation from the 80- 120% recovery range, the results are expressed with respect to nominal concentrations.

Changes in fluorescence

Unspiked mode: no statistically significant variation of fluorescence was induced by the test item at any of the tested concentrations. Spiked mode: at the highest concentration tested, the test item induced a statistically significant increase in fluorescence. This induction of 9% compared to the T3 3.25 µg/L control group remains below the threshold of 12% which is the minimal induction defining an active concentration in the XETA OECD test guideline.

III. CONCLUSIONS

Mortality did not exceed 20% in any group, and all validity criteria were met.

The mean measured concentrations were within a range to 101.3% to 80.3% (mean percent recovery), except for the lowest test concentration (0.312 µg/L) where the percent of recovery was 79% after the first 24 hours. As this does not represent a major deviation from the 80- 120% recovery range, the results can be expressed with respect to nominal concentrations.

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Unspiked mode: no statistically significant variation of fluorescence was induced by the test item at any of the tested concentrations.

Spiked mode: at the highest concentration tested, the test item induced a statistically significant increase in fluorescence. This induction of 9% compared to the T3 3.25 µg/L control group remains below the threshold of 12% which is set as the minimal induction defining an active concentration in the XETA according to the OECD Draft Test Guideline.

It can therefore be concluded that the test item fluopyram (technical substance) does not show thyroid activity in the *Xenopus* Embryonic Thyroid Assay (XETA).

Assessment and conclusion by applicant:

The study and its data are considered as supplementary data with no use in risk assessment.

The study was conducted prior to the publication of OECD TG 248 (2019). It therefore follows the draft OECD TG dated 2018 where the recommended test duration was 48 h (instead of 72 h in OECD TG 248, 2019), and the temperature was held at 26°C (instead of 21°C in OECD TG 248, 2019).

The study does not fully meet the validity criteria as an induction lower than the TG threshold of 70% (53.3 %) was recorded between the T4 control group and the test medium control in one of the three runs. This is considered as a minor deviation.

The data from this study do not support that the observed effects of fluopyram result from a T-mediated endocrine mode of action.

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Data Point:	KCA 8.2.3/02
Report Author:	[redacted]
Report Year:	2020
Report Title:	XETA analysis report - Fluopyram
Report No:	P-2019-0152
Document No:	M-684884-01-1
Guideline(s) followed in study:	Xenopus Eleutheroembryonic Thyroid Assay (XETA) OECD TG 248
Deviations from current test guideline:	Current Guideline: OECD 248 (2019) Deviation: None
Previous evaluation:	No, not previously submitted submitted after the legal deadline
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The objective of the *Xenopus* Eleutheroembryonic Thyroid Assay (XETA) assay is to detect potential activity of the test item on the thyroid axis. The XETA detects thyroid active molecules acting through various mechanisms of action including TH receptors agonists and antagonists, modulators of TH metabolism (including deiodinase inhibitors), modulators of TH clearance (including UDPGT modulators), and modulators of TH transport *via* interaction with TH plasma binding proteins. The purpose of this test was to measure the capacity of Fluopyram to activate or inhibit the transcription of a genetic construct (THb/ZIP-GFP *X. laevis* eleutheroembryos) either directly through binding to the thyroid receptor (TR) or modifying the binding of thyroid hormones (TH) to the TR, or indirectly by modifying the amount of TH available to activate the TR and thereby transcription of the TH/bZIP-GFP construct.

Nominal concentrations of 1.7, 5 and 15 mg a.s./L were evaluated for fluorescence. The highest test concentration was selected based upon the results of solubility and survival pre-tests showing that this concentration corresponds to the solubility limit in the test medium and results in non-lethal signs of toxicity. The exposure levels were verified analytically. Arithmetic mean measured concentrations were 1.24, 3.75 and 13.4 mg a.s./L for 1.7, 5 and 15 mg a.s./L, respectively, corresponding to a mean recovery of 79.1%.

Fluopyram showed no indication of endocrine activity for the thyroid modality at the lowest concentrations of 1.24 and 3.75 mg a.s./L. At the highest concentration of 13.4 mg a.s./L, a statistically significant fluorescence increases above 12% (test guideline threshold), indicating a pro-thyroid activity, is detected. No toxicity was observed on the embryos at the two lowest concentrations, but signs of toxicity were observed at the highest test item concentration. Therefore, it does not appear that the increase in fluorescence is due to an endocrine mechanism.

I. MATERIALS AND METHODS

Test material	Fluopyram technical Specification No.: 102000017196 Batch ID: PFV187P078 Purity: 99 % w/w
Guideline(s) adaptation	None
Test species	*Xenopus laevis*
Organism age at study initiation	Eleutheroembryo

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/ size at study initiation	Stage 45 according to the Nieuwkoop & Faber development table (Nieuwkoop & Faber 1994).
Number of runs	3 independent runs
Organisms per concentration	60 (20 per run)
Exposure	Duration: 72 hours Medium renewal frequency: every 24 hours
Environmental test conditions	Temperature: 21°C Illumination: none pH (recorded in the highest test concentration): 7.5
Test medium	Evian® water
Solvent	Dimethylsulfoxide (0.01 %)
Nominal concentrations tested	1.7, 5 and 15 mg a.s./L
Mean measured test concentrations	1.24, 3.75 and 13.4 mg a.s./L for 1.7, 5 and 15 mg a.s./L, respectively
Mean recovery	72.9, 74.9 and 89.3% (mean 79.1%) for 1.24, 3.75 and 13.4 mg a.s./L, respectively
Parameters Measured / Observations	Macroscopic observation of malformations and survival after 24, 48 and 72 hours. Reading of fluorescence using Leica Macrofluo at test termination (72 hours).

In the XETA, the test item is tested in two modes corresponding to two levels of activity of the thyroid axis placing the eleutheroembryos in two different physiological states:

- In the unspiked mode, the eleutheroembryos are exposed to the test item diluted in the test medium. In this unstimulated mode, only pro-thyroid activity, *i.e.* activation of the thyroid axis through one or several of the mechanisms listed above (see executive summary), can be detected if directly induced by the test item. Activation of the thyroid axis induces an increase in the fluorescence of the eleutheroembryos.
- In the spiked mode, the eleutheroembryos are exposed to the test item diluted in the test medium and supplemented with 3.25 µg/L of the thyroid hormone T3 (triiodothyronine). In this stimulated mode, increase or decrease in the fluorescence of eleutheroembryos can be detected corresponding to pro- or anti-thyroid activities respectively, *i.e.* activation or inhibition of the thyroid axis through one or several of the mechanisms listed above (see executive summary).

II. RESULTS AND DISCUSSION

Validity criteria:

According to OECD TG 248 (2019), for the test to be valid, the following validity criteria should be met for each run and for the pool of the three runs:

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Table 8.2.3- 3: Validity criteria according to OECD TG 248 (2019)

Validity criteria	Required	Obtained			
		Run 1	Run 2	Run 3	Mean
Mean fluorescence of the 3.25 µg/L T3 group higher than mean fluorescence of the test medium control group.	≥ 20%	65%	71%	52%	64%
Statistically significant induction of fluorescence in the T4 control group as compared to the test medium control.	≥ 70%	144 %	139 %	133 %	139 %
Coefficient of variation of fluorescence intensity measured for the test medium control.	≤ 30 %	9 %	8 %	6 %	10 %
Initial pH of the exposure solutions for each renewal.	6.5-8.5	7.5	7.5	7.5	7.5
Mortality in each control group.	≤ 10%	<10 %	0 %	<10 %	≤ 10 % [A]
Percentage of malformed organisms in each control group.	≤ 10%	0 %	0%	0%	0 %

[A] One dead embryo was recorded in the test Evian® water control group + DMSO after 72h of exposure in the first run/ one dead embryo was recorded in the T3 control group at the test termination in the third run.

Chemical analysis

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 SANTE/2020/12830, Rev.1.

The entire volume of the exposure medium corresponding to the nominal test concentrations of 1.7, 5 and 15 mg a.s./L was sampled for analytical verification. Acetonitrile was added prior to store the samples at -20°C before analysis. The concentration of the stock solutions was also verified.

Fluopyram concentrations in the test medium, T3 and T4 controls were all below the limit of quantification (LOQ = 0,0165 mg/L), indicating the absence of cross contamination between exposed and control conditions.

Arithmetic mean measured concentrations were 1.24, 3.75 and 13.4 mg a.s./L for 1.7, 5 and 15 mg a.s./L, respectively, corresponding to a mean recovery of 79.1% (range of 72.9 to 89.3%).

The mean percent recovery in the stock solutions in run 1 and 3 was 98%. The mean percent recovery in the stock solution of the run 2 was determined several times; the results were 49, 51.1 and 46.5%, corresponding to a mean recovery of 48.9%. This low recovery value in the stock solution of the run 2 is explained by a technical error during the sampling where 500 µL of acetonitrile were added and resulted in a dilution of the final concentration by 1/2. Taking into account this dilution factor and using the average of 48.9%, we can estimate a recovery rate of 97.3% of the stock solution for the second run.

Overall, the estimated average total recovery for the stock solutions used in the three runs was 97.3%.

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<u>Biological results</u>

Observations:

Over the test period, no mortality, malformations, nor immobile embryos were observed. On the other hand, signs of toxicity (stress behavior as shown by the emission of dejections after 24, 48 and 72 hours) were observed at the highest test item concentration in the survival pre-test. During this pre-test, suspended particles were also observed at the highest concentration, indicating that the solubility limit in the test medium was reached.

Fluorescence measurement:

Unspiked mode:

No statistically significant variation of fluorescence greater than 12% was induced by the test item at any of the concentrations tested.

Spiked mode:

At the highest concentration tested, the test item induced a statistically significant increase greater than 12 % (+17.6 %) in fluorescence.

Table 8.2.3- 4: Test results. Mean measured concentrations are given in bracket for the nominal test concentrations of 1.7, 5 and 5 mg a.s./L

Fluopyram test concentrations (mg a.s./L):	**1.7 (1.24)**	**5 (3.75)**	**15 (13.4)**
Unspiked mode [A] (Activation of the thyroid axis)	No active concentrations		
T3-spiked mode [B] (Activation/inhibition of the thyroid axis)	No active concentrations		+17.6 % Significant increase in fluorescence (p = 0.001)

[A] In the unspiked mode. An active concentration is defined as a concentration giving a statistically significant fluorescence increase of 12 % or greater compared to the test medium control.

[B] In the T3-spiked mode. An active concentration is defined as a concentration giving a statistically significant fluorescence increase or decrease of 12 % or greater compared to the T3 control.

III. CONCLUSIONS

At the nominal concentrations of 1.7 and 5 mg a.s./L, Fluopyram does not show indication of endocrine activity for the thyroid modality under unspiked and spiked conditions.

At the highest nominal concentration of 15 mg a.s./L (mean measured concentration corresponding to 13.4 mg a.s./L), the test item induced a statistically significant increase in fluorescence of 17.6 % (P value = 0.001) compared to the T3 control group, indicating a pro-thyroid activity.

No toxicity was observed on the embryos at any test concentrations during the definitive test, but signs of toxicity were observed at the highest test concentration of 15 mg a.s./L in the survival pre-test. However, due to the clear observation of signs of toxicity at this concentration during the pre-survival test and due to the fact that this concentration is close to the solubility limit, it does not appear that the test conditions including the physiological status of the embryos were met to link the increase in fluorescence to an endocrine mechanism.

<u>**Assessment and conclusion by applicant:**</u>

The study and its data are considered as supplementary data with no use in risk assessment.

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The data from this study do not support that the observed effects of fluopyram result from a T-mediated endocrine mode of action.

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Data Point:	KCA 8.2.3/03
Report Author:	[redacted]
Report Year:	2021
Report Title:	Fluopyram: Amphibian metamorphosis assay for the detection of thyroid active substances
Report No:	149A-312
Document No:	M-761814-01-1
Guideline(s) followed in study:	OECD 231; U.S. EPA OPPTS 890.1100
Deviations from current test guideline:	Current guideline: OECD 231, U.S.EPA OPPTS 890.1100 Deviation: None
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 evaluate the potential for Fluopyram to interfere with the normal function of the hypothalamic-pituitary-thyroid (HPT) axis of the African clawed frog (*Xenopus laevis)* during a 21-day exposure period under flow-through test conditions. Endpoints that were evaluated for endocrine disruption of the HPT axis included development stage, wet weight, snout-to-vent length, hind-limb length, normalized hind-limb length, and thyroid gland histology. Survival and gross morphological abnormalities were also assessed throughout the test.

Xenopus laevis tadpoles were exposed to a series of three test concentrations, a negative (dilution water) control and solvent control (0.10 mL/L DMF) under flow-through conditions. Nominal test concentrations were 1.4, 4.1 and 13 mg a.s./L. Mean measured concentrations were determined from samples of test water collected approximately weekly during the exposure period.

In the definitive test, precipitates were observed in the test chambers of the high treatment group, therefore all samples were analyzed before and after centrifugation. Analytical recoveries from the centrifuged samples were comparable to the non-centrifuged results. The mean measured concentrations over 21 days were 1.4, 3.9 and 7.7 mg/L representing 98, 95 and 59% of nominal concentrations.

Biological observations at day 7 confirmed the highest test concentration as being lethal, with 98.8% survival. Survival on day 21 in the negative control, solvent control, 1.4, and 3.9 mg/L treatment groups was 100%. The highest test concentration, corresponding to 13 mg/L nominal, is the Maximum Tolerated Concentration (MTC). Amongst the surviving tadpoles at this concentration, one smaller individual and one weaker individuals were noted on day 11 and throughout the remainder of the test; this indicates additional sublethal effects at the MTC.

All statistically significant effects observed in the AMA were associated with signs of overt toxicity and reduced survival at the highest test concentration (13 mg/L_{nom} = 7.7 mg/L_{mm}) being the MTC. The only exception is reduced hind-limb length that was statistically significant in the 3.9 mg/L treatment group

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at day 21. Without thyroid histopathology and delayed development, the decrease in hind-limb length in the AMA with fluopyram appears as a finding that is not thyroid-related.

In conclusion, the data from this study do not support that the observed effects of fluopyram result from a T-mediated endocrine mode of action.

I. MATERIALS AND METHODS

Test material	Fluopyram technical Specification No.: 102000017196 Origin Batch No.: PTE197P141 Batch code: AE C656948-01-22 Purity: 98.6 % w/w
Guideline(s) adaptation	None
Test species	African Clawed Frog (*Xenopus laevis*)
Organism age at study initiation / size at study initiation	Tadpoles at NF Stage 51 at initiation
Number of replicates per test group	4
Number of organisms per replicate	20
Number of organisms per concentration	80
Exposure	Duration: 21 days, flow-through
Environmental test conditions	Temperature: 22 ± 1°C Illumination: 12 hours light, 12 hours dark with 30 minutes transition pH (recorded in the highest test concentration): 7.8 – 8.2 Dissolved oxygen range: ≥5.9 mg/L (≥ 72% of air saturation) Light intensity range: 600 to 870 lux at water surface
Test medium	Well water
Solvent	HPLC-grade DMF
Nominal concentrations tested	1.4, 4.1, 13 mg a.s./L
Mean measured test concentrations	1.4, 3.9, 7.7 mg a.s./L
Mean recovery	91 to 103% of nominal concentrations in the 1.4 and 4.1 mg a.s./L treatment groups and 37 to 70% of nominal concentrations in the 13 mg a.s./L treatment group
Parameters Measured / Observations	Survival General Behavior Developmental Stage Body Weight Snout-to-Vent Length Hind-Limb Length Normalized Hind-Limb Length (normalized by dividing by snout-to-vent length) Thyroid gland histopathology

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II. RESULTS AND DISCUSSION

Table 8.2.3- 5: Validity criteria

Criterion	Required	Obtained
Dissolved oxygen concentration throughout exposure period	≥ 40 %	≥ 72 %
Water temperature	± 1 °C between vessels 22 ± 1 °C during exposure	21.7 – 22.6 [1]
Minimum median stage of the control tadpoles at the end of the test[2]	≥ 57	57
The 10th and 90th percentile of the developmental stage distribution of control tadpoles	Within 4 stages	Within 4 stages
Test concentrations were consistent over the course of the study	≤20 % CV over the 21-day test	CV = 2.64% for 1.4 mg/L, CV = 2.71% for 4.1 mg/L, CV = 21.9% for 13 mg/L
Survival in control group	≥ 90 %	100 %
Mortality in any control replicate	≤ 2	0
Non-control test concentrations with overt toxicity	≤ 2	1
Replicates across the test that were compromised	≤ 2	0

[1] Temperature monitored continuously in one negative control replicate ranged from 20.6 to 22.2 ºC, measured to the nearest 0.1 ºC.
[2] Tadpoles that had developed beyond stage 60 by Day 21 were excluded from analyses of length and weight.

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 SANTE/2020/12830, Rev.1.

Based on mean measured test concentrations:

African clawed frog (*Xenopus laevis*) tadpoles were exposed to Fluopyram at mean measured concentrations of 1.4, 3.9 and 7.7 mg a.s./L for 21 days. The endpoints evaluated to determine if the test substance might impact the hypothalamus-pituitary-thyroid (HPT) axis of tadpoles were survival, developmental stage, wet weight, snout-to-vent length, hind-limb length, normalized hind-limb length and thyroid gland histopathology.

Survival in the pooled control, 1.4, 3.9 and 7.7 mg a.s./L treatment groups was 100, 100, 100 and 98.8%. There were no statistically significant or treatment-related effects on survival in any treatment group in comparison to the pooled control on either Day 7 or Day 21 ($p > 0.05$). Since the high test concentration resulted in mortality that was <10%, this can be considered as the maximum tolerated concentration (MTC).

Tadpoles in the negative control group, solvent control group, 1.4 and 3.9 mg a.s./L treatment groups appeared normal throughout the test. Tadpoles in the 7.7 mg a.s./L treatment group appeared normal with the exception of 2 small tadpoles noted on Day 11 and throughout the remainder of the test.

There were treatment-related decreases in the developmental stage, wet weight, snout-to-vent length, hind-limb length and normalized hind-limb length in the 7.7 mg a.s./L treatment group on Day 7. There was a treatment-related decrease in developmental stage in the 7.7 mg a.s./L treatment group on Day 21 and a treatment-related decrease in hind-limb length and normalized hind-limb length in the 3.9 and 7.7

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mg a.s./L treatment groups on Day 21. There were no other significant changes seen in any of the treatment groups relative to the pooled control group weight or snout-to-vent length on Day 21.

The sole finding of histopathological analysis was the slightly decreased presence of follicular cell hypertrophy in the frogs of the 7.7 mg a.s./L group in comparison to the frogs of the solvent control.

The histological and biological results of this study indicate that the treatment-related effects are indicative of sub-lethal toxicity.

III. CONCLUSIONS

African clawed frog (*Xenopus laevis*) tadpoles were exposed to Fluopyram at mean measured concentrations of 1.4, 3.9 and 7.7 mg a.s./L for 21 days. The endpoints evaluated to determine if the test substance might impact the hypothalamus-pituitary-thyroid (HPT) axis of tadpoles were survival, developmental stage, wet weight, snout-to-vent length, hind-limb length, normalized hind-limb length and thyroid gland histopathology.

In the 7.7 mg a.s./L treatment group, the survival rate was 98.8% at test termination, as compared to 100% in all other groups including the controls. Furthermore, two tadpoles showed signs of sublethal toxicity at the high test level. These results indicate that 7.7 mg a.s./L corresponds to the Maximum Tolerated Concentration (MTC) according to OECD TG 231.

In the 7.7 mg a.s./L treatment group, the treatment-related decreases in developmental stage, wet weight, snout-to-vent length, hind-limb length and normalized hind-limb length on Day 7 and the treatment-related decrease in developmental stage and hind-limb length and normalized hind-limb length on Day 21 are associated with sign of sublethal toxicity (two small tadpoles) and mortality that was <10 %.

There was a treatment-related decrease in hind-limb length and normalized hind-limb length in the 3.9 mg a.s./L treatment groups on Day 21. There were no other significant changes seen in any of the treatment groups relative to the pooled control group weight or snout-to-vent length on Day 21.

Exposure to the highest test level was associated with a slightly decreased prevalence of follicular cell hypertrophy. However, concomitant reductions in the NF stage score and somatic growth suggest a non-endocrine causation.

The histological and biological results indicate that the treatment-related effects observed at the Maximum Tolerated Concentration of 7.7 mg/L are indicative of systemic toxicity and do not result from an endocrine mode of action.

Assessment and conclusion by applicant:

According to OECD GD 150 (2018), the Amphibian Metamorphosis Assay (OECD TG 231) is designed as a screen for thyroid activity in amphibians, and not to provide information on endocrine activity for use in assessing the environmental risks of an individual chemical based on a predicted environmental concentration/predicted no-effect concentration (PEC/PNEC) approach. Furthermore, the use of only three concentrations of test chemical precludes the reliable establishment of a no-observed-effect-concentration/x% effect concentration (NOEC/LOEC).

The study and its data are therefore considered as supplementary data with no use in risk assessment.

The data from this study do not support that the observed effects of fluopyram result from a T-mediated endocrine mode of action.

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Data Point:	KCA 8.2.3/04
Report Author:	████
Report Year:	2021
Report Title:	Fluopyram technical - Short-term reproduction assay with fathead minnow (Pimephales promelas)
Report No:	13798.6503
Document No:	M-762527-01-1
Guideline(s) followed in study:	OECD 229 (2012) OCSPP 890.1350 (2009)
Deviations from current test guideline:	Current guideline: OECD 229 (2012), OCSPP 890.1350 (2009) Deviation: None
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 empirically identify the potential for fluopyram technical to interact with the normal function of the hypothalamus-pituitary-gonadal (HPG) endocrine axis in fathead minnow. The endpoints evaluated were fecundity (number of eggs per female per reproductive day), fertilization success, nuptial tubercle score, blood plasma vitellogenin (VTG) concentration, gonadal somatic index (GSI), survival, and histopathological findings. The results of this study are based on mean measured concentrations of fluopyram technical.

Nominal concentrations for the short-term reproduction assay with fathead minnow (*Pimephales promelas*) were selected based on preliminary testing and in consultation with the Study Sponsor. The nominal concentrations selected for the reproduction assay were 0.16, 0.57, and 2.0 mg/L. Results of the analyses demonstrated that the measured concentrations approximated nominal concentration, were relatively consistent for the duration of the exposure period, and maintained the expected concentration gradient. Mean measured fluopyram technical concentrations ranged from 97 to 110% of nominal concentration and defined the treatment levels tested as 0.17, 0.60, and 1.9 mg/L. The coefficient of variance (% CV) for the measured concentrations ranged from 15 to 16 %.

No abnormal observations in behavior such as hyperventilation, loss of equilibrium, uncoordinated swimming, atypical quiescence, and feeding abstinence were noted in any treatment level or the control during daily observations. Appearance of all fish throughout the exposure was within expectations for adult, spawning fathead minnow. A significant reduction in fecundity was detected in the 1.9 mg/L treatment level. A significant reduction in male tubercle scores was detected in the 0.60 and 1.0 mg/L treatment levels. There were no treatment-related histopathological or other findings in this study.

The data from this study do not support that the observed effects of fluopyram result from an EAS-mediated endocrine mode of action.

I. Materials and Methods

Test material	Fluopyram technical Specification No.: 102000017196 Origin Batch No: PFV187P078 Purity: 99 % w/w
Guideline(s) adaptation	None
Test species	Fathead Minnow (*Pimephales promelas*)

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Organism age at study initiation / size at study initiation	Age at initiation of pre-exposure period: approximately 25 weeks old
Number of test groups	3 test concentrations and a negative (water) control
Number of replicate per test groups	4
Number of organisms per replicate	6 (2 males and 4 females)
Number of organisms per concentration	24
Exposure	Duration: 21 days, flow-through
Environmental test conditions	Temperature: 24-26 °C Illumination: 16 hours light, 8 hours dark with a 15-30 min transition period pH (recorded in the highest test concentration): 6.9-7.5 Dissolved oxygen range: 6.05 to 8.16 mg/L (73.5 to 98.9 % of saturation) Light intensity range: 52 to 97 footcandles (560 to 1040 lux)
Test medium	Well water
Solvent	None
Nominal concentrations tested	0.16, 0.57, 2.0 mg/L
Mean measured test concentrations	0.17, 0.60, 1.9 mg/L
Mean recovery	100, 110 and 97 % (mean 102 %) for 0.16, 0.57 and 2.0 mg a.s./L, respectively
Parameters Measured / Observations	Fecundity (number of eggs/female/day) Fertilization success Nuptial tubercle score Blood plasma vitellogenin (VTG) concentration GSI (Gonadal Somatic Index) Survival Gonad histopathology

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 SANTE/2020/12830, Rev.1.

Prior to the start of the definitive exposure, samples from each replicate of each treatment level and control solution were collected and analyzed for fluopyram technical concentration. In addition, a sample of the diluter stock solution was also analyzed. Results of the pretest analyses were used to verify that sufficient quantities of fluopyram technical were being delivered and maintained in the exposure aquaria prior to test initiation. The diluter system, which prepared and delivered the test solutions to the exposure aquaria functioned properly throughout the exposure, and all exposure solutions were observed to be clear and colorless. No undissolved test substance was observed in the diluter system.

The study protocol meets the requirements of OCSPP Guideline 890.1350 and OECD Guideline 229 for the fish short-term reproduction assay.

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II. Results and Discussion

Table 8.2.3- 6: Validity criteria according to OECD 229

Criterion	Required	Obtained
Test concentrations maintained at ≤20% CV (variability of measured test concentration) over the 21-day test, per treatment level	≤20 %	15 % to 16 %
Control mortality	≤10 %	0 %
Water temperature throughout the exposure	25 ± 2 °C	24 to 26 °C
Dissolved oxygen air saturation for the duration of testing	≥60 %	≥73.5 %

Results of the analyses demonstrated that the measured concentrations approximated nominal concentration, were relatively consistent for the duration of the exposure period, and maintained the expected concentration gradient. Mean measured fluopyram technical concentrations ranged from 97 to 110 % of nominal concentration and defined the treatment levels tested as 0.17, 0.60, and 1.9 mg/L. The coefficient of variance (% CV) for the measured concentrations ranged from 15 to 16 %.

No abnormal behavior or notable changes in secondary sex characteristics were observed in either sex throughout the 21-day study. No other abnormal observations (*e.g.*, body color (light or dark), coloration patterns, body shape, size of dorsal nape pad in males, or ovipositor size in females) were observed during the exposure period or at study termination in any of the treatment levels or the control. The results of the statistical analyses, gonadal staging, and histopathology evaluation are presented below.

Table 8.2.3- 7: Test results for test concentrations of 0.17, 0.6 and 1.9 mg a.s./L

Endpoints	Mean Measured Concentration (mg a.s./L)		
	0.17	0.60	1.9
Male Survival	-	-	-
Female Survival	-	-	-
Combined Male and Female Survival	-	-	-
Fecundity	-	-	↓
Fertilization Success	-	-	-
Nuptial Tubercle Score	-	↓	↓
Male GSI	-	-	-
Female GSI	-	-	-
Male VTG (including outliers)	-	-	-
Female VTG (including outliers)	-	-	-
Male Gonadal Staging	NF	NF	NF
Male Gonad Histopathology Findings	NF	NF	NF
Female Gonadal Staging	NF	NF	NF
Female Gonad Histopathology Findings	NF	NF	NF

- Endpoint not statistically different from controls.
↓ Statistical analysis determined endpoint to be significantly reduced compared to the control.
NF: No findings related to fluopyram technical exposure.

A significant reduction in fecundity was detected in the 1.9 mg/L treatment level. A significant reduction in male tubercle scores was detected in the 0.60 and 1.0 mg/L treatment levels. There were no other treatment-related findings in the study.

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There were no treatment-related histopathological findings in this study. In the testes of males, the mean testicular stage scores of negative control and treated groups were generally comparable.

In the ovaries of females, prevalences and severities of increased oocyte atresia and post-ovulatory follicles were either comparable between negative control and treated groups, or slight differences occurred in individual groups, e.g., decreased prevalence of oocyte atresia in 0.60 mg/L females, and increased prevalence and abundance of post-ovulatory follicles in 0.17 mg/L females. These minor single-group differences, which displayed non-monotonic patterns of dose-response, are attributed to biological variability unrelated to treatment. The mean ovarian stage scores of negative control and treated groups were generally comparable.

III. CONCLUSIONS

Since the effects on tubercle scores and fecundity are not associated to changes in VTG levels and gonad histology that are specific diagnostic indicators of an endocrine mechanism of action, the data from this study do not support that the observed effects of fluopyram result from an endocrine mode of action.

Assessment and conclusion by applicant:

As the Amphibian Metamorphosis Assay (OECD TG 231), the Fish Short-Term Reproduction Assay (OECD TG 229) assay is primarily designed as a screen for *in vivo* endocrine activity in fish and also provides information on adverse effects on fecundity which could be used in characterising the hazards of an individual chemical based on a predicted environmental concentration/predicted no-effect concentration approach.

However, the use of only three concentrations of test chemical precludes the reliable establishment of a no-observed-effect-concentration/x% effect concentration (NOEC/LOEC).

The study and its data are therefore considered as supplementary data with no use in risk assessment.

The data from this study do not support that the observed effects of fluopyram result from an EAS-mediated endocrine mode of action.

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Assessment of potential endocrine-disrupting properties of fluopyram in non-target aquatic vertebrates according to Commission Regulation (EU) 2018/605 (excerpt from Appendix I)

Data Point:	KCA 8.2.3/05
Report Author:	
Report Year:	2021
Report Title:	Appendix I - Assessment of the endocrine disrupting properties of the active substance fluopyram in accordance with Commission Regulation (EU) 2018/605
Report No:	M-764022-01-1
Document No:	M-764022-01-1
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

The analysis of potential endocrine-disrupting properties of fluopyram has been conducted according to a weight-of-evidence approach following the recommendations of the ECHA-EFSA Guidance for the identification of endocrine disruptors (ECHA-EFSA, 2018). It has been reported using Appendix I of EFSA's administrative guidance on submission of dossiers (EFSA, 2019) and is accompanied by the Excel file, completed in line with the template for reporting the available information relevant for ED assessment (Appendix E.1 to the Guidance) and submitted as a supplement to this document.

Table 8.2.3- 8: Outline of dataset considered for the assessment of potential endocrine-disrupting properties of fluopyram in non-target aquatic vertebrates

Type of toxicity	Species / Assay	Duration	Used for T/EAS modality	Reference	Matrix ID *
In vitro assay	ER Binding Assay based on OECD TG 493	2 hours	E	M-632859-01-1 M-632695-01-1	17 16
	Stably Transfected Human ERα Transcriptional Activation Assay (ER STTA) based on OECD TG 455	2 hours	E	M-632695-01-1	16
	AR Binding Assay based on OECD TG 458	24 hours	A	M-632697-01-1	18
	Aromatase Assay based on US EPA OCSPP 890.1200	5 minutes	S	M-632696-01-1	19
	Thyroperoxidase activity (TPO)	1 minute	T	M-299276-01-1	20
	Phase I enzyme induction in rat hepatocytes	96 hours 7 days	EATS	M-450157-01-1 M-759019-01-1	36 37
	Phase II enzyme induction in rat hepatocytes	7 days	EATS	M-759019-01-1	37
Non-animal *in vivo* mechanistic studies	*Xenopus laevis* (eleutheroembryos) / XETA (draft OECD TG, 2018)	48 hours	T	M-632157-01-1 **	38
	Xenopus laevis (eleutheroembryos) / XETA (OECD TG 248, 2019)	72 hours	T	M-684884-01-1	39
Chronic studies in non-mammalian vertebrates	*Xenopus laevis* (tadpoles) / AMA (OECD TG 231)	21 days	T	M-761814-01-1	40
	Fathead minnow (*Pimephales promelas*) / FELST (OECD TG 210)	33 days	T	M-279440-01-1	41

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	Fathead minnow (*Pimephales promelas*) / FSTRA (OECD TG 229)	21 days	EAS	M-762527-01-1	42

* by reference to Appendix E1
** This study does not fully meet the validity criteria of OECD TG 248 (2019)(see KCA 8.2.3/01).

ED assessment for T-modality in non-target aquatic organisms

T-mediated adversity

It may be considered that T-mediated adversity has not been sufficiently investigated because only an OECD level-4 study according to OECD TG 210 (Fish Early Life-Stage Test, FELST) is available. The OECD level-3 study conducted according to OECD TG 231 (Amphibian Metamorphosis Assay, AMA) also contains parameters that are relevant for T-mediated adversity such as development time and growth parameters (snout-vent length and body weight).
The studies conducted with aquatic vertebrates (FELST and AMA) show no evidence of endocrine-related adverse effects on metamorphosis, larval development and growth, which are population-relevant parameters.

T-mediated endocrine activity

In addition to Appendix I, a summary of the assessment of T-mediated endocrine activity has been prepared in the context of the submission of confirmatory information in November 2020:

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Data Point:	KCA 8.2.3/06
Report Author:	[redacted]
Report Year:	2020
Report Title:	Statement - Summary of the weight-of-evidence-based assessment of potential thyroid-related endocrine-disrupting properties of fluopyram in non-target vertebrates other than mammals
Report No:	M-755900-01-1
Document No:	M-755900-01-1
Guideline(s) followed in study:	none
Deviations from current test guideline:	Current Guideline: not applicable
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	Yes

T-mediated endocrine activity has been sufficiently investigated in non-mammalian aquatic vertebrates. *In vitro* and *in vivo* mechanistic studies show no direct interaction of fluopyram with thyroid-related mechanisms: fluopyram did not inhibit TPO and was inactive in the XETA (OECD TG 248) at concentrations below the limit of solubility (15 mg/L_{nom}; 13.4 mg/L_{mm}).
In addition, the AMA (OECD TG 231) did not show substance-related T-mediated effects at fluopyram concentrations below the functional limit of solubility (13 mg/L_{nom}; 7.7 mg/L_{mm}) which also corresponded to the Maximum Tolerated Concentration (MTC) in this study.

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According to the ECHA-EFSA ED Guidance (2018), "*if the T-mediated parameters foreseen to be investigated in an Amphibian Metamorphosis Assay (AMA, OECD TG 231 (OECD, 2009c)) are negative, this would be sufficient to support that T-mediated adversity is unlikely because no T-related endocrine activity has been observed*". For fluopyram, the negative AMA therefore confirms that T-mediated adversity is unlikely.

Conclusion on the assessment of T-modality in non-target aquatic organisms

Table 8.2.3- 9: Selection of the relevant scenario for T-modality in non-target organisms

Adversity based on T-mediated parameters	Positive mechanistic OECD CF level 2/3 test	Scenario	Next step of the assessment	Scenario selected
No (sufficiently investigated)	Yes/No	1a	Conclude: ED criteria not met because there is not "**T-mediated**" adversity	
Yes (sufficiently investigated)	Yes/No	1b	Perform MoA analysis	
No (not sufficiently investigated)	Yes	2a (i)	Perform MoA analysis (additional information may be needed for the analysis)	
No (not sufficiently investigated)	No (sufficiently investigated)	2a (ii)	Conclude: ED criteria not met because **no T-mediated endocrine activity** observed	**X**
No (not sufficiently investigated)	No (not sufficiently investigated)	2a (iii)	Generate missing level 2 and 3 information. Alternatively, generate missing "EATS-mediated" parameters. Depending on the outcome move to corresponding scenario	
Yes (not sufficiently investigated)	Yes/No	2b	Perform MoA analysis	

Based on scenario 2a (ii), the endocrine activity was sufficiently investigated for T-modality. Because no T-mediated endocrine activity was observed and T-mediated adversity is unlikely, it can be concluded that <u>fluopyram does not meet the ED criteria for the T-modality in non-target aquatic vertebrates</u>.

ED assessment for EAS-modalities in non-target aquatic organisms

EAS-mediated adversity

It can be considered that EAS-mediated adversity has not been sufficiently investigated because only an OECD level 3 study conducted to OECD TG 229 (FSTRA) is available. The FSTRA contains parameters that are relevant for EAS-mediated adversity such as growth and reproductive parameters. In the FSTRA, fluopyram did not affect growth and fertility, and decreased fecundity at the highest test concentration (1.9 mg a.s./L) was not associated with changes in any of the other parameters measured in female fish. The study data therefore provide no supportive evidence for EAS-mediated adverse effects in fish.

EAS-mediated endocrine activity

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EAS-mediated endocrine activity has been sufficiently investigated in non-mammalian aquatic vertebrates. Investigation of EAS-mediated endocrine activity in *in vitro* (screening assays for E, A and S modalities) and *in vivo* mechanistic (vitellogenin level in the FSTRA) data indicated that fluopyram had no endocrine activity *via* the E, A and S modalities.
Consistently, there was no indication of EAS-mediated endocrine activity in the FSTRA. In particular, unaffected female and male gonad histology confirms the absence of EAS-mediated effects *in vivo*.

Conclusion on the assessment of EAS-modalities in non-target aquatic organisms

Table 8.2.3- 10: Selection of the relevant scenario for EAS-modalities in non-target organisms

Adversity based on EAS-mediated parameters	Positive mechanistic OECD CF level 2/3 test	Scenario	Next step of the assessment	Scenario selected
No (sufficiently investigated)	Yes/No	1a	Conclude: ED criteria not met because there is not "**EAS-mediated**" adversity	
Yes (sufficiently investigated)	Yes/No	1b	Perform MoA analysis	
No (not sufficiently investigated)	Yes	2a (i)	Perform MoA analysis (additional information may be needed for the analysis)	
No (not sufficiently investigated)	No (sufficiently investigated)	2a (ii)	Conclude: ED criteria not met because **no EAS-mediated endocrine activity** observed	X
No (not sufficiently investigated)	No (not sufficiently investigated)	2a (iii)	Generate missing level 2 and 3 information. Alternatively, generate missing "EATS-mediated" parameters. Depending on the outcome move to corresponding scenario	
Yes (not sufficiently investigated)	Yes/No	2b	Perform MoA analysis	

As the endocrine activity was sufficiently investigated for EAS-modalities, scenario 2a(ii) applies and it can be concluded that fluopyram does not meet the ED criteria for EAS modalities in non-target aquatic vertebrates.

Assessment and conclusion by applicant:
It is concluded that fluopyram does not meet the ED criteria for non-target aquatic organisms according to Commission Regulation (EU) 2018/605.

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CA 8.2.4 Acute toxicity to aquatic invertebrates

CA 8.2.4.1 Acute toxicity to Daphnia magna

Active substance fluopyram

Data Point:	KCA 8.2.4.1/01
Report Author:	████
Report Year:	2006
Report Title:	Acute toxicity of AE C656948 (tech.) to the waterflea Daphnia magna in a static laboratory test system
Report No:	EBGMP046
Document No:	M-278709-01-1
Guideline(s) followed in study:	OECD guideline 202, (2004); EEC Directive 92/69/EWG, part C.2 (1992); U.S. EPA Pesticide Assessment Guidelines, Subdivision E, § 72-2 (1982), OPPTS Guideline 850.1010 public draft 1996 (modified); JMAFF 12-Nousan No. 8147 (2000); U.S. EPA FIFRA paragraph 72 2; Canadian PMRA Ref.: DACO 9.3.2; EU Council Directive 91/414/EEC (1991)
Deviations from current test guideline:	Current Guideline: 202 (2004) Deviations: None. All validity criteria were met.
Previous evaluation:	yes, evaluated and accepted in DAR (2011)
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 daphnids (*Daphnia magna*) under static conditions to determine the 48 – hour EC_{50}. First-instar neonate daphnids (< 24 hours old) were exposed to fluopyram in groups of 30 (6 replicates of 5 organisms per test level) to the nominal concentrations of 3.05, 4.88, 7.81, 12.5 and 20.0 mg a.s./L. Additionally, a control and solvent control were included. Immobilisation and sub-lethal behavioural effects were determined after 24 and 48 hours.

Concentrations of fluopyram were verified by HPLC – UV on day 0 and 2. Measured concentrations were in the 80 - 98 % range of nominal concentrations and no residues were found in the control samples higher than 0.1029 mg a.s./L, which was used as the lowest standard concentration during this study.

The study fulfils all validity criteria of OECD 202 guideline.

No immobility or other effects on behaviour were observed in all concentrations.

The endpoint based on nominal concentrations was: EC_{50} – 48 hours (95 % C.I.): > 20 mg a.s./L (n.d.).

I. MATERIAL AND METHODS

Test material	Fluopyram (AE C656948) Specification No.: 102000012455 Batch No.: 08528/0002 Purity.: 94.7 % w/w
Guideline(s) adaptation	None specified

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Test species	Water flea (*Daphnia magna*)
Organism age/size at study initiation	First instar neonates, less than 24 hours old
Test solutions	Nominal concentrations: 3.05 - 4.88 - 7.81 - 12.5 - 20.0 mg a.s./L Mean measured concentrations were between 80 and 98 % of nominal concentrations Control: water Solvent control: dimethylformamide (100 µL/L) Evidence of undissolved material: not reported
Replication	No. of vessels per concentration (replicates): 6 No. of vessels per control (replicates): 6 No. of vessels per solvent control (replicates): 6
Organisms per replicate	No. of organisms per vessel: 5
Exposure	Static Total exposure duration: 48 hours
Feeding during test	None
Test conditions	Temperature: 20.7 - 21.0 °C Photoperiod: 16 hours light, 8 hours dark Light intensity: max. 1500 lux pH: 8.1 - 8.2 Water hardness: 231 mg/L as $CaCO_3$ (at test start) Dissolved oxygen: 7.5 - 8.4 mg/L Conductivity: 570 µS/cm (at test start) Alkalinity: 53 mg/L as $CaCO_3$/L (at test start)
Parameters Measured / Observations	Observations for immobility and sub-lethal behavioural effects were made after 24 and 48 hours of exposure. Prior to test initiation, conductivity, total hardness and alkalinity of the dilution media (Elendt M7) were determined. The dissolved oxygen and pH values were measured in the freshly prepared test solutions of each treatment level and control and repeatedly in the pooled replicates of the aged media at test termination (day 2). Environmental (air) temperature and temperature of the test media inside one vessel of the untreated control and of the highest test concentration were continuously recorded during exposure by a computer controlled measurement system Light intensity was measured at start of the study as „diffuse light“ immediately above the exposure vessels with a photometer.
Chemical analysis	Samples were taken at test start from freshly prepared batch solutions and at test end from composite solutions from all replicate samples. The chemical analyses were performed by high-performance liquid chromatograph (HPLC) – UV.
Data analysis	Since no treatment related effects were evident at any tested concentration, statistical evaluations were not performed.

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II. RESULTS AND DISCUSSION

Table 8.2.4.1- 1: Validity criteria

Validity criteria acc. to OECD 202	Required	Obtained
Mortality in control during test	≤ 10 %	0 %
Dissolved oxygen concentration at the end of the test	≥ 3 mg/L	8.2 - 8.4 mg/L

Analytical results:

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 SANTE/2020/12830, Rev.1.

Recoveries on day 0 and 2 ranged from 80 to 98 % of nominal values. The biological results are based on nominal concentrations.

No residues of fluopyram were detected in the control samples higher than 0.1029 mg a.s./L, which was used as the lowest standard concentration during this study.

Table 8.2.4.1- 2: Analytical results

Nominal concentration [mg a.s./L]	Measured concentration [mg a.s./L]		% of nominal	
	Day 0 (New)	Day 2 (Aged)	Day 0 (New)	Day 2 (Aged)
3.05	2.91	2.98	96	98
4.88	4.53	4.72	93	97
7.81	6.74	7.28	86	93
12.5	10.4	11.5	83	92
20.0	16.0	18.0	80	90

Biological results:

Observations

No immobility or other effects on behaviour were observed in all test concentrations and the controls.

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Table 8.2.4.1- 3: Immobilisation of daphnids

Nominal concentration [mg a.s./L]	No. of immobilized (cumulative %)	
	Exposure time	
	24 h	48 h
Control	0 (0)	0 (0)
Solvent control	0 (0)	0 (0)
3.05	0 (0)	0 (0)
4.88	0 (0)	0 (0)
7.81	0 (0)	0 (0)
12.5	0 (0)	0 (0)
20.0	0 (0)	0 (0)

III. Conclusion

The study meets the validity criteria according to OECD 202 and the endpoints based on nominal concentrations were:

EC_{50} – 48 hours (95 % C.I.):	**> 20 mg a.s./L** (n.d.)
EC_{50} – 24 hours (95 % C.I.):	> 20 mg a.s./L (n.d.)

Assessment and conclusion by applicant:

The study and its data are considered as acceptable and reliable for use in risk assessment.

The endpoint is: EC_{50} (48 hours) > 20 mg a.s./L

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Metabolite fluopyram-7-hydroxy

Data Point:	KCA 8.2.4.1/02
Report Author:	
Report Year:	2020
Report Title:	Daphnia sp. acute immobilisation test (following OECD 202 & OCSPP 850.1010) on BCS-AA10065 (7- hydroxy-fluopyram)
Report No:	RRCo-000776 01
Document No:	M-759029-01-1
Guideline(s) followed in study:	GUIDELINE OECD 202 (13 April 2004) Daphnia sp. Acute Immobilisation Test and OCSPP 850.1010: Aquatic Invertebrate Acute Toxicity Test, Freshwater Daphnids (December 2016).
Deviations from current test guideline:	Current Guideline: OECD 202 (2004) Deviations: None. All validity criteria were met.
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 test was performed with daphnids (*Daphnia magna*) under static conditions to determine the 48 – hour EC_{50}. First-instar neonate daphnids (< 24 hours old) were exposed to fluopyram-7-hydroxy to the single nominal concentration of 88.7 mg p.m./L with 4 replicates of 5 organisms. Additionally, an untreated control was included. Immobilisation and sub-lethal behavioural effects were determined after 24 and 48 hours.

Concentrations of fluopyram-7-hydroxy were verified by HPLC-MS/MS on day 0 and 2. Measured concentrations were in the 96 – 103 % range of nominal concentrations and no residues were found in the control samples. The limit of quantification was 0.010 mg p.m./L and limit of detection 0.033 mg p.m./L.

The study fulfils all validity criteria of OECD 202 guideline.

No immobility or other effects on behaviour were observed in the single test concentration and the control.

The endpoint based on nominal concentrations was: EC_{50} – 48 hours (95 % C.I.): > 88.7 mg p.m./L (n.d.).

I. MATERIAL AND METHODS

Test material	Fluopyram-7-hydroxy Batch No.: SES12367-10-8 Purity: 99.4 % w/w
Guideline(s) adaptation	None specified
Test species	Water flea (*Daphnia magna*)
Organism age/size at study initiation	First instar neonates, between 6 and 24 hours old

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Test solutions	Nominal concentration: 88.7 mg p m./L Corresponding measured concentrations were between 96 and 103 % of nominal concentrations Control: untreated medium Evidence of undissolved material: not reported
Replication	No. of vessels per concentration (replicates): 4 No. of vessels per control (replicates): 4
Organisms per replicate	No. of organisms per vessel: 5
Exposure	Static Total exposure duration: 48 hours
Feeding during test	None
Test conditions	Temperature: 20.0 – 20.3 °C Photoperiod: 16 hours light, 8 hours dark Light intensity: 97 - 102 lux pH: 7.6 – 7.8 Water hardness: 140 – 250 mg/L as $CaCO_3$ (culture medium) Dissolved oxygen: 7.6 - 8.2 mg/L Conductivity: not reported Alkalinity: not reported
Parameters Measured / Observations	Observations for immobility and sub-lethal behavioural effects were made after 24 and 48 hours of exposure. Dissolved oxygen and pH values were measured at test initiation and termination in one vessel per concentration. The temperature was continuously recorded during the test. Prior to test initiation total hardness was determined in the culture medium. Light intensity was measured at test initiation and termination.
Chemical analysis	The analytical samples were sampled at test start in the test solutions preparations and after 48 hours in the content of the vessels pooled by replicate. The chemical analyses were performed by using a High-performance liquid chromatograph (HPLC-MS/MS).
Data analysis	Since no treatment related effects were observed, a statistical evaluation was not performed.

II. RESULTS AND DISCUSSION

Table 8.2.4.1- 4: Validity criteria

Validity criteria acc. to OECD 202	Required	Obtained
Mortality in control during test	≤ 10 %	0 %
Dissolved oxygen concentration at the end of the test	≥ 3 mg/L	8.2 mg/L

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Analytical results:

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 SANTE/2020/12830, Rev.1.

Recoveries on day 0 and 2 ranged from 96 to 103 % of nominal values. The biological results are based on nominal concentrations.

No residues of fluopyram-7-hydroxy were detected in the control samples. The limit of quantification was 0.010 mg p.m./L and limit of detection 0.033 mg p.m./L.

Table 8.2.4.1- 5: Analytical results

Nominal concentration [mg p m./L]	Measured concentration [mg p.m./L]		% of nominal	
	Day 0	Day 2	Day 0	Day 2
88.7	85.538	91.37	96	103

Biological results:

Observations

No immobility or other effects on behaviour were observed in the single test concentration at 88.7 mg p.m./L and in the control.

Table 8.2.4.1- 6: Immobilisation of daphnids

Nominal concentration [mg p m./L]	No. of immobilized (cumulative %)	
	Exposure time	
	24 h	48 h
Control	0 (0)	0 (0)
88.7	0 (0)	0 (0)

III. CONCLUSION

The study meets the validity criteria according to OECD 202 and the endpoints based on nominal concentrations were:

EC_{50} – 48 hours (95 % C.I.):	**> 88.7 mg p.m./L** (not applicable)
EC_{50} – 24 hours (95 % C.I.):	> 88.7 mg p.m./L (not applicable)
LOEC – 48 hours: highest concentration without an effect	> 88.7 mg p.m./L
NOEC – 48 hours: highest concentration without an effect	88.7 mg p.m./L

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Assessment and conclusion by applicant:

The study and its data are considered as acceptable and reliable for use in risk assessment.

The endpoint is: EC_{50} (48 hours) > 88.7 mg p.m./L

Metabolite trifluoroacetic acid (TFA)

Data Point:	KCA 8.2.4.1/03
Report Author:	[redacted]
Report Year:	1992
Report Title:	The acute toxicity of sodium trifluoroacetate to Daphnia magna
Report No:	C047203
Document No:	M-247890-01-1
Guideline(s) followed in study:	OECD: no. 202; USEPA (=EPA): 72-2
Deviations from current test guideline:	Current Guideline: OECD 202 (2004) Deviations: The study comprised 3 replicates of 10 organisms per test level instead of 4 replicates of 5 organisms. These deviations were not expected to have impacted the study results. All validity criteria were met.
Previous evaluation:	yes, evaluated and accepted in flurtamone RAR (2017)
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 daphnids (*Daphnia magna*) under static conditions to determine the 48 – hour EC_{50}. First-instar neonate daphnids (< 24 hours old) were exposed to sodium trifluoroacetate in groups of 30 (3 replicates of 10 organisms per test level) to the single nominal concentration of 1200 mg p.m./L. Immobilisation and sub-lethal behavioural effects were determined after 24 and 48 hours.

Concentrations of sodium trifluoroacetate were verified by means of ion chromatography on day 0 and 2. Measured concentrations were in the 101 – 102 % range of nominal concentrations and no residues were found in the control samples above the limit of detection (LOD: 0.02 mg p.m./L).

The study fulfils all validity criteria of OECD 202 guideline.

No immobility or other effects on behaviour were observed in the single test concentration of 1200 mg p.m./L and in the control.

The endpoint based on nominal concentration of sodium trifluoroacetate was: EC_{50} – 48 hours (95 % C.I.): > 1200 mg p.m./L (not applicable).

The converted endpoint based on nominal concentrations of trifluoroacetic acid was: EC_{50} – 48 hours (95 % C.I.): > 1008 mg p.m./L (not applicable).

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I. MATERIAL AND METHODS

Test material	Sodium trifluoroacetate Batch No.: ACA9135AB Purity.: 99 % 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 concentration: 1200 mg p.m./L Mean measured concentration: 1215 mg p m./L Control: ISO-water Evidence of undissolved material: not reported
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: 19.4 - 20.5 °C Photoperiod: 16 hours light, 8 hours dark Light intensity: max. 1500 lux pH: 7.7 - 8.0 Water hardness: about 250 mg/L as $CaCO_3$ Dissolved oxygen: 8.4 - 8.6 mg/L Conductivity: not reported Alkalinity: not reported
Parameters Measured / Observations	Observations for immobility and sub-lethal behavioural effects were made after 24 and 48 hours of exposure. The temperature, dissolved oxygen and pH values were measured at test start and termination in one vessel per concentration. Prior to test initiation total hardness was determined. Also, light intensity was measured, however time point was not reported.
Chemical analysis	Duplicate samples were taken at test start from the test solution and at test end from composite solutions from the pooled samples of all replicates. The chemical analyses were performed by means of ion chromatography.
Data analysis	The EC_{50} (48h) was calculated using a probit analysis (procedure PROBIT of SAS). The NOEC was determined using Fisher's exact test.

II. RESULTS AND DISCUSSION

Table 8.2.4.1- 7: Validity criteria

Validity criteria acc. to OECD 202	Required	Obtained
Mortality in control during test	≤ 10 %	0 %
Dissolved oxygen concentration at the end of the test	≥ 3 mg/L	8.4 - 8.5 mg/L

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Analytical results:

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 SANTE/2020/12830, Rev.1.

Recoveries on day 0 and 2 ranged from 101 to 102 % of nominal values. The biological results are based on the nominal concentration.

No residues of sodium trifluoroacetate were detected in the control sample above the limit of detection (LOD: 0.02 mg p.m./L).

Table 8.2.4.1- 8: Analytical results

Nominal concentration [mg p.m./L]	Measured concentration [mg p m./L]		% of nominal [A]		Mean measured concentration [mg p.m./L]	% of nominal [A]
	Day 0	Day 2	Day 0	Day 2		
1200	1220	1210	102	101	1215	101

[A] Not given in report. Calculations based on measured concentrations on day 0 and day 2.

Biological results:

Observations

No immobility or other effects on behaviour were observed in the single test concentration (1200 mg p.m./L) and the control.

Table 8.2.4.1- 9: Immobilisation of daphnids

Nominal concentration [mg p.m./L]	No. of immobilized (cumulative %)	
	Exposure time	
	24 h	48 h
Control	0 (0)	0 (0)
1200	0 (0)	0 (0)

III. Conclusion

The study meets the validity criteria according to OECD 202 and the endpoints based on nominal concentrations of sodium trifluoroacetate were:

EC_{50} – 48 hours (95 % C.I.):	**> 1200 mg p m./L [A]** (not applicable)
NOEC – 48 hours: highest concentration without an effect	1200 mg p m./L [A]

p.m.: pure metabolite referring to sodium trifluoroacetate

[A] Based on the molecular weights, a concentration of 1200 mg sodium trifluoroacetate/L corresponds to 1008 mg trifluoroacetate acid/L. As the study was conducted with sodium trifluoroacetate which is the sodium salt of trifluoroacetic acid, the endpoint was converted to trifluoroacetic acid with factor 0.84.

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Assessment and conclusion by applicant:

The study and its data are considered as acceptable and reliable for use in risk assessment.

The endpoint is: EC_{50} (48 hours) > 1200 mg p.m./L (sodium trifluoroacetate) corresponding to > 1008 mg p.m./L (trifluoroacetic acid)

CA 8.2.4.2 Acute toxicity to an additional aquatic invertebrate species

Active substance fluopyram

Data Point:	KCA 8.2.4.2/01
Report Author:	[redacted]
Report Year:	2008
Report Title:	AEC656948 - Toxicity to marine amphipods (Leptocheirus plumulosus) during a 10-day sediment exposure
Report No:	13798.6240
Document No:	M-297751-01-1
Guideline(s) followed in study:	OPPTS Draft Guideline 850.1740
Deviations from current test guideline:	Current Guideline: OPPTS Guideline 850.1740 Deviations: The age of the test organism was not reported (immature amphipods). The temperature of overlying water was between 23 – 26 °C and thus below the minimum 24 °C as recommended in OPPTS 850.1740. The overlying water volume was 750 mL and thus slightly below the recommended 800 mL. The light duration was 24 hours and not 12 hours as recommended. The light intensity ranged between 500 and 830 lux and thus below the minimum 540 lux as recommended. No aeration was provided although there were static conditions. The salinity of overlying water was 20 - 24 ‰ and thus outside the recommended range of 20 ± 3 ‰. These deviations were not expected to have impacted the study results. All validity criteria were met.
Previous evaluation:	yes, evaluated and accepted in DAR (2011)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

A chronic study was performed with estuarine amphipod (*Leptocheirus plumulosus*) under static conditions of the overlying water for a period of 10 days. Fluopyram was applied at nominal sediment concentrations of 5.8, 14, 26, 51 and 100 mg a.s./kg. A water control and a solvent control were also included. The test comprised 5 replicates of 20 organisms for each group. Mortality and abnormal behaviour were recorded daily. At test termination (day 10), the total number of surviving amphipods was determined in each test vessel.

Concentrations of fluopyram in sediment, pore water and overlying water were verified by LSC on days 0 and 10 for each concentration and controls. Measured sediment concentrations were in the 92 - 125 % range of nominal concentrations. In the control and solvent control the mean measured sediment concentrations of total [^{14}C]- residues as fluopyram equivalents were reported to be between < 0.017

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and < 0.038 mg a.s./kg. The mean measured sediment concentrations were: 5.8, 14, 26, 51 and 100 mg a.s./kg.

The study fulfils all validity criteria of the OPPTS Guideline 850.1740.

Amphipod survival showed no statistical significance in any test concentration and the controls.

The endpoints based on mean measured sediment concentrations were: NOEC - 10 days: 100 mg a.s./L and LOEC- 10 days: > 100 mg a.s./L.

I. MATERIAL AND METHODS

Test material:	Non-radiolabelled test substance: Fluopyram Spec. No.: 102000012455 Batch No: 08528/0002 Purity: 94.7 % w/w	Radiolabelled test substance: [^{14}C]-fluopyram- pyridyl-2,6 Sample ID: BECH 2168 Batch No: not reported Radiochemical purity: > 99 %
Guideline(s) adaptation	None specified	
Test species:	Estuarine Amphipod *(Leptocheirus plumulosus)*	
Culturing conditions	Amphipods were maintained in plastic culture tubs with a 1- to 2-cm deep layer of 0.25 mm sieved marine sediment and 7 to 8 L of 20 ppt seawater. The dissolved oxygen concentration was 7.1 mg/L and the temperature ranged between 22 and 27 °C. Culture water was the same water used as overlying water during the test. No mortality was observed in the test population 48 hours prior to test initiation.	
Organism age/ size at study initiation:	Juvenile amphipods, size range: 2 - 4 mm	
Preparation of spiked sediment	A 9 mL volume of each dosing stock solution was applied to 0.05 kg of fine silica sand placed in glass Petri dishes. The solvent was allowed to evaporate off the sand for 45 minutes. The dry sand, containing the test substance, was then added to 2.5 kg of wet natural sediment. The jars with sediment were sealed and rolled at approximately 15 rpm for four hours at room temperature. Following the four hours of rolling the jars were stored upright at 4 °C overnight. Sediments were allowed to equilibrate for 14 days. Once a week during the equilibration period and prior to addition into the replicate exposure vessels (test day –1), the jars were mixed on the rolling mill for an additional two hours at room temperature to ensure the sediment was homogeneous.	
Test concentrations	Nominal sediment concentrations 6.0, 12, 24, 47, 95 mg a.s./kg Corresponding mean measured sediment concentrations: 5.8 – 14 – 26 – 41 – 100 mg a.s./kg Control: water Solvent control: acetone (9 ml acetone per 0.0500 kg of fine silicia sand) Evidence of undissolved material: Stock solutions were clear and with no visible undissolved test substance.	
Replication:	No. of vessels per concentration (replicates): 5 No. of vessels per control (replicates): 5 No. of vessels per solvent control (replicates): 5 In addition 4 replicates were maintained for chemical analysis.	
Organisms per replicate:	No. of organisms per vessel: 20	
Exposure:	Static conditions of the overlying water (spiked sediment test) Total exposure duration: 10 days	

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Feeding during test	No feeding during test.
Test conditions:	Water temperature: overlying water: 24 - 26 °C (continuous measurement in water bath revealed 24 - 26 ºC); pore water: 21 - 22 °C Photoperiod: 24 h light Light intensity: 500 - 830 lux pH: overlying water: 7.7 - 8.3; pore water: 6.9 - 7.3 Total ammonia as nitrogen: overlying water: 57 - 190 mg/L; pore water: 170 - 440 mg/L Water hardness: not reported Dissolved oxygen: 4.6 - 7.5 mg/L (corresponding to 62 - 102.3 % of saturation; based on 25 °C, salinity of 20 ‰) Conductivity: not reported Salinity: 20 - 24 ‰ Sediment volume: 175 mL (approx. 2 cm layer) Overlying water volume: 725 mL Depth of sediment and overlying water: not reported
Sediment	Natural freshwater sediment (collected from Little Harbor Beach, Wareham, Massachusetts, USA): % organic carbon: 3.7 % sand: 80 % silt: 10 % clay: 10 pH: 8.2 % moisture at 1/3 bar: 46.2 %
Parameters Measured / Observations	Temperature, pH, dissolved oxygen concentration and salinity were measured in the overlying water of each replicate vessel of each treatment level and control used for biological monitoring at test initiation and termination. On test days 1 to 9 temperature, dissolved oxygen, pH and salinity were measured in one alternating replicate each day. In addition, temperature was continuously monitored in an auxiliary vessel in the temperature controlled water bath. Total ammonia concentration of the overlying water was monitored at test initiation and test termination in each treatment level and control solution from a sample of overlying water from each treatment and control. In addition, total ammonia, temperature and pH were measured on days 0 and 10 in the additional replicates established for pore water measurements. All vessels were examined at test initiation and at 24-hour intervals thereafter, until test termination (day 10). Observations of mortality and abnormal behaviour were made and the physical characteristics of the test samples were recorded. At test termination (day 10), the total number of surviving amphipods was determined in each test vessel by sieving the entire volume of sediment to remove all surviving amphipods.
Sampling for chemical analysis	During the in-life phase of the definitive study, sediment, pore water and overlying water samples were removed and analysed for total [^{14}C]-residue concentration on test days 0 and 10. On day 0, samples were removed and analysed from replicate vessels “F” of all treatments and controls, while on day 10, samples were removed and analysed from replicate “G” of all treatment levels and controls. Chemical analysis of sediment, pore water and overlying water samples were performed by liquid scintillation counting (LSC).
Data analysis:	A t-Test was conducted for the survival data to compare the performance of the control organisms with that of the solvent control organisms. During the study, the t-Test indicated no significant difference between control and solvent control survival data. Therefore, data from all dose levels was compared to the pooled control data. Chi-Square Test for normality was conducted to compare the observed sample distribution with a normal distribution

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	As a check on the assumption of homogeneity of variance implicit in parametric statistics, data were analyzed using Bartlett's Test. The survival data passed the test for homogeneity. For this study, the survival data met the assumptions for normal distribution and homogeneity, therefore, Bonferroni's t-Test was used to establish treatment effects on amphipod survival. All statistical analyses were used to establish, at the 95 % level of certainty, the lowest test concentration that showed a statistically significant effect (Lowest-Observed-Effect Concentration, LOEC) and the highest test concentration that showed no statistically significant difference (No-Observed-Effect Concentration, NOEC) from the control data. TOXSTAT® Version 3.5 was used to calculate the LOEC and NOEC values. The LC_{50} is the estimated measured sediment concentration of the test substance which produces 50 % mortality in the test population of amphipods at test termination. During this study, no concentration tested resulted in ≥ 50 % mortality, therefore, the LC_{50} value was empirically estimated to be greater than the highest mean measured sediment concentration tested.

II. Results and Discussion

Table 8.2.4.2- 1: Validity criteria

Validity criteria according to OPPTS 850.1740	**Required**	**Obtained**
Survival of amphipods in controls	≥ 80 %	99 % (control and solvent control)
Dissolved oxygen saturation	> 4.4 mg/L (> 60 % saturation)	4.6 - 7.5 mg/L (62 - 102 % of saturation)

Analytical results:

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 SANTE/2020/12830, Rev.1.

Measured sediment concentrations on day 1 and day 10 ranged between 92 and 125 % of the nominal concentrations (see table below). Results are based on mean measured sediment concentrations.

In the control and solvent control samples the mean measured sediment concentrations of total [^{14}C]-residues as fluopyram equivalents were reported to be between < 0.017 and < 0.038 mg a.s./kg.

Furthermore, measured concentrations in overlying water and pore water are shown below.

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Table 8.2.4.2- 2: Analytical results: Measured concentrations of total [^{14}C]-residues in sediment

Nominal sediment concentration [mg a.s./kg]	Measured concentration [mg a.s./kg]		% of nominal [A]		Mean measured sediment concentration [mg a.s./kg] [B]	Mean % of nominal
	Day 0	Day 10	Day 0	Day 10		
Control	< 0.038	< 0.017	-	-	-	-
Solvent control	< 0.038	< 0.017	-	-	-	-
6.0	5.5	6.2	92	103	5.8	97
12	12	15	100	125	14	110
24	24	28	100	117	26	110
47	49	54	104	115	51	110
95	91	110	96	116	100	110

[A] Not given in study report. Calculated on the basis of nominal and measured concentrations.
[B] Mean measured values were calculated using the rounded values presented in this table.

Table 8.2.4.2- 3: Analytical results: Measured concentration of total [^{14}C]-residues, measured by LSC analysis, in pore and overlying water samples during the 10-day toxicity test

Nominal sediment concentration [mg a.s./kg]	Day 0	Day 10
Pore water - measured concentration [mg a.s./L]		
Control	< 0.0057	< 0.0056
Solvent control	< 0.0057	< 0.0056
6.0	0.53	0.33
12	1.1	0.84
24	2.2	1.6
47	4.5	3.3
95	7.8	7.2
Overlying water - measured concentration [mg a.s./L]		
Control	< 0.0022	< 0.0022
Solvent control	< 0.0022	< 0.0022
6.0	0.041	0.17
12	0.089	0.37
24	0.16	0.69
47	0.34	1.4
95	0.64	2.6

Biological results:

Following 10 days of exposure, amphipod survival in both the control and solvent control averaged 99 %. Statistical analysis determined no significant difference between control and solvent control survival. At test termination (test day 10), survival observed among amphipods exposed to the 5.8, 14, 26, 51 and 100 mg a.s./kg treatment levels was 99, 95, 94, 97 and 98 % respectively.

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Table 8.2.4.2- 4: Results for survival after 10 days

Mean measured sediment concentration [mg a.s./kg]	Mean % survival (± SD)
Control	99 (± 2)
Solvent control	98 (± 3)
Pooled control	99 (± 2)
5.8	99 (± 2)
14	95 (± 5)
26	94 (± 8)
51	97 (± 7)
100	98 (± 3)

SD: Standard deviation

III. CONCLUSION

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

Endpoint	Midge survival
LC_{50} - 10 days (95 % C.I.):	> 100 mg a.s./kg (n.a.)
LOEC - 10 days: lowest concentration with an effect	> 100 mg a.s./kg
NOEC - 10 days: highest concentration without an effect	**100 mg a.s./kg**

n.a.: not applicable; LC_{50} value was empirically estimated, therefore, corresponding 95 % confidence interval could not be calculated.

Assessment and conclusion by applicant:

The study and its data are considered as acceptable and reliable for use in risk assessment.

The endpoint is: NOEC (10 days) = 100 mg a.s./kg

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Data Point:	KCA 8.2.4.2/02
Report Author:	████████████████████
Report Year:	2006
Report Title:	AE C656948: A 96-hour shell deposition test with the eastern oyster (Crassostrea virginica)
Report No:	149A-222
Document No:	M-282691-01-1
Guideline(s) followed in study:	U.S. EPA OPPTS Number 850.1025
Deviations from current test guideline:	Current Guideline: OCSPP 850.1025 oyster Deviations: The valve height at test start ranged between 29.5 - 40.5 mm and thus slightly lower than the recommended range of 30-50 mm. The light intensity was 177 lux at test initiation, and thus below the recommended range of 540-1080 lux. The TOC was not reported. These deviations were not expected to have impacted the study results. All validity criteria were met.
Previous evaluation:	yes, evaluated and accepted in DAR (2011)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The Eastern oysters (*Crassostrea virginica*) were exposed to fluopyram under flow through conditions to determine the 96 hour-EC_{50}. The following nominal concentrations were included in the study: 0.038, 0.075, 0.15, 0.30 and 0.60 mg a.s./L. There was 1 replicate with 20 organisms for each test concentration and the controls (control and solvent control). Biological observations were made after 4, 24, 48, 72 and 96 hours.

Concentrations of fluopyram technical were verified by HPLC – UV detection at test initiation (0 hour) after 24, 48 and 96 hours. Measured concentrations were in the 68 - 99 % range of nominal concentrations and no residues were found in the control and solvent control samples above the LOQ (0.025 mg a.s./L). The biological results were reported based on the mean measured concentrations of 0.032, 0.070, 0.14, 0.27 and 0.44 mg a.s./L.

The study fulfils all validity criteria of OPPTS 850.1025 guideline.

There were no mortalities among oysters in any treatment or control group during the test. All oysters appeared normal throughout the 96-hour exposure period.

The endpoints based on mean measured concentrations were: EC_{50} – 96 hours: > 0.44 mg a.s./L and NOEC – 96 hours: 0.44 mg a.s./L.

I. MATERIAL AND METHODS

Test material	Fluopyram (AE C656948) Specification No.: 102000012455 Batch No: 08528/0002 Purity: 94.7 % w/w
Guideline(s) adaptation	None specified
Test species	Eastern oyster (*Crassostrea virginica*)
Acclimation	10 days prior testing No mortality during 10 days before test initiation

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Organism age/size at study initiation	The oysters were of similar age and had a mean length of 33.6 ± 2.7 mm (n = 20).
Test solutions	Nominal concentrations: 0.038 – 0.075 – 0.15 – 0.30 – 0.60 mg a.s./L (corrected for purity) Mean measured concentrations: 0.032 – 0.070 – 0.14 – 0.27 – 0.44 mg a.s./L Control: filtered saltwater Solvent control: dimethylformamide (0.1 mL/L) Evidence of undissolved material: At test initiation, all solutions in mixing chambers and test chambers appeared clear and colourless. At test termination, all the solutions in the diluter mixing chambers appeared as a clear and light green solution, due to the algal feed. The solutions in the test chambers appeared as a slightly cloudy and green solution as well as dark green precipitate on bottom, due to the algal feed and oyster waste.
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: 20
Exposure	Flow through (at least 1 L of test solution per oyster per hour) Total exposure duration: 96 hours
Feeding during test	Suspension of marine microalgae (Reed Mariculture, Campbell, California) provided continuously at a rate of 5.8×10^9 cells/oyster/day
Test conditions	Temperature: 20.0 - 21.5 °C Photoperiod: 16 hours light, 8 hours dark; with a 30-minute transition period Light intensity: 177 lux (at test start) Type of light: fluorescent light bulbs pH: 7.9 - 8.2 Dissolved oxygen: 5.8 - 7.9 mg/L (≥ 72 % of saturation; 60 % of saturation corresponds to a dissolved oxygen concentration of 4.8 mg/L at salinity of 20 ‰) Salinity: 20 ‰
Parameters Measured / Observations	Oysters were inspected visually after approx. 4, 24, 48, 72 and 96 hours to determine the numbers of mortalities and the numbers of individuals exhibiting sub-lethal signs of toxicity. At the end of the test, the longest finger of new shell growth on each oyster was measured. Temperature was measured in each test chamber at test initiation and termination. In addition, continuous measurements of the temperature were made in the control test chamber. Dissolved oxygen was measured in each test chamber at test initiation and after 24, 48, 72 and 96 hours, pH was measured in each test chamber at test initiation, after 48 hours and at test termination. Salinity was measured in each test chamber at test initiation and termination. Light intensity was determined at test initiation
Chemical analysis	Three days prior to test initiation, samples were collected from each treatment and control group. During test samples were collected from each test chamber and mixing chamber for each treatment and control group at approximately 0, 24, 48 and 96 hours. Further samples of the stock solutions were collected after 24 hours due to low recoveries on day 0. All samples were analysed using high performance liquid chromatography (HPLC) with UV detection (220 nm).
Data analysis	Negative control and solvent control shell deposition data were compared using an appropriate t-test. The EC_{50} value, the concentration of test substance that would inhibit shell deposition by 50 % relative to the pooled control, was determined by a visual assessment of data due to no inhibition in shell growth. The shell deposition data were evaluated for normality and homogeneity of variance using the Chi-Square test and Levene's test, respectively. Since the data passed the assumptions of normality and homogeneity, the data in the treatment groups were compared to the pooled control data

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	using analysis of variance (ANOVA) and Bonferroni's t-test to identify any significant differences (5). The no-observed-effect-concentration (NOEC) was determined from the statistical analysis of the data and an assessment of the concentration-response pattern. Statistical analyses were conducted using the TOXSTAT® computer program.

II. RESULTS AND DISCUSSION

Table 8.2.4.2- 5: Validity criteria

Validity criteria acc. to OPPTS 850.1025	Required	Obtained
Mortality in control during test	≤ 10 %	0 % (in control and solvent control)
Dissolved oxygen concentration during test	≥ 60 %	≥ 73 % (≥ 5.8 mg/L)
New shell growth in control oysters (control and solvent control)	≥ 2 mm	Control: 1.9 – 6.8 mm Solvent control: 3.2 – 7.2 mm
Evidence that the concentration of the substance being tested has been satisfactorily maintained over the test period	-	Fulfilled [A]
No evidence of spawning during the test	-	Fulfilled

[A] Considered fulfilled. During the test the recoveries were slightly lower than the minimum required 80 % (68.2 to 98.9 % of nominal concentrations. However, the pre-test diluter verification samples from the test chambers revealed recoveries between 85 and 94 % of nominal concentrations. Furthermore, the measured concentrations in the stock solutions ranged between 96.0 and 96.7 % of nominal concentrations. Therefore, the correct concentrations were being delivered to the diluter.

Analytical results:

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 SANTE/2020/12830, Rev.1.

Analysis of the test concentrations ranged in all test concentrations between 68.2 and 98.9 % of the nominal concentrations (see table below). At the highest test concentration slightly lower recoveries ranging between 68.2 and 87.1 % of nominal concentration were determined, indicating the test was conducted near the limit of solubility in saltwater. The recoveries for the 4 lowest test concentrations (0.038 to 0.30 mg a.s./L) were between 74.7 and 98.9 % of nominal concentration. Therefore, biological results were based mean measured concentrations.

No residues of fluopyram were found in the control samples above the limit of quantification (LOQ: 0.0250 mg a.s./L).

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Table 8.2.4.2- 6: Analytical results

Nominal concentration [mg a.s./L]	Measured concentration [mg a.s./L]				% of nominal				Mean measured concentration [mg a.s./L]	% of nominal
	0 h [A]	24 h	48 h	96 h	0 h [A]	24 h	48 h	96 h		
0.038	0.0360	0.0311	0.0310	0.0283	94.7	81.7	81.7	74.5	0.032	84
0.075	0.0742	0.0684	0.0699	0.0680	98.9	91.2	93.2	90.6	0.070	93
0.15	0.137	0.136	0.143	0.136	91.6	90.3	95.1	90.8	0.14	93
0.30	0.286	0.255	0.275	0.244	95.4	85.1	91.6	81.4	0.27	90
0.60	0.523	0.409	0.423	0.418	87.1	68.2	70.5	69.7	0.44	73

[A] 0-hour sample results initially were low, ranging from <LOQ to approximately 70 % of nominal, due to an elevated curve in the linear regression analysis, which caused the samples to appear to yield lower recoveries. The samples were reanalysed after approximately one month of ambient storage and the results are presented. The initial 0-hour sample results were excluded from calculation of the mean measured concentrations.

Biological results:

Observations

There were no mortalities or clinical signs of toxicity observed at any concentration tested.

There was no statistically significant difference between the control and solvent control data (p>0.05). Therefore, growth inhibition in the treatment groups was evaluated on the basis of the pooled control data.

Table 8.2.4.2- 7: Shell deposition and shell growth inhibition

Mean measured concentration [mg a.s./L]	Mean shell deposition at 96 h ± SD [A] [mm]	% Inhibition of shell growth [B]
Control	4.6 ± 1.3	-
Solvent Control	5.4 ± 1.1	-
Pooled Control	5.0 ± 1.2	-
0.032	4.7 ± 1.3	6.0
0.070	5.1 ± 1.6	-2.0
0.14	5.0 ± 1.2	0.0
0.27	5.0 ± 1.3	0.0
0.44	5.1 ± 1.6	-2.0

[A] Mean and standard deviation for 20 oysters

[B] % inhibition from the pooled control

III. CONCLUSION

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

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EC_{50} – 96 hours (95 % C.I.): (based on shell deposition and mortality)	**> 0.44 mg a.s./L** (n.d.)
NOEC – 96 hours: highest concentration without an effect	0.44 mg a.s./L

Assessment and conclusion by applicant:

The study and its data are considered as acceptable and reliable for use in risk assessment.

The endpoint is: EC_{50} (96 hours) > 0.44 mg a.s./L

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Data Point:	KCA 8.2.4.2/03
Report Author:	[redacted]
Report Year:	2007
Report Title:	AE C656948 - A 96-hour flow-through acute toxicity test with the saltwater mysid (Americamysis bahia)
Report No:	149A-221
Document No:	M-282839-01-2
Guideline(s) followed in study:	OPPTS Guideline 850.1035
Deviations from current test guideline:	Current Guideline: OCSPP 850.1035 acute mysid Deviations: The TOC was not reported. This missing information was not expected to have impacted the study results. All validity criteria were met.
Previous evaluation:	yes, evaluated and accepted in DAR (2011)
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 saltwater mysids (*Americamysis bahia*) under flow through conditions to determine the 96 hour-LC_{50}. Juvenile saltwater mysids (< 24 hours old) were exposed to fluopyram in groups of 20 (two replicates of 10 mysids) to the nominal concentrations of 0.038, 0.075, 0.15, 0.30, and 0.60 mg a.s./L for a period of 96 hours. Additionally, a control and solvent control were included. Biological observations were made after 3, 24, 48, 72 and 96 hours.

Concentrations of fluopyram were verified by HPLC at test initiation (0 hour) and after 48 and 96 hours. Measured concentrations were in the 78 - 117% range of nominal concentrations and no residues were found in the control and solvent control samples above the LOQ (0.0250 mg a.s./L). The biological results were reported based on mean measured concentrations which were: 0.043, 0.079, 0.15, 0.28 and 0.50 mg a.s./L.

The study fulfils all validity criteria of OPPTS 850.1035 guideline.

All mysids in the negative and solvent control groups appeared normal throughout the test. All mysids in the 4 highest test concentration (0.043, 0.079, 0.15 and 0.28 mg a.s./L, mean measured concentrations) also appeared normal throughout the test, with no mortalities or sublethal signs of toxicity noted. In the highest test concentration (0.50 mg a.s./L, mean measured concentration), there were two mysids (10 %) missing and presumed dead by test termination. All surviving mysids appeared normal.

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The endpoints based on mean measured concentrations were: LC_{50}-96 hours: > 0.50 mg a.s./L and NOEC-96 hours: 0.28 mg a.s./L.

I. Material and methods

Test material	Fluopyram (AE C656948) Specification No.: 102000012455 Batch No.: 08528/0002 Purity: 94.7 % 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: 0.038 – 0.075 – 0.15 – 0.30 – 0.60 mg a.s./L Mean measured concentrations: 0.043 – 0.079 – 0.15 – 0.28 – 0.50 mg a.s./L Control: filtered saltwater Solvent control: dimethylformamide (0.1 mL/L) Evidence of undissolved material: No visible precipitates were observed. All test solutions appeared clear and colourless in the test chambers and in the diluter mixing chambers at test initiation and termination.
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	Flow-through (22 volume additions of test water (turnovers) every 24 hours, 110 L per day) Total exposure duration: 96 hours
Feeding during test	Live brine shrimp *Artemia nauplii* added to each test vessel once daily.
Test conditions	Temperature: 24.0 - 25.0 °C Photoperiod: 16 hours light / 8 hours dark with a 30-minute transition period. Light intensity: 779 lux (at test start) pH: 8.0 Dissolved oxygen: 5.5 - 6.9 mg/L (≥ 75 % saturation; 60 % corresponds to a dissolved oxygen concentration of 4.4 mg/L at a salinity of 20 ‰.) Salinity: 20 ‰ (at test start and termination)
Parameters Measured / Observations	Biological observations were made approximately 3, 24, 48, 72 and 96 hours after test initiation. Temperature was measured in each test chamber at test start and after 96 hours and in addition continuously during the test in one negative control test chamber. pH and dissolved oxygen concentration were measured in alternating replicate test vessels of each treatment and control group at test start and after 24, 48, 72 and 96 hours. Salinity was measured in the dilution water at the beginning and end of the test. Light intensity was determined at test initiation at the surface of the water of one representative test chamber.
Chemical analysis	Three days prior to test initiation, samples were collected from one test chamber of each treatment and control group. Due to low recoveries in the highest test concentration (0.60 mg a.s./L) an additional sample was collected on day 0. During test, samples for

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	analysis were taken from alternating replicate test chambers at test initiation (0 hour) and after 48 and 96 hours. After 96 hours, a sample was collected from each replicate of the second highest test concentration (0.075 mg a.s./L) to confirm the concentration since the test chamber and compartment were replaced on day 3 in one of the replicates due to the presence of a biological film. All samples were analysed using high performance liquid chromatography (HPLC).
Data analysis	There was < 50 % mortality in all treatment groups at test termination, so LC_{50} values could not be statistically calculated. Therefore, the 24, 48, 72 and 96-hour 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

Table 8.2.4.2- 8: Validity criteria

Validity criteria acc. to OPPTS 850.1035	Required	Obtained
Mortality in control during test	≤10 %	0 % (control and solvent control)

Analytical results:

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 SANTE/2020/12830, Rev.1.

Analysis of the test concentration at 0 and 96 hours ranged between 78 and 117 % of the nominal concentrations (see table below). Biological results were based on mean measured concentrations.

No residues of fluopyram were found in the control and solvent control samples above the limit of quantification (LOQ 0.0250 mg a.s./L).

Table 8.2.4.2- 9: Analytical results

Nominal concentration [mg a.s./L]	Mean measured concentration [mg a.s./L]			% of nominal			Mean measured concentration [mg a.s./L]	Mean % of nominal
	0 h	48 h	96 h	0 h	48 h	96 h		
0.038	0.0446	0.0435	0.0410	117	115	108	0.043	113
0.075	0.0795	0.0797	0.0793 [A]	106	106	106 [A]	0.079	105
0.15	0.157	0.154	0.147	105	102	89.0	0.15	100
0.30	0.309	0.257	0.261	103	85.5	86.9	0.28	93
0.60	0.527	0.512	0.469	87.8	85.3	78.1	0.50	83

[A] Not given in report. Calculations based on measured concentrations of two replicates. Samples were analysed from both replicates at 96 hours due to replacement of the test chamber and compartment in replicate B on day 3.

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Biological results:

Observations

All mysids in the negative and solvent control groups appeared normal throughout the test.

All mysids in the 4 highest test concentration (0.043, 0.079, 0.15 and 0.28 mg a.s./L, mean measured concentrations) also appeared normal throughout the test, with no mortalities or sublethal signs of toxicity noted. In the highest test concentration (0.50 mg a.s./L, mean measured concentration), there were two mysids (10 %) missing and presumed dead by test termination. All surviving mysids appeared normal.

Table 8.2.4.2- 10: Cumulative Mortality during the test

Mean measured concentration [mg a.s./L]	Cumulative mortality [%]				
	Exposure time				
	4 h	24 h	48 h	72 h	96 h
Control	0	0	0	0	0
Solvent Control	0	0	0	0	0
0.043	0	0	0	0	0
0.079	0	0	0	0	0
0.15	0	0	0	0	0
0.28	0	0	0	0	0
0.50	0	0	0	10	10

III. CONCLUSION

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

LC_{50} – 96 hours (95 % C.I.):	**> 0.50 mg a.s./L** (n.d.)
NOEC – 96 hours: highest concentration without an effect	0.28 mg a.s./L

Assessment and conclusion by applicant:

The study and its data are considered as acceptable and reliable for use in risk assessment.

The endpoint is: LC_{50} (96 hours) > 0.50 mg a.s./L

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CA 8.2.5 Long-term and chronic toxicity to aquatic invertebrates

CA 8.2.5.1 Reproductive and development toxicity to Daphnia magna

Active substance fluopyram

Data Point:	KCA 8.2.5.1/01
Report Author:	██████
Report Year:	2008
Report Title:	Influence of AE C656948 (tech.) on development and reproductive output of the waterflea Daphnia magna in a static renewal laboratory test system
Report No:	EBGMP047
Document No:	M-282102-02-1
Guideline(s) followed in study:	OECD-211, dated September 21, 1998: Daphnia magna Reproduction Test, EEC Directive 92/69/EWG, part C.20, U.S. EPA-Pesticide Assessment Guidelines, Subdivision E, §72-4, dated October, 1982: ...aquatic invertebrate life-cycle studies, U.S. EPA- OPPTS Guideline 850.1300, dated April 1996: Daphnid chronic toxicity test- public Draft; US EPA FIFRA paragraph 72-4; Canadian PMRA Ref.: DACO 9.3.3; EU Council Directive 91/414/EEC (1991)
Deviations from current test guideline:	Current Guideline: OECD 211 (2012) Deviations: None. All validity criteria were met.
Previous evaluation:	Yes, evaluated and accepted in DAR (2011)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

A chronic test was performed with *Daphnia magna* under static-renewal conditions (approx. 3 water renewals per week) for 21 days. Fluopyram was applied at nominal concentrations of 80, 200, 500, 1250 and 3125 µg a.s./L. Additionally, a control and solvent control were included. The test comprised 10 replicates of 1 organism for each group. Observations for sub-lethal effects and survival (immobilisation) were made daily. Reproductive output (neonates counts) were determined at the time of first brood release and then daily. Growth determinations (length and dry weight) were made at the end of the exposure.

Concentrations of fluopyram were verified by HPLC at test start and on day 2, 9, 12, 19 and 21 for each concentration and control. Measured concentrations were in the 92 - 104 % range of nominal concentrations and no residues were found in the control and solvent control samples above 5.14 µg a.s./L, which was used as the lowest standard concentration during the study. The mean measured concentrations were: 80, 197, 496, 1222 and 2996 µg a.s./L.

The study fulfils all validity criteria of OECD 211 guideline.

There were no immobilisations of adult daphnids in any test concentration and the controls (control and solvent control). Regarding the data for lengths on day 21, there was a statistically significant effect in the highest test concentration (3125 µg a.s./L, nominal test concentration) compared to the pooled control group. The time to first brood ranged from 9 to 10 days with no statistically significant differences. Statistical analysis regarding neonates per adult total reproduction and reproduction per day indicated a significant effect in the highest test concentration (3125 µg a.s./L, nominal test concentration).

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The endpoints based on nominal concentrations were: NOEC- 21 days: 1250 µg a.s./L and LOEC- 21 days: 3125 µg a.s./L. The MATC (Maximum Acceptable Toxicant Concentration), calculated as the geometric mean between NOEC and LOEC, was 1976 µg a.s./L.

I. Material and Methods

Test material:	Fluopyram (AE C656948) Specification No.: 102000012455 Batch no.: 08528/0002 Purity: 94.7 % 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: 80 – 200 – 500 – 1250 – 3125 µg a.s./L Corresponding mean measured concentrations: 80 – 197 – 496 – 1222 and 2996 µg a.s./L Control: water Solvent control: dimethylformamide (0.1 mL/L) Evidence of undissolved material: not reported
Replication:	No. of vessels per concentration (replicates): 10 No. of vessels per control (replicates): 10
Organisms per replicate:	No. of organisms per vessel: 1
Exposure:	Static-renewal conditions (approx. 3 water renewals per week) Total exposure duration: 21 days
Test Vessel Loading:	100 mL of test solution/daphnid
Feeding during test	Green algae (*Desmodesmus subspicatus*) were fed a daily amount of 0.2 mg total organic carbon per test vessel with 100 mL (corresponding to 1 x 10^8 cells/L). On day 2 the three fold amount was fed for the first weekend where no feeding occurred.
Test conditions:	Temperature: 20.6 - 21.0 °C Photoperiod: 16 h light, 8 h dark Light intensity: max. 1500 lux pH: 7.8 - 8.3 Water hardness: 231 - 249 mg/L as $CaCO_3$ (13 - 14 °dH, calculated based on 1 °dH= 17.8 mg/L as $CaCO_3$) Dissolved oxygen: 7.6 - 9.5 mg/L (≥ 85 % of saturation, in control: 85 - 105 % of saturation) Conductivity: 574 - 581 µS/cm Alkalinity: 53 mg/L as $CaCO_3$
Parameters Measured / Observations	Prior to each preparation of test concentrations, pH, conductivity, total hardness and alkalinity of the used dilution media (ELENDT M7) were measured. Content of dissolved oxygen, pH, total hardness and alkalinity were measured in the freshly prepared test solutions of each treatment concentration and controls and repeatedly in the aged media at the end of each two or three days lasting exposure interval (pooled replicates). Temperature of the test media was measured inside one vessel of the untreated control, of the exposure concentration 200 µg a.s./L and of the highest test concentration at start and

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	end of each renewal interval. Light intensity was measured at start of the study as „diffuse light“. Observations for sub-lethal effects and survival (immobilisation) were made daily. Reproductive output (neonates counts) were determined at the time of first brood release and then daily. Growth determinations (length and dry weight) were made at the end of the exposure.
Chemical analysis	For analytical verification of the test item concentrations, duplicate samples of the freshly prepared test media have been taken on day 0, 9 and day 19 from bulk preparation for each treatment level and controls prior to introducing the food and test animals. For additional stability measurements the aged replicates were pooled on days 2, 12 and 21 and samples then taken from this pooled media for analysis. The chemical analyses were performed by high-performance liquid chromatograph (HPLC).
Data analysis:	Reproductive output data and parental body mass and body length data were analysed on variance homogeneity (Cochran's Test) and normal distribution (Kolmogorroff-Smirnov Test) on a 5 % level of significance using the treatment levels and pooled controls as co-variates. Parametric procedures involved subjecting reproduction data to a standard one way analysis of variance (ANOVA). If significant differences among the means were indicated, multiple comparison procedures (e.g. Dunnett's multiple t-test procedure or, in case of monotonous decrease of responses, adequate step down trend-tests (e.g. Williams multiple sequential t-test procedure) were performed on a 5 % level of significance ($p < 0.05$ / one-sided [smaller] probability), to indicate which treatment groups differed significantly from the control. For non-parametric procedures, the Mann-Whitney-Wilcoxon U-test for independent samples was applicable. Alternatively, corresponding multiple comparison procedure with Bonferroni-Correction is applicable. All statistical procedures were carried out by using the ToxRat Professional© Software (Vers.2.09, ToxRat Solutions GmbH, Germany).

II. RESULTS AND DISCUSSION

Table 8.2.5.1- 1: Validity criteria

Validity criteria acc. to OECD 211	Required	Obtained
Mortality of the parent animals in control at the end of the test	≤ 20 %	0 %
Mean number of living offspring produced per parent animal surviving in control at the end of the test	> 60	185.2 (control) 170.1 (solvent control)

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Analytical results:

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 SANTE/2020/12830, Rev.1.

Recoveries over the test period ranged between 92 to 104 % of the nominal concentrations (see table below). Results reported are based on nominal concentrations of the test substance.

No residues of fluopyram were found in the control and solvent control samples above 5.14 µg a.s./L, which was used as the lowest standard concentration during the study.

Table 8.2.5.1- 2: Analytical results

Nominal concentration [µg a.s./L]	Measured concentration [µg a.s./L]						Mean measured concentration [µg a.s./L] [A]
	Day 0 New	Day 2 Aged	Day 9 New	Day 12 Aged	Day 19 New	Day 21 Aged	
80	83	80	78	78	81	80	80
200	201	202	192	196	194	199	197
500	505	510	496	498	489	476	496
1250	1247	1255	1159	1212	1224	1235	1222
3125	3005	3154	2864	2991	2992	2971	2996
	% of nominal						Mean % of nominal [A]
	Day 0 New	Day 2 Aged	Day 9 New	Day 12 Aged	Day 19 New	Day 21 Aged	
80	104	100	98	98	101	100	100
200	101	101	96	98	97	100	99
500	101	102	99	100	98	95	99
1250	100	100	93	97	98	99	98
3125	96	101	92	96	96	95	96

[A] Not given in report. Calculated on the basis of given measured data.

Biological results:

Observations

There were no immobilisations of adult daphnids in any test concentration and the controls (control and solvent control).

Also no treatment related effects on parental behaviour were observable during the course of exposure. Nevertheless, five parental animals were identified as males. These animals were excluded from any statistical evaluation.

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Table 8.2.5.1- 3: Immobility after 21 days of exposure

Nominal concentration [µg a.s./L]	No. of immobilised daphnids (% [A])
Control	0 (0)
Solvent Control	0 (0)
80	0 (0)
200	0 (0)
500	0 (0)
1250	0 (0)
3125	0 (0)

[A] % Immobilisation refers to an initial number of 10 daphnids.

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Growth

The data for all study endpoints revealed homogeneity between both control groups. Thus, the data from both control groups were pooled and all data from treatment groups were related to the pooled control data.

Regarding the results for lengths on day 21, there was a statistically significant effect in the highest test concentration (3125 µg a.s./L, nominal test concentration) compared to the pooled control group.

Table 8.2.5.1- 4: Dry weight and length of adult daphnids after 21 days of exposure

Nominal concentration [µg a.s./L]	Dry weight		Length	
	Mean ± SD [mg]	% Inhibition from pooled control	Mean ± SD [mm]	% Inhibition from pooled control
Control	0.85 ± 0.1	NA	4.8 ± 0.1	NA
Solvent Control	0.96 ± 0.1	NA	4.8 ± 0.2	NA
Pooled control	0.91 ± 0.1	NA	4.8 ± 0.1	NA
80	0.95 ± 0.1	+4.15	4.8 ± 0.2	+1.0
200	0.80 ± 0.1	-11.65	4.8 ± 0.2	-0.5
500	0.84 ± 0.1	-7.45	4.8 ± 0.1	± 0.0
1250	0.90 ± 0.1	-1.21	4.8 ± 0.2	± 0.0
3125	0.87 ± 0.2	-4.26	4.5 ± 0.4	-6.1 *

NA: Not applicable
SD: Standard deviation
* Statistically significant difference from pooled controls (verified by Dunnett's multiple t-test Procedure on a 5 % level of significance at one-sided [smaller] probability).

Reproduction data

The reproduction data revealed homogeneity between both control groups. Therefore, both control groups were pooled and the treatment groups were compared to the pooled control.

The time to first brood ranged from 9 to 10 days with no statistically significant differences. Statistical analysis regarding neonates per adult total reproduction and reproduction per day indicated a significant effect in the highest test concentration (3125 µg a.s./L, nominal test concentration). Observations revealed a high affection rate for offspring from the highest test concentration of 3125 µg a.s./l. The

total living offspring of 611 individuals derived from 8 parent animals, included 49 affected animals. Further 225 dead neonates were counted and excluded from reproduction statistics.

The 4 lowest test concentrations (80, 200, 500 and 1250 µg a.s./L) caused no effects on the behaviour of the corresponding offspring.

Table 8.2.5.1- 5: Time to first brood and mean young per reproduction day

Nominal concentration [µg a.s./L]	Time to 1st brood [days]	Total young per adult	Number of reproduction days	Number of living neonates per adult reproduction day
	Mean ± SD			
Control	9.3 ± 0.88	185.2 ± 24.7	13.6 ± 0.88	13.8 ± 2.8
Solvent control	9.6 ± 1.42	170.1 ± 23.1	13.3 ± 1.42	12.9 ± 2.3
Pooled control	9.5 ± 1.17	177.3 ± 24.4	13.4 ± 1.17	13.3 ± 2.5
80	9.3 ± 1.02	192.6 ± 38.3	13.6 ± 1.01	14.3 ± 3.2
200	10.2 ± 1.57	156.8 ± 43.7	12.7 ± 1.57	12.5 ± 3.7
500	9.7 ± 0.83	174.6 ± 38.8	13.2 ± 0.83	13.3 ± 3.3
1250	9.5 ± 1.51	162.0 ± 31.6	13.4 ± 1.51	12.3 ± 3.1
3125	9.3 ± 1.30	76.4 ± 32.6 *	13.6 ± 1.3	5.6 ± 2.4 *
	% Inhibition compared to pooled control			
Control	-	-	-	-
Solvent control	-	-	-	-
80	-1.4	+8.6	-	+7.2
200	+7.6	-11.5	-	-6.2
500	+2.1	- 1.5	-	-0.5
1250	+0.2	- 8.6	-	-7.6
3125	-2.2	-56.9 *	-	-57.7 *

SD: Standard deviation
* Denotes statistically significant difference from pooled controls (verified by multiple sequential t-test procedure after Williams and Dunnett's multiple t-test Procedure on a 5 % level of significance at one-sided [smaller] probability).

III. CONCLUSION

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

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Endpoint	Immobili-sation (adult)	Time to first brood	Neonates per adult reproduction day	Cumulative neonates per adult	Adult body length	Adult dry weight
NOEC -21 days [µg a.s./L]: highest concentration without an effect	**≥ 3125**	**≥ 3125**	**1250**	**1250**	**1250**	**≥ 3125**
LOEC -21 days [µg a.s./L]: lowest concentration with an effect	> 3125	> 3125	3125	3125	3125	> 3125
MATC [µg a.s./L]: maximum acceptable toxicant concentration	-	-	1976			-
EC_{10} -21 days [µg a.s./L] [A]	Not determined [B]					
EC_{20} -21 days [µg a.s./L] [A]	Not determined [B]					
EC_{50} -21 days [µg a.s./L] [A]	Not determined [B]					

[A] Recalculated to fulfil the data requirements set in regulation 283/2013. Please refer to recalculation M-758376-01-1.
[B] Due to the lacking concentration/response the ECx could not be determined.

Assessment and conclusion by applicant:

The study and its data are considered as acceptable and reliable for use in risk assessment.

The endpoint is: NOEC (21 days) = 1.25 mg a.s./L (based on reproduction)

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Data Point:	KCA 8.2.5.1/02
Report Author:	[redacted]
Report Year:	2020
Report Title:	Re-evaluation of the Influence of AE C656948 (tech.) on development and reproductive output of the waterflea Daphnia magna in a static renewal laboratory test system (Bruns, E., 2007; M-282102-02-1)
Report No:	M-758376-01-1
Document No:	M-758376-01-1
Guideline(s) followed in study:	--
Deviations from current test guideline:	Current Guideline: not applicable Deviations: not applicable
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	Yes

Summary

In the existing report, EC_{10} and EC_{20} values were statistically determined.

EC_{10} and EC_{20} values were calculated for the chronic *Daphnia magna* study (M-282102-02-1) to fulfil the data requirements according to regulation EU 283/2013. Additionally, the validity criteria were re-evaluated according to the current guideline OECD 201 (2011).

The recalculations were performed with the software ToxRat Professional (Version 3.2.1) with the nominal concentrations provided in the report.

A calculation of the effect concentration (ECx) endpoints for cumulative hatch was done based on a Weibull analysis, however, it was found to have a missing dose-response relationship so no EC_x values were presented. No further analyses were done due to an insufficient response in each endpoint, so no additional calculations were presented.

Assessment and conclusion by applicant:

The study and its data are considered as acceptable and reliable for use in risk assessment.

The relevant endpoint from the report is: NOEC (21 days) = 1.25 mg a.s./L (based on reproduction)

According to the AGD, EC_{10} are preferred endpoints for risk assessment. However, due to a missing dose-response relationship, no EC_{10} values could be calculated.

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Metabolite trifluoroacetic acid (TFA)

Data Point:	KCA 8.2.5.1/03
Report Author:	[redacted]
Report Year:	2010
Report Title:	Influence of 30 % w/w sodium trifluoroacetate aqueous solution to Daphnia magna in a semi-static reproduction test
Report No:	55911221
Document No:	M-615126-01-1
Guideline(s) followed in study:	OECD Guideline 211, adopted October 03, 2008, Commission Regulation (EC) No 440/2008, C.20, 2008
Deviations from current test guideline:	Current Guideline: OECD 211 (2012) Deviations: The pH in media ranged between 7.9 and 9.2 and thus higher than the maximum recommended pH of 9 recommended in OECD 211. This deviation is not expected to have impacted the study results. All validity criteria were met.
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 test was performed with *Daphnia magna* under static-renewal conditions (approx. 3 water renewals per week) for 21 days. Sodium trifluoroacetate aqueous solution was applied at nominal concentrations of 1.0, 3.2, 10.0, 32.0 and 100 mg test item/L, corresponding to 0.3, 0.96, 3.0, 9.6 and 30 mg p.m./L. Additionally, a control was included. The test comprised 10 replicates of 1 organism for each group. Observations for sub-lethal effects and survival (immobilisation) were made daily. Reproductive output (neonates counts) were determined at the time of first brood release.

Concentrations of sodium trifluoroacetate were verified by IC measurements at test start and on day 3, 5, 12, 14 and 19 only in the highest test concentration (100 mg test item/L) and the control. Measured concentrations were in fresh and aged test media in the 102 - 117 % range of nominal concentrations and no residues were found in the control samples above the limit of quantification (50 mg test item/L). The limit of detection was 0.21 mg p.m./L. Therefore, the biological results are based on nominal concentrations of the test item.

The study fulfils all validity criteria of OECD 211 guideline.

There were no particular signs of intoxication observed at the test animals during the test in any test concentration and the control. In the control and all test concentrations all Daphnia survived until the end of the test after the exposure time of 21 days. There were no immobile or dead neonates observed in any treatment level. No significant toxic effect of the test item on the mean reproduction rate was determined up to and including the highest test concentration of nominal 100 mg test item/L, corresponding to 30 mg p.m./L. The first young Daphnia released from their parent animals were recorded in the all test concentrations at the observation on day 9. In the control, the first offspring was recorded at day 10.

The endpoints based on nominal concentrations of sodium trifluoroacetate aqueous solution and of sodium trifluoroacetate were: NOEC - 21 days: ≥ 100 mg sodium trifluoroacetate aqueous solution/L, corresponding to ≥ 30 mg p.m./L and LOEC - 21 days: 100 mg sodium trifluoroacetate aqueous solution/L, corresponding to 30 mg p.m./L (trifluoroacetate).

The converted endpoints based on nominal concentrations of trifluoroacetic acid were: NOEC - 21 days: ≥ 25.2 mg p.m./L and LOEC - 21 days: 25.2 mg p.m./L.

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I. MATERIAL AND METHODS

Test material:	30 % w/w Sodium trifluoroacetate aqueous solution Batch No.: 10JGR1448 Purity: 30 ± 0.3 % w/w; according to certificate of analysis
Guideline(s) adaptation	None specified
Test species:	Water flea (*Daphnia magna)* clone 5
Organism Age at Experimental Start:	Age at study initiation: Female 1st instar neonates less than 24 h old (2 to 19,25 h)
Acclimation	Not necessary since the breeding is performed in the same kind of test water.
Test solutions	Nominal concentrations: 1.0 – 3.2 – 10.0 – 32.0 – 100 mg sodium trifluoroacetate aqueous solution/L (corresponding to 0.3 – 0.96 – 3.0 – 9.6 – 30 mg p.m./L) Corresponding measured concentrations ranged between 102 and 117 % of nominal concentrations.: Controls: reconstituted water Evidence of undissolved material: There were no remarkable observations. The test medium appeared clear.
Replication:	No. of vessels per concentration (replicates): 10 individual replicates No. of vessels per control (replicates): 10 individual replicates
Organisms per replicate:	No. of organisms per vessel: 1 for the individual replicates
Exposure:	Semi-static test, renewal on days 3, 5, 7, 10, 12, 14, 17 and 19 of the exposure period Total exposure duration: 21 days
Test Vessel Loading:	60 mL of test solution / daphnid
Feeding during test	Green algae (*Desmodesmus subspicatus*) daily: The fed daily amounts of algal TOC/*Daphnia* were as follows: Days 0-3: 0.10 mg algal TOC/Daphnia Days 4-7: 0.15 mg algal TOC/Daphnia Days 8-20: 0.20 mg algal TOC/Daphnia
Test conditions:	Temperature: 21 °C in fresh media and 20 – 21 °C in aged media Photoperiod: 16 h light, 8 h:dark Light intensity: 1000 - 11000 lux (measured once during the test) pH: 7.9 - 8.2 in fresh medium, 7.8 – 9.2 in aged medium Water hardness: 250 mg/L as $CaCO_3$ Dissolved oxygen:: 8.2 – 8.8 mg O_2/L in fresh media, 8 – 10.1 mg O_2/L in aged media (corresponding to 97 - 104 % of saturation in fresh media, 88 – 111 % in aged media) Conductivity: not reported
Parameters Measured / Observations	The pH-values, dissolved oxygen concentrations and water temperatures were measured in all test concentrations and the control in all freshly prepared and aged test media. In the aged test media, measurement was performed in the pooled test vessels of each concentration and the control. The mortality of the test animals and the number of young daphnids were recorded each day. Dead animals and offspring were removed at the same times.

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Sampling for chemical analysis	Duplicate samples from the freshly prepared test media of the highest test concentration (100 mg test item/L) and the control were taken on day 3, 12 and 14. Aged test media of the highest test concentration (100 mg test item/L)and control media were sampled in duplicate on day 5, 14 and 17. These stability control treatments lasted between 48 and 72 hours (on weekend). The concentrations of sodium trifluoroacetate were only measured in the duplicate test medium samples of the highest test concentration (100 mg test item/L) from all sampling dates. From the control samples only one of the duplicate samples was analysed from each sampling date. The other test media were not analysed, since they were below the NOEC determined in this test. The chemical analyses were performed by IC measurements using a calibration curve.
Data analysis:	The NOEC and the LOEC for the reproduction rate and age at first reproduction were evaluated by the Williams t-test. The EC_{50} (21 d) of the reproduction rate could not be determined by due to absence of toxicity of the test item. The software used to perform the statistical analysis was ToxRat Professional, Version 2.10, ToxRat® Solutions GmbH, 2009.

II. Results and Discussion

Table 8.2.5.1- 6: Validity criteria

Validity criteria	Required	Obtained
Mortality of the parent animals in control at the end of the test	≤ 20 %	0 %
Mean number of living offspring produced per parent animal surviving in control at the end of the test	≥ 60	101

Analytical results:

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 SANTE/2020/12830, Rev.1.

Recoveries over the test period ranged in the freshly prepared test media between 102 to 117 % and in the aged test media between 106 to 112 % of the nominal test concentrations. Therefore, the biological results are based on nominal concentrations of the test item.

No residues of sodium trifluoroacetate were measured in the control samples above the limit of quantification (50 mg test item/L). The limit of detection was 0.21 mg p.m./L.

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Table 8.2.5.1- 7: Analytical results

Nominal concentrations		Mean measured concentrations [A] [mg p.m./L]					
[mg test item/L]	[mg p m./L]	Day 3 New	Day 5 Aged	Day 12 New	Day 14 Aged	Day 14 New	Day 17 Aged
1.0	0.3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
3.2	0.96	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
10	3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
32	9.6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
100	30	30.66	32.08	33.05	33.74	35.06	33.25
		% of nominal [A]					
1.0	0.3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
3.2	0.96	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
10	3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
32	9.6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
100	30	102	107	110	113	117	111

n.d. not determined, since below NOEC

[A] Not given in report. Calculations based on the measured replicate samples on each sampling day.

Biological results:

Observations

No particular signs of intoxication were observed at the test animals during the test.

Adult survival

In the control and all test concentrations all Daphnia survived until the end of the test after the exposure time of 21 days.

Table 8.2.5.1- 8: Immobility after 21 days of exposure

Nominal concentration		No. of immobilised daphnids (% [A])
[mg test item/L]	[mg p.m./L]	
Control	Control	0 (0)
1.0	0.3	0 (0)
3.2	0.96	0 (0)
10	3	0 (0)
32	9.6	0 (0)
100	30	0 (0)

[A] % Immobilisation refers to an initial number of 10 daphnids.

Reproduction data

There were no immobile or dead neonates observed in any treatment level.

No significant toxic effect of the test item on the mean reproduction rate was determined up to and including the highest test concentration of nominal 100 mg test item/L (Williams t-test, one-sided, α =

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0.05). The first young Daphnia released from their parent animals were recorded in the all test concentrations at the observation on day 9. In the control, the first offspring was recorded at day 10.

Table 8.2.5.1- 9: Day of first brood and mean young per reproduction day

Nominal concentration		Day of 1st brood	Total young per adult	Total young per adult compared to control [%]	Mean number of living neonates per adult and day ± S.D. [A]
[mg test item/L]	[mg p.m./L]				
Control	Control	10	1011	-	101 (± 25.3)
1.0	0.3	9	1041	103	104 (± 18.1)
3.2	0.96	9	1091	108	109 (± 18.7)
10	3	9	1040	103	104 (± 18.9)
32	9.6	9	1016	100	102 (± 22.9)
100	30	9	1050	104	105 (± 14.7)

S.D Standard deviation
[A] Mean and standard deviation from maximum 10 replicates

III. CONCLUSION

The study meets the validity criteria and the endpoints based on nominal concentrations of sodium trifluoroacetate aqueous solution and of sodium trifluoroacetate were:

Endpoint	Nominal concentrations	
	[mg sodium trifluoroacetate aqueous solution/L]	[mg sodium trifluoroacetate/L]
EC_{50} - 21 days 95 % C.I.) (based on reproduction):	> 100 (not determined)	> 30 [B] (not determined)
EC_{20} - 21 days [µg p.m./L]	Not determined [A]	
EC_{10} - 21 days [µg p m./L]	Not determined [A]	
LOEC - 21 days: lowest concentration with an effect (based on parent survival and reproduction)	> 100	> 30 [B]
NOEC - 21 days: highest concentration without adverse effects (based on parent survival and reproduction)	**≥ 100**	**≥ 30** [B]

[A] Due to the lacking concentration/response the ECx could not be determined.
[B] Based on the molecular weights, a concentration of 30 mg sodium trifluoroacetate/L corresponds to 25.2 mg trifluoroacetate acid/L. As the study was conducted with sodium trifluoroacetate which is the sodium salt of trifluoroacetic acid, the endpoint was converted to trifluoroacetic acid with factor 0.84.

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Assessment and conclusion by applicant:

The study and its data are considered as acceptable and reliable for use in risk assessment.

The endpoints are:

NOEC (21 days) ≥ 100 mg sodium trifluoroacetate aqueous solution/L

NOEC (21 days) ≥ 30 mg p.m./L (sodium trifluoroacetate) corresponding to ≥ 25.2 mg p.m./L (trifluoroacetic acid).

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

No additional studies were performed.

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CA 8.2.5.3 Development and emergence in Chironomus riparius

Active substance fluopyram

Data Point:	KCA 8.2.5.3/01
Report Author:	
Report Year:	2008
Report Title:	AEC656948 - Life-cycle toxicity test exposing midges (Chironomus tentans) to a test substance applied to sediment under static-renewal conditions following EPA test methods
Report No:	13798.6212
Document No:	M-298809-01-1
Guideline(s) followed in study:	EPA Test Method 100.5
Deviations from current test guideline:	Current Guideline: EPA test method 100.5 Deviations: The variation of ammonia during the test was 93 % (0.10 - 2.7 mg/L as N) and thus higher than 50% variation as recommended in the guideline. The daily measured values of temperature was not reported. This deviation was not expected to have impacted the study results. All validity criteria were met.
Previous evaluation:	yes, evaluated and accepted in DAR (2011)
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 influence of fluopyram on emergence and development of *Chironomus tentans* during 54 days under static-renewal conditions of the overlying water (spiked sediment exposure). First instar larvae of *C. tentans* were exposed to nominal sediment concentrations of 7.5, 15, 30, 60 and 120 mg/kg. Additionally a water control and a solvent control were included. The test comprised 20 replicates of 12 midge larvae for each group. The sex, time point of emergence and number of emerged midges was recorded daily during the period of emergence. Survival of individual flies (male and female) was recorded daily until death. Reproductive/oviposit chambers for each treatment level and control were checked daily for dead adults and egg masses and dead flies were removed daily.

Concentrations of fluopyram were verified by LSC in the overlying water, pore water and the sediment on day 0, 20 and 54. Measured sediment concentrations in the treatments on day -1, 20 and day 54 ranged between 57.5 and 112 %. Biological results are based on mean measured sediment concentrations.

The study fulfils all validity criteria of the EPA test method 100.5.

For survival and in percent emergence there was a statistically significant difference at the two highest test concentrations (50 and 98 mg a.s./kg, mean measured sediment concentration) compared to the pooled control. Regarding growth (ash-free dry weight per midge larvae) a statistically significant difference was determined in the highest test concentration (98 mg a.s./kg, mean measured sediment concentration) compared to the pooled control. Regarding mean development rate a statistically significant difference was determined at the mean measured sediment concentrations of 13 and 98 mg a.s./kg compared to the pooled control. Although statistically significant, the difference in mean development rate in the treatment level of 13 mg a.s./kg was not considered to be biologically relevant due to the lack of a similar observation at the next two higher treatment levels. Regarding mean number of total eggs per female there was no significant difference in any of the treatment levels compared to

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the solvent control. Although statistically significant, the difference in mean percent hatch at the mean measured sediment concentration of 50 mg a.s./kg was not considered to be biologically relevant due to the lack of a similar observation at the next higher treatment level.

The endpoints based on mean measured sediment concentrations were: NOEC – 20 days (based on survival): 26 mg a.s./kg, NOEC – 20 days (based on growth): >98 mg a.s./kg, NOEC – 54 days (based on emergence rate): 26 mg a.s./kg, NOEC – 54 days (based on development rate): 50 mg a.s./kg and NOEC – 54 days (based on time to death for mated individuals, number of total eggs per female and percent hatch): 98 mg a.s./kg.

I. MATERIAL AND METHODS

Test material	Non-radiolabelled test substance: Fluopyram tech. Specification No.: 102000012455 Origin Batch No.: 08528/0002 Purity: 94.7 % w/w	Radiolabelled test substance: [^{14}C]-fluopyram-pyridyl-2,6 Sample ID: BECH 2168 Batch No: not reported Radiochemical purity: > 99 % w/w
Guideline(s) adaptation	None specified	
Test species	*Chironomus tentans*	
Culturing conditions	Not specified	
Organism age/size at study initiation	First instar larvae (3 hours old)	
Test concentrations	Nominal sediment concentrations: 7.5 - 15 - 30 - 60 - 120 mg a.s./kg Corresponding mean measured sediment concentrations: 6.4 - 13 - 26 - 50 - 98 mg a.s./kg Control: water Solvent control: Acetone Evidence of undissolved material: The non-radiolabelled stock solution was observed to be dark amber in colour with no visible undissolved test substance present following preparation. All further five individual dosing stock solutions had no visible undissolved substance, but were different in colours showing amber, light amber or clear and yellow in colour.	
Replication:	No. of vessels per concentration (replicates): 12 No. of vessels per control (replicates): 12 No. of vessels per solvent control (replicates): 12 8 additional replicate vessels were maintained: – 4 replicate vessels (M-P) were established on test day 10 for production of auxiliary males during the emergence phase of the test. – 4 (Q-T) replicates were maintained for the purpose of the chemical analysis.	
Organisms per replicate	No. of organisms per vessel: 12	
Exposure	Static-renewal (350 mL per vessel per day, 2 overlying volume replacements per vessel per day) Spiked sediment exposure Total exposure duration: 54 days	
Feeding during test	The midge larvae were fed a diet consisting of a finely ground flaked fish suspension (4 mg/mL). During the exposure, the food was introduced at a rate of 1.5 mL of flaked fish food suspension per test vessel, once daily.	
Test conditions	Temperature: 21- 25 °C (overlying water) Photoperiod: 16 h light, 8 h dark	

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	Light intensity: 400 - 630 lux pH: 6.2 - 7.2 (overlying water) Water hardness: 32 - 52 mg/L CaCO3 (overlying water) Dissolved oxygen: 2.5 - 8.6 mg O_2/L (corresponding to 28 - 91 % of saturation, calculated based on a temperature of 21 °C and barometric pressure of 760 mm Hg) Sediment volume: 146 g (100 mL, 4 cm layer) Overlying water volume: 175 mL Depth of sediment and overlying water: not reported (275 mL)
Sediment	Artificial sediment according to OECD Guideline No. 218 (OECD, 2004) % organic carbon: 2.2 % quartz sand: 75.0 (fine and coarse sand) % silt: 4 % clay: 21 pH: 6.4 % moisture at 1/3 bar (water holding capacity): 14.7 %
Parameters Measured / Observations	Chemical-physical parameters: One day prior to test initiation, test day 10 (initiation of the male auxiliary replicates), test day 20 and test termination, dissolved oxygen concentration, temperature and pH were measured in the overlying water of each replicate vessel of each treatment level and control used for biological monitoring (A-L; except at termination where no measurements were done in replicates F, G, K and L). On the remaining test days, dissolved oxygen and temperature were measured in one alternating replicate each day. In addition, the temperature was continuously monitored in an auxiliary vessel throughout the study. Total hardness, alkalinity, specific conductivity and the ammonia concentration of the overlying water were monitored at test initiation, test day 10, test day 20 and test termination in each treatment level and control solution from a composite sample (replicates A-L; except at termination where no measurements were done in replicates F, G, K and L). Biological parameters: Midge were examined in all test vessels at test initiation and at 24-hour intervals thereafter, until test termination (day 54). Daily observations of mortality (larvae or pupae) on the sediment surface and abnormal behaviour were made. Midge larval survival and growth (as ash-free dry weight) were determined prior to test day 20 using 4 of the 12 replicate test vessels. The sex, time point of emergence and number of emerged midges was recorded daily during the period of emergence starting on day 18. Larvae which did not yet mature were not taken into account for emergence rates and development time respectively development rate. Emerged male and female flies from each treatment level were held individually and survival was recorded daily until death. Reproductive/oviposit chambers for each treatment level and control were checked daily for dead adults and egg masses and dead flies were removed daily. The number of eggs produced in each primary egg mass laid by female flies in each treatment level and control by replicate were counted the day the egg mass was laid, using the ring method: Five rings of eggs in each egg mass were selected at about equal distances along the length of the egg mass and the number of eggs in these five rings was then counted.
Sampling for chemical analysis	The concentrations in the overlying water, pore water and the sediment of the test item were analysed on day 0, day 20 and termination. The chemical analysis was conducted with LSC.
Data analysis	TOXSTAT® Version 3.5 was used to calculate the EC_{50} value using the Inhibition Concentration Method. Analyses were performed using the mean replicate organism response in each treatment group rather than individual response values. All statistical analyses were conducted at the 95 % level of certainty except in the case of Shapiro-Wilks' Test or Chi-Square Test and Bartlett's Test in which the 99 % level of certainty was applied.

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II. RESULTS AND DISCUSSION

Table 8.2.5.3- 1: Validity criteria (EPA test method 100.5)

Validity criteria according to EPA test method 100.5	Required	Obtained
Emergence in control	≥ 50 %	78 % (control) 83 % (solvent control)
Average survival in the controls at the end of the test	≥ 70 %	88 % (control) 94 % (solvent control)
Average size (ash-free dry weight) in the control sediment	≥ 0.48 mg	1.59 mg (control) 1.47 mg (solvent control)
Dissolved oxygen in the overlying water	> 2.5 mg O_2/L	3.8 - 8.1 mg O_2/L
Daily mean test temperature	23 ± 1 °C	21 – 25 °C [A] (during test)
Instantaneous temperature	23 ± 3 °C	21 – 25 °C

[A] Considered fulfilled as daily temperature readings outside the recommended range appeared on some days in a few test concentrations (day 5: 21.4 °C, day 20: 21.3 °C, day 38: 21.4 °C, day 47: 24.6 – 24.7 °C and on day 54: 24.5 - 24.6 °C).

Table 8.2.5.3- 2: Validity criteria (OECD 218)

Validity criteria according to OECD 218	Required	Obtained
Emergence rate in the controls at the end of the test	≥ 70 %	78 % (control) 83 % (solvent control)
Emergence period in the controls *(C. tentans)*	Between day 20 and 65	Between day 19 and 35 (control), between day 18 and 41 (solvent control)
Oxygen content (at the end)	≥ 60 %	28 - 91 [A] % of saturation (2.5 - 8.1 mg O_2/L, during test)
Water pH of the overlying water	6 - 9	6.2 -7.2
Water temperature of the overlying water	Should not differ by more than ±1°C	21 – 25 °C [B]

[A] Not given in report. Calculated based on a temperature of 21 °C and barometric pressure of 760 mm Hg.
[B] Considered fulfilled as daily temperature readings outside the recommended range appeared on a few days (day 5: 21.4 °C, day 20: 21.3 °C, day 38: 21.4 °C, day 47: 24.6 to 24.7 °C and on day 54: 24.5 to 24.6 °C).

<u>Analytical results:</u>

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 SANTE/2020/12830, Rev.1.

Measured sediment concentrations on day -1, 20 and day 54 ranged between 57.5 and 112 % of nominal sediment concentrations (see table below). Biological results are based on mean measured sediment concentrations.

In the control and solvent control, the mean measured sediment concentrations of total [^{14}C]- residues as AE1170437 equivalents were reported to be between < 0.027 and < 0.051 mg a.s./kg.

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Table 8.2.5.3- 3: Analytical results for total [14C]-residues in sediment

Nominal sediment concentration [mg a.s./kg]	Measured concentration [mg a.s./kg]			% of nominal A			Mean measured sediment concentration [mg a.s./kg] B	Mean % of nominal
	Day -1	Day 20	Day 54	Day -1	Day 20	Day 54		
Control	< 0.051	< 0.027	< 0.027	-	-	-	-	-
Solvent control	< 0.044	< 0.027	< 0.027	-	-	-	-	-
7.5	7.5	5.3	6.4	100	70.7	85.3	6.4	85
15	14	14	11	93.3	93.3	73.3	13	88
30	32	24	23	107	80.0	76.7	26	87
60	67	43	40	112	71.7	66.7	50	83
120	120	100	69	100	83.3	57.5	98	82

A Not given in study report. Calculated on the basis of nominal and measured concentrations.
B Mean measured values were calculated using the actual analytical results and not the rounded values (two significant figures) presented in this table.

The measured concentrations in overlying water and pore water were presented below.

Table 8.2.5.3- 4: Analytical results for total [14C]-residues in pore water

Nominal sediment concentration [mg a.s./kg]	Pore water - measured concentration [mg a.s./L]		
	Day -1	Day 20	Day 54
Control	< 0.0093	< 0.0077	< 0.0072
Solvent control	< 0.0097	< 0.0078	< 0.0079
7.5	1.1	0.57	0.49
15	2.8	1.9	1.2
30	5.8	3.6	2.8
60	14	8.4	5.6
120	21	19	13

Table 8.2.5.3- 5: Analytical results for total [14C]-residues in overlying water

Nominal sediment concentration [mg a.s./kg]	Overlying water - measured concentration [mg a.s./L]		
	Day -1	Day 20	Day 54
Control	< 0.0029	< 0.0028	< 0.0028
Solvent control	< 0.0029	< 0.0028	< 0.0028
7.5	0.063	0.033	0.0083
15	0.12	0.040	0.012
30	0.29	0.14	0.028
60	0.57	0.31	0.062
120	0.93	1.3	0.13

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Biological results:

Observations

Start of emergence was at day 18 and 19 for the controls and all test concentrations.

There were no significant difference (t-Test) between control and solvent control survival and growth; therefore, control and solvent control data were pooled.

Statistical analysis (Bonferroni's Test) demonstrated a statistically significant difference in survival among midge exposed at the two highest test concentrations (50 and 98 mg a.s./kg, mean measured sediment concentration) compared to the pooled control organisms. Regarding growth (ash-free dry weight per midge larvae) a statistically significant difference was determined in the highest test concentration (98 mg a.s./kg, mean measured sediment concentration) as compared to the pooled control.

Table 8.2.5.3- 6: Mean percent survival and mean ash-free dry weight per larvae on day 20

Mean measured sediment concentration [mg a.s./kg]	Mean % survival (SD) Day 20	Mean Ash-Free dry weight per larvae (SD) Day 20 [mg]
Control	88 (8)	1.59 (016)
Solvent control	94 (8)	1.47 (0.19)
Pooled control [A]	91 (8)	1.53 (0.18)
6.4	94 (8)	1.48 (0.31)
13	96 (5)	1.37 (0.26)
26	92 (7)	1.41 (0.19)
50	48 (17) *	1.40 (0.20)
98	71 (20) *	1.18 (0.19) *

SD: Standard deviation
[A] Controls were pooled as no significant difference between control and solvent control was determined.
* Statistically different (≤ 0.05) compared to the pooled control data, based on Bonferroni's Test

There were no significant difference (t-Test) between control and solvent control percent emergence, therefore, control and solvent control data were pooled.

Statistical analysis (Bonferroni's Test) demonstrated a statistically significant difference in percent emergence in the two highest test concentrations (50 and 98 mg a.s./kg, mean measured sediment concentration) as compared to the pooled control. Regarding mean development rate a statistically significant difference was determined at the mean measured sediment concentrations of 13 and 98 mg a.s./kg as compared to the pooled control. Although statistically significant, the difference in mean development rate in the treatment level of 13 mg a.s./kg was not considered to be biologically relevant due to the lack of a similar observation at the next two higher treatment levels.

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Table 8.2.5.3- 7: Influence on emergence and development rate after 54 days

Mean measured sediment concentration [mg a.s./kg]	Number of introduced larvae	Number of emerged midges	Mean emergence of inserted larvae			Development rate (pooled sex) [1/d]
			Total [%]	Male [%]	Female [%]	
Control	96	75	78	26.4	25.7	0.0392
Solvent control	96	80	83	27.1	28.5	0.0410
Pooled control A	96		81			0.0401
6.4	96	66	69	31.9	13.9	0.0455
13	96	74	77	22.2	29.2	0.0361 **
26	96	75	78	22.2	29.9	0.0396
50	96	48	50 *	19.4	13.9	0.0369
98	96	50	52 *	17.4	17.4	0.0293 *

SD: Standard deviation
A Controls were pooled as no significant difference between control and solvent control was determined.
* Statistically different compared to the pooled control data based on Bonferroni's Test.
** Although statistically different compared to the pooled control data, not considered to be biologically relevant due to the lack of a similar response at the next two higher concentrations.

Statistical analysis (Bonferroni's Test) determined no significant difference in total mean number of days to death in any of the treatment levels tested as compared to the pooled control.

Regarding mean number of total eggs per female there was no significant difference in any of the treatment levels tested as compared to the solvent control (939 eggs per female).

Statistical analysis (Kruskal-Wallis Test) determined a significant difference in mean percent hatch in the treatment level of 50 mg a.s./kg as compared to the pooled control. Although statistically significant, the difference in mean percent hatch in the 50 mg a.s./kg was not considered to be biologically relevant due to the lack of a similar observation at the next higher treatment level.

Table 8.2.5.3- 8: Mean days to death, mean number of total eggs and mean percent hatch

Mean measured sediment concentration [mg a.s./kg]	Mean days to Death [days]				Mean number of total eggs/ female (SD)	Mean % hatch (SD)
	Unmated males	Mated males	Mated females	Total		
Control	2.5	3.9	4.4	4.1	796 (146)	99 (1.0)
Solvent control	3.3	4.1	4.4	4.3	1082 (151)	99 (0.5)
Pooled control A	2.9	4.0	4.4	4.2	939 (206)	99 (0.8)
6.4	2.8	3.6	4.3	3.9	1049 (260)	97 (1.8)
13	2.7	4.8	4.5	4.5	960 (227)	98 (1.5)
26	2.7	4.3	4.4	4.4	1038 (141)	98 (0.8)
50	2.8	3.7	4.2	4.0	1175 (308)	95 (4.3) **
98	2.4	4.6	5.1	5.0	853 (341)	99 (1.0)

SD: Standard deviation
A Controls were pooled as no significant difference between control and solvent control was determined.
** Although statistically different compared to the pooled control data based on Kruskal-Wallis Test, not considered to be biologically relevant due to the lack of a similar response at the next higher concentration.

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III. CONCLUSION

The study meets the validity criteria of the EPA test method 100.5 and the endpoints based on mean measured sediment concentrations were:

Table 8.2.5.3- 9: Endpoints for survival and growth after 20 days

Endpoint	Midge survival	Midge growth
LC_{50} / EC_{50} – 20 days (95 % C.I.):	> 98 mg a.s./kg (n.a.)	98 mg a.s./kg (n.a.)
LC_{10} / EC_{10} – 20 days	Not determined [A]	Not determined [A]
LOEC – 20 days: lowest concentration with an effect	50 mg a.s./kg	98 mg a.s./kg
NOEC – 20 days: highest concentration without an effect	**26 mg a.s./kg**	50 mg a.s./kg

C.I.: Confidence interval

n.a.: Not applicable as LC/EC_{50} value was empirically estimated; therefore, corresponding 95 % confidence intervals could not be calculated.

[A] Not determined due to mathematical reasons and a lack of a clear dose response pattern. Please refer to recalculation M-758550-01-1

Table 8.2.5.3- 10: Endpoints for emergence rate, development rate, time to death for mated individuals, number of eggs per female and % hatch after 54 days

Endpoint	Emergence rate	Development rate	Time to death for mated individuals	Number of eggs per female	Egg masses, % hatch
LOEC – 54 days: lowest concentration with an effect	50 mg a.s./kg	98 mg a.s./kg	>98 mg a.s./kg	>98 mg a.s./kg	>98 mg a.s./kg
NOEC – 54 days: highest concentration without an effect	**26 mg a.s./kg**	50 mg a.s./kg	98 mg a.s./kg	98 mg a.s./kg	98 mg a.s./kg
54 day- EC_{10}:	Not determined [A]				
54 day- EC_{20}:	Not determined [A]				
54 day- EC_{50}:	Not determined [A]				

[A] Not determined due to mathematical reasons and a lack of a clear dose response pattern. Please refer to recalculation M-758550-01-1

Assessment and conclusion by applicant:

The study and its data are considered as acceptable and reliable for use in risk assessment.

The endpoint is: NOEC (54 days) = 26 mg a.s./kg (based on emergence rate)

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Data Point:	KCA 8.2.5.3/02
Report Author:	[redacted]
Report Year:	2020
Report Title:	Re-evaluation of AEC656948 - Life-cycle toxicity test exposing midges (Chironomus tentans) to a test substance applied to sediment under static-renewal conditions following EPA test methods (Putt, A., 2008; M-298809-01-1)
Report No:	M-758550-01-1
Document No:	M-758550-01-1
Guideline(s) followed in study:	None
Deviations from current test guideline:	Current Guideline: not applicable Deviations: not applicable
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Not applicable
Acceptability/Reliability	Yes

Summary

EC_{10} and EC_{20} values were evaluated for the chronic Midges (*Chironomus tentans*) and report M-298809-01-1 to fulfil the data requirements according to regulation EU 283/2013. Additionally, the validity criteria were re-evaluated according to the current guideline OECD 201 (2011).

The NOEC and LOEC endpoints determined for Midge survival, Midge growth, percent emergence, development rate, time to death for mated individuals, number of eggs per female, and percent hatch are presented in the original report M-298809-01-1. No further analyses were done due to an insufficient response in each endpoint, as stated below.

For Midge survival, a NOEC of 26 mg/kg was reported and at this concentration the survival was better than in the controls. Additionally, there was no clear dose response pattern in the concentrations above the NOEC, therefore, no EC_{10} was calculated.

For Midge growth, there was less than 10% of a difference to the controls at the NOEC (50 mg/kg), and the values below the NOEC are in the range of the NOEC, as well. A proper EC_{10} could not be calculated.

For the development rate, at 50 mg/L the developmental rate was 0.0369, while for the pooled controls the value is 0.0410 (standard deviation = 0.0039). This is a 7.98 % difference compared to the controls at the NOEC. An EC_{10} calculation will not be robust as there is not a clear dose-response relationship.

For percent emergence, the NOEC was found to be 26 mg/kg, with a difference to the controls of 3.7 %. At 50 mg/kg, the survival was reduced by 38.3 % while at the highest test concentration it was reduced by 35.8 %. This plateau did not allow for a dose-response curve fitting and since the effect at the NOEC was a < 10 % difference to controls, we propose to stay with the NOEC.

For the time to death for mated individuals, there was no effect up to the highest test concentration so no EC_{10} calculation was possible.

For the number of eggs per female, the variability of the endpoint results does not allow for a reliable calculation of an EC_{10}. The mean number of total eggs at the highest concentration is < 10% difference to the pooled controls, therefore a reasonable EC_{10} calculation is not possible. The NOEC is below a 10 % difference to the pooled controls and should be used instead of an EC_{10}.

For percent hatch, the highest concentration resulted in the same number as the pooled controls, thus no effect was observed up to and including the highest test concentration and an EC_{10} could not be calculated.

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Assessment and conclusion by applicant:

The study and its data are considered as acceptable and reliable for use in risk assessment.

The endpoint from the report is: NOEC (54 days) = 26 mg a.s./kg (based on emergence rate)

According to the AGD, EC_{10} are preferred endpoints for risk assessment. However, the statistical evaluation showed that no EC_{10} values could be calculated as the data for midge survival, midge growth, development rate, time to death and percent hatch for mated individuals showed no clear dose-response relationship. For the parameters percent emergence and number of eggs per female the NOEC showed a < 10 % difference to controls, therefore, it was proposed to use the NOEC in the risk assessment.

CA 8.2.5.4 Sediment dwelling organisms

Data Point:	KCA 8.2.5.4/01
Report Author:	[redacted]
Report Year:	2008
Report Title:	Chironomus riparius 28-day chronic toxicity test with fluopyram (tech.) in a water-sediment system using spiked water
Report No:	EBGMP121
Document No:	M-298266-01-1
Guideline(s) followed in study:	OECD Guideline 219: "Sediment-Water Chironomid Toxicity Test Using Spiked Water" (adopted 13 April 2004); Equivalent to US EPA OPPTS Guideline No. 850.1790 (SUPP)
Deviations from current test guideline:	Current Guideline: OECD 219 (2004) Deviations: The spacing factor between all concentrations was in the 2.3-10 range and thus higher than 2 as recommended in OECD 2019. No chemical analysis of the sediment was performed. These deviations were not expected to have impacted the study results. All validity criteria were met.
Previous evaluation:	yes, evaluated and accepted in DAR (2011)
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 influence of the test item on emergence and development of *Chironomus riparius* during 28 days under static conditions of the overlying water in a water-sediment-system. First instar larvae of *Chironomus riparius* were exposed to nominal concentrations of 0.0139, 0.139, 0.320, 1.39, 3.20 and 32.0 mg a.s./L. Additionally a water control and a solvent control were included. There were 4 replicates with 20 animals per test concentration, control and solvent control. The sex, time point of emergence and number of emerged midges was recorded daily during the period of emergence.

Concentrations of fluopyram were verified by HPLC-UV and HPLC-MS/MS in the overlying water and pore water on day 0, 7 and 28 in the highest, medium and lowest test concentration and on day 0 in the controls. Additional samples of the overlying water were analysed on day 7 and 28 in the test concentrations of 0.139, 0.32 and 1.39 mg a.s./L. Measured concentrations in overlying water on day 0 were in the 92 - 97 % range of nominal concentrations. During the exposure phase the recoveries decreased on day 7 to 50.1- 60.3 % and on day 28 to 20.9- 24.0 %. Recoveries in the pore water over

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time ranged between 0.4 and 0.9 % of nominal concentrations. Biological results are based on nominal concentrations.

The study fulfils all validity criteria of the OECD Guideline 219.

A statistical significant difference on emergence ratio was determined at the two highest nominal test concentrations (3.20 and 32.0 mg a.s./L) compared to the pooled control. For the development rate a statistical significant difference was evaluated at the highest nominal test concentration (32.0 mg a.s./L).

The endpoints based on nominal concentrations were: NOEC- 28 days: 1.39 mg a.s./L, LOEC- 28 days: 3.20 mg a.s./L, EC_{15}- 28 days: 1.37 mg a.s./L, EC_{10}- 28 days: 0.54 mg a.s./L and EC_{50} - 28 days: >32.0 mg a.s./L.

I. Material and Methods

Test material	Fluopyram (AE C656948) Specification No.: 102000017196 Origin Batch No.: PFV064E001 Purity: 97.5 % w/w
Guideline(s) adaptation	Not specified
Test species	*Chironomus riparius*
Culturing conditions	Temperature: 20 °C Photoperiod: 16 h light, 8 h dark Light intensity: 500 - 1000 lux Medium used for breeding was the same as used in the test (Elendt medium M7). During rearing, the midges were fed with green algae and an aqueous suspension of a plant material based fish food (Tetra Phyll®).
Organism age/size at study initiation	First instar larvae
Test concentrations	Nominal concentrations: 0.0139 – 0.139 – 0.32 – 1.39 – 3.20 – 32.0 mg a.s./L Control: water Solvent control: dimethylformamide (0.1 mL/L) Evidence of undissolved material: Not reported.
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 organisms per vessel: 20
Exposure	Static test system of the overlying water Spiked water exposure Total exposure duration: 28 days
Feeding during test	During the study the larvae were fed at least about three times per week with a commercial ornamental fish food extract (trade name Tetra Phyll®). About 0.5- 1 mg Tetra Phyll®/ larvae/day) was added to each test container.
Test conditions	Temperature: 20.1 – 20.5 °C (overlying water) Photoperiod: 16 h light, 8 h dark Light intensity: 500 -1000 lux pH: 7.7 - 8.5 (overlying water) Water hardness: 267.0 - 302.6 mg/L $CaCO_3$ (overlying water) Dissolved oxygen: 7.4 - 8.9 mg O_2/L (corresponds to 81.6- 98 % of saturation, calculations based on a temperature of 20.1 °C and a barometric pressure of 760 mm Hg) Sediment volume: 140 g (1.5 cm layer) Overlying water volume: 380 mL (6.0 cm layer)

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	Depth of sediment and overlying water: 7.5 cm Aeration: Test vessels were aerated during the equilibration phase. The aeration of the water was stopped for 24 hours after insertion of test organisms and re-started just before application of the test item. Aeration was provided through a glass Pasteur pipette situated about 2.5 cm above the sediment layer throughout the complete study over 28 days.
Sediment	Artificial sediment: % organic carbon: 1.9 % quartz sand: 75 % sphagnum peat: 4 - 5 (pH 2 - 4) % kaolinite: 20 % calcium carbonate: 1 pH: 7 ± 0.5
Parameters Measured / Observations	Temperature in the overlying water was recorded hourly in one control vessel, and once a week in the additional test vessels. One day prior to the start of the study and later on once a week, the pH in the overlying water phase of the additional test vessels for water parameter measurements of the test levels incl. control(s) were measured. At the end of the test (day 28) pH was measured in all test vessels additionally. Dissolved oxygen in the overlying water phase of the additional test vessels for water parameter measurements of each test level incl. control(s) was measured twice per week. At the end of the test (day 28) dissolved oxygen concentration was measured in all test vessels additionally. Measurements of total hardness and of ammonia of the control and the highest test concentration (water phase) were performed on day 0 and day 28. The test vessels were observed at least three times per week to make a visual assessment of any behavioural differences compared to the control. The sex, time point of emergence and number of emerged midges was recorded daily during the period of emergence. As only fully emerged adults are relevant for the endpoints of this study, larvae which did not yet mature were not taken into account for emergence rates and development time. To determine number and sex of emerged adults, the covering plates of each test container were carefully moved and the midges, which mostly stayed at the sides of the vessels, were enumerated; after identification of the sex (male midges have feathered antennae) midges were removed.
Sampling for chemical analysis	The concentrations of the test item in the overlying water and pore water were analysed on day 0 (one hour after application), 7 and 28 at the highest, medium and lowest test concentration and on day 0 in the controls. Additional samples of the overlying water were analysed on day 7 and 28 in the test concentrations of 0.139, 0.32 and 1.39 mg a.s./L. The chemical analysis was conducted with HPLC – UV and HPLC – MS/MS.
Data analysis	ECx values and confidence intervals after 28 days were calculated by probit (or logit, weibit, etc.) analysis or in case of failure by non-parametric methods from the appropriate parameters (endpoints), using a commercial program. The LOEC determinations from the appropriate parameters (endpoints) were done using the ANOVA procedure (α = 0.05, one sided) and properly selected multiple t-tests from a commercial program. In case of a limit test (comparison of control and one treatment group only) the STUDENT t-test can be used. Calculations were carried out using Microsoft Excel® datasheets. Calculations were performed using rounded values. All further statistical evaluations were carried out by using the commercial program ToxRat Professional®.

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II. RESULTS AND DISCUSSION

Table 8.2.5.4- 1: Validity criteria

Validity criteria according to OECD 219	Required	Obtained
Emergence rate in the controls at the end of the test	≥ 70 %	95 %
Emergence period in the controls	Between day 12 and 23	Between day 14 and 23
Oxygen content	≥ 60 %	81.6 - 98 % ($7.4 - 8.9$ mg O_2/L)
Water pH of the overlying water	6 - 9	7.7 - 8.5
Water temperature of the overlying water	Should not differ by more than ± 1 °C	20.1 - 20.5 °C

Analytical results:

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 SANTE/2020/12830, Rev.1.

Recoveries in the overlying water on day 0 in the lowest and highest test concentration (0.0139 and 3.20 mg a.s./L) ranged between 92 and 97 % (see table below). Since the highest test concentration (32.0 mg a.s./L) exceeded the topical water solubility of 16 mg a.s./L, this concentration was excluded from all average calculations of day 0, 7 and 28 for the overlying water and pore water. Results reported are based on nominal concentrations.

No residues of fluopyram were found in the control and solvent control samples above 1.096 µg a.s./L, which was used as the lowest standard concentration during the study.

Table 8.2.5.4- 2: Analytical results for overlying water

Nominal concentration [mg a.s./L]	Overlying water: Measured concentration [µg a.s./L]			% of nominal		
	Day 0	Day 7	Day 28	Day 0	Day 7	Day 28
Control	< 1.096	n.a.	n.a.			
Solvent control	< 1.096	n.a.	n.a.			
0.0139	12.8	6.97	3.18	92.1	50.1	22.9
0.139	n.a.	74.2	29.0	-	53.4	20.9
0.32	n.a.	173	76.8	-	54.1	24.0
1.39	n.a.	745	305	-	53.6	21.9
3.20	3.108	1.930	0.740	97.1	60.3	23.1
32.0 [A]	5.520	8.030	5.489	17.2	25.1	17.2

n.a. Not analysed
[A] Due to a maximum water solubility of 16 mg fluopyram/L, the analytical results of the test concentrations of 32.0 mg a.s./L were excluded for the average calculation.

Recoveries in the pore water of the sediment at the test concentrations of 0.0139 and 3.20 mg a.s./L were between 0.4 and 1.7 % of nominal concentrations (see table below).

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Table 8.2.5.4- 3: Analytical results for pore water

Nominal concentration [mg a.s./L]	Pore water Measured concentration [µg a.s./L]			% of nominal		
	Day 0	Day 7	Day 28	Day 0	Day 7	Day 28
Control	< 1.096	n.a.	n.a.	-	-	-
Solvent control	< 1.096	n.a.	n.a.	-	-	-
0.0139	0.809	2.64	1.82	0.4	1.1	0.9
0.139	n.a.	n.a.	n.a.	-	-	-
0.32	n.a.	n.a.	n.a.	-	-	-
1.39	n.a.	n.a.	n.a.	-	-	-
3.20	239	781	440	0.4	1.7	0.8
32.0 [A]	1.477	2.990	2.683	0.3	0.6	0.5

n.a. Not analysed.
[A] Due to a maximum water solubility of 16 mg fluopyram/L, the analytical results of the test concentrations of 32.0 mg a.s./L were excluded for the average calculation.

Biological results:

Observations

Start of emergence was at day 14 for the controls and all test concentrations.

Regarding distribution between sexes no statistically significant difference was determined compared to the assumption of 50 % females and 50 % males (using Chi²-Test). Therefore, male and female results were pooled for further statistical analyses to increase the statistical power.

Table 8.2.5.4- 4: Influence on emergence and development rate after 28 days (based on nominal concentrations of the test item in the overlying water)

Nominal concentration [mg a.s./L]	Number of introduced larvae	Number of emerged midges	Emergence of inserted larvae			Development rate (pooled sex) [1/d]
			Total [%]	Male [%]	Female [%]	
Control	80	76	91.9	- [A]	- [A]	-
Solvent control	80	71	88.8	- [A]	- [A]	
Pooled control	160	147	95.0	43.8	51.2	0.0604
0.0139	80	71	88.8	33.8	55.0	0.0588
0.139	80	63	78.8	32.5	46.3	0.0608
0.32	80	70	87.5	45.0	42.5	0.0621
1.39	80	66	82.5	38.8	43.8	0.0607
3.20	80	62	77.5	35.0	42.5	0.0600
32.0	80	39	48.8	26.3	22.5	0.0525

[A] Emergence ratio of individual sexes not performed due to practical reasons (Introduction of the same number of female and male organisms as larvae into the individual test beakers not possible).

There was no statistically significant difference (α = 0.050) between the control and the solvent control for emergence rate (pooled sex) (using Chi²-2 × 2 Test) and for the development rate (pooled sex).

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Therefore, the two controls were pooled for further statistical analyses to increase the statistical power.

Regarding the emergence ratio a statistical significant difference ($\alpha = 0.05$) was determined at the two highest nominal test concentrations (3.20 mg a.s./L and 32.0 mg a.s./L) compared to the pooled control.

For the development rate a statistical significant difference ($\alpha = 0.05$) compared to the pooled control was evaluated at the highest nominal test concentration (32.0 mg a.s./L).

III. CONCLUSION

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

Endpoint	Emergence ratio (pooled sex)	Development rate (pooled sex)
NOEC – 28 days: highest concentration without an effect	**1.39 mg a.s./L**	**3.20 mg a.s./L**
LOEC – 28 days: lowest concentration with an effect	3.20 mg a.s./L	32.0 mg a.s./L
EC_{10} – 28 days (95 % C.I.):	0.54 mg a.s./L (0.00 – 2.50 mg a.s./L)	24.79 mg a.s./L (0.50 – 32.89 mg a.s./L)
EC_{15} – 28 days (95 % C.I.):	1.37 mg a.s./L (0.01 – 6.40 mg a.s./L)	> 32.0 mg a.s./L [A] (n.d.)
EC_{20} – 28 days (95 % C.I.):	2.89 mg a.s./L (0.12 – 21.9 mg a.s./L)	> 32.0 mg a.s./L [A] (n.d.)
EC_{50} – 28 days (95 % C.I.):	> 32.0 mg a.s./L [A] (n.d.)	> 32.0 mg a.s./L [A] (n.d.)

A Not determined due to mathematical reasons
n.d. Not determined due to mathematical reasons
C.I.: Confidence interval

Reliability assessment (EFSA 2015)

The following table provides the reliability indicators for EC_{10} values for *Chironomus riparius*.

Biological endpoints	EC_{10} [mg a.s./L]	95% CL	NW	Relationship $EC_{10}/EC_{20/50}$
Emergence Ratio	0.54	0.00 – 2.5	4.630 (bad)	EC_{20}, low < EC_{10} < EC_{50}, low (medium)
Development Rate	24.79	0.5 – 32.89	1.307 (poor)	-[A]

[A] It is not possible to determine as the EC_{20} confidence interval could not be calculated.

Assessment and conclusion by applicant:

The study and its data are considered as acceptable and reliable for use in risk assessment.

The endpoints are:

NOEC (28 days) = 1.39 mg a.s./L (based on emergence ratio) and EC_{10} (28 days) = 0.54 mg a.s./L (based on emergence ratio)

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Data Point:	KCA 8.2.5.4/02
Report Author:	[redacted]
Report Year:	2008
Report Title:	AEC656948 - Toxicity to estuarine amphipods (Leptocheirus plumulosus) during a 28-day sediment exposure
Report No:	13798.6211
Document No:	M-298810-02-1
Guideline(s) followed in study:	Guidelines series 850: Sediment testing: Whole sediment chronic (marine) Equivalent to US EPA OPPTS Guideline No. 850.1350 (SEP)
Deviations from current test guideline:	Current Guideline: OCSPP 850.1350 Deviations: There were 3 volume replacements per week and thus not the minimum 5 volume replacements per day as recommended in OCSPP 850.1350. The number of mysids was 100 per concentration and thus higher than the recommended number of 40 per test concentration. There were 20 mysids per replicate and thus higher than the maximum of 5 organism per replicate. The spacing factor between test concentrations was 2.5 and thus above the maximum factor of 2.0. The light duration of 16 hours was slightly higher than the recommended 14 hours light. No transition period was maintained. No chemical analysis was performed on day 7 and 21 as recommended in OCSPP 850.1350. The concentration of solvent was not reported. These deviations were not expected to have impacted the study results. All validity criteria were met.
Previous evaluation:	Yes, evaluated and accepted in DAR (2011)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

A chronic study was performed with estuarine amphipod (*Leptocheirus plumulosus*) with a renewal of the overlying water three times weekly for a period of 28 days. Fluopyram was applied at nominal sediment concentrations of 2.6, 6.4, 16, 40 and 100 mg a.s./kg. A water control and a solvent control were also included. The test comprised 5 replicates of 20 organisms for each group. Mortality and abnormal behaviour were recorded daily. At test termination (day 28), the total number of surviving amphipods was determined in each test vessel.

Concentrations of fluopyram in sediment, pore water and overlying water were verified by LSC on days 0, 14 and 28 for each concentration and control. Measured sediment concentrations were in the 92 - 110 % range of nominal concentrations. In the control and solvent control, the mean measured sediment concentrations of total [^{14}C]- residues as fluopyram equivalents were reported to be between < 0.0089 and < 0.017 mg a.s./kg. Results are based on mean measured sediment concentrations of 2.6, 6.8, 15, 38 and 94 mg a.s./kg.

The study fulfils all validity criteria of the OPPTS Guideline 850.1740.
There was no statistically significant difference in survival in any of the treatment levels tested compared to the pooled control. For growth (dry weight) among amphipods there was a statistical significant reduction in the highest test concentration (94 mg a.s./kg, mean measured sediment concentration) compared to the pooled control. For reproduction there were no statistically significant difference in any of the treatment levels tested compared to the solvent control.

The endpoints based on mean measured sediment concentrations were: NOEC – 28 days (based on survival and reproduction): 94 mg a.s./kg and NOEC – 28 days (based on growth): 38 mg a.s./kg.

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I. MATERIAL AND METHODS

Test material:	<u>Non-radiolabelled test substance:</u> Fluopyram (AE C656948) Specification No.: 102000012455 Batch No: 08528/0002 Purity: 94.7 %	<u>Radiolabelled test substance:</u> [^{14}C]-fluopyram-pyridyl-2,6 Batch No: not reported Radiochemical purity: > 99 %
Guideline(s) adaptation	None specified	
Test species:	Estuarine Amphipod *(Leptocheirus plumulosus)*	
Culturing conditions	Amphipods were maintained in plastic culture tubs with a 1- to 2-cm deep layer of 0.25 mm sieved marine sediment and 7 to 8 L of 20 ppt seawater. The dissolved oxygen concentration was 6.9 mg/L and the temperature ranged between 23 and 26 °C. Culture water was the same water used as overlying water during the test. No mortality was observed in the test population 48 hours prior to test initiation.	
Organism age/ size at study initiation:	Juvenile amphipods, size range: 0.6 to 0.25 mm	
Preparation of spiked sediment	A 9 mL volume of each dosing stock solution was applied to 0.05 kg of fine silica sand placed in glass Petri dishes. The solvent was allowed to evaporate off the sand for 120 minutes. The dry sand, containing the test substance, was then added to 2.75 kg of wet sediment. The jars with sediment were sealed and rolled at approximately 15 rpm for four hours at room temperature. Following the four hours of rolling the jars were stored upright at 4 °C overnight. Sediments were allowed to equilibrate for a 14- day period in the refrigerator. Once a week during the equilibration period and prior to addition into the replicate exposure vessels (test day -1), the jars were mixed on the rolling mill for an additional two hours at room temperature to ensure the sediment was homogeneous.	
Test concentrations	Nominal sediment concentrations: 2.6 – 6.4 – 16 – 40 – 100 mg a.s./kg Corresponding mean measured sediment concentrations: 2.6 – 6.8 – 15 – 38 – 94 mg a.s./kg Control: water Solvent control: acetone (9 ml acetone per 0.0500 kg of fine silica sand) Evidence of undissolved material: No visible undissolved test substance was observed.	
Replication:	No. of vessels per concentration (replicates): 5 No. of vessels per control (replicates): 5 No. of vessels per solvent control (replicates): 5 In addition, 5 replicates were maintained for chemical analysis.	
Organisms per replicate:	No. of organisms per vessel: 20	
Exposure:	Renewal of the overlying water (spiked sediment test) Total exposure duration: 28 days During the 28-day study, the overlying water was renewed by siphoning 400 mL of the overlying water out of the test vessel and replacing it with 400 mL of fresh overlying water three times per week. The siphoning and replacement of overlying water was done so as not to disturb the sediment layer.	
Feeding during test	The amphipods were fed a diet consisting of a flaked fish food suspension (10 mg/mL). During the exposure, each vessel was fed three times per week, following renewal of the overlying water. On test days 0 through 13, each exposure vessel was fed 2.0 mL of flaked fish food suspension. On days 14 through 27, 4.0 mL of flaked fish food suspension was added to each vessel.	

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Test conditions:	Water temperature: overlying water: 24 - 26 °C (continuous measurement in water bath revealed 24 - 26 ºC); pore water: 19 - 20 °C Photoperiod: 16 hours light: 8 hours darkness Light intensity: 800 - 980 lux pH: overlying water: 7.4 - 8.2; pore water: 6.5 - 6.9 Total ammonia as nitrogen: overlying water: 17 - 18 mg/L (day 0), ≤ 1.0 (day 28); pore water: 110 - 120 mg/L (day 0), ≤ 10 (day 28) Water hardness: not reported Dissolved oxygen: 4.5 - 7.3 mg/L (corresponding to 61.4 - 99.5 % of saturation; calculation based on a temperature of 25 °C and a salinity of 20 ‰) Conductivity: not reported Salinity: 20 - 28 ‰ Sediment volume: 175 mL (approx. 2 cm layer) Overlying water volume: 725 mL Depth of sediment and overlying water: not reported
Sediment	Natural marine sediment (collected from Little Harbor Beach, Wareham, Massachusetts, USA): % organic carbon: 5.6 % sand: 35.8 % silt: 34.6 % clay: 29.6 pH: 7.6 % moisture at 1/3 bar: 53.7 %
Parameters Measured / Observations	Temperature, pH, dissolved oxygen concentration and salinity were measured in the overlying water of each replicate vessel of each treatment level and control used for biological monitoring at test initiation and termination. On test days 1 through 27, dissolved oxygen, salinity, pH and temperature were measured in one alternating replicate each day. In addition, the temperature was continuously monitored in an auxiliary vessel in the temperature controlled water bath. Ammonia concentration (as nitrogen) of the overlying water was monitored at test initiation in each treatment level and control solution from a composite sample (replicates G through J). Ammonia concentration (as nitrogen) in the overlying water was monitored at test termination in each treatment level and control solution from a sample of replicate I. In addition, at test initiation and test termination, salinity, pH, temperature and ammonia (as nitrogen) concentration were measured in a pore water sample of each treatment level and control. All vessels were examined at test initiation and at 24-hour intervals thereafter, until test termination (day 28). Observations of mortality and abnormal behaviour were made and the physical characteristics of the test samples were recorded. At test termination (day 28), the total number of surviving amphipods was determined in each test vessel by sieving the entire volume of sediment to remove all surviving amphipods. Young amphipods were removed from the sieve and transferred to a labelled sample jar. Reproduction was determined as the number of young per surviving adult amphipod in each replicate vessel. Growth was also determined at test termination
Sampling for chemical analysis	During the in-life phase of the definitive study, sediment, pore water and overlying water samples were removed and analysed for total [^{14}C]-residue concentration on test days 0, 14 and 28. On day 0, samples were removed and analyzed from replicate vessels F of all treatment levels and controls. On day 14, samples were removed and analyzed from replicate vessels H of all treatment levels and controls while on day 28, samples were removed and analyzed from replicate J of all treatment levels and controls. Chemical analysis of sediment, pore water and overlying water samples were performed by liquid scintillation counting (LSC).
Data analysis:	Shapiro-Wilks' Test for normality was conducted to compare the observed sample distribution with a normal distribution. The assumption that observations are normally

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	distributed must be validated before subsequent analyses, following parametric procedures, can be performed. If the data are not normally distributed, then a non-parametric procedure is used for subsequent analyses. Analysis of the survival and reproduction data met this assumption of normal distribution. As a check on the assumption of homogeneity of variance implicit in parametric statistics, data were analyzed using Bartlett's Test. The survival and reproduction data passed the test for homogeneity. A t-Test was conducted for the survival, growth and reproduction data to compare the performance of the control organisms with that of the solvent control organisms in order to determine if there were any statistically significant positive or negative effects. During this study, the t-Test for survival and growth data indicated no significant difference between control and solvent control data. For this study, data from all dose levels was compared to the pooled control data to determine treatment level effects for survival and growth endpoints. Data from all dose levels was compared to the solvent control data to determine treatment level effects for reproduction. For this study, the survival and reproduction data met the assumptions for normal distribution and homogeneity. Bonferroni's t-Test was used to establish treatment effects on amphipod survival. Dunnett's Test was used to establish treatment effects on amphipod reproduction. Growth data did not meet the assumption for normal distribution or homogeneity. Therefore, Kruskal-Wallis' Test was used to determine treatment level effects for amphipod growth. All statistical analyses were used to establish, at the 95 % level of certainty, the lowest test concentration that showed a statistically significant effect (Lowest-Observed-Effect Concentration, LOEC) and the highest test concentration that showed no statistically significant difference (No-Observed-Effect Concentration, NOEC) from the appropriate control data. TOXSTAT® Version 3.5 was used to calculate the LOEC and NOEC values.

II. RESULTS AND DISCUSSION

Table 8.2.5.4- 5: Validity criteria

Validity criteria according to OPPTS 850.1740	Required	Obtained
Survival of amphipods in controls	≥ 80 %	84 % (control) 87 % (solvent control)
Dissolved oxygen saturation	> 4.4 mg/L (≥ 60 % saturation)	4.5 - 7.3 mg/L (61 - 99.5 % of saturation)

Analytical results:

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 SANTE/2020/12830, Rev.1.

Measured sediment concentrations on day 0 ranged between 120 and 172 % of the nominal concentrations. On day 14 and 28 concentrations ranged between 63 and 88 % of the nominal concentrations (see table below). Results are based on mean measured sediment concentrations.

In the control and solvent control samples the mean measured sediment concentrations of total [^{14}C]-residues as fluopyram equivalents were reported to be between < 0.0089 and < 0.017 mg a.s./kg.

Furthermore, measured concentrations in overlying water and pore water are shown below.

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Table 8.2.5.4- 6: Analytical results: Measured concentrations of total [^{14}C]-residues in sediment

Nominal sediment concentration [mg a.s./kg]	Measured concentration [mg a.s./kg]			% of nominal A			Mean measured sediment concentration B [mg a.s./kg]	Mean % of nominal B
	Day 0	Day 14	Day 28	Day 0	Day 14	Day 28		
Control	< 0.0092	< 0.0091	< 0.017	n.a.	n.a.	n.a.	n.a.	n.a.
Solvent control	< 0.0092	< 0.0089	< 0.017	n.a.	n.a.	n.a.	n.a.	n.a.
2.6	3.7	2.0	2.1	142	77	81	2.6	99
6.4	11	4.7	4.9	172	73	77	6.8	110
16	23	12	10	144	75	63	15.0	92
40	59	30	25	148	75	63	38.0	96
100	120	88	72	120	88	72	94	94

NA: Not Applicable

B Mean measured and percent recovery values were calculated using the actual analytical results and not the rounded values (two significant figures) presented in this table.

A Not given in study report. Calculated on the basis of nominal and measured concentrations.

Table 8.2.5.4- 7: Analytical results: Measured concentration of total [^{14}C]-residues, measured by LSC analysis, in pore and overlying water samples during the 28-day toxicity test

Nominal sediment concentration [mg a.s./kg]	Day 0	Day 14	Day 28
	Pore water - measured concentration [mg a.s./L]		
Control	< 0.0025	< 0.0026	< 0.0024
Solvent control	< 0.0025	< 0.0026	< 0.0024
2.6	0.19	0.12	0.098
6.4	0.56	0.32	0.25
16	1.3	0.87	0.52
40	3.7	2.3	1.5
100	7.6	6.0	4.1
	Overlying water - measured concentration [mg a.s./L]		
Control	< 0.00097	< 0.00098	< 0.00097
Solvent control	< 0.00097	< 0.00097	< 0.00097
2.6	0.022	0.041	0.016
6.4	0.063	0.11	0.057
16	0.15	0.24	0.16
40	0.40	0.72	0.35
100	0.78	1.6	0.79

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Biological results:

For survival and growth, statistical analysis determined no significant difference between control and solvent control data. Therefore, data from all dose levels was compared to the pooled control.

There were no statistical significant difference (Dunnett's Test) in survival in any of the treatment levels tested compared to the pooled control.
For growth (dry weight) among amphipods there was a statistical significant reduction (Kruskal-Wallis' Test) in the highest test concentration (94 mg a.s./kg, mean measured sediment concentration) compared to the pooled control.

Treatment level effects for reproduction was compared to the solvent control data. For reproduction there were no statistical significant difference (Dunnett's Test) in any of the treatment levels tested compared to the solvent control.

Table 8.2.5.4- 8: Results for survival after 10 days

Mean measured sediment concentration [mg a.s./kg]	Mean % survival (± SD)	Average Dry Weight/Amphipod (± SD) [mg]	Average Number of Offspring/Amphipod (± SD)
Control	84 (± 7)	2.24 (± 0.05)	9 (± 1)
Solvent control	87 (± 8)	2.38 (± 0.13)	6 (± 1)
Pooled control	86 (± 7)	2.31 (± 0.11)	n.a.
2.6	90 (± 12)	2.14 (± 0.28)	5 (± 1)
6.8	94 (± 7)	1.98 (± 0.18)	5 (± 3)
15.0	90 (± 5)	2.10 (± 0.10)	7 (± 2)
38.0	88 (± 8)	2.20 (± 0.20)	8 (± 2)
94	90 (± 14)	1.69 (± 0.56) *	7 (± 4)

SD: Standard deviation
n.a.: Not Applicable. Treatment data was compared to the solvent control for this endpoint.
* Statistically different (≤ 0.05) compared to the pooled control data, based on Kruskal-Wallis' Test.

III. CONCLUSION

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

Endpoint	Survival	Growth	Reproduction
LC_{50} - 28 days (95 % C.I.):	> 94 mg a.s./kg (n.a. [A])	> 94 mg a.s./kg (n.a. [A])	> 94 mg a.s./kg (n.a. [A])
LOEC - 28 days: lowest concentration with an effect	> 94 mg a.s./kg	94 mg a.s./kg	> 94 mg a.s./kg
NOEC - 28 days: highest concentration without an effect	**94 mg a.s./kg**	**38 mg a.s./kg**	**94 mg a.s./kg**

[A] Not applicable as LC_{50} value was empirically estimated; therefore, corresponding 95 % confidence interval could not be calculated.

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Assessment and conclusion by applicant:

The study and its data are considered as acceptable and reliable for use in risk assessment.

The endpoint is: NOEC (28 days) = 38 mg a.s./kg (based on growth)

CA 8.2.6 Effects on algal growth

CA 8.2.6.1 Effects on growth of green algae

Active substance fluopyram

Data Point:	KCA 8.2.6.1/01
Report Author:	[redacted]
Report Year:	2007
Report Title:	Toxicity of AE C656948 technical to the green alga Pseudokirchneriella subcapitata
Report No:	EBGMP048
Document No:	M-286541-01-1
Guideline(s) followed in study:	FIFRA Guideline 123-2 (1982); OPPTS Guideline 850.5400 (2006 draft); OECD Guideline 201 (2006)
Deviations from current test guideline:	Current Guideline: OECD 201 (2006) Deviations: The pH increase in the control was 2.9 units and thus higher than the maximum 1.5 units as recommended in OECD 201. The light intensity was 4273 - 4715 lux and thus lower than the minimum 4440 lux recommended. These deviations were not expected to have impacted the study results. All validity criteria were met.
Previous evaluation:	yes, evaluated and accepted in DAR (2011)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The green alga *Pseudokirchneriella subcapitata* were exposed to fluopyram under static conditions for 96 hours. Algal cultures with an initial nominal cell count of approximately 1.0 × 10^4 cells/mL were used to test the nominal concentrations of 0.102, 0.256, 0.640, 1.60, 4.00 and 10.0 mg a.s./L. Additionally a control and solvent control were included. There were 3 replicates for each test concentration and control. At 24 hour-intervals, the cell density (cells/mL) of each culture was counted.

Concentrations of fluopyram were verified by HPLC – UV on day 0 and day 4 for each concentration and control. Measured concentrations were in the 88 - 98 % range of nominal concentrations and no residues were found in the control and solvent control samples above the LOQ (0.005 mg a.s./L). The biological results are based on the mean measured concentrations of 0.093, 0.241, 0.584, 1.46, 3.78 and 9.53 mg a.s./L.

The study fulfils all validity criteria of OECD 201 guideline.

No physical abnormalities in the controls or treatment groups during the study are reported.

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The endpoints based on mean measured concentrations were: 72 hours – E_rC_{50}: 8.9 mg a.s./L, 72 hours – E_bC_{50}: 3.97 mg a.s./L and 72 hour – E_yC_{50}: 4.26 mg a.s./L.

I. Material and Methods

Test material	Fluopyram (AE C656948) Specification No.: 102000012455 Batch No.: 08528/0002 Purity: 94.7 % w/w
Guideline(s) adaptation	None specified.
Test species	Green algae *Pseudokirchneriella subcapitata*
Culturing conditions	In-house, 3 day old batch culture in log phase growth held under test conditions
Test solutions	Nominal concentrations: 0.102 – 0.256 – 0.640 – 1.60 – 4.00 – 10.0 mg a.s./L Corresponding mean measured concentration: 0.093 – 0.241 – 0.584 – 1.46 – 3.78 – 9.53 mg a.s./L Control: untreated medium Solvent control: Dimethylformamide (0.1 mL/L) Evidence of undissolved material: No precipitates were observed during exposure.
Replication	No. of vessels per concentration (replicates): 3 No. of vessels per control/solvent control (replicates): 3
Exposure	Static Total exposure duration: 96 hours
Initial cells density	1 × 10^4 cells/mL in each test group
Test conditions	Temperature: 23.4 - 24.3 °C Photoperiod: 24 hours light Light intensity: 4273 - 4715 lux Type of light: Cool white fluorescents pH of controls: 7.3 - 9.9 (0 - 96 hours) Conductivity: 84 - 94 µmhos/cm Growth medium same as culture medium: Yes
Parameters Measured / Observations	pH and conductivity were measured on day 0 and 4. Temperature was measured hourly via a calibrated data logger plus daily manual records via a calibrated thermometer. Also, light intensity was measured; however time point was not reported. Each day, density was determined in the three test replicates at each test concentration using a model ZI Beckman Coulter® particle counter and a hemocytometer.
Sampling for chemical analysis	Samples for analysis of test substance were taken at test initiation (0 hour) from batch prepared solutions for each test concentration and at test termination (96 hours) from composite samples from each test concentration. Samples were analysed by using a high-performance liquid chromatograph (HPLC) – UV.
Data analysis	Raw or transformed data from treatment groups were compared to controls for mortality and homogeneity of variance using the Shapiro-Wilks test and Levene's test of equal variance, respectively. If normality and homogeneity of variance were demonstrated for the raw or transformed values, then parametric analyses were conducted using analysis of variance (ANOVA) followed by Dunnett's test. If normality and/or homogeneity of variance were not demonstrated on raw or transformed values, nonparametric procedures were used. The ranks of the raw values were determined, and then an analysis of variance and a one-tailed Dunnett's test were performed on these ranks. The 72 or 96-hour EC_{50}, and the respective 95 % confidence intervals, was calculated with help of regressions analysis for cell density, cumulative biomass, and growth rate. All statistical analyses were performed using the SAS computer software package.

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II. RESULTS AND DISCUSSION

Table 8.2.6.1- 1: Validity criteria

Validity criteria acc. to OECD 201 (adopted 2006)	Required	Obtained
The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period.	≥ 16	Approx. 117 (pooled control and solvent control)
The mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3) in the control cultures must not exceed 35 %.	≤ 35 %	24 %
The coefficient of variation of average specific growth rates during the 72-hour test period in replicate control cultures must not exceed 7 %.	≤ 7 %	3 %

Analytical results:

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 SANTE/2020/12830, Rev.1.

Recoveries on day 0 and day 4 ranged between 88 and 98 % of nominal concentrations (see table below). The biological results are based on mean measured concentrations of fluopyram.

No residues of fluopyram were measured in the control and solvent control samples above the limit of quantification (LOQ: 0.005 mg a.s./L).

Table 8.2.6.1- 2: Analytical results

Nominal concentration [mg a.s./L]	Measured concentration [mg a.s./L]		% of nominal		Mean measured concentration [mg a.s./L]	Mean % of nominal
	Day 0 (New)	Day 4 (Old)	Day 0 (New)	Day 4 (Old)		
0.102	0.097	0.090	95	88	0.093	92
0.256	0.244	0.238	95	93	0.241	94
0.64	0.600	0.567	94	89	0.584	91
1.6	1.49	1.44	93	90	1.46	92
4.0	3.76	3.80	94	95	3.78	94
10.0	9.79	9.27	98	93	9.53	95

Biological results:

Observations:

No physical abnormalities were observed in the controls or any test concentration during the study.

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Table 8.2.6.1- 3: Cell density

Mean measured concentration [mg a.s./L]	Mean cell density [x 10^4 cells/mL]				% Inhibition of cell density [A] at 96 h
	24 h	48 h	72 h	96 h	
Control	4.15	31.52	118.18	198.24	-
Solvent Control	4.12	30.50	115.56	168.52	-
Pooled Controls	-	-	-	183.4	-
0.093	4.09	30.21	96.10	138.76	24.3
0.241	3.96	31.22	110.22	157.99	13.9
0.584	3.93	28.09	93.50	160.51	12.5
1.46	3.91	28.46	109.88	177.98	3.0
3.78	3.55	16.46	67.30	123.91	32.4
9.53	1.26	1.39	1.75	1.33	99.3 *

* Statistically significant difference from control (Dunnett's one-tailed test; $p \leq 0.05$).

[A] % Inhibition=100-((Treatment group parameter mean/pooled control parameter mean)*100).

Table 8.2.6.1- 4: Algae growth rate

Mean measured concentration [mg a.s./L]	Mean growth rate [A] [1/h]		% Inhibition of average specific growth rate [B]	
	0 - 72 h	0 - 96 h	0 - 72 h	0 - 96 h
Control	0.066228	0.054736	-	-
Solvent Control	0.065800	0.052902	-	-
Pooled Controls	0.066014	0.053819	-	-
0.093	0.063139	0.051114	4.4	5.0
0.241	0.065227	0.052691	1.2	2.1
0.584	0.062788	0.052884	4.9	1.7
1.46	0.065148	0.053432	1.3	0.7
3.78	0.058395	0.049888	11.5 *	7.3
9.53	0.007536	0.002968	88.6 *	94.5 *

* Statistically significant difference from control (Dunnett's one-tailed test; $p \leq 0.05$).

[A] Growth rate [1/h] is calculated from the cell density data.

[B] % Inhibition = 100-((Treatment group parameter mean/pooled control parameter mean)*100).

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Table 8.2.6.1- 5: Biomass

Mean measured concentration [mg a.s./L]	Cumulative biomass [A]		% Inhibition of cumulative biomass [B]	
	0 - 72 h	0 - 96 h	0 - 72 h	0 - 96 h
Control	2214.2	5987.2	-	-
Solvent Control	2157.6	5542.7	-	-
Pooled Controls	2185.9	5764.9	-	-
0.093	1916.4	4710.8	12.3	18.3
0.241	2106.8	5301.4	3.6	8.0
0.584	1830.4	4854.5	16.3 *[C]	15.8
1.46	2035.4	5465.6	6.9	5.2
3.78	1227.8	3498.4	43.8 *	39.3 *
9.53	24.52	37.48	98.9 *	99.3 *

* Statistically significant difference from control (Dunnett's one-tailed test; $p \leq 0.05$).
[A] Cumulative biomass is equal to the area under the growth curve.
[B] % Inhibition =100-((Treatment group parameter mean/pooled control parameter mean)*100).
[C] Not considered to be biologically significant. This is justified since the data did not follow a monotonic trend (the next highest test concentration (1.46 mg a.s./L) was not statistically significant). Further, the 96 hour analysis of this endpoint showed no statistical effect at this test concentration.

III. Conclusion

The study meets the validity criteria and the 0 - 72 and 0 - 96 hours endpoints based on mean measured concentrations were:

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Results – 0 to 72 hours	
E_rC_{50} - 72 hours (95 % CI):	**8.9 mg a.s./L** (not determined [A])
E_rC_{20} -72 hours (95 % C.I.):	7.7 mg a.s./L (not determined [A])
E_rC_{10} -72 hours (95 % C.I.):	7.1 mg a.s./L (not determined [A])
E_bC_{50} - 72 hours (95 % CI):	**3.97 mg a.s./L** (3.92 - 4.02 mg a.s./L [A])
E_yC_{50} - 72 hours (95 % CI): [B]	4.26 (2.59 – 8.96)
E_yC_{20} -72 hours (95 % C.I.): [B]	2.72 (< 0.102 – 3.62)
E_yC_{10} -72 hours (95 % C.I.): [B]	2.16 (< 0.102 – 3.11)
LOEC - 72 hours: lowest concentration with an effect (based on growth rate and biomass)	3.78 mg a.s./L
NOEC - 72 hour: highest concentration without an effect (based on growth rate and biomass)	1.46 mg a.s./L
NOE_yC - 72 hours: [B] highest concentration without an effect	1.6 mg a.s./L
Results – 0 to 96 hours	
E_rC_{50} - 96 hours (95 % CI):	**9.5 mg a.s./L** (not determined [A])
E_bC_{50} - 96 hours (95 % CI):	**4.07 mg a.s./L** (3.83 - 4.31 mg a.s./L)
LOEC - 96 hours: lowest concentration with an effect (based on growth rate and cell density (standing crop))	9.53 mg a.s./L
LOEC - 96 hours: lowest concentration with an effect (based on biomass)	3.78 mg))./L
NOEC - 96 hours: highest concentration without an effect (based on growth rate and cell density (standing crop))	3.78 mg a.s./L
NOEC - 96 hours: highest concentration without an effect (based on biomass)	1.46 mg a.s./L

[A] Reliable confidence intervals could not be determined as the calculated E_rC_{50} values were very near the highest level tested, which was the functional limit of solubility in the testing system.
[B] Please refer to recalculation document by M-757659-01-1

Reliability assessment (EFSA 2015)

The following table provides reliability indicators for EC_{10} values.

Biological endpoints	EC_{10} [mg a.s./L]	95% CL	NW	Relationship $EC_{10}/EC_{20/50}$
Growth Rate	7.1	-[A]	-[B]	-[B]
Yield	2.16	<0.102 – 3.11	1.393 (poor)	EC_{20}, low < EC_{10} < EC_{50}, low (medium)

[A] The confidence interval could not be defined.
[B] Could be calculated as confidence intervals could not be determined.

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Assessment and conclusion by applicant:

The study and its data are considered as acceptable and reliable for use in risk assessment.

The endpoint is: E_rC_{50} (72 hours) = 8.9 mg a.s./L

Metabolite fluopyram-lactam

The metabolite fluopyram-lactam is not considered to be a relevant metabolite for fluopyram. Full details on the rationale are given in the MCA 7.2.1.2.

The UV-VIS absorption spectrum of a solution containing fluopyram showed one maximum at 270 nm (abs 0.1149) and a shoulder at 216 nm (abs 0.3623). The absorption of light by fluopyram terminates at above 292 nm and does not extend into the range of wavelengths relevant for the environment. It can be concluded that the direct interaction of fluopyram in aqueous solution with sunlight is unlikely.

While in the rather artificial photo-transformation study of fluopyram in pure sterile buffer of pH 7, slow but continuous photolytic degradation of fluopyram was observed and the degradation product found was fluopyram-lactam at > 5% AR, confirmation is given by a study considering photolytic degradation of fluopyram in natural water, where degradation of fluopyram was relatively slow under environmental conditions, and no degradation product was detected at > 5% AR and the maximum amount of lactam was 1.0 to 1.2% of AR, only. Since this study is regarded as highest tier of information, it can be concluded that photolytic transformation will only be a minor contributor to the overall fate of fluopyram under natural outdoor conditions, and no photolytic metabolite has to be included in residue definition for aquatic risk assessments.

However, since the study was already submitted previously, a study summary is presented below, although it is formally not required.

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Data Point:	KCA 8.2.6.1/02
Report Author:	[redacted]
Report Year:	2008
Report Title:	Pseudokirchneriella subcapitata growth inhibition test with fluopyram-lactame
Report No:	EBGMP155
Document No:	M-298668-01-1
Guideline(s) followed in study:	OECD Guideline 201:"Freshwater Alga and Cyanobacteria, Growth Inhibition Test" (March 23, 2006); Equivalent to US EPA OPPTS Guideline No. 850.SUPP
Deviations from current test guideline:	Current Guideline: OECD 201 (2006) Deviations: None. All validity criteria were met.
Previous evaluation:	yes, evaluated and accepted in DAR (2011)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The green alga *Pseudokirchneriella subcapitata* were exposed to fluopyram-lactame (BCS-AA10634) under static conditions for 72 hours. Algal cultures with an initial nominal cell count of approximately 1.0 × 10^4 cells/mL were used to test the nominal concentrations 0.0959, 0.241, 0.602, 1.50, 3.76 and 9.40 mg p.m./L. Additionally a control was included. There were 3 replicates for each test concentration and 6 replicates for the control. At 24 hour-intervals, the cell density (cells/mL) of each culture was counted.

Concentrations of fluopyram-lactame were verified by HPLC – UV on day 0 and day 3 for each concentration and control. Measured concentrations were in the 94 - 99 % range of nominal concentrations and no residues were found in the control samples above 0.01 mg p.m./L which was used as the lowest standard concentration during this study. The biological results are based on nominal test concentrations.

The study fulfils all validity criteria of OECD 201 guideline.

No physical abnormalities were observed in the control or any test concentration during the study.

The endpoints based on nominal concentrations were: 72 hours – E_rC_{50}: > 9.4 mg p.m./L, 72 hours – E_bC_{50}: > 9.4 mg p.m./L and 72 hour – E_yC_{50}: > 9.4 mg p.m./L.

I. MATERIAL AND METHODS

Test material	fluopyram-lactame (BCS-AA10634) Batch ID.: SES 10037-8-1 Purity: 94 % w/w
Guideline(s) adaptation	None specified.
Test species	Green algae *Pseudokirchneriella subcapitata*
Culturing conditions	7 – 10 days old stock culture was transferred into a cotton plugged Erlenmeyer flask containing nutrient medium once every week.
Test solutions	Nominal concentrations: 0.0959 – 0.241 – 0.602 – 1.50 – 3.76 – 9.40 mg p m./L Corresponding mean measured concentration: 94.4 – 236 – 583 – 1465 – 3644 – 8870 mg p m./L Control: untreated medium Evidence of undissolved material: not reported
Replication	No. of vessels per concentration (replicates): 3 No. of vessels per control (replicates): 6

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Exposure	Static Total exposure duration: 72 hours
Initial cells density	1 × 10^4 cells/mL in each test group
Test conditions	Temperature: 21.6 - 22.8 °C Photoperiod: 24 hours light Light intensity: 5000 - 6750 lux Type of light: Cool white fluorescents lamps pH of controls: 8.1 - 9.5 (0 - 72 hours) Conductivity: not reported Growth medium same as culture medium: Yes
Parameters Measured / Observations	The pH was measured at each observation time in all test levels and the control. Temperature was measured hourly via a calibrated data logger. Also, light intensity was measured, however time point was not reported. Cell numbers per volume (as a surrogate for biomass per volume) and possible alterations in algae cells such as unusual cell size were estimated by direct algae cell counting under a microscope at a magnification of 400 times.
Sampling for chemical analysis	Samples for analysis of test substance were taken on day 0 and 3. At exposure termination, the contents of all replicate vessels were combined. Samples were analysed by using a high-performance liquid chromatograph (HPLC) – UV.
Data analysis	Probit analysis using linear max. likelihood regression was used for EC_x-value estimation. LOEC/ NOEC determinations were done using the ANOVA procedure and properly selected multiple t-tests. Calculations were done with Microsoft Excel sheets and the further statistical evaluations with the commercial program ToxRat Professional (version 2.09).

II. RESULTS AND DISCUSSION

Table 8.2.6.1- 6: Validity criteria

Validity criteria acc. to OECD 201 (adopted 2006)	Required	Obtained
The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period.	≥ 16	Approx. 105
The mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3) in the control cultures must not exceed 35 %.	< 35 %	16.2 %
The coefficient of variation of average specific growth rates during the 72-hour test period in replicate control cultures must not exceed 7 %.	< 7 %	2.3 %

Analytical results:

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 SANTE/2020/12830, Rev.1.

Recoveries on day 0 and day 3 ranged between 94 and 99 % of nominal concentrations (see table below). The biological results are based on nominal test concentrations.

No residues of fluopyram-lactame were measured in the control samples above 0.01 mg p.m./L which was used as the lowest standard concentration during this study.

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Table 8.2.6.1- 7: Analytical results

Nominal concentration [mg p m./L]	Measured concentration [µg p m./L]		% of nominal	
	Day 0 (New)	Day 3 (Old)	Day 0 (New)	Day 3 (Old)
0.0959	0.951	0.0937	99	98
0.241	0.237	0.235	98	98
0.602	0.584	0.582	97	97
1.500	1.449	1.481	97	99
3.76	3.649	3.638	97	97
9.4	8.818	8.921	94	95

Biological results:

Observations:

No observations reported.

Table 8.2.6.1- 8: Cell density

Nominal concentration [mg p m./L]	Mean cell density [x 10^4 cells/mL]		
	24 h	48 h	72 h
Control	5.8	21.8	105.3
0.0959	4.0	20.0	101.3
0.241	6.2	22.2	108.2
0.602	5.2	21.8	107.8
1.500	4.8	23.8	107.7
3.76	4.2	19.3	115.0
9.4	5.0	23.7	106.2

Table 8.2.6.1- 9: Algae growth rate

Nominal concentration [mg p.m./L]	Mean growth rate [A] [1/d]	% Inhibition of average specific growth rate [B]
	0 - 72 h	0 - 72 h
Control	1.551	-
0.0959	1.536	1.0
0.241	1.560	-0.6
0.602	1.556	-0.3
1.500	1.559	-0.5
3.76	1.581	-2.0
9.4	1.555	-0.3

[A] Calculated from the cell density data.
[B] -% inhibition: increase in growth relative to the control

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III. CONCLUSION

The study meets the validity criteria and the 0 - 72 endpoints based on nominal concentrations were:

Results – 0 to 72 hours	
E_rC_{50} - 72 hours (95 % CI):	**> 9.40 mg p.m./L** (n.d.)
E_rC_{20} -72 hours (95 % C.I.):	> 9.40 mg p.m./L (n.d.)
E_rC_{10} -72 hours (95 % C.I.):	> 9.40 mg p.m./L (n.d.)
E_bC_{50} - 72 hours (95 % CI):	**> 9.40 mg p.m./L** (n.d.)
E_bC_{20} -72 hours (95 % C.I.):	> 9.40 mg p.m./L (n.d.)
E_bC_{10} -72 hours (95 % C.I.):	> 9.40 mg p.m./L (n.d.)
E_yC_{50} - 72 hours (95 % CI): [A]	**> 9.40 mg p.m./L** **(n.d.)**
E_yC_{20} - 72 hours (95 % CI): [A]	> 9.40 mg p.m./L (n.d.)
E_yC_{10} - 72 hours (95 % CI): [A]	> 9.40 mg p.m./L (n.d.)
LOEC - 72 hours: lowest concentration with an effect (based on growth rate and biomass)	> 9.4 mg p m./L
NOEC - 72 hours: highest concentration without an effect (based on growth rate, biomass and yield [A])	≥ 9.4 mg p m./L

n.d.: Not determined due to mathematical reasons or inappropriate data
[A] Please refer to recalculation document by M-757718-01-1

Assessment and conclusion by applicant:

The study and its data are considered as supplementary data with no use in risk assessment.

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Recalculation of endpoints for the active substance fluopyram

Data Point:	KCA 8.2.6.1/03
Report Author:	[redacted]
Report Year:	2020
Report Title:	Statistical evaluation (non-GLP) of the study M-286541-01-1 (Banman, C. S., Lam, C. V., 2007, EBGMP048) on the chronic toxicity of AE C656948 technical to Pseudokirchneriella subcapitata (currently known as: Raphidocelis subcapitata) under static conditions
Report No:	112463
Document No:	M-757659-01-1
Guideline(s) followed in study:	--
Deviations from current test guideline:	Current Guideline: not applicable Deviations: not applicable
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	Yes

Summary

In the existing report M-286541-01-1, endpoints for yield were statistically determined at 72 h.

A statistical evaluation addressing the calculation of valid 72-h EC_{10}, EC_{20}, and EC_{50} values as well as NOEC values for yield was conducted to fulfil the data requirements according to regulation EU 283/2013. Furthermore, the validity criteria for the study were re-evaluated according to the current guideline OECD 201 (2011).

The recalculations were performed with the software ToxRat Professional (Version 3.3.0) with the nominal concentrations provided in the report.

A control and a solvent control (dimethylformamide) were tested in parallel. The data from both were pooled for statistical testing.

Models providing best fit to the respective data were selected and are as follows: In order to derive Effect Concentrations that have 10, 20 and 50 % effects on yield of the test subjects (EC_{10}, EC_{20}, and EC_{50}), a probit analysis using linear maximum likelihood regression was performed.

NOEC was determined by Williams Multiple Sequential t-test Procedure (one-sided smaller, $p = 0.05$). To test for normal distribution and variance homogeneity, a Shapiro-Wilk's test and a Levene's test were performed respectively.

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Table 8.2.6.1- 10: Re-calculated EC_{10}, EC_{20}, EC_{50} and NOEC values based on nominal concentrations

Endpoint	Fluopyram (AE C656948) [mg a.s./L] Yield
72 hours - EC_{10} (95 % C.I.)	2.16 (< 0.102 – 3.11)
72 hours - EC_{20} (95 % C.I.)	2.72 (< 0.102 – 3.62)
72 hours - EC_{50} (95 % C.I.)	4.26 (2.59 – 8.96)
72 hours - NOEC	1.6

C.I.: Confidence interval

Assessment and conclusion by applicant:

The data are considered as supplementary data with no use in risk assessment.

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Recalculation of endpoints for the metabolite fluopyram-lactam

As described in detail under KCA 8.2.6.1/02 the metabolite fluopyram-lactam is not considered to be a relevant metabolite for fluopyram. However, since the original study report was submitted previously, a recalculation of the algal endpoints is presented below, although it is formally not required.

Data Point:	KCA 8.2.6.1/04
Report Author:	[redacted]
Report Year:	2020
Report Title:	Statistical evaluation (non-GLP) of the study M-298668-01-1 (Dorgerloh, M., 2008, EBGMP155) on the chronic toxicity of fluopyram-lactame to Pseudokirchneriella subcapitata (currently known as: Raphidocelis subcapitata) under static conditions
Report No:	M-757718-01-1
Document No:	M-757718-01-1
Guideline(s) followed in study:	None
Deviations from current test guideline:	Current Guideline: not applicable Deviations: not applicable
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	Yes

Summary

In the existing report M-298668-01-1, endpoints for yield were statistically determined at 72 h.

A statistical evaluation addressing the calculation of valid 72-h EC_{10}, EC_{20}, and EC_{50} values as well as NOEC values for yield was conducted to fulfill the data requirements according to regulation EU 283/2013. Furthermore, the validity criteria for the study were re-evaluated according to the current guideline OECD 201 (2011).

The recalculations were performed with the software ToxRat Professional (Version 3.3.0) with the nominal concentrations provided in the report.

Due to lack of inhibition compared to the control, no further statistical analysis was performed.

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Table 8.2.6.1- 11: Re-calculated EC_{10}, EC_{20}, EC_{50} and NOEC values based on nominal concentrations

Endpoint	Fluopyram-lactam [mg p m./L]
	Yield
72 hours - EC_{10} (95 % C.I.)	n.d.
72 hours - EC_{20} (95 % C.I.)	n.d.
72 hours - EC_{50} (95 % C.I.)	n.d.
72 hours - NOEC	≥ 9.40

C.I.: Confidence interval
n.d.: Not determined

Assessment and conclusion by applicant:

The data is considered as supplementary data with no use in risk assessment.

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Metabolite fluopyram-7-hydroxy

Data Point:	KCA 8.2.6.1/05
Report Author:	
Report Year:	2020
Report Title:	Evaluation of Pseudokirchneriella subcapitata's growth inhibition (following OECD 201 & OCSPP850.4500) on BCS-AA10065 (7-hydroxy-fluopyram)
Report No:	RRCo-000775_01
Document No:	M-758708-01-1
Guideline(s) followed in study:	GUIDELINE OECD 201 (23 March 2006) Freshwater Alga and Cyanobacteria, Growth Inhibition Test OCSPP 850.4500: Algal Toxicity (June 2012)
Deviations from current test guideline:	Current Guideline: OECD 201 (2006) Deviations: None. All validity criteria were met.
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 green alga *Pseudokirchneriella subcapitata* were exposed to fluopyram-7-hydroxy (BCS-AA10065) under static conditions for 96 hours. Algal cultures with an initial nominal cell count of approximately 1.0×10^4 cells/mL were used to test the nominal concentrations 5.4, 10.8, 21.6, 43.2 and 86.4 mg p.m./L. Additionally a control was included. There were 4 replicates for each test concentration and the control. At 24 hour-intervals, the cell density (cells/mL) of each culture was counted.

Concentrations of fluopyram-7-hydroxy were verified by HPLC – MS/MS on day 0, day 3 and day 4 for each concentration and control. Measured concentrations were in the 92 - 110 % range of nominal concentrations and no residues were found in the control samples. The limit of detection and the limit of quantification were 0.0033 mg p.m./L and 0.01 mg p.m./L, respectively. The biological results are based on nominal test concentrations.

The study fulfils all validity criteria of OECD 201 guideline.

No physical abnormalities were observed in the control or any test concentration during the study.

The 0-72 hours endpoints based on nominal concentrations were: 72 hours – E_rC_{50}: 20.9 mg p.m./L and 72 hour – E_yC_{50}: 13.0 mg p.m./L.

The 0-96 hours endpoints based on nominal concentrations were: 96 hours – E_rC_{50}: 21.1 mg p.m./L, 72 hours – E_bC_{50}: 12.6 mg p.m./L and 72 hour – E_yC_{50}: 13.7 mg p.m./L.

I. MATERIAL AND METHODS

Test material	Fluopyram-7-hydroxy (BCS-AA10065) Batch ID.: T15F019 Purity: 99.67 % w/w
Guideline(s) adaptation	None specified.
Test species	Green algae *Pseudokirchneriella subcapitata*
Culturing conditions	In-house, 4-day old batch culture in log phase growth held under test conditions
Test solutions	Nominal concentrations: 5.4 – 10.8 – 21.6 – 43.2 – 86.4 mg p.m./L

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	Measured concentration ranged between 92.0 and 109.8 % of nominal concentrations Control: untreated medium Evidence of undissolved material: not reported
Replication	No. of vessels per concentration (replicates): 4 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 ± 2 °C Photoperiod: 24 hours light Light intensity: 4440 - 8880 lux Type of light: Not reported pH of controls: 7.6 – 7.5 (0 - 96 hours) Conductivity: Not reported Growth medium same as culture medium: Yes
Parameters Measured / Observations	The pH was measured at test start and after 96 hours in all treatments. Temperature and light intensity were determined; however, time point was not reported. Cell numbers per volume (as a surrogate for biomass per volume) was measured every day in each flask using an electronic particle counter. At test end after 96 hours, any abnormal appearance of the algae was determined by microscopic observation.
Sampling for chemical analysis	Samples for analysis of test substance were taken on day 0, day 3 and 4. The analytical samples were sampled on day 0 in the test solutions preparations and on day 3 and 4 in the content of the vessels pooled by replicate. Samples were analysed by using a high-performance liquid chromatograph (HPLC) – MS/MS.
Data analysis	The NOEC and LOEC was determined by comparison of treatment means using analysis of variance (ANOVA) techniques (parametric or non-parametric) and pot hoc test. EC_{10}/ EC_{20}/ EC_{50} values for yield, growth rate area under the curve, and confidence limits were obtained by calculation according to Regtox Macro ("Vindimian"), using the Hill model. Calculations were done with Microsoft Excel sheets and the further statistical evaluations with the R software.

II. RESULTS AND DISCUSSION

Table 8.2.6.1- 12: Validity criteria

Validity criteria acc. to OECD 201 (adopted 2006)	Required	Obtained
The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period.	≥ 16	111
The mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3) in the control cultures must not exceed 35 %.	< 35 %	18.7 %
The coefficient of variation of average specific growth rates during the 72-hour test period in replicate control cultures must not exceed 7 %.	< 7 %	1.09 %

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Analytical results:

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 SANTE/2020/12830, Rev.1.

Recoveries on day 0, day 3 and day 4 ranged between 92.0 and 109.8 % of nominal concentrations (see table below). The biological results are based on nominal test concentrations.

No residues of fluopyram-7-hydroxy were measured in the control samples. The limit of detection and the limit of quantification were 0.0033 mg p.m./L and 0.01 mg p.m./L, respectively.

Table 8.2.6.1- 13: Analytical results

Nominal concentration [mg p.m./L]	Measured concentration [µg p.m./L]			% of nominal [A]		
	Day 0	Day 3	Day 4	Day 0	Day 3	Day 4
5.4	4.966	5.455	5.653	92.0	101.0	104.7
10.8	10.645	11.483	10.694	98.6	106.3	99.0
21.6	21.338	21.892	22.381	98.8	101.4	103.6
43.2	41.834	44.885	46.122	96.8	103.9	106.8
86.2	87.267	89.622	94.673	101.2	104.0	109.8

[A] Not given in report. Calculations based on measured concentrations on each sampling day.

Biological results:

Observations:

No abnormal appearance of the algae at the start and at the end of the test was recorded.

Table 8.2.6.1- 14: Cell density

Nominal concentration [mg p m./L]	Mean cell density [x 10^4 cells/mL]			
	24 h	48 h	72 h	96 h
Control	3.825	16.593	110.800	459.350
5.4	3.538	16.005	109.685	439.675
10.8	3.096	13.490	85.313	365.400
21.6	1.289	1.646	1.897	3.259
43.2	1.186	1.197	1.277	1.467
86.2	1.241	1.197	1.425	1.461

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Table 8.2.6.1- 15: Algae growth rate and the percent inhibition

Nominal concentration [mg p m./L]	Mean growth rate [A] [1/d]	% Inhibition of average specific growth rate [B]
	0 - 96 h	0 - 96 h
Control	1.53	-
5.4	1.52	0.73
10.8	1.47	3.74
21.6	0.29	80.79
43.2	0.10	93.76
86.2	0.09	93.83

[A] Calculated from the cell density data.
[B] -% inhibition: increase in growth relative to the control

Table 8.2.6.1- 16: Yield and cumulative biomass and the corresponding percent inhibitions

Nominal concentration [mg p.m./L]	Mean yield [A] [cell/mL]	% Inhibition of yield [B]	Cumulative biomass [C]	% Inhibition of biomass [C]
	0 - 96 h	0 - 96 h	0 - 96 h	0 - 96 h
Control	4583500	-	4200888	-
5.4	4386750	4.29	3455659	24.61
10.8	3644000	20.50	2810981	38.67
21.6	22594	99.51	29625	99.35
43.2	4666	99.90	8929	99.81
86.2	4612	99.90	10940	99.76

[A] Calculated from the cell density data.
[B] -% inhibition: increase relative to the control
[C] Cumulative biomass is equal to the area under the growth curve (area $\times 10^6$).

III. Conclusion

The study meets the validity criteria and the 0 - 72 hours endpoints as well as the 0-96 hours endpoints based on nominal concentrations were:

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Results – 0 to 72 hours	
E_rC_{50} - 72 hours (95 % CI):	**20.9 mg p m./L** (20.8 – 21.1 mg p.m./L)
E_rC_{20} -72 hours (95 % C.I.):	20.4 mg p m./L (20.2 – 20.6 mg p.m./L)
E_rC_{10} -72 hours (95 % C.I.):	20.2 mg p m./L (19.9 – 20.3 mg p.m./L)
E_yC_{50} - 72 hours (95 % C.I.):	**13.0 mg p.m./L** (12.3 – 13.9 mg p.m./L)
E_yC_{20} - 72 hours (95 % C.I.):	10.2 mg p m./L (9.6 – 10.9 mg p m./L)
E_yC_{10} - 72 hours (95 % C.I.): [A]	8.9 mg p.m./L (8.1 – 9.7 mg p.m./L)
$LOE_{r/y}C$ - 72 hours: lowest concentration with an effect (based on growth rate and yield)	10.8 mg p m./L
$NOE_{r/y}C$ - 72 hours: highest concentration without an effect (based on growth rate and yield)	5.4 mg p m./L
Results – 0 to 96 hours	
E_rC_{50} - 96 hours (95 % C.I.):	**21.1 mg p m./L** (20.9 – 21.2 mg p.m./L)
E_rC_{20} - 96 hours (95 % C.I.):	20.7 mg p m./L (20.2 – 20.7 mg p.m./L)
E_rC_{10} - 96 hours (95 % C.I.):	20.4 mg p m./L (19.8 – 20.5 mg p.m./L)
E_yC_{50} - 96 hours (95 % C.I.):	**13.7 mg p.m./L** (13.7 – 13.7 mg p.m./L)
E_yC_{20} - 96 hours (95 % C.I.):	10.9 mg p m./L (10.9 – 10.9 mg p.m./L)
E_yC_{10} - 96 hours (95 % C.I.):	9.6 mg p.m./L (9.6 – 9.6 mg p.m./L)
E_bC_{50} - 96 hours (95 % C.I.):	**12.6 mg p m./L** (12.2 – 12.8 mg p.m./L)
E_bC_{20} - 96 hours (95 % C.I.):	10.1 mg p m./L (9.2 – 10.5 mg p m./L)
E_bC_{10} - 96 hours (95 % C.I.):	8.9 mg p.m./L (7.8 – 9.4 mg p.m./L)
$LOE_{r/y}C$ - 96 hours: lowest concentration with an effect (based on growth rate and yield)	10.8 mg p m./L
$NOE_{r/y}C$ - 96 hours: highest concentration without an effect (based on growth rate and yield)	5.4 mg p m./L
LOE_bC - 96 hours: lowest concentration with an effect (based on area under growth curve)	5.4 mg p.m./L
NOE_bC - 96 hours: highest concentration without an effect (based on area under growth curve)	< 5.4 mg p.m./L

Reliability assessment (EFSA 2015)

The following table provides reliability indicators for EC_{10} values for *Pseudokirchneriella subcapitata.*

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Biological endpoints	EC_{10} [mg p.m./L]	95% CL	NW	Relationship $EC_{10}/EC_{20/50}$
Growth Rate	20.2	19.9 – 20.3	0.020 (excellent)	EC_{20}, low < EC_{10} < EC_{50}, low (medium)
Yield	8.9	8.1 – 9.7	0.180 (excellent)	EC_{10} < EC_{20}, low (high)

Assessment and conclusion by applicant:

The study and its data are considered as acceptable and reliable for use in risk assessment.

The endpoint is: E_rC_{50} (72 hours) = 20.9 mg p.m./L

Metabolite trifluoroacetic acid (TFA)

Data Point:	KCA 8.2.6.1/06
Report Author:	[redacted]
Report Year:	1993
Report Title:	The toxicity of sodium trifluoroacetate to the alga Selenastrum capricornutum at low concentrations
Report No:	C047121
Document No:	M-247818-02-1
Guideline(s) followed in study:	OECD: 201 (1984)
Deviations from current test guideline:	Current Guideline: OECD 201 (2006) Deviations: Four test concentrations were used instead of a minimum of 5 recommended in the OECD 201 guideline. The spacing factor was 3.33 and thus higher than the maximum recommended factor of 3.2. Cell density was 0.64 x 104 instead of 1.0 x 104 cells/mL. These deviations were not expected to have impacted the study results. All validity criteria were met.
Previous evaluation:	yes, evaluated and accepted in flurtamone RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The green alga *Pseudokirchneriella subcapitata* (formerly known as *Selenastrum capricornutum,* currently known as *Raphidocelis subcapitata*) were exposed to sodium trifluoroacetate under static conditions for 72 hours. Algal cultures with an initial nominal cell count of approximately 0.64 × 10^4 cells/mL were used to test the nominal concentrations 0.036, 0.12, 0.36, and 1.20 mg p.m./L. Additionally a control was included. There were 4 replicates for each test concentration and for the control. At 24 hour intervals, the cell density (cells/mL) of each culture was counted.

Concentrations of the stock solution and of the highest test concentration of sodium trifluoroacetate were verified by ion chromatography on day 0 and day 3. Measured concentrations were in the 92 - 100 % range of nominal concentrations. The control samples were not analysed. The biological results are based on nominal test concentrations.

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The study fulfils all validity criteria of OECD 201 guideline.

No physical abnormalities were observed in the control or any test concentration during the study.

The endpoints based on nominal concentrations of sodium trifluoroacetate were: 72 hours – E_rC_{50}: > 1.20 mg p.m./L, 72 hours – E_bC_{50}: > 1.20 mg p.m./L and 72 hours – E_yC_{50}: > 1.20 mg p.m./L.

The converted endpoints based on nominal concentrations of trifluoroacetic acid were: 72 hours – E_rC_{50}: > 1.01 mg p.m./L, 72 hours – E_bC_{50}: > 1.01 mg p.m./L and 72 hours – E_yC_{50}: > 1.01 mg p.m./L.

I. MATERIAL AND METHODS

Test material	Sodium trifluoroacetate Batch ID.: ACA9135AB Purity: > 99 % w/w
Guideline(s) adaptation	None specified.
Test species	Green algae *Pseudokirchneriella subcapitata* (formerly known as *Selenastrum capricornutum* currently known as *Raphidocelis subcapitata*)
Culturing conditions	The preculture used for this study was transferred into fresh algae medium 4 days prior to test initiation.
Test solutions	Nominal concentrations: 0.036 – 0.12 – 0.36 – 1.2 mg p m./L Control: untreated medium Evidence of undissolved material: not reported
Replication	No. of vessels per concentration (replicates): 4 No. of vessels per control (replicates): 4
Exposure	Static Total exposure duration: 72 hours
Initial cells density	0.64 × 10^4 cells/mL in each test group
Test conditions	Temperature: 24 - 25 °C Photoperiod: 24 hours light Light intensity: 6200 lux Type of light: fluorescents lamps pH of controls: 7.2 - 7.6 Conductivity: not reported Growth medium same as culture medium: not reported
Parameters Measured / Observations	The pH was measured at day 0 and 3 in one replicate of each test concentration. Temperature was measured continuously in the shaking incubator during the experiment. Also, light intensity was measured, however time point was not reported. At day 1, 2 and 3 the absorption at 750 nm was measured in all replicates, using a spectrophotometer (Hitachi U-2000) and a cuvette with a pathlength of 5 cm. Cell densities were determined on day 1, 2 and 3 using a calibration line. At test termination on day 3, the condition of the algae was examined by microscope and any abnormalities were recorded.
Sampling for chemical analysis	At test initiation 4 samples were taken of the stock solution and two samples from the highest test concentration (1.2 mg p.m./L). At the end of the test one sample was taken from each replicate of the highest concentration (1.2 mg p m./L). No chemical analysis was performed for the control samples. Samples were analysed by using an ion chromatograph.
Data analysis	The NOEC was determined at the highest concentration that does not show a statistically significant response (Williams, 1972) in comparison to the control. The NOEC based on growth rate data was not determined, because the NOEC, based on areas under the growth curve (biomass integral), was a much more sensitive parameter.

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II. Results and discussion

Table 8.2.6.1- 17: Validity criteria

Validity criteria acc. to OECD 201 (adopted 2006)	Required	Obtained
The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period.	≥ 16	195 [A]
The mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3) in the control cultures must not exceed 35 %.	≤ 35 %	11.9 % [A]
The coefficient of variation of average specific growth rates during the 72-hour test period in replicate control cultures must not exceed 7 %.	≤ 7 %	1.25 % [A]

[A] Not given in report. Calculations based cell densities on day 0, 2 and 3.

Analytical results:

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 SANTE/2020/12830, Rev.1.

Analysis of the stock solution and the highest test concentration (1.2 mg p.m./L) on day 0 and day 3 revealed recoveries between 92 and 100 % of nominal concentrations (see table below). The biological results are based on nominal test concentrations.

The control samples were not analysed.

Table 8.2.6.1- 18: Analytical results

Nominal concentration [mg p m./L]	Measured concentration [mg p m./L]		% of nominal	
	Day 0	Day 3	Day 0	Day 3
237 [A] (stock solution)	225	-	95	-
	225	-	95	-
	225	-	95	-
	230	-	97	-
1.2 [B]	1.1	1.2	92	100
	1.2	1.2	100	100
	-	1.2	-	100
	-	1.2	-	100

[A] Nominal concentration of the stock solution
[B] Nominal concentration of the highest test concentration

Biological results:

Observations:

No effect of sodium trifluoroacetate on the appearance of the algae was observed.

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Table 8.2.6.1- 19: Cell density

Nominal concentration [mg p m./L]	Mean cell density [×10⁶ cells/mL]		
	24 h	48 h	72 h
Control	0.035	0.252	1.25
0.036	0.034	0.243	1.24
0.12	0.034	0.243	1.26
0.36	0.033	0.213	1.11
1.2	0.031 [A]	0.173 [A]	0.911 [A]

[A] Based on three replicates instead of 4 due to an outlier of the growth data in one replicate.

Table 8.2.6.1- 20: Algae growth rate

Nominal concentration [mg p.m./L]	Mean growth rate [A] [1/d]	% Inhibition of average specific growth rate [B]
	0 - 72 h	0 - 72 h
Control	1.76	
0.036	1.76	0.057
0.12	1.76	- 0.28
0.36	1.72	2.3
1.2	1.65 [C]	6.1 [C]

[A] Calculated from the cell density data.
[B] -% inhibition: increase in growth relative to the control
[C] Based on three replicates instead of 4 due to an outlier of the growth data in one replicate.

Table 8.2.6.1- 21: Biomass

Nominal concentration [mg p m./L]	Cumulative biomass [A]	% Inhibition of cumulative biomass [B]
	0 - 72 h	0 - 72 h
Control	0.894	-
0.036	0.885	1.0
0.12	0.891	0.34
0.36	0.783	12
1.2	0.639 [C]	29 [C]

[A] Cumulative biomass is equal to the area under the growth curve (area $\times 10^6$).
[B] % Inhibition =100-((Treatment group parameter mean/control parameter mean)*100).
[C] Based on three replicates instead of 4 due to an outlier of the growth data in one replicate.

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III. CONCLUSION

The study meets the validity criteria and the 0 - 72 endpoints based on nominal concentrations of sodium trifluoroacetate were:

Results – 0 to 72 hours	
E_rC_{50} - 72 hours (95 % C.I.):	**> 1.2 mg p.m./L [B]** (n.d.)
E_rC_{20} -72 hours (95 % C.I.): [A]	**> 1.2 mg p.m./L [B]** (n.d.)
E_rC_{10} -72 hours (95 % C.I.): [A]	**> 1.2 mg p.m./L [B]** (n.d.)
E_bC_{50} - 72 hours (95 % C.I.):	**> 1.2 mg p.m./L [B]** (n.d.)
E_bC_{20} -72 hours (95 % C.I.): [A]	0.691 mg p m./L (0.479 – 0.974 mg p m./L)
E_bC_{10} -72 hours (95 % C.I.): [A]	0.292 mg p m./L (0.129 – 0.432 mg p m./L)
E_yC_{50} - 72 hours (95 % C.I.): [A]	**> 1.2 mg p.m./L [B]** (n.d.)
E_yC_{20} - 72 hours (95 % C.I.): [A]	0.736 mg p m./L (0.519 – 1.026 mg p m./L)
E_yC_{10} - 72 hours (95 % C.I.): [A]	0.324 mg p m./L (0.148 – 0.470 mg p m./L)
LOEC - 72 hours: [A] highest concentration without an effect (based on yield, growth and biomass)	0.36 mg p.m./L
NOE$_b$C - 72 hours: highest concentration without an effect (based on biomass)	0.12 mg p.m./L
NOE$_{r,y}$C - 72 hours: [A] highest concentration without an effect (based on yield and growth)	0.12 mg p.m./L

n.d.: Not determined due to mathematical reasons or inappropriate data
[A] Please refer for endpoints to recalculation document by M-762268-02-1
[B] Based on the molecular weights, a concentration of >1.2 mg sodium trifluoroacetate/L corresponds to >1.01 mg trifluoroacetate acid/L. As the study was conducted with sodium trifluoroacetate which is the sodium salt of trifluoroacetic acid, the endpoint was converted to trifluoroacetic acid with factor 0.84.

Assessment and conclusion by applicant:

The study and its data are considered as acceptable and reliable for use in risk assessment.

The endpoint is: E_rC_{50} (72 hours) > 1.2 mg p.m./L (sodium trifluoroacetate) corresponding to > 1.01 mg p.m./L (trifluoroacetic acid)

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Data Point:	KCA 8.2.6.1/07
Report Author:	[redacted]
Report Year:	2021
Report Title:	Typographical correction of re-evaluation of M-247818-01-1: The toxicity of sodium trifluoroacetate to the Alga selenastrum capricornutum at low concentrations
Report No:	M-762268-02-1
Document No:	M-762268-02-1
Guideline(s) followed in study:	None
Deviations from current test guideline:	Current Guideline: not applicable
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	Yes

Summary

A statistical evaluation addressing the calculation of valid 72-h EC_{10}, EC_{20}, and EC_{50} values as well as LOEC and NOEC values for yield, growth and biomass was conducted for M-247818-02-1 to fulfill the data requirements according to regulation EU 283/2013. Furthermore, the validity criteria for the study were re-evaluated according to the current guideline OECD 201 (2011).

The recalculations were performed with the software ToxRat Professional (Version 3.2.1) with nominal concentrations provided in the report.

In order to derive Effect Concentrations that have 10, 20 and 50 % effects on yield, growth and biomass of the test subjects (EC_{10}, EC_{20}, and EC_{50}), a probit analysis was performed.

NOEC and LOEC were determined by Williams Multiple Sequential t-test Procedure (one-sided smaller, $p = 0.05$).

Table 8.2.6.1- 22: Re-calculated EC_{10}, EC_{20}, EC_{50}, LOEC and NOEC values based on nominal concentrations

Endpoint	Sodium Trifluoroacetate ACA9135AB [mg p m./L]		
	Yield	**Growth**	**Biomass**
72 hours - EC_{10} (95 % C.I.)	0.324 (0.148 – 0.470)	>1.2	0.292 (0.129 – 0.432)
72 hours - EC_{20} (95 % C.I.)	0.736 (0.519 – 1.026)	>1.2	0.691 (0.479 – 0.974)
72 hours - EC_{50} (95 % C.I.)	3.530 (2.069 – 12.563)	>1.2	3.583 (2.058 – 13.179)
72 hours - LOEC	0.36	0.36	0.36
72 hours - NOEC	0.12	0.12	0.12

C.I.: Confidence interval

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Reliability assessment (EFSA 2015)

The following table provides reliability indicators for EC_{10} values for *Pseudokirchneriella subcapitata*.

Biological endpoints	**Method**	**EC_{10} [mg p m./L]**	**95% CL**	**NW**	**Relationship $EC_{10}/EC_{20/50}$**
Growth rate (72 h)	Probit	> 1.2	n.d.	n.d.	n.d.
Yield (72 h)	Probit	0.324	0.148 – 0.47	0.99 (fair)	$EC_{10} < EC_{20,low}$ (high)
Biomass (72 h)	Probit	0.292	0.129 – 0.432	1.04 (poor)	$EC_{10} < EC_{20,low}$ (high)

Assessment and conclusion by applicant:

The study and its data are considered as acceptable and reliable for use in risk assessment.

The endpoint is: E_rC_{50} (72 hours) > 1.2 mg p.m./L (sodium trifluoroacetate) corresponding to > 1.01 mg p.m./L (trifluoroacetic acid)

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Data Point:	KCA 8.2.6.1/08
Report Author:	[redacted]
Report Year:	1992
Report Title:	The toxicity of sodium trifluoroacetate to the alga Selenastrum capricornutum
Report No:	C047124
Document No:	M-247820-01-1
Guideline(s) followed in study:	OECD: 201 (1984)
Deviations from current test guideline:	Current Guideline: OECD 201 (2006) Deviations: Due to unexpectedly high growth of the algae the pH had increased 2.1 units and therefore, more than 1.5 units. The spacing factor was between 3 and 3.33 and thus higher than the maximum recommended factor of 3.2. During 20 minutes in the first 24 hours the temperature increased to 27 °C. These deviations were not expected to have impacted the study results. All validity criteria were met.
Previous evaluation:	yes, evaluated and accepted in flurtamone RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The green alga *Pseudokirchneriella subcapitata* (formerly known as *Selenastrum capricornutum,* currently known as *Raphidocelis subcapitata*) were exposed to sodium trifluoroacetate under static conditions for 72 hours. Algal cultures with an initial nominal cell count of approximately 1 × 10^4 cells/mL were used to test the nominal concentrations 0.36, 1.2, 3.6, 12, 36, 120, 360 and 1200 mg p.m./L. Additionally a control was included. There were 4 replicates for each test concentration and 8 for the control, including one control for determination of adsorption. At 24 hour-intervals, the

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cell density (cells/mL) of each culture was counted.

Samples from each test concentration were verified by ion chromatography on day 0 and day 3. Measured concentrations were in the 92 - 111 % range of nominal concentrations and no residues were found in the control samples above 0.1 mg p.m./L. The biological results are based on nominal test concentrations.

The study fulfils all validity criteria of OECD 201 guideline.

The control algae showed no toxicity symptoms, while the algae exposed to the highest concentration (1200 mg p.m./L) were clearly affected. The other test concentrations were not observed.

The endpoints based on nominal concentrations of sodium trifluoroacetate were: 72-hour E_rC_{50}: 160 mg p.m./L, 72- hour E_bC_{50}: > 4.8 mg p.m./L and 72-hour E_yC_{50}: 4.190 mg p.m./L.

The converted endpoints based on nominal concentrations of trifluoroacetic acid were: 72-hour E_rC_{50}: 134.4 mg p.m./L, 72- hour E_bC_{50}: > 4.03 mg p.m./L and 72-hour E_yC_{50}: 3.52 mg p.m./L.

I. MATERIAL AND METHODS

Test material	Sodium trifluoroacetate Batch ID.: ACA9135AB Purity: 99 % w/w
Guideline(s) adaptation	None specified.
Test species	Green algae *Pseudokirchneriella subcapitata (*formerly known as *Selenastrum capricornutum* currently known as *Raphidocelis subcapitata*)
Culturing conditions	The preculture used for this study was transferred into fresh algae medium 5 days prior to test initiation.
Test solutions	Nominal concentrations: 0.36 – 1.2 – 3.6 – 12 – 36 – 120 – 360 – 1200 mg p m./L Mean measured concentrations: 0.4 – 1.2 – 3.6 – 12.3 – 36 – 121 – 353 – 1160 mg p m./L Control: untreated medium Evidence of undissolved material: not reported
Replication	No. of vessels per concentration (replicates): 4 No. of vessels per control (replicates): 7
Exposure	Static Total exposure duration: 72 hours
Initial cells density	1 × 10^4 cells/mL in each test group
Test conditions	Temperature: 23.5 – 24.5 °C (slight increase to 27 °C during 20 min in the first 24 hours) Photoperiod: 24 hours light Light intensity: 7200 lux Type of light: fluorescents lamps pH of controls: 7.3 – 9.4 Conductivity: not reported Growth medium same as culture medium: Yes
Parameters Measured / Observations	The pH was measured at day 0 and 3 in one replicate of each test concentration. Temperature was measured continuously in the shaking incubator during the experiment. Also, light intensity was measured, however time point was not reported. At the start of the test the absorption was measured in one replicate per test concentration. At day 1, 2 and 3 the absorption at 680 nm was measured in all 39 replicates, using a spectrophotometer (Varian DMS 90) and a cuvette with a pathlength of 5 cm. Cell densities were determined on day 1, 2 and 3 using a calibration line. At test termination on day 3, the condition of the algae was examined by microscope in a sample of the control and in the highest test concentration (1200 mg p.m./L) and any abnormalities were recorded.

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Sampling for chemical analysis	At test initiation a sample was taken from all test solutions. At the end of the test samples were taken from each vessel and pooled per concentration. This was done in duplicate of which only one sample was analysed. Samples were analysed by using an ion chromatograph.
Data analysis	The E_bC_{50}/ E_rC_{50} values were estimated using a figure which expresses the percentage of biomass/ growth rate inhibition against the logarithm of the test substance concentration. The NOEC was determined at the highest concentration that does not show a statistically significant response (Williams, 1972) in comparison to the control. The NOEC based on growth rate data was not determined, because the NOEC, based on areas under the growth curve (biomass integral), was a much more sensitive parameter.

II. Results and Discussion

Table 8.2.6.1- 23: Validity criteria

Validity criteria acc. to OECD 201 (adopted 2006)	Required	Obtained
The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period.	> 16	176 [A]
The mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3) in the control cultures must not exceed 35 %.	< 35 %	17.1 [A]
The coefficient of variation of average specific growth rates during the 72-hour test period in replicate control cultures must not exceed 7 %.	< 7 %	0.68 [A]

[A] Not given in report. Calculations based cell densities of 7 replicates on day 0, 2 and 3.

Analytical results:

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 SANTE/2020/12830, Rev.1.

Recoveries on day 0 and day 3 ranged between 92 and 111 % of nominal concentrations (see table below). The biological results are based on nominal test concentrations.

No residues of the metabolite sodium trifluoroacetate were measured in the control samples above 0.1 mg p.m./L.

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Table 8.2.6.1- 24: Analytical results

Nominal concentration [mg p m./L]	Measured concentration [mg p m./L]		% of Nominal [A]		Mean measured concentration [mg p m./L]	% of nominal [A]
	Day 0	Day 3	Day 0	Day 3		
Control	< 0.1	< 0.1	-	-	< 0.1	-
0.36	0.4	0.4	111	111	0.4	111
1.2	1.1	1.2	92	100	1.2	100
3.6	3.5	3.6	97	100	3.6	100
12	12.6	11.9	105	99	12.3	103
36	36	35	100	97	36	100
120	120	121	100	101	121	101
360	355	350	99	97	353	98
1200	1150	1170	96	102	1160	97

[A] Not given in report. Calculations based on mean measured concentrations on day 0 and day 3

Biological results:

Observations:
At day 3 the control algae showed no toxicity symptoms, while the algae exposed to the highest concentration (1200 mg p.m./L) were clearly affected. The other test concentrations were not observed.

Table 8.2.6.1- 25: Cell density

Nominal concentration [mg p m./L]	Mean cell density [$\times 10^6$ cells/mL]		
	24 h	48 h	72 h
Control	0.045	0.351	1.76
0.36	0.042	0.309	1.60
1.2	0.037	0.230	1.12
3.6	0.036	0.202	0.887
12	0.036	0.172	0.654
36	0.032	0.126	0.366
120	0.028	0.080	0.162
360	0.029	0.055	0.084
1200	0.033	0.041	0.053

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Table 8.2.6.1- 26: Algae growth rate

Nominal concentration [mg p.m./L]	Mean growth rate [A] [1/d]	% Inhibition of average specific growth rate [B]
	0 - 72 h	0 - 72 h
Control	1.72	-
0.36	1.69	2
1.2	1.57	9
3.6	1.50	13
12	1.39	19
36	1.20	30
120	0.93	46
360	0.71	59
1200	0.56	68

A Calculated from the cell density data.
B -% inhibition: increase in growth relative to the control

Table 8.2.6.1- 27: Biomass

Nominal concentration [mg p m./L]	Cumulative biomass [A]	% Inhibition of cumulative biomass [B]
	0 - 72 h	0 - 72 h
Control	1.25	0
0.36	1.11	11
1.2	0.802	36
3.6	0.657	47
12	0.510	59
36	0.317	75
120	0.164	87
360	0.102	92
1200	0.076	94

A Cumulative biomass is equal to the area under the growth curve (area $\times 10^6$).
B % Inhibition =100-((Treatment group parameter mean/ control parameter mean)*100).

III. CONCLUSION

The study meets the validity criteria and the 0 - 72 endpoints based on nominal concentrations of sodium trifluoroacetate and trifluoroacetate acid were:

Results – 0 to 72 hours	Endpoint [mg sodium trifluoroacetate/L]
E_rC_{50} - 72 hours (95 % CI):	**160** [C, D] (n.d.)
E_rC_{20} -72 hours (95 % C.I.): [B]	10.329 (8.601 – 12.208)
E_rC_{10} -72 hours (95 % C.I.): [B]	2.239 (1.716 – 2.842)
E_bC_{50} - 72 hours (95 % CI):	**> 4.8** [C] (n.d.)

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Results – 0 to 72 hours	Endpoint [mg sodium trifluoroacetate/L]
E_bC_{20} -72 hours (95 % C.I.): [B]	0.461 (0.323 – 0.623)
E_bC_{10} -72 hours (95 % C.I.): [B]	0.130 (0.080 – 0.194)
E_yC_{50} - 72 hours (95 % C.I.): [B]	**4.190** (3.526 – 4.959)
E_yC_{20} - 72 hours (95 % C.I.): [B]	0.504 (0.366 – 0.661)
E_yC_{10} - 72 hours (95 % C.I.): [B]	0.167 (0.108 – 0.239)
NOEC - 72 hours: [B] highest concentration without an effect (based on growth, yield and biomass)	< 0.36 [A]

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

[A] The lowest test concentration at 0.36 mg p.m./L showed a statistically significant inhibition at day 3. However, as the inhibition was only 11 % the effect was considered to be of doubtful biological significance.

[B] Please refer for these endpoints to the statistical re-evaluation document M-762208-02-1

[C] Please note: As in the statistical re-calculation the calculations of EC_{10}, EC_{20}, EC_{50} and determination of NOEC values were performed with different statistical methods compared to the original report different endpoints were derived. For a conservative approach, the lowest endpoints were considered. In the original report ECx values were estimated using a figure which expresses the percentage of biomass/ growth rate inhibition against the logarithm of the test substance concentration and the NOE_bC value by Williams-test (1972). For the re-calculations ECx values were determined using probit analysis and NOEC value using Williams multiple sequential t-test.

[D] Based on the molecular weights, a concentration of 160 mg sodium trifluoroacetate/L corresponds to 134.4 mg trifluoroacetate acid/L. As the study was conducted with sodium trifluoroacetate which is the sodium salt of trifluoroacetic acid, the endpoint was converted to trifluoroacetic acid with factor 0.84.

Assessment and conclusion by applicant:

The study and its data are considered as supplementary data with no use in risk assessment.

The endpoint is: E_rC_{50} (72 hours) = 160 mg p.m./L (sodium trifluoroacetate) corresponding to 134.4 mg p.m./L (trifluoroacetic acid)

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Data Point:	KCA 8.2.6.1/09
Report Author:	[redacted]
Report Year:	2021
Report Title:	Typographical correction of re-evaluation of M-247820-01-1: The toxicity of sodium trifluoroacetate to the Alga Selenastrum capricornutum
Report No:	M-762208-02-1
Document No:	M-762208-02-1
Guideline(s) followed in study:	None
Deviations from current test guideline:	Current Guideline: not applicable
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	Yes

Summary

A statistical evaluation addressing the calculation of valid 72-h EC_{10}, EC_{20}, and EC_{50} values as well as NOEC values for yield, growth and biomass was conducted for M-247820-01-1 to fulfill the data requirements according to regulation EU 283/2013. Furthermore, the validity criteria for the study were re-evaluated according to the current guideline OECD 201 (2011).

The recalculations were performed with the software ToxRat Professional (Version 3.2.1) with nominal concentrations provided in the report.

In order to derive Effect Concentrations that have 10, 20 and 50 % effects on yield, growth and biomass of the test subjects (EC_{10}, EC_{20}, and EC_{50}), a probit analysis was performed.

NOEC was determined by Williams Multiple Sequential t-test Procedure (one-sided smaller, p = 0.05).

Table 8.2.6.1- 28: Recalculated EC_{10}, EC_{20}, EC_{50} and NOEC values based on nominal concentrations

Endpoint	Sodium Trifluoroacetate [mg p m./L]		
	Yield	Growth	Biomass
72 hours - EC_{10} (95 % C.I.)	0.167 (0.108 – 0.239)	2.239 (1.716 – 2.842)	0.130 (0.080 – 0.194)
72 hours - EC_{20} (95 % C.I.)	0.504 (0.366 – 0.661)	10.329 (8.601 – 12.208)	0.461 (0.323 – 0.623)
72 hours -EC_{50} (95 % C.I.)	4.190 (3.526 – 4.959)	192.484 (170.878 – 217.941)	5.193 (4.305 – 6.235)
72 hours -NOEC	< 0.36	< 0.36	< 0.36

C.I.: Confidence interval

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Reliability assessment (EFSA 2015)

The following table provides reliability indicators for EC_{10} values for *Pseudokirchneriella subcapitata*.

Biological endpoints	Method	EC_{10} [mg p m./L]	95% CL	NW	Relationship $EC_{10}/EC_{20/50}$
Growth rate (72 h)	Probit	2.239	1.716 – 2.842	0.50 (fair)	$EC_{10} < EC_{20, low}$ (high)
Yield (72 h)	Probit	0.167	0.108 – 0.239	0.78 (fair)	$EC_{10} < EC_{20, low}$ (high)
Biomass (72 h)	Probit	0.13	0.08 – 0.194	0.88 (fair)	$EC_{10} < EC_{20, low}$ (high)

Assessment and conclusion by applicant:

The study and its data are considered as supplementary data with no use in risk assessment.

The endpoint is: E_rC_{50} (72 hours) = 160 mg p.m./L (sodium trifluoroacetate) or = 134.4 mg p.m./L (trifluoroacetic acid)

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Data Point:	KCA 8.2.6.1/10
Report Author:	[redacted]
Report Year:	1995
Report Title:	A comparison of the toxicity of sodium trifluoroacetate, sodium difluoroacetate, sodium monofluoroacetate and sodium fluoride to the alga Scenedesmus supspicatus
Report No:	C047129
Document No:	M-247825-01-1
Guideline(s) followed in study:	OECD: 201 (1984)
Deviations from current test guideline:	Current guideline: OECD 201 (2006) Deviations: No chemical analysis performed. There were only 2 replicates instead of a minimum 4 replicates recommended by the guideline. Furthermore, the test was performed with only 4 test concentrations instead of a minimum of 5 recommended test concentrations. The spacing factor between test concentrations was 10 and thus higher than the maximum recommended factor of 3.2. The validity criteria were met except the mean coefficient of variation for section-by-section specific growth rates.
Previous evaluation:	yes, evaluated and accepted in flurtamone RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The green alga *Desmodesmus subspicatus* (formerly known as *Scenedesmus subspicatus*) were exposed to sodium trifluoroacetate under static conditions for 72 hours. Algal cultures with an initial nominal cell count of approximately 1 x 10^4 cells/mL were used to test the nominal concentrations 0,12, 1.2, 12 and 120 mg p.m./L. Additionally a control was included. There were 2 replicates for each test concentration and 6 for the control. At 24 hour-intervals the cell density (cells/mL) of each culture was counted.

No chemical analysis was conducted.

The study fulfils all validity criteria of OECD 201 guideline except for the mean coefficient of variation.

The endpoint based on nominal concentrations was: 72-hour EC_{50} (based on growth rate and biomass): >120 mg p.m./L and 72 hours NOEC (based on growth rate and biomass): >120 mg p.m./L.

I. Material and Methods

Test material	Sodium trifluoroacetate (TFA) Batch ID.: ACA9135AB Purity: 99 % w/w
Guideline(s) adaptation	None specified.
Test species	Green algae *Desmodesmus subspicatus* (formerly known as *Scenedesmus subspicatus*)
Culturing conditions	The preculture used for this study was transferred into fresh algae medium 4 days prior to test initiation.
Test solutions	Nominal concentrations: 0.12 – 1.2 –12 –120 mg p.m./L Control: untreated medium Evidence of undissolved material: not reported
Replication	No. of vessels per concentration (replicates): 2 No. of vessels per control (replicates): 6

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Exposure	Static Total exposure duration: 72 hours
Initial cells density	1×10^4 cells/mL in each test group
Test conditions	Temperature: 22.5 – 24 °C Photoperiod: 24 hours light Light intensity: 80.2 µmol/s²/m² Type of light: fluorescents lamps pH of controls: 7.2 – 7.8 (0 - 72 hours) Conductivity: not reported Growth medium same as culture medium: Yes
Parameters Measured / Observations	The pH was measured on day 0 in the algal medium and on day 3 in one replicate of each test concentration. Temperature was measured continuously in an additional test vessel in the shaking incubator. Also, light intensity was measured, however time point was not reported. At the start of the test the absorption was measured in one replicate per test concentration. At day 1, 2 and 3 the absorption at 750 nm was measured in all replicates, using a spectrophotometer (Hitachi U-2000) and a cuvette with a pathlength of 5 cm. Cell densities were determined on day 1, 2 and 3 using a calibration line.
Sampling for chemical analysis	No chemical analysis was conducted.
Data analysis	No statistical evaluation mentioned in report.

II. Results and Discussion

Table 8.2.6.1- 29: Validity criteria

Validity criteria acc. to OECD 201 (adopted 2006)	Required	Obtained
The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period.	≥ 16	60 [A]
The mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3) in the control cultures must not exceed 35 %.	< 35 %	67 [A]
The coefficient of variation of average specific growth rates during the 72-hour test period in replicate control cultures must not exceed 7 %.	< 7 %	1.95 [A]

[A] Not given in report. Calculations based cell densities on day 0, 2 and 3.

Analytical results:

No chemical analysis was conducted.

Biological results:

Observations:

No biological observations of the cells were performed.

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Table 8.2.6.1- 30: Cell density

Nominal concentration [mg p m./L]	Mean cell density [$\times 10^4$ cells/mL]		
	24 h	48 h	72 h
Control [A]	1.9	22.4	60.3
0.12	2.0	15.6	63.0
1.2	1.9	13.8	58.3
12	1.8	13.1	53.8
120	1.8	13.0	52.9

A Based on 5 control replicate test vessels since during inoculation of the test vessels at day 0 one control test vessels broke.

Table 8.2.6.1- 31: Algae growth rate

Nominal concentration [mg p.m./L]	Mean growth rate [A] [1/d]	% Inhibition of average specific growth rate [B]
	0 - 72 h	0 - 72 h
Control [C]	1.36	-
0.12	1.381	-1.12
1.2	1.355	0.80
12	1.323	3.12
120	1.322	3.17

A Not given in report. Calculated from the cell density data.
B Not given in report. Calculations based on mean growth rate using the formula: % Inhibition =100-((Treatment group parameter mean/control parameter mean)*100). Here -% inhibition means increase in growth relative to the control
C Based on 5 control replicate test vessels since during inoculation of the test vessels at day 0 one control test vessels broke.

Table 8.2.6.1- 32: Biomass

Mean measured concentration [mg p m./L]	Cumulative biomass [A, B]	% Inhibition of cumulative biomass [C]
	0 - 72 h	0 - 72 h
Control [D]	51.96	-
0.12	46.50	10.5
1.2	42.253	18.7
12	39.25	24.4
120	38.60	25.7

A Cumulative biomass is equal to the area under the growth curve (area $\times 10^4$).
B Not given in report. Calculations based on cumulative biomass of each replicate.
C Not given in report. Calculations based on mean cumulative biomass on day 3 using the formula: % Inhibition =100-((Treatment group parameter mean/control parameter mean)*100).
D Biological results for the control refer to 5 control replicates since during inoculation of the test vessels at day 0 one control test vessels broke.

III. CONCLUSION

The study meets the validity criteria except for the mean coefficient of variation and the 0 - 72 endpoints based on nominal concentrations were:

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Results – 0 to 72 hours	
EC_{50} - 72 hours (95 % CI):	**> 120 mg p.m./L** (n.d.)
E_rC_{20} -72 hours (95 % C.I.):	Not determined [A]
E_rC_{10} -72 hours (95 % C.I.):	Not determined [A]
E_bC_{50} - 72 hours (95 % CI):	Not determined [A]
E_bC_{20} -72 hours (95 % C.I.):	Not determined [A]
E_bC_{10} -72 hours (95 % C.I.):	Not determined [A]
E_yC_{50} - 72 hours (95 % C.I.):	Not determined [A]
E_yC_{20} - 72 hours (95 % C.I.):	Not determined [A]
E_yC_{10} - 72 hours (95 % C.I.):	Not determined [A]
NOEC - 72 hours: highest concentration without an effect (based on growth rate, biomass)	**120 mg p m./L**

n.d.: Not determined due to mathematical reasons or inappropriate data
[A] Due to the lacking concentration/response, the ECx values could not be determined

Assessment and conclusion by applicant:

The study and its data are considered as not acceptable and not reliable with no use in risk assessment.

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Data Point:	KCA 8.2.6.1/11
Report Author:	[redacted]
Report Year:	1996
Report Title:	The toxicity of sodium trifluoroacetate to algae
Report No:	C047126
Document No:	M-247822-01-1
Guideline(s) followed in study:	--
Deviations from current test guideline:	Current guideline: Not applicable Deviations: Not applicable
Previous evaluation:	yes, evaluated and accepted in flurtamone RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The purpose was to review the algal studies which were conducted with sodium trifluoroacetate on 11 different algal species. For these 11 different algal species the available toxicity data are discussed.

Pseudokirchneriella subcapitata (formerly *Selenastrum capricornutum*), was the most sensitive species for sodium trifluoroacetate. Based on the results of five toxicity tests with *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*) a concentration of 0.12 mg/L (120 µg/L) can be

considered a toxicity threshold concentration. Adverse effects on the growth of this species were not found at this concentration. For the remaining 10 algal species the EC_{50} values were all higher than 100 mg/L.

One semi-field study with mesocosm streams has been conducted with sodium trifluoroacetate. The long-term exposure to a mean sodium trifluoroacetate concentration of 31-32 µg/L had no effect on the algal primary production in the mesocosm stream. Severe effects on the algal species composition of the stream mesocosm were not found. Based on the results of the large number of algal laboratory toxicity tests and based on the results of the semi-field study with stream mesocosms, an exposure of an aquatic ecosystem to a sodium trifluoroacetate concentration of 0.12 mg/L has no adverse effect on the algae. Based on the available information a concentration of 0.12 mg/L can be considered a safe concentration for the algae.

I. MATERIAL AND METHODS

This is a review of algal laboratory studies which were conducted with sodium trifluoroacetate (NaTFA), including M-247818-02-1 (C047121), M-247820-01-1 (C047124) and M-247825-01-1 (C047129).

The tests reported for *Pseudokirchneriella subcapitata,* in addition to M-247818-02-1 (C047121) and M-247820-01-1 (C047124) was only a preliminary test using 2 replicates per concentration. In another test the design was also limited to 2 replicates per concentration and in addition there was a large ratio (10) between the test concentrations. In a third test the growth rate of control algae decreased during the test due to a high initial cell density (4.9 x 10^4 cells/mL).

Overall, 16 laboratory studies with sodium trifluoroacetate were conducted with the following 11 different algal species: *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*), *Chlorella vulgaris*, *Scenedesmus subspicatus*, *Chlamidomonas reinhardtii*, *Dunaliella tertiolecta*, *Euglena gracilis*, *Phaeodactylum tricornutum*, *Navicula pelliculosa*, *Skeletonema costatum*, *Anabaena flosaquae* and *Microcystis aeruginosa*. In addition, the effect of sodium trifluoroacetate on algal communities in a freshwater stream was studied.

In the following the study designs are briefly summarised.

Study 1: Chronic study with *Pseudokirchneriella subcapitata*	
Test material	Sodium trifluoroacetate
Test species	*Pseudokirchneriella subcapitata (formerly Selenastrum capricornutum)*
Source of culture and culturing conditions	Laboratory Solvay Duphar: Culture obtained from the American Type Culture Collection (Rockyille, Maryland, USA). Culturing in a 100-mL Erlenmeyer with 50 mL algal medium. Each week an inoculum of this culture was transferred to algal medium.
Test solutions	Control – 0.36 – 1.2 – 3.6 – 12 – 36 – 120 – 360 - 1200 mg/L
Replication	No. of vessels per concentration (replicates): 4 No. of vessels per control (replicates): 7
Exposure	72 hours
Initial cells density	1 × 10^4 cells/mL in each test group
Test conditions	Algal Medium: EPA medium

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Study 2: Chronic study with *Pseudokirchneriella subcapitata*	
Test material	Sodium trifluoroacetate
Test species	*Pseudokirchneriella subcapitata (formerly Selenastrum capricornutum)*
Source of culture and culturing conditions	Laboratory Solvay Duphar: Culture obtained from the American Type Culture Collection (Rockville, Maryland, USA). Culturing in a 100-mL Erlenmeyer with 50 mL algal medium. Each week an inoculum of this culture was transferred to algal medium.
Test solutions	Control - 0.036 – 0.12 – 0.36 – 1.2 mg/L
Replication	No. of vessels per concentration (replicates): 4 No. of vessels per control (replicates): 4
Exposure	72 hours
Initial cells density	0.64 × 10^4 cells/mL in each test group
Test conditions	Algal medium: EPA medium

Study 3: Chronic study with *Pseudokirchneriella subcapitata*	
Test material	Sodium trifluoroacetate
Test species	*Pseudokirchneriella subcapitata (formerly Selenastrum capricornutum)* strain ATCC 22662
Source of culture and culturing conditions	Laboratory Solvay Duphar: Culture obtained from the American Type Culture Collection (Rockville, Maryland, USA). Culturing in a 100-mL Erlenmeyer with 50 mL algal medium. Each week an inoculum of this culture was transferred to algal medium.
Test solutions	Control - 0.012 – 1.2 – 12 - 120 mg/L
Replication	No. of vessels per concentration (replicates): 2 No. of vessels per control (replicates): 6
Exposure	72 hours
Initial cells density	1.0 × 10^4 cells/mL in each test group
Test conditions	Algal medium: OECD medium

Study 4: Chronic study with *Pseudokirchneriella subcapitata*	
Test material	Sodium trifluoroacetate
Test species	*Pseudokirchneriella subcapitata (formerly Selenastrum capricornutum)* strain ATCC 22662
Source of culture and culturing conditions	Laboratory Solvay Duphar: Culture obtained from the American Type Culture Collection (Rockville, Maryland, USA). Culturing in a 100-mL Erlenmeyer with 50 mL algal medium. Each week an inoculum of this culture was transferred to algal medium.
Test solutions	Control - 0.3 – 1.0 – 3.0 – 10 – 30 – 100 – 300 - 1000 mg/L
Replication	No. of vessels per concentration (replicates): 2 No. of vessels per control (replicates): 4
Exposure	72 hours
Initial cells density	1.0 × 10^4 cells/mL in each test group
Test conditions	Algal medium: OECD medium

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Study 5: Chronic study with *Pseudokirchneriella subcapitata*	
Test material	Sodium trifluoroacetate
Test species	*Pseudokirchneriella subcapitata (formerly Selenastrum capricornutum)* strain ATCC 22662
Source of culture and culturing conditions	Laboratory Elf-Atochem (Levallois-Perret, France): Algae (reference CCAP 278/4) were received from the Center for Culture of Algae and Protozoa, Cambridge, United Kingdom.
Test solutions	Control - 0.05 – 0.5 – 2.5 – 5.0 – 10 – 50 mg/L
Replication	No. of vessels per concentration (replicates): 3 No. of vessels per control (replicates): 3
Exposure	72 hours
Initial cells density	4.9 × 10^4 cells/mL in each test group
Test conditions	Algal medium: OECD medium

Study 6: Recovery study with *Pseudokirchneriella subcapitata*	
Test material	Sodium trifluoroacetate
Test species	*Pseudokirchneriella subcapitata (formerly Selenastrum capricornutum)*
Source of culture and culturing conditions	Laboratory Elf-Atochem (Levallois-Perret, France): Algae (reference CCAP 278/4) were received from the Center for Culture of Algae and Protozoa, Cambridge, United Kingdom.
Test solutions	Control - 120 – 1200 mg/L
Replication	No. of vessels per concentration (replicates): 4 No. of vessels per control (replicates): 4
Exposure	24 hours exposure on day 0 72 hours
Initial cells density	1.0 × 10^4 cells/mL in each test group
Test conditions	Algal medium: EPA medium

Study 7: Chronic study with *Chlorella vulgaris*	
Test material	Sodium trifluoroacetate
Test species	*Chlorella vulgaris*
Source of culture and culturing conditions	Laboratory Solvay Duphar: Culture obtained from Culture Collection of Algae and Protozoa, Ambleside, United Kingdom
Test solutions	Control - 1200 mg/L
Replication	No. of vessels per concentration (replicates): 4 No. of vessels per control (replicates): 4
Exposure	72 hours
Initial cells density	1.0 × 10^4 cells/mL in each test group
Test conditions	Algal medium: special medium

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Study 8: Chronic study with *Scenedesmus subspicatus*	
Test material	Sodium trifluoroacetate
Test species	*Scenedesmus subspicatus* strain 86.81 SAG
Source of culture and culturing conditions	Culture obtained from the University of Gottingen. Institute of Plant Physiology. Culturing at Laboratory Solvay Duphar:
Test solutions	Control – 0.12 – 1.2 – 12 - 120 mg/L
Replication	No. of vessels per concentration (replicates): 2 No. of vessels per control (replicates): 5
Exposure	72 hours
Initial cells density	1.0×10^4 cells/mL in each test group
Test conditions	Algal medium: OECD medium

Study 9: Chronic study with *Chlamidomonas reinhardtii*	
Test material	Sodium trifluoroacetate
Test species	*Chlamidomonas reinhardtii*
Source of culture and culturing conditions	Culture obtained from the University of Gottingen. Institute of Plant Physiology. Culturing at Laboratory Solvay Duphar.
Test solutions	Control – 120 mg/L
Replication	No. of vessels per concentration (replicates): 6 No. of vessels per control (replicates): 6
Exposure	72 hours
Initial cells density	1.0×10^4 cells/mL in each test group
Test conditions	Algal medium: OECD medium

Study 10: Chronic study with *Dunaliella tertiolecta*	
Test material	Sodium trifluoroacetate
Test species	*Dunaliella tertiolecta* Strain 13.86
Source of culture and culturing conditions	Culture obtained from the University of Gottingen. Institute of Plant Physiology. Culturing at Laboratory Solvay Duphar.
Test solutions	Control – 124 mg/L
Replication	No. of vessels per concentration (replicates): 6 No. of vessels per control (replicates): 6
Exposure	72 hours
Initial cells density	Initial calculated absorbance: 0.010
Test conditions	Algal Medium: Special medium

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Study 11: Chronic study with *Euglena gracilis*	
Test material	Sodium trifluoroacetate
Test species	*Euglena gracilis* strain CCAP 1224/52
Source of culture and culturing conditions	Culture obtained from the Culture Collection of Algae and Protozoa. Freshwater Biological Association, The Ferry House, Ambleside, Cumbria, LA22 OLP, United Kingdom Culturing at Laboratory Solvay Duphar.
Test solutions	Control – 112 mg/L
Replication	No. of vessels per concentration (replicates): 6 No. of vessels per control (replicates): 6
Exposure	8 days
Initial cells density	Initial calculated absorbance: 0.0135
Test conditions	Algal medium: Special medium

Study 12: Chronic study with *Phaeodactylum tricornutum*	
Test material	Sodium trifluoroacetate
Test species	*Phaeodactylum tricornutum,*
Source of culture and culturing conditions	Culture obtained from the Culture Collection of Algae and Protozoa. Dunstaffnage Marine Laboratory. P.O. Box 3, Oban, Argyll. PA34 4AD, Scotland Culturing at Laboratory Solvay Duphar.
Test solutions	Control – 117 mg/L
Replication	No. of vessels per concentration (replicates): 6 No. of vessels per control (replicates): 6
Exposure	72 hours
Initial cells density	Initial calculated absorbance: 0.020
Test conditions	Algal Medium: Special medium

Study 13: Chronic study with *Navicula pelliculosa*	
Test material	Sodium trifluoroacetate
Test species	*Navicula pelliculosa,* (strain UTEX 667)
Source of culture and culturing conditions	Test performed by Zeneca Brixham
Test solutions	Control – 19 – 38 – 75 – 150 – 300 – 600 – 1200 – 2400 mg/L
Replication	No. of vessels per concentration (replicates): 3 No. of vessels per control (replicates): 6
Exposure	96 hours
Initial cells density	0.313×10^4 cells/mL in each test group
Test conditions	Algal medium: Special medium

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Study 14: Chronic study with *Skeletonema costatum*	
Test material	Sodium trifluoroacetate
Test species	*Skeletonema costatum* (strain CCAP 1077/1 C)
Source of culture and culturing conditions	Test performed by Zeneca Brixham
Test solutions	Control – 19 – 38 – 75 – 150 – 300 – 600 – 1200 – 2400 mg/L
Replication	No. of vessels per concentration (replicates): 3 No. of vessels per control (replicates): 6
Exposure	96 hours
Initial particle density	0.538 × 10^4 cells/mL in each test group
Test conditions	Algal medium: Special medium

Study 15: Chronic study with *Anabaena flosaquae*	
Test material	Sodium trifluoroacetate
Test species	*Anabaena flosaquae* (strain CCAP 1403/13A)
Source of culture and culturing conditions	Test performed by Zeneca Brixham
Test solutions	Control – 19 – 38 – 75 – 150 – 300 – 600 – 1200 – 2400 mg/L
Replication	No. of vessels per concentration (replicates): 3 No. of vessels per control (replicates): 6
Exposure	96 hours
Initial calculated absorbance	0.004 × 10^4 cells/mL in each test group
Test conditions	Algal medium: Special medium

Study 16: Chronic study with *Microcystis aeruginosa.*	
Test material	Sodium trifluoroacetate
Test species	*Microcystis aeruginosa.* (strain 46.80)
Source of culture and culturing conditions	Culture obtained from the University of Göttingen, Institute for Plant Physiology. Collection of Algal Cultures, Göttingen, Germany. Culturing at Laboratory Solvay Duphar.
Test solutions	Control – 117 mg/L
Replication	No. of vessels per concentration (replicates): 6 No. of vessels per control (replicates): 6
Exposure	96 hours
Initial calculated absorbance	0.020 in each test group
Test conditions	Algal medium: Special medium

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Study 17: Semi-field study	
Test material	Sodium trifluoroacetate
Test system	Study site: White Clay Creek (WCC), Chester Co. Pennsylvania, U.S.A. Stream mesocosms: Dimensions: 2.2 m (length), 0.20 m (width) and 0.13 m (depth) Water depth: 1.5 cm Water flow: Recirculating with continuously inflow of White Clay Creek water. Sodium trifluoroacetate was metered continuously into two stream mesocosms:
Source of culture and culturing conditions	Not reported
Test solutions	Control – 31 – 32 (mean measured concentrations) mg/L.
Replication	No. of mesocosms per concentration (replicates): 1 No. of mesocosms per control (replicates): 1
Exposure	Start of exposure: September 21,1992 End of exposure: June 4, 1993
Test conditions	Not reported
Test to study primary productivity	During the test, samples of the algae (originating from rocks in the stream mesocosm) and water samples of the same mesocosm were combined. Sodium trifluoroacetate and ^{14}C-bicarbonate was added, and the samples were incubated in the light for 2 hours to study the primary productivity (algal biomass production). The incorporation of ^{14}C into the algal biomass was studied with liquid scintillation counting. Five sodium trifluoroacetate concentrations were tested: 0 - 0.2 - 2.0 - 20 – 200 mg/l. On one date, algae samples were aspirated from sediment or scraped from rocks and the algal composition was determined

II. RESULTS AND DISCUSSION

Laboratory toxicity tests:

The laboratory toxicity tests show that *Selenastrum capricornutum* is the most sensitive algal species. Based on the results of five studies with *Selenastrum capricornutum* a concentration of 0.12 mg/L can be considered a toxicity threshold value. For the remaining 10 algal species the EC_{50} values are all higher than 100 mg/L.

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Table 8.2.6.1- 33: Endpoints of the toxicity tests with algae with sodium trifluoroacetate. The endpoints were determined at test termination (biomass integral) or were based on the whole test period (growth rate)

Species	Study type	Time scale	Endpoint: E_rC_{50} [mg/L]	NOEC (biomass integral) [mg/L]
Pseudokirchneriella subcapitata (Study 1)	chronic, static	72 h	160	< 0.36
Pseudokirchneriella subcapitata (Study 2)		72 h	-	0.12
Pseudokirchneriella subcapitata (Study 3)		72 h	14 [A]	-
Pseudokirchneriella subcapitata (Study 4)		72 h	27	0.30
Pseudokirchneriella subcapitata (Study 5)		72 h	7.7	0.15 [B]
Scenedesmus subspicatus		72 h	> 120	-
Anabaena flos-aquae		125 h	> 2400	600
Navicula pelliculosa		96 h	> 2400	600
Skeletonema costatum		96 h	> 2400	2400
Chlorella vulgaris		72 h	>1200	1200
Chlamidomonas reinhardii		72 h	>120	120
Dunaliella tertiolecta		72 h	>124	124 [C]
Euglena gracilis		192 h	>112	112
Phaedactylum tricornutum		72 h	>117	117
Microcysstis aeruginosa		144 h	> 117	117

[A] Due to the large ratio between test concentrations (10) and the low number of replicates (only 2 replicates per concentration) the value is only a rough estimate

[B] EC_{10} value

[C] The inhibition of the biomass integral was statistically significant (p=0.018)

Semi-field study:

Short term exposure to the highest concentration of 200 mg/L had no severe effect on the primary productivity. Furthermore, the long-term exposure to a mean sodium trifluoroacetate concentration of 31-32 µg/L during several months had no effect on the algal primary production in the mesocosm stream. Detrimental effects on the algal species composition of the stream mesocosm were not found.

The experiments showed that the algal excretion of photosynthetate was lower for the exposed stream mesocosm when sodium trifluoroacetate concentrations of 0 - 2 mg/L were used in the samples. This physiological effect on the algae has probably no ecological consequences for the ecosystem. There are no indications that the increased excretion is adverse for the algae. Furthermore, it should be realized that the high amount of photosynthetate in the solutions could be affected by bacteria. It is known that excretion products of the algae are taken up by bacteria.

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III. Conclusion

Pseudokirchneriella subcapitata (formerly *Selenastrum capricornutum*), was the most sensitive species for sodium trifluoroacetate. Based on the results of five toxicity tests with *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*) a concentration of **0.12 mg/L** can be considered a **toxicity threshold concentration**. Adverse effects on the growth of this species were not found at this concentration. For the remaining 10 algal species the EC_{50} values were all higher than 100 mg/L (>112 to >2400 mg/L).

The results of algal species determinations showed that a long-term exposure to a mean sodium trifluoroacetate concentration of 31-32 µg/L had no severe effect on the algal species composition of the mesocosms.

Assessment and conclusion by applicant:

The study and its data are considered as supplementary data with no use in risk assessment.

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Data Point:	KCA 8.2.6.1/12
Report Author:	[redacted]
Report Year:	2017
Report Title:	Alga, growth inhibition test - Effect of the trifluoroacetic acid on the growth of the unicellular alga Pseudokirchneriella subcapitata, according to OECD guideline 201
Report No:	M-615180-01-1
Document No:	M-615180-01-1
Guideline(s) followed in study:	OECD guidelines for the testing of chemicals, n°201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test - (adopted March 23th 2006, annex 5 corrected on July 28th 2011).
Deviations from current test guideline:	Current Guideline: OECD 201 (2006) Deviations: The light intensity was 2570 – 3790 lux and thus lower than the minimum of 4440 lux recommended in OECD 201. This deviation was not expected to have impacted the study results. All validity criteria were met.
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 green alga *Pseudokirchneriella subcapitata* were exposed to trifluoroacetic acid under static conditions for 72 hours. Algal cultures with an initial nominal cell count of approximately 1 × 10^4 cells/mL were used to test the nominal concentrations 0.9, 2.5, 7.0, 19.5, 54.7, 153.1, 428.6 and 1200 mg p.m./L, corresponding to geometric mean measured concentrations 0.9, 2.7, 7.2, 20.2, 55.6, 157.6, 438.0 and 1207 mg p.m./L. Additionally a control was included. There were 3 replicates for each test concentration and 6 for the control. At 24 hour-intervals, the cell density (cells/mL) of each culture was counted.

Samples from each test concentration were verified by ion-exchange chromatography on day 0 and day 3. Measured concentrations were in the 99 - 113 % range of nominal concentrations and no residues were found in the control samples. The limit of quantification was 0.5 mg p.m./L and the limit of detection 0.17 mg p.m./L.

The study fulfils all validity criteria of OECD 201 guideline.

Some aggregations of algal cells were noticed at the highest test concentrations (mainly in the range between 157.57 mg p.m./L and 1207 mg p.m./L (geometric mean measured concentrations), corresponding to 153.1 and 1200 mg p.m./L (nominal concentrations). On the other hand, no changes in color and cell shape were observed for any test concentration.

The endpoint based on nominal concentrations was: 72- hour E_rC_{50}: 237.07 mg p.m./L.

The endpoints based on geometric mean measured concentrations were: 72- hour E_rC_{50}: 241.95 mg p.m./L, 72- hour E_bC_{50}: 26.866 mg p.m./L and 72- hour E_yC_{50}: 18.956 mg p.m./L.

I. Material and Methods

Test material	Trifluoroacetic acid Batch ID.: SFA1702530 Purity: 99.9 % w/w
Guideline(s) adaptation	None specified.

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Test species	Green algae *Pseudokirchneriella subcapitata (*currently known as *Raphidocelis subcapitata*), Strain CCAP 278/4
Culturing conditions	In-house, 4 day old batch culture in log phase growth held under test conditions
Test solutions	Nominal concentrations: 0.9 – 2.5 – 7.0 – 19.5 – 54.7 – 153.1 – 428.6 – 1200.0 mg p.m./L Corresponding geometric mean measured concentrations: 0.9 – 2.7 – 7.2 – 20.2 – 55.6 – 157.6 – 438.0 – 1207.0 mg p.m./L Control: untreated medium Evidence of undissolved material: not reported
Replication	No. of vessels per concentration (replicates): 3 No. of vessels per control (replicates): 6
Exposure	Static Total exposure duration: 72 hours
Initial cells density	1 × 10^4 cells/mL in each test group
Test conditions	Temperature: 21.7 – 22.2 °C Photoperiod: 24 hours light Light intensity: 2570 – 3790 lux Type of light: plant growth light pH of controls: 8.3 – 8.8 Conductivity: not reported Growth medium same as culture medium: Yes
Parameters Measured / Observations	The pH was measured at day 0 and 3 in each test concentration and the control. Temperature was measured continuously during the experiment. Also, light intensity was measured, however time point was not reported. Number of algal cells was counted every 24 hours in all test replicates using an electronic particle counter. Algal cells were microscopically observed to verify the normal and healthy appearance of the inoculum culture on day 0 and after 72 hours to observe any abnormal appearance of the algae.
Sampling for chemical analysis	Samples for analysis of test substance were taken on day 0 and day 3. At test start samples were taken in one replicate of each test concentration and the control before algae addition. On day 3 samples were taken in replicates with and without algae. Chemical analysis was performed by ion-exchange chromatography with gradient elution and conductimetric detection.
Data analysis	Determination of NOEC: Statistics have been performed using Toxstat 3.5. Normality and homogeneity of the data have been checked and the significance of the effect on growth was determined, by performing a two tailed ANOVA (α-value = 0.05) followed by the Bonferroni post-hoc test (α-value = 0.05). Calculation of E_rC_x values: E_rC_{10} and E_rC_{50} 72 h values have been calculated using log-logistic regression with bootstrap estimation of 95% confidence limits.

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II. RESULTS AND DISCUSSION

Table 8.2.6.1- 34: Validity criteria

Validity criteria acc. to OECD 201 (adopted 2006)	Required	Obtained
The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period.	≥ 16	112.2
The mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3) in the control cultures must not exceed 35 %.	≤ 35 %	14.7 %
The coefficient of variation of average specific growth rates during the 72-hour test period in replicate control cultures must not exceed 7 %.	≤ 7 %	1.7 %

Analytical results:

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 SANTE/2020/12830, Rev.1.

Recoveries on day 0 and day 3 ranged between 99 and 113 % of nominal concentrations (see table below). The biological results are based on nominal test concentrations.

No residues of the metabolite trifluoroacetic acid were found in the control samples. The limit of quantification was 0.5 mg p.m./L and the limit of detection was 0.17 mg p.m./L.

Table 8.2.6.1- 35: Analytical results

Nominal conc. [mg p.m./L]	Measured concentration [mg p.m./L]			% of Nominal			Geometric mean measured conc. [mg p.m./L]	% of nominal
	Day 0	Day 3 with algae	Day 3 without algae	Day 0	Day 3 with algae	Day 3 without algae		
0.9	1.0	0.9	0.9	113	99	103	0.94	106
2.5	2.7	2.7	2.70	108	106	108	2.66	107
7.0	7.3	7.2	7.2	105	103	103	7.23	104
19.5	20.2	20.1	19.8	103	103	101	20.15	103
54.7	55.7	55.5	55.0	102	101	101	55.60	102
153.1	157.1	158.0	158.1	103	103	103	157.57	103
428.6	436.7	439.3	438.9	102	103	102	437.98	102
1200.0	1208.7	1205.4	1208.4	101	100	101	1207.04	101

Biological results:

Observations:

Some aggregations of algal cells were noticed in the three highest test concentrations (mainly in the range between 153.1 and 1200 mg p.m./L, nominal concentrations). On the other hand, no changes in color and cell shape were observed for any test concentration.

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Table 8.2.6.1- 36: Cell density

Nominal concentration [mg p m./L]	Geometric mean measured concentration [mg p m./L]	Mean cell density [×10^4 cells/mL]		
		24 h	48 h	72 h
Control	-	3.75	19.48	112.21
0.9	0.94	4.68	20.58	106.41
2.5	2.66	4.40	19.20	91.83
7.0	7.23	3.95	18.46	85.48
19.5	20.15	3.78	14.14	61.97
54.7	55.60	3.00	7.41	18.64
153.1	157.57	3.21	6.87	15.92
428.6	437.98	2.56	4.81	7.52
1200.0	1207.04	2.56	3.28	4.16

Table 8.2.6.1- 37: Algae growth rate

Nominal concentration [mg p m./L]	Geometric mean measured concentration [mg p m./L]	Mean growth rate [A] [1/d]	% Inhibition of average specific growth rate
		0 - 72 h	0 - 72 h
Control		1.573	-
0.9	0.94	1.555	1.1
2.5	2.66	1.506	4.3
7.0	7.23	1.482	5.8
19.5	20.15	1.370	12.9
54.7	55.60	0.975	38.0
153.1	157.57	0.922	41.4
428.6	437.98	0.672	57.3
1200.0	1207.04	0.475	69.8

[A] Calculated from the cell density data.

III. CONCLUSION

The study meets the validity criteria and the 0 - 72 endpoints based on nominal and geometric mean measured concentrations of trifluoroacetic acid were:

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Results – 0 to 72 hours		
Endpoint	**Nominal concentration**	**Geometric mean measured concentration**
E_rC_{50} - 72 hours (95 % CI):	**237.07 mg p.m./L** (192.21 – 289.23 mg p.m./L)	**241.95 mg p.m./L** [A,B] (195.45 – 292.66 mg p m./L)
E_rC_{20} -72 hours (95 % C.I.): [A]	Not determined	26.123 (19.113 – 33.887)
E_rC_{10} -72 hours (95 % C.I.):	**5.59 mg p.m./L** (3.00 – 9.69 mg p.m./L)	5.80 mg p m./L (3.36 – 10.06 mg p.m./L)
E_bC_{50} - 72 hours (95 % CI): [A]	Not determined	**26.866 mg p.m./L** (21.620 – 33.389 mg p m./L)
E_yC_{50} - 72 hours (95 % CI): [A]	Not determined	**18.956 mg p.m./L** (15.266 – 23.537 mg p m./L)
LOE_rC - 72 hours: highest concentration without an effect (based on growth rate)	2.5 mg p m./L [A,B]	2.7 mg p m./L [A,B]
$LOE_{y,b}C$ - 72 hours: [A] highest concentration without an effect (based on yield and biomass)	2.5 mg p.m./L [A]	2.7 mg p.m./L [A]
NOE_rC - 72 hours: highest concentration without an effect (based on growth rate)	0.9 mg p m./L [A,B]	0.9 mg p.m./L [A]
$NOE_{y,b}C$ - 72 hours: [A] highest concentration without an effect (based on yield and biomass)	0.9 mg p.m./L [A]	0.9 mg p.m./L [A]

[A] Please refer for endpoints to recalculation document M-762267-01-1

[B] Please note: As in the statistical re-calculation the calculations of EC_{10}, EC_{20}, EC_{50} and determination of NOEC/ LOEC values were performed with a different statistical method compared to the original report different endpoints were derived. For a conservative approach, the lowest endpoints were considered. In the original report ECx values were determined using log-logistic regression with bootstrap estimation of 95 % confidence limits and the NOEC values by performing a two tailed ANOVA (α-value = 0.05) followed by the Bonferroni post-hoc test. For the re-calculations ECx values were determined using probit analysis and NOEC/LOEC values using Williams multiple sequential t-test.

Assessment and conclusion by applicant:

The study and its data are considered as supplementary data with no use in risk assessment.

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Data Point:	KCA 8.2.6.1/13
Report Author:	
Report Year:	2021
Report Title:	Statement - Re-evaluation of M-615180-01-1: Effect of the trifluoroacetic acid on the growth of the unicellular alga Pseudokirchneriella subcapitata, according to OECD guideline 201
Report No:	M-762267-01-1
Document No:	M-762267-01-1
Guideline(s) followed in study:	None
Deviations from current test guideline:	Current Guideline: not applicable
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	Yes

Summary

A statistical evaluation addressing the calculation of valid 72-h EC_{10}, EC_{20}, and EC_{50} values as well as LOEC and NOEC values for yield, growth and biomass was conducted for M-615180-01-1 to fulfill the data requirements according to regulation EU 283/2013. Furthermore, the validity criteria for the study were re-evaluated according to the current guideline OECD 201 (2011).

The recalculations were performed with the software ToxRat Professional (Version 3.2.1) with geometric mean measured concentrations provided in the report.

In order to derive Effect Concentrations that have 10, 20 and 50 % effects on yield, growth and biomass of the test subjects (EC_{10}, EC_{20}, and EC_{50}), a probit analysis was performed.

NOEC and LOEC were determined by Williams Multiple Sequential t-test Procedure (one-sided smaller, p = 0.05).

Table 8.2.6.1- 38: Re-calculated EC_{10}, EC_{20}, EC_{50}, LOEC and NOEC values based on geometric mean measured concentrations

Endpoint	Trifluoroacetic Acid [mg p m./L]		
	Yield	**Growth**	**Biomass**
72 hours - EC_{10} (95 % C.I.)	2.193 (1.291 – 3.221)	7.895 (5.026 – 11.368)	2.671 (1.579 – 3.941)
72 hours - EC_{20} (95 % C.I.)	4.598 (3.115 – 6.170)	26.123 (19.113 – 33.887)	5.899 (4.007 – 7.941)
72 hours - EC_{50} (95 % C.I.)	18.956 (15.266 – 23.537)	257.553 (213.553 – 315.536)	26.866 (21.620 – 33.389)
72 hours -LOEC	2.7	2.7	2.7
72 hours -NOEC	0.9	0.9	0.9

C.I.: Confidence interval

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Reliability assessment (EFSA 2015)

The following table provides reliability indicators for EC_{10} values for *Pseudokirchneriella subcapitata*.

Biological endpoints	Method	EC_{10} [mg p m./L]	95% CL	NW	Relationship $EC_{10}/EC_{20/50}$
Growth rate (72 h)	Probit	7.895	5.026 – 11.368	0.80 (fair)	$EC_{10} < EC_{20,low}$ (high)
Yield (72 h)	Probit	2.193	1.291 – 3.221	0.88 (fair)	$EC_{10} < EC_{20,low}$ (high)
Biomass (72 h)	Probit	2.671	1.579 – 3.941	0.88 (fair)	$EC_{10} < EC_{20,low}$ (high)

Assessment and conclusion by applicant:

The study and its data are considered as supplementary data with no use in risk assessment.

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CA 8.2.6.2 Effects on growth of an additional algal species

Active substance fluopyram

Data Point:	KCA 8.2.6.2/01
Report Author:	[redacted]
Report Year:	2007
Report Title:	Toxicity of AE C656948 technical to the freshwater diatom Navicula pelliculosa
Report No:	EBGMP040
Document No:	M-289899-01-1
Guideline(s) followed in study:	FIFRA Guideline 123-2 (1982) OPPTS Guideline 850.5400 (1996 draft) OECD Guideline 201 (1984, 2004 draft)
Deviations from current test guideline:	Current Guideline: OECD 201 (2006) Deviations: None. All validity criteria were met.
Previous evaluation:	yes, evaluated and accepted in DAR (2011)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The freshwater Diatom *Navicula pelliculosa* was exposed to fluopyram under static conditions for 96 hours. Diatom cultures with an initial nominal cell count of approximately 1.0×10^4 cells/mL were used to test the nominal concentrations of 0.63, 1.25, 2.5, 5.0 and 10.0 mg a.s./L. The study design included 3 replicates for each test concentration and control (control and solvent control). At 24 hour-intervals, the cell density (cells/mL) of each culture was counted.

Concentrations of fluopyram were verified by HPLC – UV on day 0 and day 4 for each concentration and control. Measured concentrations were in the 92 - 101 % range of nominal concentrations and no residues were found in the control and solvent control samples above the LOQ (0.06 mg a.s./L). The biological results are based on the mean measured concentrations of 0.59, 1.23, 2.47, 5.01 and 10.0 mg a.s./L.

The study fulfils all validity criteria of OECD 201 guideline.

No physical abnormalities were observed in the controls or any test concentration during the study.

The endpoints based on mean measured concentrations were: 72 hours– E_rC_{50} (95 % C.I.): 9.08 mg a.s./L (8.96 - 9.20 mg a.s./L); 72 hours- E_bC_{50} (95 % C.I.): 5.62 mg a.s./L (5.57 - 5.68 mg a.s./L) and 72 hours- E_yC_{50} (95 % C.I.): 5.64 mg a.s./L (5.07– 6.21 mg a.s./L).

I. MATERIAL AND METHODS

Test material	Fluopyram (AE C656948) Specification No.: 102000012455 Batch No.: 08528/0002 Purity: 94.7 % w/w
Guideline(s) adaptation	None specified.
Test species	*Navicula pelliculosa* UTEX

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Culturing conditions	In-house 3 day old batch culture held under test conditions.
Test solutions	Nominal concentrations: 0.63 – 1.25 – 2.5 – 5.0 – 10.0 mg a.s./L Corresponding mean measured concentration: 0.59 – 1.23 – 2.47 – 5.01 – 10.0 mg a.s./L Control: untreated medium Solvent control: Dimethylformamide (0.1 mL/L) Evidence of undissolved material: Precipitates were observed in the stock solutions prepared at 15 and 20 mg a.s./L. No precipitate were observed during exposure.
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
Initial cells density	1×10^4 cells/mL in each test group
Test conditions	Temperature: 23.7- 24.0 °C Photoperiod: 24 hour light Light intensity: 3972 - 4682 lux Type of light: cool white fluorescents pH of controls: 7.4 – 8.4 (0 – 96 h) Conductivity: 129 – 144 µmhos/cm Growth medium same as culture medium: Yes
Parameters Measured / Observations	pH and conductivity were measured at study start and end. Temperature was measured hourly via a calibrated datalogger plus daily manual records via a calibrated thermometer. Each day, density was determined in the three test replicates at each concentration and the control by manual counts via light microscope and hemocytometer slide. Cellular observations were done by visual inspection via light microscope.
Sampling for chemical analysis	Samples for analysis of test substance were taken at test initiation (0 hour) from batch prepared solutions and at test termination (96 hours) from composite samples from each test solution. Samples were analysed by using a High-performance liquid chromatograph (HPLC) – UV.
Data analysis	Raw or transformed data from treatment groups were compared to controls for normality and homogeneity of variance using the Shapiro-Wilks test and Levene's test of equal variance, respectively. If normality and homogeneity of variance were demonstrated for the raw or transformed values, then parametric analyses were conducted using analysis of variance (ANOVA) followed by Dunnett's test. If normality and/or homogeneity of variance were not demonstrated on raw or transformed values, nonparametric procedures were used. The ranks of the raw values were determined, and then an analysis of variance and a one-tailed Dunnett's test were performed on these ranks. The 72 or 96-hour EC_{50}, and the respective 95 % confidence intervals, was calculated with help of regressions analysis for cell density, cumulative biomass, and growth rate. All statistical analyses were performed using the SAS computer software package.

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II. RESULTS AND DISCUSSION

Table 8.2.6.2- 1: Validity criteria

Validity criteria acc. to OECD 201 (adopted 2006)	Required	Obtained
The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 0 to 72 hour test period.	≥ 16	Approx. 184 (pooled controls)
The mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3) in the control cultures must not exceed 35 %.	≤ 35 %	30 %
The coefficient of variation of average specific growth rates during the 0 to 72 hour test period in replicate control cultures must not exceed 10 %.	≤ 10 %	2 %

Analytical results:

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 SANTE/2020/12830, Rev.1.

Recoveries on day 0 and day 4 were between 92 and 101 % of nominal concentration (see table below). The biological results are based on mean measured concentrations of fluopyram.

No residues of fluopyram were measured in the control and solvent control samples above the limit of quantification (LOQ: 0.06 mg a.s./L).

Table 8.2.6.2- 2: Analytical results

Nominal concentration [mg a.s./L]	Measured concentration [mg a.s./L]		% of nominal		Mean measured concentration [mg a.s./L]	Mean % of nominal
	Day 0 (New)	Day 4 (Old)	Day 0 (New)	Day 4 (Old)		
0.63	0.60	0.58	95	92	0.59	94
1.25	1.25	1.20	100	96	1.23	98
2.5	2.48	2.45	99	98	2.47	99
5.0	5.06	4.96	101	99	5.01	100
10.0	10.0	9.93	100	99	10.0	100

Biological results:

Observations:

No physical abnormalities were observed in the controls or any test concentration during the study.

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Table 8.2.6.2- 3: Cell density

Mean measured concentration [mg a.s./L]	Mean cell density [x 10^4 cells/mL]				% Inhibition [A] at 96 h
	24 h	48 h	72 h	96 h	
Control	3.35	33.3	174.2	254.83	-
Solvent control	3.58	33.1	193.0	262.08	-
Pooled controls	-	-	-	258.46	-
0.59	3.44	29.6	194.9	249.00	3.7
1.23	3.60	32.0	164.1	246.92	4.5
2.47	3.56	32.0	168.2	261.58	-1.2
5.01	2.59	20.3	117.1	218.83	15.3 *
10.0	1.79	5.48	8.26	11.81	95.4 *

* Statistically significant from pooled control (Dunnett's one-tailed test; $p \leq 0.05$)
[A] % Inhibition = 100-((Treatment group parameter mean / pooled control parameter mean)*100)

Table 8.2.6.2- 4: Biomass

Mean measured concentration [mg a.s./L]	Cumulative biomass [A]		% Inhibition [B]	
	0 - 72 h	0 - 96 h	0 - 72 h	0 - 96 h
Control	2909.9	8033.9	-	-
Solvent control	3136.1	8573.1	-	-
Pooled controls	3023.0	8303.5	-	-
0.59	3071.7	8374.7	-1.6	-0.9
1.23	2762.5	7670.5	8.6	7.6
2.47	2809.4	7942.4	7.1	4.3
5.01	1895.2	5902.2	37.3 *	28.9 *
10.0	213.6	430.5	92.9 *	94.8 *

* Statistically significant difference from pooled control (Dunnett's one-tailed test; $p \leq 0.05$)
[A] Cumulative biomass is equal to the area under the growth curve
[B] % Inhibition = 100-((Treatment group parameter mean / pooled control parameter mean)*100)

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Table 8.2.6.2- 5: Algae growth rate

Mean measured concentration [mg a.s./L]	Mean growth rate [A] [1/h]		% Inhibition [B]	
	0 - 72 h	0 - 96 h	0 - 72 h	0 - 96 h
Control	0.071641	0.057709	-	-
Solvent Control	0.073044	0.058002	-	-
Pooled Controls	0.072343	0.057856	-	-
0.59	0.073219	0.057471	-1.2	0.7
1.23	0.070800	0.057377	2.1	0.8
2.47	0.071147	0.057964	1.7	-0.2
5.01	0.066150	0.056122	8.6*	3.0*
10.0	0.029288	0.025660	59.5*	55.6*

* Statistically significant difference from pooled control (Dunnett's one-tailed test; $p \leq 0.05$)
[A] Growth rate is calculated from the cell density data.
[B] % Inhibition=100-((Treatment group parameter mean/pooled control parameter mean)*100).

III. CONCLUSION

The study meets the validity criteria and the 0-72 and 0-96 hours endpoints based on mean measured concentrations were:

Results – 0 to 72 hours	
E_rC_{50} – 72 hours (95 % C.I.):	**9.08 mg a.s./L** (8.96 - 9.20 mg a.s./L)
E_rC_{20} - 72 hours (95 % C.I.):	6.41 mg a.s./L (6.09 - 6.73 mg a.s./L)
E_rC_{10} - 72 hours (95 % C.I.):	5.23 mg a.s./L (4.85 - 5.60 mg a.s./L)
E_bC_{50} - 72 hours (95 % C.I.):	**5.62 mg a.s./L** (5.57 - 5.68 mg a.s./L)
E_yC_{50} - 72 hours (95 % CI): [A]	5.64 mg a.s./L (5.07– 6.21 mg a.s./L)
E_yC_{20} - 72 hours (95 % CI): [A]	4.24 mg a.s./L (3.02 – 4.85 mg a.s./L)
E_yC_{10} - 72 hours (95 % CI): [A]	3.58 mg a.s./L (2.14 – 4.30 mg a.s./L)
LOEC - 72 hours: lowest concentration with an effect (based on growth rate and biomass)	5.01 mg a.s./L
NOEC - 72 hour: highest concentration without an effect (based on growth rate and biomass)	2.47 mg a.s./L
NOE_yC - 72 hours: [A] highest concentration without an effect (based on yield)	2.5 mg a.s./L

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Results – 0 to 96 hours	
E_bC_{50} - 96 hours (95 % C.I.):	**5.90 mg a.s./L** (5.85 to 5.95 mg a.s./L)
EC_{50} - 96 hours (based on cell density) (95 % C.I.):	6.43 mg a.s./L (6.31 - 6.54 mg a.s./L)
LOEC - 96 hours: lowest concentration with an effect (based on growth rate and biomass, cell density (standing crop))	5.01 mg a.s./L
NOEC - 96 hours: highest concentration without an effect (based on growth rate ng crop))	2.47 mg a.s./L

[A] Please refer to recalculation document by M-757699-01-1

Reliability assessment (EFSA 2015)

The following table provides reliability indicators for EC_{10} values for *Navicula pelliculosa*.

Biological endpoints	EC_{10} [mg a.s./L]	95% CL	NW	Relationship $EC_{10}/EC_{20/50}$
Growth Rate	5.23	4.85 – 5.60	0.143 (excellent)	EC_{10} < EC_{20}, low (high)
Yield	3.58	2.14 – 4.30	0.603 (fair)	EC_{20}, low < EC_{10} < EC_{50}, low (medium)

Assessment and conclusion by applicant:

The study and its data are considered as acceptable and reliable for use in risk assessment.

The endpoint is: E_rC_{50} (72 hours) = 9.08 mg a.s./L

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Data Point:	KCA 8.2.6.2/02
Report Author:	[redacted]
Report Year:	2007
Report Title:	Toxicity of AE C656948 technical to the blue-green algae Anabaena flos-aquae
Report No:	EBGMP049
Document No:	M-287287-01-1
Guideline(s) followed in study:	FIFRA Guideline 123-2 (1982) OPPTS Guideline 850.5400 (1996 draft) OECD Guideline 201 (2006)
Deviations from current test guideline:	Current Guideline: OECD 201 (2006) Deviations: The pH increase in the control was 2.5 units and thus higher than the maximum 1.5 units as recommended in OECD 201. The light intensity was 1938 - 2336 lux and thus outside the recommended range of 4440 - 8880 lux. These deviations were not expected to have impacted the study results. All validity criteria were met except for the mean coefficient of variation for section-by-section specific growth rates based on the solvent control data. However, the mean coefficient of variation for section-by-section specific growth rates based on the pooled control data is only slightly above the maximum value of 35 % requested by the guideline and considered fulfilled as it is difficult to meet with this filamentous growing species.
Previous evaluation:	yes, evaluated and accepted in DAR (2011)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	No

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Executive Summary

The Blue green algae *Anabaena flos-aquae* was exposed to fluopyram under static conditions for 96 hours. Algae cultures with an initial nominal cell count of approximately 1.0 × 10^4 cells/mL were used to test the nominal concentrations of 0.63, 1.25, 2.5, 5.0 and 10.0 mg a.s./L. The study design included 3 replicates for each test concentration and control (control and solvent control). At 24 hour-intervals, the cell density (cells/mL) of each culture was counted.

Concentrations of fluopyram were verified by HPLC – UV on day 0 and day 4 for each concentration and control. Measured concentrations were in the 81 - 98 % range of nominal concentrations and no residues were found in the control and solvent control samples above the LOQ (0.06 mg a.s./L). The biological results are based on the mean measured concentrations of 0.55, 1.18, 2.33, 4.50 and 9.69 mg a.s./L.

The study fulfils the validity criteria of OECD 201 guideline except for the mean coefficient of variation for section-by-section specific growth rates based on the solvent control data. However, the mean coefficient of variation for section-by-section specific growth rates based on the pooled control data is only slightly above the maximum value of 35 % requested by the guideline and considered fulfilled as it is difficult to meet with this filamentous growing species.

As the biomass data (0-72 hour, 0-96 hour) was evaluated on the basis of the pooled control data the endpoints can be considered valid. For the growth rate the 0-72 and 0-96 hour data were evaluated on the basis of the solvent control and pooled control data, respectively. Therefore, only the growth rate data for the 0-96 h period can be considered valid.

No physical abnormalities were observed in the controls or any test concentration during the study.

The endpoints based on mean measured concentrations were: 72 hours – E_bC_{50}: > 9.69 mg a.s./L and 72 hours – E_yC_{50}: > 9.69 mg a.s./L.

I. MATERIAL AND METHODS

Test material	Fluopyram (AE C656948) Specification No.: 102000012455 Batch No.: 08528/0002 Purity: 94.7 % w/w
Guideline(s) adaptation	None specified.
Test species	Blue green algae, *Anabaena flos-aquae* UTEX
Culturing conditions	In-house 4 day old batch culture held under test conditions.
Test solutions	Nominal concentrations 0.63 – 1.25 – 2.5 – 5.0 – 10.0 mg a.s./L Corresponding mean measured concentration: 0.55 – 1.18 – 2.33 – 4.50 – 9.69 mg a.s./L Control: untreated medium Solvent control: Dimethylformamide (0.1 mL/L) Evidence of undissolved material: No precipitates were observed during exposure.
Replication	No. of vessels per concentration (replicates): 3 No. of vessels per control/solvent control (replicates): 3
Exposure	Static Total exposure duration: 96 hours
Initial cells density	1 × 10^4 cells/mL in each test group
Test conditions	Temperature: 23.6 - 24.2 °C (datalogger) Photoperiod: 24 hours light Light intensity: 1938 - 2336 lux Type of light: Cool white fluorescents pH of controls: 7.4 - 9.9 (0 - 96 h) Conductivity: 83 - 94 µmhos/cm Growth medium same as culture medium: Yes
Parameters Measured / Observations	pH and conductivity were measured on day 0 and 4. Temperature was measured hourly via a calibrated datalogger plus daily manual records via a calibrated thermometer. Each day, density was determined in the three test replicates at each test concentration using a light microscope and a hemocytometer slide. Cellular observations were done by visual inspection via light microscope.
Sampling for chemical analysis	Samples for analysis of test substance were taken at test initiation (0 hour) from batch prepared solutions for each test concentration and at test termination (96 hours) from composite samples from each test concentration. Samples were analysed by using a High-performance liquid chromatograph (HPLC) – UV.
Data analysis	Raw or transformed data from treatment groups were compared to controls for normality and homogeneity of variance using the Shapiro-Wilks test and Levene's test of equal variance, respectively. If normality and homogeneity of variance were demonstrated for the raw or transformed values, then parametric analyses were conducted using analysis of variance (ANOVA) followed by Dunnett's test. If normality and/or homogeneity of variance were not demonstrated on raw or transformed values, nonparametric procedures were used. The ranks of the raw values were determined, and then an analysis of variance and a one-tailed Dunnett's test were performed on these ranks. The EC_{50}, and the respective 95 % confidence intervals, was calculated with help of regressions analysis for cell density, cumulative biomass, and growth rate.

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	All statistical analyses were performed using the SAS computer software package.

II. RESULTS AND DISCUSSION

Table 8.2.6.2- 6: Validity criteria

Validity criteria acc. to OECD 201 (adopted 2006)	Required	Obtained 0 – 72 hours
The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period.	≥ 16	45 (solvent control) 50 (pooled control) [A]
The mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2, 2-3) in the control cultures must not exceed 35 %.	< 35 %	45 % (solvent control) [A,B] 37 % (pooled control) [B]
The coefficient of variation of average specific growth rates during the 72-hour test period in replicate control cultures must not exceed 10 %.	< 10 %	2.4 % (solvent control) [A] 5.1 % (pooled control)

[A] Not given in report. Calculations based on cell density data.
[B] Considered fulfilled as difficult to meet with this filamentous growing species. Due to the filamentous growth of this algal species maintaining exponential growth during the entire study period is difficult and rarely achieved, generally as a result of a lag phase during the first 24 hours of the study. However, the alga grew well during the study period and multiplied by a factor of 45 far exceeding the growth criteria of 16x. The fact that this growth criterion was not met is not uncharacteristic when testing with this species and slightly exceeding this did not impact the quality of the study.

Analytical results:

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 SANTE/2020/12830, Rev.1.

Recoveries on day 0 and day 4 were in the range from 81 to 98 % of nominal concentrations (see table below). The biological results are based on mean measured concentrations of fluopyram.

No residues of fluopyram were measured in the control and solvent control samples above the limit of quantification (LOQ 0.06 mg a.s./L).

Table 8.2.6.2- 7: Analytical results

Nominal concentration [mg a.s./L]	Measured concentration [mg a.s./L]		% of nominal		Mean measured concentration [mg a.s./L]	Mean % of nominal
	Day 0 (New)	Day 4 (Old)	Day 0 (New)	Day 4 (Old)		
0.63	0.59	0.51	94	81	0.55	88
1.25	1.23	1.13	98	91	1.18	94
2.5	2.30	2.35	92	94	2.33	93
5.0	4.31	4.70	86	94	4.50	90
10.0	9.68	9.70	97	97	9.69	97

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Biological results:

Observations:

No physical abnormalities were observed in the controls or any test concentration during the study.

Regarding cell density (standing crop, 0-96 hour) the control and solvent control were not significantly different from each other. Therefore, the cell density in the treatment groups was evaluated on the basis of the pooled control data.

Table 8.2.6.2- 8: Cell density

Mean measured concentration [mg a.s./L]	Mean cell density [x 10^4 cells/mL]				% Inhibition[A] at 96 h
	24 h	**48 h**	**72 h**	**96 h**	
Control	3.43	18.69	62.19	206.92	-
Solvent Control	2.92	16.49	45.32	188.25	-
Pooled Controls	-	-	-	197.58	-
0.55	2.13	14.72	50.93	203.33	-2.9
1.18	2.90	19.93	71.42	213.58	-8.1
2.33	3.52	20.90	63.17	188.83	4.4
4.50	2.95	19.62	65.13	188.92	4.4
9.69	2.30	19.79	51.96	219.25	-11.0

[A] % Inhibition=100-((Treatment group parameter mean/pooled control parameter mean)*100)

Regarding biomass (0-72 hour, 0-96 hour) the control and solvent control were not significantly different from each other. Therefore, the biomass in the treatment groups was evaluated on the basis of the pooled control data.

Table 8.2.6.2- 9: Biomass

Mean measured concentration [mg a.s./L]	Mean cumulative biomass [A]		% Inhibition [B]	
	0 - 72 h	**0 - 96 h**	**0 - 72 h**	**0 - 96 h**
Control	1217.2	4422.6	-	-
Solvent Control	949.6	3728.5	-	-
Pooled Controls	1083.4	4075.6	-	-
0.55	955.5	3982.7	11.8	2.3
1.18	1344.8	4740.8	-24.1	-16.3
2.33	1284.3	4284.3	-18.5	-5.1
4.50	1263.3	4287.8	-16.6	-5.2
9.69	1093.7	4324.2	-0.9	-6.1

[A] Cumulative biomass is equal to the area under the growth curve.
[B] % Inhibition=100-((Treatment group parameter mean/pooled control parameter mean)*100).

Regarding 0-72 hour growth rate the control and solvent control were significantly different from each other. Therefore, the 0-72 hour growth rate in the treatment groups was evaluated on the basis of the solvent control data.

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Regarding 0-96 hour growth rate the control and solvent control were not significantly different from each other. Therefore, the 0-96 hour growth rate in the treatment groups was evaluated on the basis of the pooled control data.

Table 8.2.6.2- 10: Algae growth rate

Mean measured concentration [mg a.s./L]	Mean growth rate [A] [1/h]		% Inhibition [B]	
	0 - 72 h	0 - 96 h	0 - 72 h [B]	0 - 96 h [C]
Control	0.057271	0.055522	-	-
Solvent Control	0.052930	0.054555	-	-
Pooled Controls [C]	0.055100	0.055039	-	-
0.55	0.054536	0.055144	-3.0	-0.2
1.18	0.059020	0.055832	-11.5	-1.4
2.33	0.057509	0.054449	-8.7	1.1
4.50	0.057984	0.054580	-9.5	0.8
9.69	0.054779	0.056060	-3.5	-1.9

[A] Growth rate [1/h] is calculated from the cell density data.
[B] % Inhibition=100-((Treatment group parameter mean/solvent control parameter mean)*100)
[C] % Inhibition=100-((Treatment group parameter mean/pooled control parameter mean)*100).

III. Conclusion

The study meets the validity criteria except for the mean coefficient of variation for section-by-section specific growth rates based on the solvent control data. However, the mean coefficient of variation for section-by-section specific growth rates based on the pooled control data is only slightly above the maximum value of 35 % requested by the guideline and considered fulfilled as it is difficult to meet with this filamentous growing species.

As the biomass data (0-72 hour, 0-96 hour) was evaluated on the basis of the pooled control data the endpoints can be considered valid. For the growth rate the 0-72 and 0-96 hour data were evaluated on the basis of the solvent control and pooled control data, respectively. Therefore, only the growth rate data for the 0-96 h period can be considered valid.

The 0-72 and 0-96 hours endpoints based on mean measured concentrations were:

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Results – 0 to 72 hours	
E_rC_{50} – 72 hours (95 % C.I.): [D]	**> 9.69 mg a.s./L** (not determined [A])
E_rC_{20} – 72 hours (95 % C.I.): [D]	> 9.69 mg a.s./L (n.d.)
E_rC_{10} – 72 hours (95 % C.I.): [D]	> 9.69 mg a.s./L (n.d.)
E_bC_{50} – 72 hours (95 % C.I.):	**> 9.69 mg a.s./L** (not determined [A])
E_yC_{50} - 72 hours (95 % CI): [B, D]	**> 9.69 mg a.s./L** (not determined [C])
E_yC_{20} - 72 hours (95 % CI): [B, D]	Not determined [C]
E_yC_{10} - 72 hours (95 % CI): [B, D]	Not determined [C]
LOEC – 72 hour: lowest concentration with an effect (based on growth rate and biomass)	> 9.69 mg a.s./L
NOEC – 72 hours: highest concentration without an effect (based on growth rate and biomass)	9.69 mg a.s./L
Results – 0 to 96 hours	
E_rC_{50} – 96 hours (95 % CI):	**> 9.69 mg a.s./L** (not determined [A])
E_bC_{50} – 96 hours (95 % C.I.):	> 9.69 mg a.s./L (not determined [A])
LOEC – 96 hours: lowest concentration with an effect (based on growth rate and biomass)	> 9.69 mg a.s./L
NOEC – 96 hours: highest concentration without an effect (based on growth rate and biomass)	9.69 mg a.s./L

A Not determined as above functional limit of solubility.
B Please refer to recalculation document by M-757698-01-1
C Not determined due to mathematical reasons. For yield after 72 hours, no decrease was observed at any tested concentration compared to the solvent control. On the contrary, an increase in yield was observed in every tested concentration. The highest test concentration of 10 mg a.s./L showed a 15 % increase of yield compared to the pooled control.
D Endpoints considered not valid as the 0-72 hour data were evaluated on the basis of the solvent control for which the validity criterion "mean coefficient of variation for section-by-section specific growth rates" was not met.

Assessment and conclusion by applicant:

The study and its data are considered as supplementary data with no use in risk assessment.

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Data Point:	KCA 8.2.6.2/03
Report Author:	[redacted]
Report Year:	2007
Report Title:	Toxicity of AE C656948 technical to the saltwater diatom Skeletonema costatum
Report No:	EBGMP050
Document No:	M-287289-01-1
Guideline(s) followed in study:	FIFRA Guideline 123-2 (1982) OPPTS Guideline 850.5400 (2006 draft) OECD Guideline 201 (2006)
Deviations from current test guideline:	Current Guideline: OECD 201 (2006) Deviations: The inoculum was a 5 day old batch culture and not between 2 and 4 days as recommended in OECD 201. Light duration was 14 hours instead a recommended continuous light. These deviations were not expected to have impacted the study results. All validity criteria were met.
Previous evaluation:	yes, evaluated and accepted in DAR (2011)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The saltwater diatom *Skeletonema costatum* was exposed to fluopyram under static conditions for 96 hours. Diatom cultures with an initial nominal cell count of approximately 1.0×10^4 cells/mL were used to test the nominal concentrations of 0.063, 0.125, 0.250, 0.50 and 1.0 mg a.s./L. The study design included 3 replicates for each test concentration and control (control and solvent control). At 24 hour-intervals, the cell density (cells/mL) of each culture was counted.

Concentrations of fluopyram were verified by HPLC – UV on day 0 and day 4 for each concentration and control. Measured concentrations were in the 101 - 130 % range of nominal concentrations and no residues were found in the control and solvent control samples above the LOQ (0.006 mg a.s./L). The biological results are based on the mean measured concentrations of 0.080, 0.152, 0.302, 0.621 and 1.13 mg a.s./L.

The study fulfils all validity criteria of OECD 201 guideline.

No physical abnormalities were observed in the controls or any test concentration during the study.

The endpoints based on mean measured concentrations were: 72 hour – E_rC_{50}: > 1.13 mg a.s./L, 72 hour – E_bC_{50}: > 1.13 mg a.s./L and 72 hours- E_yC_{50}: > 1.13 mg a.s./L.

I. MATERIAL AND METHODS

Test material:	Fluopyram (AE C656948) Specification No.: 102000012455 Batch No.: 08528/0002 Purity: 94.7 % w/w
Guideline(s) adaptation	None specified.
Test species	Saltwater Diatom *Skeletonema costatum* Strain CCAP1077/5
Culturing conditions	In-house 5 day old batch culture held under test conditions

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Test solutions	Nominal concentrations 0.063 – 0.125 – 0.250 – 0.50 – 1.0 mg a.s./L Corresponding mean measured concentrations: 0.080 – 0.152 – 0.302 – 0.621 – 1.13 mg a.s./L Control: untreated medium Solvent control: Dimethylformamide (0.1 mL/L) Evidence of undissolved material: No precipitates were observed during exposure.
Replication	No. of vessels per concentration (replicates): 3 No. of vessels per control/solvent control (replicates): 3
Exposure	Static Total exposure duration: 96 hours
Initial cells density	1 × 10^4 cells/mL in each test group
Test conditions	Temperature: 20.1 - 21.6 °C Photoperiod: 16 hours light, 8 hours dark Light intensity: 4030 - 4850 lux Type of light: Cool white fluorescents pH of controls: 7.7 - 8.4 (0 - 96 h) Salinity: 26 ‰ (on day 0 and day 4) Conductivity: not reported Growth medium same as culture medium: Yes
Parameters Measured / Observations	pH and salinity was measured on day 0 and 4. Temperature was measured hourly via a calibrated datalogger plus daily manual records via a calibrated thermometer. Density was determined daily in the three test replicates at each test concentration using a light microscope and a hemocytometer. Cellular observations were done by visual inspection via light microscope.
Sampling for chemical analysis	Samples for analysis of test substance were taken at test initiation (0 hour) from batch prepared solutions for each test concentration and at test termination (96 hours) from composite samples from each test concentration. Samples were analysed by using a High-performance liquid chromatograph (HPLC) – UV.
Data analysis	Raw or transformed data from treatment groups were compared to controls for normality and homogeneity of variance using the Shapiro-Wilks test and Levene's test of equal variance, respectively. If normality and homogeneity of variance were demonstrated for the raw or transformed values, then parametric analyses were conducted using analysis of variance (ANOVA) followed by Dunnett's test. If normality and/or homogeneity of variance were not demonstrated on raw or transformed values, nonparametric procedures were used. The ranks of the raw values were determined, and then an analysis of variance and a one-tailed Dunnett's test were performed on these ranks. The 72 or 96-hour EC_{50}, and the respective 95% confidence intervals, was calculated with help of regressions analysis for cell density, cumulative biomass, and growth rate. All statistical analyses were performed using the SAS computer software package.

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II. Results and Discussion

Table 8.2.6.2- 11: Validity criteria

Validity criteria acc. to OECD 201 (adopted 2006)	Required	Obtained
The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hours test period.	≥ 16	22.4 (pooled control and solvent control)
The mean coefficient of variation for section-by-section specific growth rates in the control cultures must not exceed 35 %.	≤ 35 %	16 %
The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures must not exceed 10 %.	≤ 10 %	2.1 %

Analytical results:

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 SANTE/2020/12830, Rev.1.

Recoveries on day 0 and day 4 were between 101 and 130 % of nominal concentrations (see table below). The biological results are based on mean measured concentrations of fluopyram.

No residues of fluopyram were found in the controls above the limit of quantification (LOQ: 0.006 mg a.s./L).

Table 8.2.6.2- 12: Analytical results

Nominal concentration [mg a.s./L]	Measured concentration [mg a.s./L]		% of nominal		Mean measured concentration [mg a.s./L]	Mean % of nominal
	Day 0 (New)	Day 4 (Old)	Day 0 (New)	Day 4 (Old)		
0.063	0.080	0.079	128	126	0.080	127
0.125	0.162	0.143	129	114	0.152	122
0.25	0.324	0.281	130	112	0.302	121
0.5	0.62	0.62	124	124	0.621	124
1.0	1.25	1.01	125	101	1.13	113

Biological results:

Observations:

No physical abnormalities were observed in the controls or treatment groups during the study.

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Table 8.2.6.2- 13: Cell densities during study

Mean measured concentration [mg a.s./L]	Mean cell density [x 10^4 cells/mL]				% Inhibition [A] at 96 h
	24 h	48 h	72 h	96 h	
Control	2.16	6.33	21.33	68.08	-
Solvent Control	2.13	6.60	23.49	75.67	-
Pooled Controls				71.88	-
0.080	2.01	5.57	19.36	61.17	14.9
0.152	2.57	7.46	29.63	85.67	-19.2
0.302	2.52	6.56	24.72	81.00	-12.7
0.621	2.31	5.00	16.15	48.50	32.5 *[B]
1.13	2.20	6.95	26.71	74.83	-4.1

* Statistically significant difference from pooled control (Dunnett's one-tailed test; p = 0.05).
[A] % Inhibition=100-((Treatment group parameter mean/pooled control parameter mean)*100).
[B] Statistical effect not considered to be biologically relevant since this effect does not follow a dose response curve (cell density in the highest treatment level was consistently higher than in the pooled control group).

Table 8.2.6.2- 14: Biomass

Mean measured concentration [mg a.s./L]	Cumulative biomass [A]		% Inhibition [B]	
	0 - 72 h	0 - 96 h	0 - 72 h	0 - 96 h
Control	399.8	1448.8	-	-
Solvent Control	431.3	1597.1	-	-
Pooled Controls	415.5	1522.9	-	-
0.080	354.3	1296.6	14.7	14.9
0.152	547.0	1906.5	-31.6	-25.2
0.302	454.5	1699.2	-9.4	-11.6
0.621	309.3	1061.0	25.6	30.3
1.13	480.2	1674.7	-15.6	-10.0

[A] Cumulative biomass is equal to the area under the growth curve.
[B] % Inhibition=100-((Treatment group parameter mean/pooled control parameter mean)*100).

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Table 8.2.6.2- 15: Algae growth rate

Mean measured concentration [mg a.s./L]	Growth rate [A] [1/h]		% Inhibition [B]	
	0 - 72 h	0 - 96 h	0 - 72 h	0 - 96 h
Control	0.042484	0.043942		
Solvent Control	0.043664	0.045000		
Pooled Controls	0.043074	0.044471		
0.080	0.040991	0.042808	6.1	3.7
0.152	0.046901	0.046303	-7.4	-4.1
0.302	0.044139	0.045644	-1.1	-2.6
0.621	0.038514	0.039900	11.8	10.3 *[C]
1.13	0.045003	0.044858	-3.1	-0.9

* Statistically significant difference from pooled control (Dunnett's one-tailed test; $p = 0.05$)
[A] Growth rate [1/h] is calculated from the cell density data.
[B] % Inhibition =100-((Treatment group parameter mean/pooled control parameter mean)*100)
[C] Statistical effect not considered to be biologically relevant since this effect does not follow a dose response curve (cell density in the highest treatment level was consistently higher than in the pooled control group).

III. Conclusion

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

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Results – 0 to 72 hours	
E_rC_{50} – 72 hours (95 % C.I.)	**> 1.13 mg a.s./L** (n.d.)
E_rC_{20} - 72 hours (95 % C.I.):	> 1.13 mg a.s./L (n.d.)
E_rC_{10} - 72 hours (95 % C.I.):	> 1.13 mg a.s./L (n.d.)
E_bC_{50} – 72 hours (95 % C.I.)	> 1.13 mg a.s./L (n.d.)
E_yC_{50} - 72 hours (95 % C.I.): [A]	**> 1.13 mg a.s./L (n.d.)**
E_yC_{20} - 72 hours (95 % CI): [A]	> 1.13 mg a.s./L (n.d.)
E_yC_{10} - 72 hours (95 % CI): [A]	> 1.13 mg a.s./L (n.d.)
LOEC – 72 hours: lowest concentration with an effect (based on biomass and growth rate)	> 1.13 mg a.s./L
NOEC – 72 hour: highest concentration without an effect (based on biomass and growth rate)	1.13 mg a.s./L
NOEyC – 72 hours: [A] highest concentration without an effect	≥ 1.13 mg a.s./L
Results - 0 to 96 hours	
E_bC_{50} – 96 hours (95 % C.I.)	> 1.13 mg a.s./L (n.d.)
EC_{50} – 96 hours (based on cell density) (95% C.I.):	> 1.13 mg a.s./L (n.d.)
LOEC – 96 hours: lowest concentration with an effect (based on based on cell density (standing crop), biomass and growth rate)	> 1.13 mg a.s./L
NOEC – 96 hours: highest concentration without an effect (based on cell density (standing crop), biomass and growth rate)	1.13 mg a.s./L

n.d.: not determined
[A] Please refer to recalculation document by M-757680-01-1

Reliability assessment (EFSA 2015)

The following table provides reliability indicators for EC_{10} values for *Skeletonema costatum.*

Biological endpoints	EC_{10} [mg a.s./L]	95% CL	NW	Relationship $EC_{10}/EC_{20/50}$
Growth Rate	>1.13	-[A]	-[B]	-[B]
Yield	>1.13	-[A]	-[B]	-[B]

[A] The confidence interval could not be defined.
[B] Could be calculated as confidence intervals could not be determined.

Assessment and conclusion by applicant:

The study and its data are considered as acceptable and reliable for use in risk assessment.

The endpoint is: E_rC_{50} (72 hours) > 1.13 mg a.s./L

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Recalculation of endpoints for the active substance fluopyram

Data Point:	KCA 8.2.6.2/04
Report Author:	[redacted]
Report Year:	2020
Report Title:	Statistical evaluation (non-GLP) of the study M-289899-01-1 (Banman, C. S., Lam, C. V., 2007, EBGMP040) on the chronic toxicity of AE C656948 technical to the freshwater diatom Navicula pelliculosa under static conditions
Report No:	M-757699-01-1
Document No:	M-757699-01-1
Guideline(s) followed in study:	None
Deviations from current test guideline:	Current Guideline: not applicable Deviations: not applicable
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	Yes

Summary

In the existing report M-289899-01-1, endpoints for yield were statistically determined at 72 h.

A statistical evaluation addressing the calculation of valid 72-h EC_{10}, EC_{20} and EC_{50} values as well as NOEC values for yield was conducted to fulfill the data requirements according to regulation EU 283/2013. Furthermore, the validity criteria for the study were re-evaluated according to the current guideline OECD 201 (2011).

The recalculations were performed with the software ToxRat Professional (Version 3.3.0) with the nominal concentrations provided in the report.

The control and solvent control (dimethylformamide) data were pooled for statistical testing.

Models providing best fit to the respective data were selected and are as follows: In order to derive Effect Concentrations that have 10, 20 and 50 % effects on yield of the test subjects (EC_{10}, EC_{20}, and EC_{50}), a 3-parameter logistic CDF using non-linear regression was performed.

NOEC was determined by Williams Multiple Sequential t-test Procedure (one-sided smaller, p = 0.05). To test for normal distribution and variance homogeneity, a Shapiro-Wilk's test and a Levene's test were performed respectively.

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Table 8.2.6.2- 16: Re-calculated EC_{10}, EC_{20}, EC_{50} and NOEC values based on nominal concentrations

Endpoint	Fluopyram (AE C656948) [mg a.s./L]
	Yield
72 hours - EC_{10} (95 % C.I.)	3.58 (2.14 – 4.30)
72 hours - EC_{20} (95 % C.I.)	4.24 (3.02 – 4.85)
72 hours -EC_{50} (95 % C.I.)	5.64 (5.07 – 6.21)
72 hours -NOEC	2.5

C.I.: Confidence interval

Assessment and conclusion by applicant:

The study and its data are considered as acceptable and reliable for use in risk assessment.

The endpoint is: E_rC_{50} (72 hours) = 9.08 mg a.s./L

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Data Point:	KCA 8.2.6.2/05
Report Author:	
Report Year:	2020
Report Title:	Statistical evaluation (non-GLP) of the study M-287287-01-1 (Banman, C. S., Lam, C. V., 2007, EBGMP049) on the chronic toxicity of AE C656948 technical to blue-green algae Anabaena flos-aquae under static conditions
Report No:	M-757698-01-1
Document No:	M-757698-01-1
Guideline(s) followed in study:	None
Deviations from current test guideline:	Current Guideline: not applicable Deviations: not applicable
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	Yes

Summary

In the existing report M-287287-01-1 endpoints for yield were statistically determined at 72 h.

A statistical evaluation addressing the calculation of valid 72-h EC_{10}, EC_{20}, and EC_{50} values as well as NOEC values for yield was conducted to fulfill the data requirements according to regulation EU 283/2013. Furthermore, the validity criteria for the study were re-evaluated according to the current guideline OECD 201 (2011).

The recalculations were performed with the software ToxRat Professional (Version 3.3.0) with the nominal concentrations provided in the report.

A control and a solvent control (dimethylformamide) were tested in parallel. As there was a statistically significant difference between the control and the solvent control (p = 0.366), for statistical testing the data was compared against the solvent control.

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Due to lack of inhibition compared to solvent control and due to breach of validity criteria, no further statistical analysis was performed.

Due to the breach of the validity criteria no further statistical assessment was conducted. However, for yield after 72 hours, no decrease was observed at any tested concentration compared to the solvent control. On the contrary, an increase in yield was observed in every tested concentration. The highest test concentration of 10 mg a.s./L showed a 15 % increase of yield compared to the pooled control.

Assessment and conclusion by applicant:
The study and its data are considered as not acceptable and not reliable with no use in risk assessment.

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Data Point:	KCA 8.2.6.2/06
Report Author:	[redacted]
Report Year:	2020
Report Title:	Statistical evaluation (non-GLP) of the study M-287289-01-1 (Banman, C. S., Lam, C. V., 2007, EBGMP050) on the chronic toxicity of AE C656948 technical to the saltwater diatom Skeletonema costatum under static conditions
Report No:	112463
Document No:	M-757680-01-1
Guideline(s) followed in study:	--
Deviations from current test guideline:	Current Guideline: not applicable Deviations: not applicable
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	Yes

Summary

In the existing report M-287289-01-1 endpoints for yield were statistically determined at 72 h.

A statistical evaluation addressing the calculation of valid 72-h EC_{10}, EC_{20}, and EC_{50} values as well as NOEC values for yield was conducted to fulfill the data requirements according to regulation EU 283/2013. Furthermore, the validity criteria for the study were re-evaluated according to the current guideline OECD 201 (2011).

The recalculations were performed with the software ToxRat Professional (Version 3.3.0) based on geometric mean measured concentrations (EFSA 2015).

A control and a solvent control (dimethylformamide) were tested in parallel. As there was no statistically significant difference between the control and the solvent control, for statistical testing the data of control and solvent control was pooled.

Models providing best fit to the respective data were selected and are as follows: In order to derive Effect Concentrations that have 10, 20 and 50 % effects on yield of the test subjects (EC_{10}, EC_{20}, and EC_{50}), no suitable model could be identified.

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NOEC was determined by Dunnett's Multiple t-test Procedure (one-sided smaller, $p = 0.05$). To test for normal distribution and variance homogeneity, a Shapiro-Wilk's test and a Levene's test were performed respectively.

Because no clear dose-response relationship was observed, EC_{10}, EC_{20}, and EC_{50} values could not be calculated and the 72-hour NOEC for yield of the test item in *Skeletonema costatum* was calculated to be ≥ 1.1 mg a.s./L (see the table below).

Table 8.2.6.2- 17: Re-calculated EC_{10}, EC_{20}, EC_{50} and NOEC values based on geometric mean measured concentrations

Endpoint	Fluopyram (AE C656948) [mg a.s./L]
	Yield
72 hours - EC_{10} (95 % C.I.)	not determined
72 hours - EC_{20} (95 % C.I.)	not determined
72 hours - EC_{50} (95 % C.I.)	not determined
72 hours - NOEC	≥ 1.1

C.I.: Confidence interval

Assessment and conclusion by applicant:

The study and its data are considered as acceptable and reliable for use in risk assessment.

The endpoint is: E_rC_{50} (72 hours) > 1.1 mg a.s./L

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CA 8.2.7 Effects on aquatic macrophytes

Active substance fluopyram

Data Point:	KCA 8.2.7/01
Report Author:	[redacted]
Report Year:	2021
Report Title:	Amendment no. 1 to final report - Lemna gibba G3 - Growth inhibition test with AE C656948 under static conditions
Report No:	EBGMP051
Document No:	M-283647-02-1
Guideline(s) followed in study:	OECD Guideline 221 (March 23, 2006); U.S. EPA OPPTS Guideline No. 850.4400
Deviations from current test guideline:	Current Guideline: OECD 221 (2006) Deviations: The health of the colonies was not reported. The solvent concentration of DMF was not given. This deviation was not expected to have impacted the study results. All validity criteria were met.
Previous evaluation:	yes, evaluated and accepted in DAR (2011)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

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Executive Summary

The duckweed *Lemna gibba* was exposed to fluopyram under static conditions for 7 days. 12 fronds per test vessel were used to test the nominal concentrations of 0.256, 0.640, 1.60, 4.00, and 10.0 mg a.s./L. The study design included 3 replicates for each test concentration and control (control and solvent control). Observations and frond counts were done on days 2, 5 and at test termination (day 7). At test termination, frond density for each replicate treatment and control vessels were determined.

Concentrations of fluopyram were verified by HPLC – UV on day 0 and day 7 for each concentration and controls. Measured concentrations were in the 88 - 114 % range of nominal concentrations and no residues were found in the control and solvent control samples above the lowest standard concentration (0.0208 mg a.s./L). The biological results were based on nominal concentrations.

The study fulfils all validity criteria of OECD 221 guideline.

No visual effects were observed in the three lowest test concentrations (0.256, 0.640, 1.60 mg a.s./L). In the test concentration of 4.00 mg a.s./L small fronds were observed on day 2, 5 and 7 as well as a slight chlorosis on day 5 until test end. In the highest test concentration (10.0 mg a.s./L) single fronds were observed on day 2, 5 and 7 with a slight chlorosis on day 5 and a medium chlorosis on day 7.

Endpoints based on nominal concentrations were: E_rC_{50} (based on frond numbers) (95 % C.I.): 2.51 mg a.s./L (1.88 - 3.29 mg a.s./L); E_rC_{50} (based on total frond area of plants) (95 % C.I.): 2.86 mg a.s./L (2.36 - 3.43 mg a.s./L); E_yC_{50} (based on frond number) (95 % C.I.): 2.12 mg a.s./L (n.d.) and E_yC_{50} (based on total frond area of plants) (95 % C.I.): 2.32 mg a.s./L (n.d.).

I. Material and Methods

Test material	Fluopyram (AE C656948) Specification No.: 102000012455

	Batch No.: 08528/0002 Purity: 94.7 % w/w
Guideline(s) adaptation	Not specified
Test species	Duckweed (*Lemna gibba*) strain G3
Acclimation	Inoculum pre-culture, preparation 8 days before the start of the main test. Cultivation under the same conditions as in main test
Culturing conditions	Growth medium: 20X AAP medium Light intensity: 6500 - 10000 lux Temperature of 24 ± 2 °C
Test solutions	Nominal concentrations: 0.256 – 0.640 – 1.60 – 4.00 – 10.0 mg a.s./L Mean measured recoveries based on a.s. content ranged from 88 to 111 % of nominal a.s. concentrations. Control: water Solvent control: dimethylformamide (concentration not reported) Evidence of undissolved material: not reported
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
Exposure	Static Total exposure duration: 7 days
Test conditions	Incubation chamber used: not specified Vessels: 470-mL glass dishes with 200 ml test solution Temperature: 23.7 - 24.3 °C Photoperiod: permanent light Light intensity: 6930 - 8620 lux (mean: 7740 lux) Type of light: bank light containing white fluorescent lamps pH of controls: 7.5 - 8.9 Water hardness: not reported Dissolved oxygen: not reported Conductivity: not reported Growth medium: 20X AAP
Parameters Measured / Observations	Determination of frond number and total frond area were made on days 0, 2, 5, 7 by computerized image analysis (Lemna Tec Scanalyzer). Visual observations of sub-lethal effects were performed on days 2, 5 and 7. Temperature was determined by a continuous measurement in one additional incubated glass vessel and recorded hourly by a data logger. The pH was measured in all freshly prepared and all aged test levels and the controls. The light was measured at least once during the test.
Sampling for chemical analysis	Water phase samples were analysed for the actual concentration of fluopyram present in all freshly prepared test levels on day 0 including controls, and in all aged test levels on day 7 of the exposure period. Aliquots for freshly prepared test levels for day 0, analyses were sampled from the prepared volume of each test treatment level. For sampling of aged test media, after removing of plant material from the test vessels on day 7 the contents of all replicate vessels were combined, and then a sample taken for analysis. Samples were analysed by using a high-performance liquid chromatograph (HPLC) – UV.

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Data analysis	EC_x calculations were performed by probit analysis using linear max. likelihood regression. The LOEC determinations of the appropriate parameter (inhibition) were done, using the ANOVA procedure (α = 0.05, one sided) and properly selected multiple t-tests. Calculations were carried out using Microsoft Excel® spreadsheets. All tabulated data are rounded. All further statistical evaluations were done using the commercial program ToxRat Professional version 2.09.

II. RESULTS AND DISCUSSION

Table 8.2.7- 1: Validity criteria

Validity criteria (OECD 221)	Required	Obtained
Doubling time	< 2.5 days	2.48 days (pooled controls)

<u>Analytical results:</u>

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 SANTE/2020/12830, Rev.1.

Recoveries on day 0 and 7 ranged between 88 and 111 % (see table below). Therefore, the biological results are based on nominal concentrations of fluopyram.

Fluopyram was not detected in the control and solvent control samples in a concentration higher than 0.0208 mg/L, which was used as the lowest standard concentration during this study.

Table 8.2.7- 2: Analytical results

Nominal concentration [mg a.s./L]	Mean measured concentration [mg a.s./L]		% of nominal	
	Day 0	Day 7	Day 0	Day 7
0.256	0.271	0.284	106	111
0.640	0.656	0.732	103	114
1.60	1.63	1.67	102	104
4.00	3.98	4.11	100	103
10.0	8.95	8.82	90	88

<u>Biological results:</u>

Observations

No visual effects were observed in the three lowest test concentrations (0.256, 0.640, 1.60 mg a.s./L). In the test concentration of 4.00 mg a.s./L small fronds were observed on day 2, 5 and 7 as well as a slight chlorosis on day 5 until test end. In the highest test concentration (10.0 mg a.s./L) single fronds were observed on day 2, 5 and 7 with a slight chlorosis on day 5 and a medium chlorosis on day 7.

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Table 8.2.7- 3: Results for frond number and frond area and corresponding growth rates and inhibitions

Nominal concentration [mg a.s./L]	Mean [A] (CV) frond number Day 7	Mean [A] (CV) growth rate for frond number [1/d]	Mean [A] (CV) final total frond area of plants Day 7 [mm^2]	Mean [A] (CV) growth rate for total frond area of plants [1/d]	% Inhibition	
					Mean growth rate for frond number	Growth rate for total frond area of plants
Control	84 (5.6)	0.277 (2.9)	239 (2.1)	0.263 (2.3)	-	-
Solvent control	86 (7.0)	0.281 (3.6)	261 (6.6)	0.275 (1.2)	-	-
Pooled controls	85	0.279	250	0.269	-	-
0.256	83 (8.5)	0.277 (4.4)	248 (9.3)	0.267 (3.8)	1.0	0.7
0.640	67 (11.3)	0.246 (6.7)	189 (15.8)	0.246 (8.8)	12.0	8.7
1.60	76 (9.9)	0.263 (5.5)	222 (7.3)	0.260 (7.1)	5.7	3.5
4.00	14 (33.8)	0.014 (331.6)	53 (32.1)	0.049 (59.4)	95.1 *	81.7 *
10.0	6 (20.4)	-0.109 (n.d.)	24 (15.4)	-0.059 (n.d.)	139 *	122.1 *

n.d.: Not determined due to mathematical reasons
CV: Coefficient of variation in %
A Mean value of 3 replicates
* Results which were significantly different (based on Student-t test for Homogeneous Variances with Bonferroni Adjustment; a = 0.05) from the controls

Table 8.2.7- 4: Results for yield based on frond counts and frond area and corresponding % inhibitions

Nominal test concentration [mg a.s./L]	Yield based on frond number		Yield based on frond area	
	Mean [A] (CV) Day 7	% Inhibition	Mean [A] (CV) Day 7	% Inhibition
Control	72 (6.6)	-	201 (2.8)	-
Solvent control	74 (8.1)	-	223 (6.6)	-
Pooled controls	73	-	212	-
0.256	71 (9.9)	2.1	210 (10.5)	0.9
0.640	55 (13.8)	24.0 *	156 (18.7)	26.6 *
1.60	64 (11.8)	12.1	186 (9.8)	12.2
4.00	2 (277.1)	97.7 *	16 (81.1)	92.3 *
10.0	-6 (n.d.)	108.7 *	-12 (n.d.)	105.8 *

n.d.: Not determined due to mathematical reasons
CV: Coefficient of variation in %
A Mean value of 3 replicates
* Results which were significantly different (based on Student-t test for Homogeneous Variances with Bonferroni Adjustment; a = 0.05) from the controls

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III. CONCLUSION

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

Endpoint (Day 0 - 7)	Effect on mean growth rate of frond number	Effect on mean growth rate of total frond area of plants
E_rC_{50} (95 % C.I.):	**2.51 mg a.s./L** (1.88 - 3.29 mg a.s./L)	**2.86 mg a.s./L** (2.36 - 3.43 mg a.s./L)
E_rC_{20} (95 % C.I.):	1.86 mg a.s./L (1.15 – 2.37 mg a.s./L)	2.06 mg a.s./L (1.52 – 2.47 mg a.s./L)
E_rC_{10} (95 % C.I.):	1.58 mg a.s./L (0.86 – 2.06 mg a.s./L)	1.74 mg a.s./L (1.17 – 2.14 mg a.s./L)
LOE_rC: lowest concentration with an effect	4.00 mg a.s./L	4.00 mg a.s./L
NOE_rC: highest concentration without an effect	1.60 mg a.s./L	1.60 mg a.s./L

Endpoint (Day 0 - 7)	Effect on mean yield of frond number	Effect on mean yield of frond area of plants
E_yC_{50} (95 % C.I.):	**2.12 mg a.s./L** **(n.d.)**	**2.32 mg a.s./L** **(n.d.)**
E_yC_{20} (95 % C.I.):	1.25 mg a.s./L (n.d.)	1.47 (n.d.)
E_yC_{10} (95 % C.I.):	0.94 (n.d.)	1.15 (n.d.)
LOE_yC: lowest concentration with an effect	0.640 mg a.s./L	0.640 mg a.s./L
NOE_yC: highest concentration without an effect	0.256 mg a.s./L	0.256 mg a.s./L

n.d.: Not determined due to mathematical reasons

Reliability assessment (EFSA 2015)

The following table provides reliability indicators for EC_{10} values for *Lemna gibba.*

Biological endpoints	EC_{10} [mg a.s./L]	95% CL	NW	Relationship $EC_{10}/EC_{20/50}$
Growth Rate, Frond Number	1.58	0.86 – 2.06	0.760 (fair)	EC_{20}, low < EC_{10} < EC_{50}, low (medium)
Growth Rate, Frond Area	1.74	1.17 – 2.14	0.557 (fair)	EC_{20}, low < EC_{10} < EC_{50}, low (medium)
Yield, Frond Number	0.94	-[A]	-[B]	-[B]
Yield, Frond Area	1.15	-[A]	-[B]	-[B]

[A] The confidence interval could not be defined.
[B] Could be calculated as confidence intervals could not be determined.

Assessment and conclusion by applicant:

The study and its data are considered as acceptable and reliable for use in risk assessment.

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The endpoint is: E_rC_{50} (72 hours) = 2.51 mg a.s./L

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Metabolite Fluopyram-7-hydroxy

Data Point:	KCA 8.2.7/02
Report Author:	[redacted]
Report Year:	2020
Report Title:	Evaluation of Lemna gibba's growth inhibition (following OECD 221 & OCSPP 850.4400) on BCS-AA10065 (7- hydroxy-fluopyram)
Report No:	RRCo-000777 01
Document No:	M-759030-01-1
Guideline(s) followed in study:	GUIDELINE OECD 221 (23 March 2006) Lemna sp. Growth Inhibition Test and OCSPP 850.4400: Aquatic Plant Toxicity Test Using Lemna spp. (June 2012)
Deviations from current test guideline:	Current Guideline: OECD 221 (2006) Deviations: No information on depth of test vessel reported; however, the test vessel volume was 250 mL. This missing information was not expected to have impacted the study results. All validity criteria were met.
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 duckweed *Lemna gibba* was exposed to fluopyram-7-hydroxy under static conditions for 7 days. 12 fronds per test vessel were used to test the nominal concentrations of 10.191, 5.100, 2.550, 1.275, and 0.638 mg p.m./L). The study design included 4 replicates for each test concentration and control. Observations and frond counts were done on days 2, 4 and at test termination (day 7). At the beginning of the test, 3 samples of 30 fronds from the inoculum culture, were dried and weighed to estimate the initial dry weight. At the end of the test, dry weight per replicate was determined.

Concentrations of fluopyram-7-hydroxy were verified by HPLC-MS/MS on day 0 and day 7 for each concentration and control. Measured concentrations were in the 95–127 % range of nominal concentrations and no residues were found in the control samples. The limit of quantification was 10 µg p.m./L and the limit of detection 3.3 µg p.m./L. Biological results are based on mean measured concentrations of 11.036, 5.396, 2.856, 1.404 and 0.701 mg p.m./L.

The study fulfils all validity criteria of OECD 221 guideline.

No visual effects were observed in the control and the three lowest test concentrations (0.701, 1.404 and 2.856 mg p.m./L). In the two highest test concentration slight signs of phytotoxicity (5.369 mg p.m./L) after 7 days and strong signs of phytotoxicity were observed (11.036 mg p.m./L).

Endpoints based on mean measured concentrations were: E_rC_{50} (based on frond numbers) (95 % C.I.): 9.2 mg p.m./L (8.9 – 9.5 mg p.m./L); E_rC_{50} (based on dry weight) (95 % C.I.): 12.3 mg p.m./L (11.4 – 13.6 mg p.m./L); E_yC_{50} (based on frond number) (95 % C.I.): 7.1 mg p.m./L (6.7 – 7.6 mg p.m./L) and E_yC_{50} (based on dry weight) (95 % C.I.): 8.2 mg p.m./L (7.2 – 9.5 mg p.m./L).

I. MATERIAL AND METHODS

Test material	Fluopyram-7-hydroxy Batch No.: SES12367-10-8 Purity: 99.4 % w/w

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Guideline(s) adaptation	Not specified
Test species	Duckweed (*Lemna gibba*)
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	Growth medium: 20X AAP medium Culturing under test conditions
Test solutions	Nominal concentrations: 10.191 – 5.100 – 2.550 – 1.275 – 0.638 mg p m./L Corresponding mean measured concentrations: 11.036 – 5.396 – 2.856 – 1.404 – 0.701 mg p m./L Control: untreated test medium Evidence of undissolved material: not reported
Replication	No. of vessels per concentration (replicates): 4 No. of vessels per control (replicates): 4
Organisms per replicate	No. of fronds per vessel: 12
Exposure	Static Total exposure duration: 7 days
Test conditions	Incubation chamber used: not specified Vessels: Glass container with 250 mL test solution Temperature: 24.2 – 24.6 °C Photoperiod: permanent light Light intensity: 6551 - 6902 lux (mean: 6693 lux) Type of light: not reported pH of controls: 7.4 - 8.5 Water hardness: not reported Dissolved oxygen: not reported Conductivity: not reported Growth medium: 20X AAP
Parameters Measured / Observations	The number of fronds per replicate was recorded on day 2, 4 and 7 (test termination). At the beginning of the test, 3 samples of 30 fronds from the inoculum culture were dried and weighed to estimate the initial dry weight. At the end of the test, dry weight per replicate was determined. Changes in plant development e.g. in frond size, appearance, indication of necrosis, chlorosis or gibbosity, colony break-up or loss of buoyancy, and in root length and appearance were noted. Temperature was measured every two hours. The pH of each treatment was measured at test start and test end. The light intensity was measured at the beginning of the test in 9 points over the test area.
Sampling for chemical analysis	Samples were taken on day 0 in the test solutions preparations and on day 7 in the content of the vessels pooled by replicate in all test item concentrations. Samples were analysed by HPLC-MS/MS.
Data analysis	ECx for yield and growth rate and confidence limits were obtained by calculation according to Regtox® Macro ("Vindimian"), using the Hill model. For estimation of the LOEC and NOEC, treatments means were compared using parametric or non-parametric analysis of variance (ANOVA) techniques plus post hoc tests ($p \leq 0.05$).

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II. Results and discussion

Table 8.2.7- 5: Validity criteria

Validity criteria (OECD 221)	Required	Obtained
Doubling time	< 2.5 days	2.16 days

Analytical results:

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 SANTE/2020/12830, Rev.1.

Recoveries on day 0 ranged between 95 and 101 % of nominal concentrations and on day 7 between 114 and 127 %. All biological results are based on the mean measured test concentrations.

Fluopyram-7-hydroxy was not detected in the control samples. The limit of quantification was 10 µg p.m./L and the limit of detection 3.3 µg p.m./L.

Table 8.2.7- 6: Analytical results

Nominal concentration [mg p m./L]	Measured concentration [µg p m./L]		% of nominal [A]		Mean measured concentration [mg p.m./L]	% of nominal [A]
	Day 0	Day 7	Day 0	Day 7		
0	0	0	-	-	-	
0.638	0.638	0.763	100	120	0.701	
1.275	1.285	1.522	101	119	1.404	
2.550	2.486	3.226	97	127	2.856	
5.100	5.002	5.789	98	114	5.396	
10.191	9.712	12.359	95	121	11.036	

[A] Not given in report. Calculations based on measured concentration on each sampling day.

Biological results:

Observations

No visual effects were observed in the control and the three lowest test concentrations (0.701, 1.404 and 2.856 mg p.m./L, mean measured concentrations). Slight signs of phytotoxicity after 7 days were observed in the second highest test concentration (5.369 mg p.m./L, mean measured concentration) and strong signs in the highest test concentration (11.036 mg p.m./L, mean measured concentration).

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Table 8.2.7- 7: Results for frond number and corresponding growth rates and inhibitions

Mean measured concentration [mg p m./L]	Mean [A] frond number Day 7	Mean [A] dry weight Day 7 [mg]	Growth rate for frond number 0-7 days		Growth rate for dry weight 0-7 days	
			Mean [A] [1/d]	% Inhibition [B]	Mean [A] [1/d]	% Inhibition [B]
0	113.00	177.75	0.32	-	0.65	-
0.701	104.75	157.50	0.31	3.37	0.64	2.59
1.404	109.50	171.50	0.32	1.41	0.65	0.84
2.856	101.00	99.50	0.30	5.04	0.49	24.81
5.396	85.25	131.50	0.28	12.57	0.61	6.61
11.036	25.00	67.00	0.10	67.49	0.51	21.53

[A] Mean value of 4 replicates
[B] % inhibition means decrease in growth relative to the control

Table 8.2.7- 8: Results for yield based on frond number and corresponding % inhibitions

Mean measured concentration [mg p.m./L]	Yield based on frond number Day 7		Yield based on dry weight Day 7	
	Mean [A]	% Inhibition [B]	Mean [A]	% Inhibition [B]
0	101.00	-	175.91	-
0.701	92.75	8.17	155.66	11.51
1.404	97.50	3.47	169.66	3.55
2.856	89.00	11.88	97.66	44.48
5.396	73.25	27.48	129.66	26.29
11.036	13.00	87.13	65.16	62.96

[A] Mean value of 4 replicates
[B] % inhibition means decrease in yield relative to the control

III. CONCLUSION

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

Endpoint (Day 0-7)	Effect on mean growth rate of frond number	Effect on mean growth rate of dry weight
E_rC_{50} (95 % C.I.):	**9.2 mg p m./L** (8.9 - 9.5 mg p.m./L)	**12.3 mg p m./L** (11.4 – 13.6 mg p.m./L)
E_rC_{20} (95 % C.I.)	6.5 mg p m./L (6.0 – 6.9 mg p m./L)	7.2 mg p.m./L (6.2 – 8.3 mg p.m./L)
E_rC_{10} (95 % C.I.)	5.2 mg p m./L (4.8 – 5.7 mg p.m./L	5.3 mg p.m./L (4.1 – 6.6 mg p.m./L)
LOE$_r$C: lowest concentration with an effect	2.856 mg p m./L	5.396 mg p.m./L
NOE$_r$C: highest concentration without an effect	1.404 mg p m./L	2.856 mg p.m./L

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Endpoint (Day 0 - 7)	Effect on mean yield of frond number	Effect on mean yield of dry weight
E_yC_{50} (95 % C.I.):	**7.1 mg p.m./L** (6.7 – 7.6 mg p.m./L)	**8.2 mg p m./L** (7.2 – 9.5 mg p.m./L)
E_yC_{20} (95 % C.I.):	5.0 mg p.m./L (4.6 – 5.6 mg p.m./L)	5.1 mg p m./L (3.9 – 6.4 mg p.m./L)
E_yC_{10} (95 % C.I.):	4.1 mg p.m./L (3.7 – 4.6 mg p.m./L)	3.9 mg p.m./L (2.7 – 5.1 mg p.m./L)
LOE$_y$C: lowest concentration with an effect	2.856 mg p m./L1	5.396 mg p.m./L
NOE$_y$C: highest concentration without an effect	1.404 mg p.m./L	2.856mg p.m./L

<u>Reliability assessment (EFSA 2015)</u>

The following table provides reliability indicators for EC_{10} values for *Lemna gibba*.

Biological endpoints	EC_{10} [mg p.m./L]	95% CL	NW	Relationship $EC_{10}/EC_{20/50}$
Growth Rate, Frond Number	5.2	4.8 – 5.7	0.180 (excellent)	$EC_{10} < EC_{20}$, low (high)
Growth Rate, Dry Weight	5.3	4.1 – 6.6	0.472 (good)	$EC_{10} < EC_{20}$, low (high)
Yield, Frond Number	8.9	8.1 – 9.7	0.180 (excellent)	$EC_{10} < EC_{20}$, low (high)
Yield, Dry Weight	3.9	2.7 – 5.1	0.615 (fair)	EC_{20}, low < EC_{10} < EC_{50}, low (medium)

<u>Assessment and conclusion by applicant:</u>

The study and its data are considered as acceptable and reliable for use in risk assessment.

The endpoint is: E_rC_{50} (72 hours) = 9.2 mg p.m./L

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Metabolite trifluoroacetic acid (TFA)

Data Point:	KCA 8.2.7/03
Report Author:	[redacted]
Report Year:	1993
Report Title:	Sodium trifluoroacetate: toxicity to the duckweed (Lemna gibba)
Report No:	C047215
Document No:	M-247900-01-1
Guideline(s) followed in study:	ASTM: E1415-91 (1991)
Deviations from current test guideline:	Current Guideline: OECD 221 (2006) Deviations: No information on depth of test vessel reported; however, the test vessel volume was 400 mL. Furthermore, no information on the light variation and age of the pre-culture is given. These missing information were not expected to have impacted the study results. All validity criteria were met.
Previous evaluation:	yes, evaluated and accepted in flurtamone RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The duckweed *Lemna gibba* was exposed to Sodium trifluoroacetate (TFA) under static conditions for 7 days. 12 fronds per test vessel were used to test the nominal concentrations of 2400, 1200, 600, 300, 150, 75, 38 and 19 mg p.m./L. The study design included 3 replicates for each test concentration and control. Observations and frond counts were done on days 2, 5 and at test termination (day 7). At test termination, frond density for each replicate treatment and control vessels were determined.

Concentrations of sodium trifluoroacetate were verified radiochemically in test solutions by liquid scintillation counting on day 0 and day 7 for each concentration and control. Also, ^{14}C residues in dry tissue samples were analysed at test end by liquid scintillation counting following sample oxidation. Measured concentrations in test solutions were in the 102 – 113 % range of nominal concentrations. The biological results were based on nominal concentrations of the metabolite Sodium trifluoroacetate.

The study fulfils all validity criteria of OECD 221 guideline.

There were no significant inhibitory effects on frond or weight increase, at a nominal concentration of 300 mg p.m./L. The tissues showed only slight bioconcentration of the test substance after 7 days, with bioconcentration factors ranging from 1.0 to 1.6, based on radiochemical analysis. The tissues showed only slight bioconcentration of the test substance after 7 days, with bioconcentration factors ranging from 1.0 to 1.6, based on radiochemical analysis.

Endpoints based on nominal concentrations of sodium trifluoroacetate were: E_yC_{50} (based on frond number increase) (95 % C.I.): 1100 mg p.m./L (960 – 1200 mg p.m./L) and E_yC_{50} (based on dry weight increase) (95% C.I.): 1200 mg p.m./L (780 – 1900 mg p.m./L).

The converted endpoints based on nominal concentrations of trifluoroacetic acid were: E_yC_{50} (based on frond number increase) (95 % C.I.): 924 mg p.m./L and E_yC_{50} (based on dry weight increase) (95 % C.I.): 1008 mg p.m./L.

I. Material and Methods

Test material	Sodium trifluoroacetate	Radiolabelled trifluoro-[2-^{14}C]-acetic acid

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	Batch No.: not reported Purity: 99 % w/w Density: not reported	Specific activity: 54 mCi/mmol (2.0 GBq/mmol) Radiochemical purity: 99.6 %
Guideline(s) adaptation	Not specified	
Test species	Duckweed (*Lemna gibba*) Strain G3	
Acclimation	Not reported	
Culturing conditions	M-Hoagland's medium Culturing conditions: not reported	
Test solutions	Nominal concentrations: 2400 – 1200 – 600 – 300 – 150 – 75 – 38 – 19 mg p.m./L Corresponding mean measured concentrations: 2500-1300 – 610 – 320 – 170 – 79 – 39 – 20 mg p.m./L Control: culture medium Evidence of undissolved material: not reported	
Replication	No. of vessels per concentration (replicates): 3 No. of vessels per control (replicates): 3	
Organisms per replicate	No. of fronds per vessel: 12	
Exposure	Static Total exposure duration: 7 days	
Test conditions	Incubation chamber used: not specified Vessels: 400-mL glass dishes with 160 ml test solution Temperature: 24.7 – 25.1 (manual daily recordings), 25.0 - 25.8 °C (automatically hourly recordings) Photoperiod: permanent light Light intensity: 9220 lux (on day 1) Type of light: "warm-white" lights pH of controls: 4.5 – 5.4 Water hardness: not reported Dissolved oxygen: not reported Conductivity: not reported Growth medium: M-Hoagland´s medium	
Parameters Measured / Observations	Determination of frond number was made on days 0, 2, 5 and 7. Visual observations of sub-lethal effects were performed on days 2, 5 and 7. Temperature was determined daily in one additional incubated glass vessel. The pH of each treatment was measured at test start using the excess remaining after filling the test vessels. At test end the pH was determined in two replicate test vessels. The light was measured once during the test.	
Sampling for chemical analysis	Samples of each test solution were taken at the start of the test using the excess remaining after filling the test vessels. At the end of the test on day 7, each replicate solution was analysed. The ^{14}C residues were analysed in dry tissues from each replicate vessels at test end. Water samples were analysed radiochemically by liquid scintillation counting. Tissue samples were analysed for ^{14}C residues by liquid scintillation counting following sample oxidation.	

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Data analysis	The 7-day EC_{50} and its 95 % confidence limits were calculated for the parameters frond growth and dry weight by the moving average angle method. The data for increase in frond numbers and weight increase were examined by one-way analysis of variance, and Dunnett's procedure was used to identify the concentrations causing significant inhibition (p= 0.05, one-sided) compared with the control.

II. RESULTS AND DISCUSSION

Table 8.2.7- 9: Validity criteria

Validity criteria (OECD 221)	Required	Obtained
Doubling time	< 2.5 days	2.29 days [A]

[A] Not given in report. Calculated based on frond numbers on day 0 and day 7.

Analytical results:

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 SANTE/2020/12830, Rev.1.

Recoveries on day 0 and day 7 ranged between 102 and 113 % of nominal concentrations. All biological results are based on nominal test concentrations of the metabolite Sodium trifluoroacetate.

Table 8.2.7- 10: Analytical results

Nominal concentration [mg p.m./L]	Measured concentration [mg p.m./L]		% of nominal [A]		Mean measured concentration [mg p.m./L]	% of nominal
	Day 0	Day 7	Day 0	Day 7		
19	20	20	105	105	20	105
38	39	39	103	103	39	103
75	79	79	105	105	79	105
150	170	170	113	113	170	113
300	320	320	107	107	320	107
600	610	613 [B]	102	102	610	102
1200	1300	1300	108	108	1300	108
2400	2500	2500	104	104	2500	104

[A] Not given in report. Calculations based on mean measured concentrations on day 0 and day 7.
[B] Value was corrected due to a calculation error in the original report.

Biological results:

Observations:

From day 5 onwards, plants in the three highest test concentrations (600, 1200 and 2400 mg p.m./L, nominal concentrations) exhibited pale, misshapen fronds with decreased root growth, compared with the control. There were no observed symptoms in the 5 lowest test concentrations (19, 38, 75, 150 and 300 mg p.m./L, nominal concentrations) compared to the control.

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There was no significant decrease in frond numbers compared to the control in the 5 lowest test concentrations (19, 38, 75, 150 and 300 mg p.m./L, nominal concentrations).

The number of fronds was significantly increased (p= 0.05) at the nominal concentrations of 75 and 150 mg p.m./L compared to the control. This apparent stimulation should be interpreted with caution, since there was no evidence of stimulatory effects at 100 mg p.m./L (nominal) in the preliminary range finding study.

No attempt was made to analyse the data for plant numbers, since frond number and weight increases were considered more reliable estimates of Lemna growth.

There was no significant decrease in dry weight in the 5 lowest test concentrations (19, 38, 75, 150 and 300 mg p.m./L, nominal concentrations) compared to the control.

Table 8.2.7- 11: Results for frond number, dry weight, yield based on frond number and dry weight and corresponding inhibitions

Nominal concentration [mg p m./L]	Mean [A] frond number Day 7	Mean [A] dry weight Day 7	Yield based on frond number [B] Day 7		Yield based on dry weight [C] Day 7	
			Mean [A]	% Inhibition	Mean [A]	% Inhibition
Control	100	16.4	88	-	14.4	-
19	107	18.1	95	-	16.1	-
38	110	17.4	98	-	15.4	-
75	144	21.9	132	-	19.9	-
150	147	19.7	135	-	17.7	-
300	121	14.2	109	-	12.2	15
600	68	11.1	56 *	36 *	9.1 *	37 *
1200	47	9.0	35 *	60 *	7.0 *	51 *
2400	41	7.8	29 *	67 *	5.8 *	60 *

A Mean value of 3 replicates
B No. of fronds at day 7 – No. of fronds (12) at day 0
C Dry weight at day 7 – estimated day 0 dry weight. Dry weight at day 0 estimated from control dry weight to be 2.0 mg/ 12 fronds (Calculated from day 7 determinations, (300) fronds weighing 49.3 mg)
* Significantly different from the control (α= 0.05, one-sided smaller)

Tissue residues:

The fresh/dry weight ratio determined on excess inoculum fronds was 19.0.

The BCF values ranged from 1.0 to 1.6, indicating only slight bioconcentration above the ambient water concentration.

Table 8.2.7- 12: Tissue concentrations (based on ^{14}C residues) and bioconcentration factors

Nominal concentration [mg p m./L]	Mean measured water concentration [mg p m./L]	Mean tissue concentration [mg p m./kg dry weight]	Mean tissue concentration [mg p m./kg fresh weight] [A]	BCF [B]
19	20	580	31	1.6

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38	39	1100	58	1.5
75	79	2000	110	1.4
150	170	4300	230	1.4
300	320	9100	480	1.5
600	610	15000	790	1.3
1200	1300	26000	1400	1.1
2400	2500	49000	2600	1.0

A Calculated from fresh/dry weight ratio=19

B BCF = Mean tissue concentration [mg p m./kg fresh weight] / Mean measured water concentration [mg p m./L]

Only slight bioconcentration of the test substance in tissues after 7 days, with bioconcentration factors ranging from 1.0 to 1.6, based on radiochemical analysis.

III. Conclusion

The study meets the validity criteria and the endpoints based on nominal concentrations of sodium trifluoroacetate were:

Endpoint (Day 0-7)	Effect on mean yield of frond number	Effect on mean yield of dry weight
E_yC_{50} (95 % C.I.):	**1100 mg p m./L [A]** (960 – 1200 mg p m./L)	**1200 mg p m./L** (780 – 1900 mg p.m./L)
LOE$_y$C: lowest concentration with an effect	150 mg p m./ L	150 mg p.m./ L
NOE$_y$C: highest concentration without an effect	300 mg p.m./L	300 mg p.m./L

A Based on the molecular weights, a concentration of 1100 mg sodium trifluoroacetate/L corresponds to 924 mg trifluoroacetate acid/L. As the study was conducted with sodium trifluoroacetate which is the sodium salt of trifluoroacetic acid, the endpoint was converted to trifluoroacetic acid with factor 0.84.

Assessment and conclusion by applicant:

~~The study and its data are considered as acceptable and reliable for use in risk assessment.~~

~~The endpoint is: E_yC_{50} (72 hours) = 1100 mg p.m./L (trifluoroacetate) corresponding to 924 mg p.m./L (trifluoroacetic acid).~~

This study is reliable but only provides yield endpoints. For risk assessment relevant growth rate endpoints (E_rC_{50}) it is referred to the subsequent re-calculation report.

****

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Data Point:	KCA 8.2.7/06
Report Author:	[redacted].
Report Year:	2021
Report Title:	Statement - Statistical re-evaluation of a Lemna study performed with sodium trifluoroacetate (Na-TFA) (Smyth et al. 1993; M-247900-01-1)
Report No:	M-768038-01-1
Document No:	M-768038-01-1
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 context of fluopyram EU renewal, the study with sodium trifluoroacetate (Na-TFA) and *Lemna gibba* by Smyth et al. (1993, M-247900-01-1) has been statistically re-evaluated. This re-evaluation was considered necessary because the original study report did not provide all endpoints requested by the latest version of the OECD guideline for *Lemna* testing (OECD 221, 2006) and the EU data requirements for active substances and their metabolites (Commission Regulation (EU) No 283/2013). The following endpoints are given in the study report:

- 7d E_yC_{50} for the measurement variables frond number and dry weight
- 7d NOE_yC for both measurement variables

The following endpoints were so far not available and had to be re-calculated:

- 7d E_rC_{50} for the measurement variables frond number and dry weight
- 7d NOE_rC for both measurement variables
- EC_{10} and EC_{20} values for both measurement and response variables

All recalculations were performed with the software ToxRat Professional Vers. 3.2.1, on the basis of nominal concentrations of Na-TFA as for the originally reported endpoints.

In this report, re-calculated endpoints are provided both as sodium trifluoroacetate (Na-TFA) and as trifluoroacetic acid (TFA). For conversion to the latter, a factor of 0.84 was used.

The results of the re-calculations based on nominal concentrations of **Na-TFA** are presented in the table below.

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Critical conc. [mg p m./L]		**0-7 d**
Yield for frond number (0-7 d)		
	EC_{10}	272
95%-CL	lower	160
	upper	376

95%-CL	EC_{20}	441
	lower	303
	upper	561
95%-CL	EC_{50}	1107
	lower	920
	upper	1357
	LOEC	600
	NOEC	300
Growth rate for frond number (0-7 d)		
95%-CL	EC_{10}	401
	lower	316
	upper	482
95%-CL	EC_{20}	775
	lower	671
	upper	873
95%-CL	EC_{50}	> 2400
	lower	2390
	upper	> 2400
	LOEC	600
	NOEC	300

Critical conc. [mg p.m./L]		**0-7 d**
Yield for dry weight (0-7 d)		
95%-CL	EC_{10}	184
	lower	67.0
	upper	305
95%-CL	EC_{20}	360
	lower	187
	upper	520
95%-CL	EC_{50}	1303
	lower	960
	upper	1976
	LOEC	600
	NOEC	300
Growth rate for dry weight (0-7 d)		
95%-CL	EC_{10}	367
	lower	250
	upper	477
	EC_{20}	859

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95%-CL	lower	700
	upper	1018
	EC_{50}	> 2400
95%-CL	lower	> 2400
	upper	> 2400
	LOEC	600
	NOEC	300

Neither for frond number, nor for dry weight a definitive E_rC_{50} could be calculated. This is due to the fact that the growth rate inhibitions for the two variables at the highest nominal test concentration of 2400 mg p.m./L were only 42 and 35%, respectively. The E_rC_{50} values for both measurement variables were therefore determined to be > 2400 mg p.m./L.

The re-calculated endpoints expressed as concentrations of TFA (correction factor: 0.84) are presented in the table below.

Critical conc. [mg p.m./L]		**0-7 d**
Yield for frond number (0-7 d)		
	EC_{10}	228
95%-CL	lower	134
	upper	316
	EC_{20}	370
95%-CL	lower	255
	upper	471
	EC_{50}	930
95%-CL	lower	773
	upper	1140
	LOEC	504
	NOEC	252
Growth rate for frond number (0-7 d)		
	EC_{10}	337
95%-CL	lower	265
	upper	405
	EC_{20}	651
95%-CL	lower	564
	upper	733
	EC_{50}	> 2016
95%-CL	lower	2008
	upper	> 2016
	LOEC	504

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	NOEC	252

Critical conc. [mg p m./L]		**0-7 d**
Yield for dry weight (0-7 d)		
	EC_{10}	155
95%-CL	lower	56.3
	upper	256
	EC_{20}	302
95%-CL	lower	157
	upper	437
	EC_{50}	1095
95%-CL	lower	806
	upper	1660
	LOEC	504
	NOEC	252
Growth rate for dry weight (0-7 d)		
	EC_{10}	308
95%-CL	lower	210
	upper	401
	EC_{20}	722
95%-CL	lower	588
	upper	855
	EC_{50}	> 2016
95%-CL	lower	> 2016
	upper	> 2016
	LOEC	504
	NOEC	252

Reliability assessment (EFSA 2015)

The following table provides reliability indicators for EC_{10} values for *Lemna gibba*.

Biological endpoints	**Method**	**EC_{10} [mg p m./L]**	**95% CL**	**NW**	**Relationship $EC_{10}/EC_{20/50}$**
Yield (frond number)	Probit	272	160 – 376	0.79 (fair)	$EC_{10} < EC_{20, low}$ (high)
Growth rate (frond number)	Probit	401	316 – 482	0.41 (good)	$EC_{10} < EC_{20, low}$ (high)
Yield (dry weight)	Probit	184	67 – 305	1.29 (poor)	$EC_{10} < EC_{20, low}$ (high)
Growth rate	Probit	367	250 – 477	0.62 (fair)	$EC_{10} < EC_{20, low}$

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(dry weight)	(high)

Assessment and conclusion by applicant:

This re-calculation is reliable and the relevant endpoint for risk assessment is: 7d E_rC_{50} > 2400 mg p.m./L (sodium trifluoroacetate), corresponding to >2016 mg p.m./L (trifluoroacetic acid)

Data Point:	KCA 8.2.7/04
Report Author:	[redacted]
Report Year:	2004
Report Title:	Haloacetic acids in the aquatic environment. Part I: macrophyte toxicity
Report No:	M-455787-01-1
Document No:	M-455787-01-1
Guideline(s) followed in study:	--
Deviations from current test guideline:	Current Guideline: not applicable
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	Yes

Executive Summary

Laboratory tests were conducted with 3 macrophytes (*Lemna gibba*, *Myriophyllum sibiricum*, and *Myriophyllum spicatum*) to assess the toxicity of 5 Haloacetic acids (HAAs). The HAAs in the present experiments were monochloroacetic acid (MCA), dichloroacetic acid (DCA), trichloroacetic acid (TCA), trifluoroacetic acid (TFA), and chlorodifluoroacetic acid (CDFA). MCA was the most toxic to *Myriophyllum* spp. with EC_{50} values ranging from 8 to 12.4 mg/L depending on the endpoint, followed by DCA (EC_{50} range 62-722.5 mg/L), TCA (EC_{50} range 49.5-1702.6 mg/L), CDFA (EC_{50} range 105.3 to greater than 10,000 mg/L), and with TFA (EC_{50} range 222.1 to 10,000 mg/L) the least toxic. Generally, *L. gibba* was less sensitive to HAA toxicity than *Myriophyllum* spp., with the difference in toxicity between them approximately 3-fold. The range of toxicity within *Myriophyllum* spp. was normally less than 2-fold. Statistically, plant length and node no. were the most sensitive endpoints as they had the lowest observed coefficients of variation, but they were not the most sensitive to HAA toxicity. Toxicological sensitivity of endpoints varied depending on the measure of effect chosen and the HAA, with morphological endpoints usually an order of magnitude more sensitive than pigments for all plant species. Overall, mass and root measures tended to be the most sensitive indicators of HAA toxicity.

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I. MATERIAL AND METHODS

Since the purpose of the literature review is to select literature relevant for the environmental risk assessment under Regulation (EC) No 1107/2009 for the metabolite trifluoroacetic acid (TFA), the study summary contains only the results for the compound of concern.

Test material and test organisms:

1. Test material

Test item:	Haloacetic acids (HAAs) including TFA, tested as neutralized sodium salts
Active substance(s):	See above
Chemical state and description:	Not stated
Source of test item:	TFA: Aldrich Chemicals (Milwaukee, WS, USA)
Batch number:	Not stated
Purity:	99 + % (spectrophotometric grade)
Storage conditions:	Not stated
Water solubility:	Not stated

2. Test solutions

Vehicle/solvent:	Not stated
Source of vehicle/solvent:	Not stated
Concentration of vehicle/solvent:	Not stated
Method of preparation:	Not stated
Evidence of unsolved material:	Not stated

3. Test organism(s)

Species:	*Myriophyllum spicatum* L., *M. sibiricum*, *Lemna gibba*
Common name:	Not stated
Source of test species:	Not stated

4. Culture conditions of test organism(s)

Culture medium:	*Myriophyllum* spp. cultured according to standard methods (ASTM, 1999); *L. gibba* cultured axenically according to Greenberg et al. (1992) with Hunter's media containing 10 g/l sucrose.
Temperature:	25:20°C during light and dark phases
Photoperiod:	16 h light:8 h
Light intensity:	Not stated
pH:	pH 5.8
Oxygen saturation:	Not stated
Acclimatisation prior to testing:	The test conditions appear to be similar to the culture conditions, thus acclimatization was not necessary. However, approximately 10 days prior to a *L. gibba* toxicity test, plants were transferred from growth media containing sucrose to media without sucrose. This was done so that the plants would switch from heterotrophic to autotrophic energy production.
Observations during acclimatisation:	Not stated

Study design and methods:

1. Test procedure

Test system:

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Test concentration(s):	*Myriophyllum* spp.: 10, 30, 100, 300, 1000, 3000, 10,000 mg/L. *Lemna gibba*: 10, 30, 100, 300, 1000, 3000 mg/L
Control(s):	Yes: Test media without test item
Number of replicates:	*Myriophyllum* spp.: Controls: n = 10; exposed plants: n = 5 per treatment. L*emna gibba*: Controls: n = 5; treated plants: n = 3

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Test conditions: *Myriophyllum* spp.: Conducted axenically in the environ-mental growth chamber for 14 days and under the environmental conditions described above. All plants were trimmed to a 3 cm apical length so that all plants would have the same initial status, with no roots or side shoots evident. Range-finding studies were conducted and used to determine the final range of concentrations chosen for the definitive tests (see above). At the end of the 14-day test period, plants were evaluated for several parameters (see below).
Lemna: Each test solution (see above) was transferred to a 10-ml plastic Petri dish and two plants, each with four fronds, for a total of eight fronds, were introduced and monitored. Tests were conducted in the growth chamber for 7 days and under environmental conditions described above.

Medium renewal: *Myriophyllum* spp.: No renewal reported
Lemna gibba: Test solutions were changed on day 3 and 5 to maintain consistent levels of the compound under study.

Frequency of test item application: See above

Test duration: *Myriophyllum* spp.: 14 days
Lemna gibba: 7 days

Endpoints: *Myriophyllum* spp.: Plant length, node number, root number, total root length, longest root length, wet mass, drymass, and chlorophyll a, chlorophyll b, and carotenoid concentrations
Lemna gibba: frond number, colony number, wet mass, frond mass, frond growth rate and chlorophyll a, chlorophyll b, and total chlorophyll concentrations.

Statistics: Regression analysis: Data evaluated from toxicity testing with all three plant species were evaluated using non-linear regression techniques described in Stephenson et al. (2000). Only new growth (e.g., shoot length, wet/dry mass, nodes) was used in the models so that a more sensitive and conservative estimate of toxicity was obtained.
NOEC / LOEC calculations: NOEC and LOEC were calculated with a one-way ANOVA in a completely randomized design in SAS Version 8.2 (SAS Institute, Cary NC, USA) using General Linear Models with no adjustments for new growth as was done for the nonlinear regression analysis.

2. Measurements during the test

Water/medium parameters: Not stated

3. Sampling

Sampling frequency: *Myriophyllum* spp.: Endpoints were evaluated at the end of the test (after 14 days).
Lemna gibba: Not stated / most probably endpoints were only evaluated at the end of the test (after 7 days)

Transport/storage of samples: Not stated

4. Chemical analysis

Guideline/protocol: Concentrations of HAAs could not be verified analytically due to interference by the growth media with the ion chromatographic methods used to quantify the HAAs in other studies

Method: See above

Pre-treatment of samples: See above

Conduction: See above

Reference item: See above

Recovery: See above

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Limit of detection: See above
Limit of quantification: See above

II. Results and Discussion

1. Validity criteria:

Not stated

2. Analytical findings:

Concentrations of Haloacetic acids (HAAs) could not be verified analytically due to interference by the growth media with the ion chromatographic methods used to quantify the HAAs in other studies.

3. Other measurements:

Please refer to point 4 'Biological findings'. Measurement of other parameters was not reported.

4. Biological findings:

TFA was the least toxic compound to *Myriophyllum* spp. with EC_{50} values ranging from 222.1 to > 10000 mg/L depending on the endpoint. *L. gibba* was less sensitive to TFA toxicity than *Myriophyllum* spp., with EC_{50} values ranging from 618.3 to > 3000 mg/L. Overall, mass and root measures tended to be the most sensitive indicators of HAA toxicity.

Table 8.2.7- 13: (taken from Hanson & Solomon, 2004): Laboratory-derived EC_x values with 95 % confidence intervals for 14 day *Myriophyllum sibiricum* toxicity tests with TFA

Endpoint	EC_{10}	EC_{25}	EC_{50}	Model	Variables	r^2
Plant length	31.8 (0, 64.1)	145.9 (53.0, 238.7)	765.0 (444.7, 1085.3)	Logistic	$t = 4.943$ $x = 765.001$ $b = 0.691$	0.88
Node number	97.1 (0, 203.2)	392.2 (121.1, 633.3)	1583.6 (897.5, 2269.7)	Logistic	$t = 17.876$ $x = 1583.553$ $b = 0.787$	0.83
Root number	90.5 (24.0, 157.0)	251.7 (130.5, 372.9)	700.0 (477.9, 922.1)	Logistic	$t = 8.446$ $x = 700.020$ $b = 1.074$	0.91
Root length	81.7 (18.7, 144.7)	166.9 (83.6, 250.1)	340.7 (224.4, 456.9)	Logistic	$t = 34.163$ $x = 340.657$ $b = 1.539$	0.88
Longest root length	91.0 (26.2, 155.9)	237.2 (126.1, 348.3)	618.1 (425.6, 810.70	Logistic	$t = 6.806$ $x = 618.135$ $b = 1.147$	0.91
Wet mass	36.3 (3.5, 69.1)	113.8 (45.8, 181.8)	357.0 (216.3, 497.6)	Logistic	$t = 436.060$ $x = 356.991$ $b = 0.961$	0.88
Dry mass	21.9 (0, 52.7)	184.1 (12.5, 255.6)	822.6 (354.0, 1291.2)	Logistic	$t = 73.885$ $x = 822.621$ $b = 0.606$	0.80
Chlorophyll *a*	4460.3 (1849.8, 7070.7)	7890.4 (6082.0, 9698.8)	13,958.4 (9702.7, 18214.2)	Logistic	$t = 0.749$ $x = 13958.416$ $b = 1.926$	0.66
Chlorophyll *b*	> 10,000	> 10,000	> 10,000	nc[a]	nc	nc
Carotenoids	> 10,000	> 10,000	> 10,000	nc	nc	nc

[a] The effect measure could not be calculated for these endpoints.

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Table 8.2.7- 14: (taken from Hanson & Solomon, 2004):: Laboratory-derived EC_x values with 95 % confidence intervals for 14 day *Myriophyllum spicatum* toxicity tests with TFA

Endpoint	EC_{10}	EC_{25}	EC_{50}	Model	Variables	r^2
Plant length	43.4 (15.7, 71.1)	196.2 (115.3, 227.1)	886.6 (654.9, 1118.3)	Logistic	$t=6.698$ $x=886.599$ $b=0.728$	0.95
Node number	53.8 (1.6, 106.0)	225.8 (84.1, 367.6)	947.9 (570.5, 1325.3)	Logistic	$t=18.201$ $x=947.871$ $b=0.766$	0.87
Root number	88.5 (7.9, 169.1)	243.2 (97.9, 388.4)	668.0 (404.5, 931.6)	Logistic	$t=7.142$ $x=668.032$ $b=1.087$	0.87
Root length	37.9 (15.8, 59.9)	91.7 (56.8, 126.7)	222.1 (166.1, 278.2)	Logistic	$t=31.467$ $x=222.137$ $b=1.242$	0.95
Longest root length	52.4 (23.8, 81.0)	129.3 (83.0, 175.5)	318.8 (242.4, 395.1)	Logistic	$t=7.731$ $x=318.790$ $b=1.217$	0.93
Wet mass	41.8 (8.8, 74.8)	114.4 (55.0, 173.8)	312.9 (205.0, 420.8)	Logistic	$t=377.373$ $x=312.908$ $b=1.092$	0.90
Dry mass	46.3 (0, 95.4)	144.5 (51.6, 237.3)	450.3 (265.1, 635.5)	Logistic	$t=72.078$ $x=450.311$ $b=0.966$	0.77
Chlorophyll *a*	672.4 (0, 1478.7)	5052.5 (2343.9, 7761.2)	37,965.4 (2877.0, 73053.7)	Logistic	$t=0.0963$ $x=37965.380$ $b=0.545$	0.68
Chlorophyll *b*	>10,000	>10,000	>10,000	nc[a]	nc	nc
Carotenoids	>10,000	>10,000	>10,000	nc	nc	nc

[a] The effect measure could not be calculated for these endpoints.

Table 8.2.7- 15: (taken from Hanson & Solomon, 2004): Laboratory-derived EC_x values with 95 % confidence intervals for 7 day *Lemna gibba* toxicity tests with TFA

Endpoint	EC_{10}	EC_{25}	EC_{50}	Model	Variables	r^2
Frond number	388.8 (306.9, 470.8)	512.3 (407.9, 616.8)	884.0 (654.3, 1113.6)	Hormetic	$t=59.415$ $h=0.011$ $x=883.961$ $b=0.829$	0.94
Colony number	541.1 (407.2, 675.0)	693.2 (516.3, 870.1)	1140.4 (757.5, 1524.3)	Hormetic	$t=17.876$ $h=0.009$ $x=1140.419$ $b=0.897$	0.87
Wet mass	192.8 (104.1, 281.5)	298.5 (191.0, 406.0)	618.3 (421.1, 815.5)	Hormetic	$t=265.412$ $h=0.009$ $x=618.269$ $b=0.662$	0.91
Frond mass	11.2 (0, 44.2)	506.6 (0, 1189.8)	22965.3 (0, 70230.3)	Logistic	$t=3.940$ $x=22965.257$ $b=0.288$	0.71
Growth rate	445.2 (342.8, 547.6)	790.4 (638.5, 942.3)	2505.2 (1761.1, 3249.3)	Hormetic	$t=0.445$ $h=0.017$ $x=2505.208$ $b=0.361$	0.95
Chlorophyll *a*	>3000	>3000	>3000	nc[a]	nc	
Chlorophyll *b*	>3000	>3000	>3000	nc	nc	
Total chlorophyll	>3000	>3000	>3000	nc	nc	

[a] The effect measure could not be calculated for these endpoints.

Table 8.2.7- 16: (taken from Hanson & Solomon, 2004): NOEC for *Myriophyllum sibiricum* exposed to HAAs including TFA. Values in brackets are the percent change from control as either stimulation (+) or inhibition (-) for untransformed data.

Endpoint	MCA	DCA	TCA	TFA	CDFA
Plant length	10 (−44)[a]	10 (−7)[b]	10 (+1)	100 (−6)	30 (−7)
Node number	10 (−38)[a]	10 (−2)	10 (−4)	100 (+1)	30 (−5)
Root mumber	5 (−22)[a]	100 (−41)[a]	100 (−51)[a]	100 (−7)	300 (−58)[a]
Root length	5 (−32)[a]	100 (−51)[a]	100 (−57)[a]	100 (−12)[a]	300 (−76)[a]
Longest root length	5 (−14)[a]	100 (−34)[c]	30 (−19)	100 (−3)	300 (−45)[a]
Wet mass	2.5 (−4)	10 (−9)	3 (+7)	100 (−10)[b]	10 (+4)
Dry mass	5 (−17)	10 (−11)	10 (−9)	100 (−7)	10 (+2)
Chlorophyll *a*	10 (−54)[a]	100 (+4)	1000 (−49)[a]	3000 (−5)	1000 (0)
Chlorophyll *b*	10 (−58)[a]	100 (+6)	1000 (−34)[a]	3000 (+113)	3000 (−5)
Carotenoids	10 (−53)[a]	100 (+4)	1000 (−31)[a]	3000 (0)	3000 (−1)

Values in parentheses are the percentage change from control as either stimulation (+) or inhibition (−) for untransformed data.
[a] This analysis was run as a non-parametric Kruskal–Wallis on Ranks.
[b] The data were ln transformed.
[c] The data were square transformed.

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Table 8.2.7- 17: (taken from Hanson & Solomon, 2004): NOEC for *Myriophyllum sibiricum* exposed to HAAs including TFA. Values in brackets are the percent change from control as either stimulation (+) or inhibition (-) for untransformed data.

Endpoint	MCA	DCA	TCA	TFA	CDFA
Plant length	5 (−6)	10 (−4)	30 (−20)[a]	30 (−5)	10 (+1)
Node number	5 (−6)	10 (−7)	3 (0)	100 (−2)	30 (−5)
Root number	2.5 (−12)	10 (−23)[a]	10 (−4)	100 (−18)	300 (−18)[a]
Root length	5 (−33)[a]	3 (−3)	10 (−17)[a]	30 (−7)[a]	30 (−24)[b]
Longest root length	10 (−49)[a]	10 (−13)	300 (−43)	30 (−1)	300 (−49)[a]
Wet mass	5 (−17)[b]	3 (−1)	10 (−12)	30 (−3)	10 (0)
Dry mass	10 (−45)[a]	3 (+4)	10 (−4)	100 (−16)	30 (−5)
Chlorophyll *a*	10 (−31)[a]	300 (−18)	30 (−15)	300 (−5)	1000 (−13)
Chlorophyll *b*	10 (−30)[a]	300 (−4)	300 (−20)	> 10000 (0)	3000 (−10)
Carotenoids	10 (−32)[a]	300 (−8)	300 (−20)	≥10000 (+2)	3000 (−5)

[a] This analysis was run as a non-parametric Kruskal–Wallis on Ranks.
[b] The data were square root transformed.

Table 8.2.7- 18: (taken from Hanson & Solomon, 2004): NOEC for *Lemna gibba* exposed to HAAs including TFA. Values in brackets are the percent change from control as either stimulation (+) or inhibition (-) for untransformed data.

Endpoint	MCA	DCA	TCA	TFA	CDFA
Frond number	10 (−6)	30 (+9)	30 (+8)	300 (+5)	30 (+1)
Colony number	10 (−3)	100 (−21)	100 (−19)	<1000	100 (+2)[c]
Wet mass	3 (−9)	50 (−16)	100 (−17)	100 (+6)	30 (0)[c]
Frond mass	3 (−12)	25 (0)	> 800 (+19)[b]	30 (−11)	100 (−11)
Growth rate	10 (−3)	50 (+5)	30 (+9)[c]	300 (+3)	30 (0)
Chlorophyll *a*	20 (−16)[a]	400 (0)[a]	nc[d]	3000 (+9)[b]	1000 (+5)
Chlorophyll *b*	20 (−7)[a]	400 (0)[a]	nc[d]	3000 (+7)	1000 (+4)
Total chlorophyll	20 (−14)[a]	400 (0)[a]	nc[d]	3000 (+9)[b]	1000 (+5)

[a] This analysis was run as a non-parametric Kruskal–Wallis on Ranks.
[b] The data were reciprocal transformed.
[c] The data were square transformed.
[d] Only the 100 mg/l TCA showed a significant difference from control with concentrations on both sides not being significantly different from controls.
[e] The data were ln transformed.

III. CONCLUSION

Under the conditions of this study, the overall lowest 14-day EC_{50} of *Myriophyllum* spp. was 222.1 mg TFA/L (based on root length) and the NOEC was established at 30 mg TFA/L. For *Lemna gibba*, the overall lowest 7-day EC_{50} was 618.3 mg TFA/L (based on wet mass) and the NOEC was established at 30 mg TFA/L (based on front mass). In conclusion, tested HAAs including TFA do not exhibit a high degree of toxicity to *Myriophyllum* spp. or *L. gibba* under laboratory conditions. In general, *L. gibba* was less sensitive to TFA toxicity than *Myriophyllum* species.

Assessment and conclusion by applicant:

The study and its data are considered as not acceptable and not reliable with no use in risk assessment.

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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 Fluopyram (AE C656948) to Xenopus laevis under static conditions
Report No:	EBGMY002
Document No:	M-395416-01-1
Guideline(s) followed in study:	OPPTS 850.SUPP
Deviations from current test guideline:	Current Guideline: No guideline available. Deviations: Validity criteria consider the methodologies from USEPA: OCSPP Guideline 850.1075, USEPA-FIFRA, 40 CFR, Part 158, Guideline No. 72-1, and OECD Guideline 203. All validity criteria were met.
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 test was performed with the African clawed frog (*Xenopus laevis*) in a static system. Tadpoles were exposed to Fluopyram in groups of 10 (three replicates of 10 tadpoles) to the single nominal concentration of 5.0 mg a.s./L. Mortalities and sub-lethal behavioural effects were observed after 6, 24 and 48 hours.

Concentrations of fluopyram were verified by LC-MS/MS on days 0 and 2. Recoveries on day 0 and day 2 were in the 81-91 % range of nominal concentrations and no residues were found in the control and solvent control samples above the LOQ (0.05 mg a.s./L).

The study fulfils all validity criteria of OECD 203 guideline.

No sub-lethal were observed in the control, solvent control and in the single test concentration of 5.0 mg a.s./L during the test.

The endpoints based on nominal concentrations were: LC_{50} - 48 hours (95 % C.I.) > 5.0 mg a.s./L (not applicable), LOEC-48 hours: > 5.0 mg a.s./L and NOEC-48 hours: > 5.0 mg a.s./L.

I. MATERIAL AND METHODS

Test material	Fluopyram (AE C656948) Specification No.: 102000012455 Batch No.: 08528/0002 Purity: 94.7 % w/w
Guideline(s) adaptation	Not specified
Test species	African clawed frog (*Xenopus laevis,* tadpoles)
Acclimation	At least 4 days to test conditions. Health during acclimation: less than 5 % mortality during holding period.
Organism age/size	Organism length (mean ± S.D.): 15.2 ± 1.11 mm (measured at experimental start)

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Test solutions	Nominal concentration: 5.0 mg a.s./L Mean measured concentration: 4.2 mg a.s./L Control: water Solvent control: Dimethylformamide (0.1 mL DMF/ L) Evidence of undissolved material: Not reported.
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 Total exposure duration: 48 hours
Feeding during test	None
Test conditions	Vessels: 8.4 L glass dishes with 7 L test solution Temperature 21.3 – 21.7 °C Photoperiod: 16 hours light, 8 hours dark; with 30-minute transition period Light quality: cool white fluorescent lamps Light intensity: 665 – 980 lux pH: 8.3 – 8.4 Water hardness: 164 - 178 mg/L Alkalinity: 119 - 127 mg/L Dissolved oxygen: 8.2 - 8.9 mg/L (92 - 98 % of saturation) Conductivity: 450 - 460 µmhos/cm
Parameters Measured / Observations	Mortalities and sub-lethal behavioural effects were observed after 6, 24 and 48 hours. Temperature was recorded daily via manual recordings and additional measurements were done hourly via a calibrated thermometer. The pH and dissolved oxygen were measured daily in each tank with surviving test organisms. Alkalinity and hardness were measured on day 0 and day 2 in tanks with surviving organisms. Light intensity was determined at test start.
Sampling for chemical analysis	Test solution samples were collected for analysis on study day 0 and day 2. The water samples were analysed with Liquid Chromatograph/ Tandem Mass Spectrometry system (LC/MS/MS).
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.

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II. RESULTS AND DISCUSSION

Table 8.2.8- 1: Validity criteria

Validity criteria [A]	Required	Obtained
Mortality rate during domestication period	≤ 5 %	≤ 5 %
Mortality in control during test	≤ 10 %	0 %
Dissolved oxygen saturation	≥ 5.8 mg/L	8.2 - 8.9 mg/L
pH of test solution during the test	Maintained at constant pH value	8.3 – 8.4

[A] No formal English guideline exists for this test protocol. Validity criteria consider the methodologies from USEPA, OCSPP Guideline 850.1075, USEPA-FIFRA, 40 CFR, Part 158, Guideline No. 72-1C and OECD Guideline 203.

Analytical results:

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 SANTE/2020/12830, Rev.1.

Recoveries on day 0 and day 2 were between 83 and 91 % (see table below). Therefore, biological results are based on nominal concentrations of fluopyram.

No residues of fluopyram were found on day 0 and day 2 in the control and solvent control samples above the limit of quantification (0.05 mg a.s./L).

Table 8.2.8- 2: Analytical results

Nominal concentration [mg a.s./L]	Day 0		Day 2		Mean measured concentration [mg a.s./L]	% of nominal
	Measured concentration [mg a.s./L]	% of nominal	Measured concentration [mg a.s./L]	% of nominal		
5.0	4.57 [A]	91	4.13	83	4.2	85

[A] Not given in report. Calculations based on 2 replicate samples.

Biological results:

Observations:

No sub-lethal were observed in the control, solvent control and in the single test concentration of 5.0 mg a.s./L during the test.

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Table 8.2.8- 3: Survival and observations

Nominal concentration [mg a.s./L]	Observations		
	6 h	**24 h**	**48 h**
Control	30 N	30 N	30 N
Solvent Control	30 N	30 N	30 N
5.0	30 N	30 N	30 N

N: Normal

Table 8.2.8- 4: Cumulative number of dead and % mortality

Nominal concentration [mg a.s./L]	4 h		24 h		48 h	
	Cumulative number of dead	**% Mortality**	**Cumulative number of dead**	**% Mortality**	**Cumulative number of dead**	**% Mortality**
Control	0	0	0	0	0	0
Solvent Control	0	0	0	0	0	0
5.0	0	0	0	0	0	0

III. Conclusion

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

LC_{50} - 48 hours (95 % C.I.):	**5.0 mg a.s./L** (not applicable)
LOEC - 48 hours: lowest concentration with an effect	> 5.0 mg a.s./L
NOEC - 48 hours: highest concentration without an effect	≥ 5.0 mg a.s./L

Assessment and conclusion by applicant:

The study and its data are considered as acceptable and reliable for use in risk assessment.

The endpoint is: LC_{50} (48 hours) > 5.0 mg a.s./L

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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 fluopyram or the solo formulation FLU SC 500 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)
- Toxicity to honeybee larvae under laboratory conditions following repeated exposure (OECD 239)
- Honeybee colonies and brood development – Semi-Field (OECD GD 75)

For the fluopyram metabolites fluopyram-benzamide and fluopyram-pyridyl-acetic acid studies on acute oral toxicity to honeybees under laboratory conditions have been conducted. For the fluopyram metabolites fluopyram-pyridyl-carboxylic acid and fluopyram-7-hydroxy studies on acute oral and contact toxicity to honeybees under laboratory conditions have been conducted. In addition, a chronic oral toxicity test (10-day feeding) as per OECD Guideline No. 245 as well as a chronic larvae laboratory study (repeated exposure) as per OECD Guidance Document No. 239 were carried out for fluopyram-benzamide. Additional information on the rationale for testing of the above metabolites is provided below.

The studies are summarised below and a full list of the relevant ecotoxicological endpoints for fluopyram is presented in the following table.

Table 8.3.1- 1: Ecotoxicological endpoints and effect values – bee toxicity studies with fluopyram technical and formulated product to bees

Test substance	Test species/ study type	Endpoint		References
Fluopyram tech.	*Apis mellifera* acute test	LD_{50} oral (48 h) LD_{50} contact (48 h)	> 102.3 µg a.s./bee > 100 µg a.s./bee	███ (2005) M-261594-01-1 KCA 8.3.1.1.1/01 KCA 8.3.1.1.2/01
	Bombus terrestris, acute test	LD_{50} oral (48 h)	> 92.5 µg a.s./bumble bee	███ 2015) M-542447-01-1 KCA 8.3.1.1.1/06
	Bombus terrestris, acute test	LD_{50} contact (48 h)	> 100 µg a.s./bumble bee	███ (2015) M-510849-01-1 KCA 8.3.1.1.2/04
	Bombus terrestris, acute test	LD_{50} oral (48 h) LD_{50} contact (48 h)	> 90.5 µg a.s./bumble bee > 100 µg a.s./bumble bee	███ (2021) M-763123-01-1 KCA 8.3.1.1.1/07 KCA 8.3.1.1.2/05

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Test substance	Test species/ study type	Endpoint	References
	Apis mellifera, larva 22-day repeated feeding test	NOEC ≥ 520 mg a.s./kg diet NOED ≥ 80.1 µg a.s./larva EC_{10} 390 mg a.s./kg diet ED_{10} 60.1 µg a.s./larva EC_{20} 511 mg a.s./kg diet ED_{20} 78.7 µg a.s./larva EC_{50} > 520 mg a.s./kg diet ED_{50} > 80.1 µg a.s./larva	(2018) M-617279-01-1 KCA 8.3.1.3/01
	Apis mellifera, Semi field honey bee feeding study with post-exposure field observation period	Overall, no adverse acute, short-term and long-term effects on mortality, colony strength and colony development, brood development, food storage, honey bee behaviour, queen survival, overall hive vitality and colony health, as well as on overwintering performance after continuous exposure of honey bee colonies under confined conditions to a fluopyram-concentration of 10000 µg a.s./kg diet for a period of 6 consecutive weeks during springtime/early summer.	(2016) M-549350-01-2 KCA 8.3.1.3/03
FLU SC 500	*Apis mellifera*, 10-day oral feeding test	LDD_{50} > 81.4 µg a.s./bee/day NOEDD 81.4 µg a.s./bee/day LC_{50} > 3333 mg a.s./kg diet NOEC 3333 mg a.s./kg diet	(2015) M-540072-01-1 KCA 8.3.1.2/01 KCP 10.3.1.2/01
Fluopyram-benzamide	*Apis mellifera*, acute test	LD_{50} oral (48 h) 22.6 µg p.m./bee	(2016) M-532472-01-2 KCA 8.3.1.1.1/02
	Apis mellifera, 10-day oral feeding test	LDD_{50} 8.58 µg p.m./bee/day NOEDD 1.17 µg p.m./bee/day LC_{50} 199 mg p.m./kg diet NOEC 26.5 mg p.m./kg diet	(2020) M-688788-01-1 KCA 8.3.1.1.1/02
	Apis mellifera, larva 22-day repeated feeding test	NOEC 12.7 mg p.m./kg diet NOED 2.0 µg p.m./larva EC_{10} 13.0 mg p.m./kg diet ED_{10} 2.1 µg p.m./larva EC_{20} 41.1 mg p.m./kg diet ED_{20} 6.5 µg p.m./larva EC_{50} > 79.3 mg p.m./kg diet ED_{50} > 12.6 µg p.m./larva	(2020) M-704606-01-1 KCA 8.3.1.3/02
Fluopyram-pyridyl-acetic acid	*Apis mellifera*, acute test	LD_{50} oral (48 h) > 109.8 µg p.m./bee	(2015) M-532426-01-1 KCA 8.3.1.1.1/03
Fluopyram-pyridyl-carboxylic acid	*Apis mellifera*, acute test	LD_{50} oral (48 h) > 110.9 µg p.m./bee LD_{50} contact (48 h) > 100 µg p.m./bee	(2016) M-566365-01-1 KCA 8.3.1.1.1/04 KCA 8.3.1.1.2/02
Fluopyram-7-hydroxy	*Apis mellifera*, acute test	LD_{50} oral (48 h) > 51.3 µg p.m./bee LD_{50} contact (48 h) > 250 µg p.m./bee	(2020) M-758325-01-1 KCA 8.3.1.1.1/05 KCA 8.3.1.1.2/03

a.s.: active substance
p.m.: pure metabolite

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Further considerations regarding metabolites

The metabolites fluopyram-benzamide, fluopyram-pyridyl-acetic acid, fluopyram-pyridyl-carboxylic acid, and fluopyram-7-hydroxy were tested for their acute toxicity to honey bees. Based on plant metabolism studies (see MCA Section 6, Point 6.2.1), the metabolism, distribution and expression of residues was examined in grapes, potatoes, beans, bell pepper, wheat, and a wheat-swiss chard-turnip rotation (confined rotational crop study). While the assessed plant parts generally did not include bee-relevant matrices (i.e. nectar, pollen, flowers), some extrapolation, for example from fruit or seed to floral tissues, could be made. Table 8.3.1-2 summarizes the information from plant metabolism studies to indicate the %TRR (% total radioactive residues) and actual concentrations (in mg/kg) of fluopyram and its metabolites in fruit and grain that might allow for an extrapolation to bee-relevant matrices, such as floral structures.

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Table 8.3.1-2 Summary of percent total radioactive residues (%TRR) and residue concentrations (in mg/kg) for fluopyram (parent) and its metabolites fluopyram-pyridyl-acetic acid, fluopyram-pyridyl-carboxylic acid, fluopyram-benzamide, and fluopyram-7-hydroxy in various plant matrices which may serve as bridging matrices for bee-relevant plant components (i.e. flowers, nectar, and pollen)

Matrix	Fluopyram (Parent)		Fluopyram-pyridyl-acetic acid		Fluopyram-pyridyl-carboxylic acid		Fluopram-benzamide		Fluopyram-7-hydroxy	
	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
Grapes [B]	97.6	1.82	-	-	-	-	0.7	0.01	0.3	<0.01
Grapes [A]	95.8	1.63	-	-	0.9	0.02	-	-	0.3	<0.01
Green beans [B]	93.9	1.31	-	-	-	-	-	-	-	-
Green beans [A]	99.3	3.86	-	-	-	-	-	-	-	-
Succulent beans [B]	11.4	<0.01	-	-	-	-	51.6	0.036	4	<0.01
Succulent beans [A]	4.8	<0.01	29.5	0.05	31	0.05	-	-	4	<0.01
Red bell pepper fruit [B]	48.9	0.019	-	-	-	-	16.1	0.006	9	0.003
Red bell pepper fruit [A] 4X	32.8	0.049	9.8	0.015	19.5	0.029	-	-	3.7	0.006
Rice kernels [B]	85.0	0.172	-	-	-	-	4.8	0.01	1.1	0.002
Rice kernels [A]	88.3	0.230	-	-	2.8	0.007	-	-	0.9	0.002
Wheat grain 1st rotation [B]	61.9	0.104	-	-	-	-	4.1	0.007	2.7	0.005
Wheat grain 1st rotation [A]	33.4	0.137	-	-	55.9	0.23	-	-	1.8	0.008
Wheat grain 2nd rotation [B]	37.1	0.02	-	-	-	-	3.3	0.002	1.3	0.001
Wheat grain 2nd rotation [A]	20.4	0.015	-	-	16.4	0.012	-	-	0.8	0.001
Wheat grain 3rd rotation [B]	28.4	0.007	-	-	-	-	5.9	0.001	3.4	0.001
Wheat grain 3rd rotation [A]	31	0.012	-	-	28.6	0.011	-	-	2.3	0.001
Maximum values	99.3	**3.86**	29.5	**0.05**	55.9	**0.23**	51.6	**0.036**	9	**0.008**

[A] pyridyl label
[B] phenyl label

- For grapes (phenyl label) please see M-282177-01-1;
- For grapes (pyridyl label) please see M-282460-01-1;
- For green and succulent beans (phenyl label) please see M-283161-02-1;
- For green and succulent beans (pyridyl label) please see M-299067-01-1;
- For red bell pepper fruit (phenyl label) please see M-298790-01-1;
- For red bell pepper fruit (pyridyl label: 4X drip application rate) please see M-298741-01-1;
- For rice kernels (phenyl label) please see M-615284-01-1;
- For rice kernels (pyridyl label) please see M-615282-01-1;
- For wheat grain from a rotational study (phenyl label) please see M-297921-01-1;
- For wheat grain from a rotational study (pyridyl label) please see M-298035-01-1.

The metabolite pattern appeared similar in all primary crops, independent of application technique, as outlined in MCA Section 6, Point 6.2.1, with fluopyram parent appearing as the main component of the detected residues. In addition, significant residues (>10% TRR) of fluopyram-benzamid-pyridyl acetic acid and fluopyram-pyridyl-carboxylic acid were found in some of the fruiting structure matrices. As such, these metabolites were assessed for their potential acute toxicity to honey bees. In particular, oral toxicity tests were considered as any exposure would only occur via ingestion of pollen and nectar following the degradation of fluopyram and distribution of these metabolites to floral tissues or fruiting structures. While fluopyram-7-hydroxy does not appear in significant amounts (<10% TRR) in the below matrices, it is nonetheless considered as a major metabolite in soil. Acute toxicity testing was carried out to proactively address any potential transport into plant tissues and the resultant residues in relevant matrices.

The acute toxicity endpoints for fluopyram-pyridyl-acetic acid, fluopyram-pyridyl-carboxylic acid and fluopyram-7-hydroxy indicated no increased toxicity relative to the active substance fluopyram, with endpoints exceeding 100 µg p.m./bee. The acute oral endpoint of fluopyram-7-hydroxy (LD_{50} > 51.3 µg p.m./bee) represented the only exception, as non-GLP solubility tests indicated low solubility of the metabolite in the sucrose feeding diet of adult honey bees and the highest tested dose of 50 µg p.m./bee only led to actual mortality of 5%. The combination of high toxicity endpoints suggesting metabolites of similar toxicity as parent, and low residues in plant matrices based on highly conservative metabolism studies suggest similar and comparable risk profiles between fluopyram and its metabolites fluopyram-pyridyl-acetic acid, fluopyram-pyridyl-carboxylic acid and fluopyram-7-hydroxy. As fluopyram occurred as the main component of measured residues, the risk assessment for fluopyram is considered to cover the above-mentioned metabolites as well.

In the case of fluopyram-benzamide, the resultant acute oral toxicity endpoint (LD_{50} = 22.6 µg p.m./bee) was significantly lower than the corresponding endpoint of the active substance fluopyram (LD_{50} > 102.3 µg a.s./bee). Consequently, a chronic oral toxicity test (10-day feeding) as per OECD Guideline No. 245 as well as a chronic larvae laboratory study (repeated exposure) as per OECD Guidance Document No. 239 to address potential chronic toxicity to honey bees and effects on honey bee development and other honey bee life stages, respectively, in accordance with the data requirements as set out in Commission Regulation (EU) No. 283/2013 were carried out. The findings of these studies are described below.

Chronic adult toxicity

A 10-day laboratory feeding study investigating the effects of fluopyram-benzamide was conducted to assess chronic toxicity to honey bees in accordance with OECD Guideline No. 245. The test comprised 6 test item treatment groups with nominal doses of 0.128, 0.319, 0.798, 1.99, 4.99, and 12.5 µg p.m./bee/day (actual intake of 0.160, 0.396, 1.17, 2.50, 6.34, and 12.5 µg p.m./bee/day after correction for recovery), corresponding to nominal concentrations of 3.28, 8.19, 20.5, 51.2, 128, and 320 mg p.m./kg diet. The $LDD_{50/20/10}$ were determined as 8.58, 5.21, and 3.74 µg p.m./bee/day, respectively. The NOEDD was identified at 1.17 µg product/bee/day (NOEC of 20.5 mg p.m./kg diet). Daily dosing with 8.58 µg p.m./bee over 10 days (total dose = 85.8 µg p.m./bee) thus did not induce higher mortality compared to a single acute oral exposure at 22.6 µg p.m./bee. Therefore, study results do not indicate delayed or cumulative toxicity effects following chronic exposure to fluopyram-benzamide compared with acute testing. In addition, the NOEC exceeds the highest measured residue level of plant metabolism studies by a factor of 570, or two orders of magnitude. In a semi-field study (M-435338-01-1; KCP 10.3.1.5/01) assessing the effects of two subsequent foliar application of Fluopyram + Trifloxystrobin SC 500 (250+250) onto *Phacelia tanacetifolia* at BBCH 59-61 and 64-65 at a rate of 560 mL test item in 400 L water/ha (corresponding to 140 g fluopyram/ha), maximum measured residues of fluopyram-benzamide in flowers, nectar and pollen ranged between <LOQ (equivalent to 0.01 mg/kg) and 0.017 mg/kg. Thus, the NOEC exceeds the maximum measured field residue in bee-relevant matrices by a factor of 1206, or three orders of magnitude, thus indicating no increased risk to bees as a result of potential exposure to this metabolite.

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Chronic larval toxicity/effects on brood

A honey bee larval toxicity test assessing the effect of fluopyram-benzamide on adult emergence following repeated feeding exposure was conducted to address effects on immature honey bee life stages and their development. The 22-day laboratory dose-response test assessed larval and pupal survival as well as adult emergence, following exposure to nominal concentrations of 79.3, 31.7, 12.7, 5.1, 2.0, and 0.8 mg p.m./kg diet. The matching cumulative doses were 12.6, 5.0, 2.0, 0.80, 0.32, and 0.13 µg p.m./larva. The 22-day NOED (emergence) was determined to be 2.0 µg p.m./larva (corresponding NOEC of 12.7 mg p.m./kg diet), indicating no risk to honey bee development. The NOEC exceeds the highest measured residue level of plant metabolism studies by a factor of 353, or two orders of magnitude. In a semi-field study (M-435338-01-1; KCP 10.3.1.5/01) assessing the effects of two subsequent foliar application of Fluopyram + Trifloxystrobin SC 500 (250+250) onto *Phacelia tanacetifolia* at BBCH 59-61 and 64-65 at a rate of 560 mL test item in 400 L water/ha (corresponding to 140 g fluopyram/ha), maximum measured residues of fluopyram-benzamide in flowers, nectar and pollen ranged between <LOQ (equivalent to 0.01 mg/kg) and 0.017 mg/kg. Thus, the NOEC exceeds the maximum measured field residue in bee-relevant matrices by a factor of 747, or two orders of magnitude, thus indicating no increased risk to bee larvae as a result of potential exposure to this metabolite.

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CA 8.3.1.1 Acute toxicity to bees

CA 8.3.1.1.1 Acute oral toxicity

Honeybees

Active substance fluopyram

Data Point:	KCA 8.3.1.1.1/01
Report Author:	[redacted]
Report Year:	2005
Report Title:	Effects of AE C656948 (acute contact and oral) on honey bees (Apis mellifera L.) in the laboratory
Report No:	24851035
Document No:	M-261594-01-1
Guideline(s) followed in study:	OECD Guideline 213, 21.09.1998 ; OECD Guideline 214, 21.09.1998; U.S. EPA OPPTS Guideline No. 850.3020
Deviations from current test guideline:	Current Guidelines: OECD 213 (1998) and OECD 214 (1998). Deviations from OECD Guideline 213: The use of a concentration of 5 % solvent was essential, to obtain the maximum dose rate of 100 µg/bee. This deviation to the guideline did not affect the outcome of the study. No further deviations to the current OECD Guideline 213 occurred. All validity criteria were met. Deviations from OECD Guideline 214: An application volume of 5 µL was chosen in deviation to the guideline-specified value of 1 µL to ensure reliable dispersion. This deviation is not expected to have impacted the study results. All validity criteria were met.
Previous evaluation:	yes, evaluated and accepted in DAR (2011)
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 fluopyram to the honey bee (*Apis mellifera* L.). Mortality of bees was used as the toxic endpoint. Sublethal effects, such as changes in behaviour, were also assessed.

Under laboratory conditions 50 worker bees were exposed for 48 hours to a single dose of 100.0 µg a.s./bee by topical application (contact limit test) and to a single dose of 100.0 µg a.s./bee by feeding (oral limit test; actual dose based on the intake of the test item was 102.3 µg a.s./bee).

The acute contact and oral test comprised a water control group and a solvent control group. In the oral test bees of all treatment groups were fed with commercial ready-to-use syrup. In both tests a toxic reference item (dimethoate) was included.

In the contact toxicity test the LD_{50} value (48 h) of fluopyram was > 100.0 µg a.s./bee. The oral LD_{50} value (48 h) of fluopyram was > 102.3 µg a.s./bee.

The study fulfils all validity criteria of current Guidelines OECD 213 (1998) and OECD 214 (1998).

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I. MATERIAL AND METHODS

Test item: Fluopyram, specification No.: 10200012455; batch No.: 08528/0002; TOX. No: 07118-00; purity: 95.5 % w/w).

Test species: Honey bee *(Apis mellifera* L.); female worker bees from a healthy and queen-right colony.

Test design: Under laboratory conditions 50 worker bees were exposed for 48 hours to a single dose of 100.0 µg a.s./bee by topical application (contact limit test) and to a single dose of 100.0 µg a.s./bee by feeding (oral limit test; actual dose based on the actual intake of the test item was 102.3 µg a.s./bee).

The controls used for the contact and oral test were tap water (water control group) and pure acetone (solvent control group). In the oral test, commercial ready-to-use syrup (Apiinvert; 30 % Saccharose, 31 % Glucose, 39 % Fructose) was fed to the bees of all treatment groups. As a toxic reference dimethoate (Perfekthion EC 400, 400.0 g/L nominal, 392.1 g/L analytical) was applied at nominal dose levels of 0.30, 0.20, 0.15 and 0.10 µg dimethoate/bee in the contact and oral test.

In the contact and oral toxicity test each treatment group (test item, water control, solvent control and reference item) comprised 5 replicates including 10 bees each.

Application in the contact test: In the contact toxicity test the test item was dissolved in acetone and applied in one 5 µL droplet onto the dorsal thorax of bees using a Burkard - Applicator. For the controls, one 5 µL droplet of tap water with 1 % Adhäsit (100 g/L Marlopon nominal; improves spreading of the test droplet on the water-repellent hairs on the thorax of bees), pure acetone and toxic standard in acetone, were applied. A 5 µL droplet was chosen in deviation to the guideline recommendation of a 1 µL droplet, since a higher volume ensured a more reliable dispersion of the test item. Bees were shortly anaesthetized with CO_2 until they were immobilized immediately before application.

Application in the oral test: The test item and toxic standard dilutions in acetone were mixed with commercial syrup (Apiinvert) in order to receive a relation of 1 part solvent solution plus 19 parts syrup. For the control, the same portion of syrup and acetone/water was used. This diet was offered in syringes which were weighed before and after introduction into the cages. After a maximum of 4 hours the test item treated diet was completely ingested by the bees and afterwards replaced by fresh, untreated sugar syrup *ad libitum*.

Dose levels:

Nominal doses of the test item:	100.0 µg a.s./bee (contact limit test),
	100.0 µg a.s./bee (oral limit test)
Actual dose of the test item (oral test):	102.3 µg a.s./bee (based on the actual food intake)
Nominal doses of the reference item:	0.30, 0.20, 0.15 and 0.10 µg dimethoate/bee (contact test),
	0.30, 0.15, 0.08 and 0.04 µg dimethoate/bee (oral test)

Actual doses of the reference item (oral test): 0.27, 0.15, 0.08 and 0.04 µg dimethoate/bee

Test conditions: Temperature: 25 °C, relative humidity: 54 - 70 %; photoperiod: 24 h darkness (except during observations).

Statistics: Results obtained from the honey bees treated with the test item were compared to those obtained from the control in both the contact and oral tests. The contact and oral median lethal dose (LD_{50}) along with the 95 % confidence limits was calculated by Probit analysis according to the maximum likelihood method (Finney 1971). The calculation of statistical significance and the LD_{50} was performed using the computer program ToxRat Professional, Version 2.09 (ToxRat® Solutions GmbH).

Dates of work: July 19th to July 23rd, 2005

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II. RESULTS AND DISCUSSION

Biological findings:

Contact Test:

In the contact toxicity test 2.0 % mortality was observed at 100.0 µg a.s./bee after 48 hours. No mortality occurred in the solvent control and the water control, respectively. No test item related behavioural effects were observed at any time.

Table 8.3.1.1.1- 1: Mortality and behavioural abnormalities of the bees in the contact toxicity test

Treatment group	After 4 h		After 24 h		After 48 h	
	Mortality	Behav. abnorm.	Mortality	Behav. abnorm.	Mortality	Behav. abnorm.
	Mean [%]		Mean [%]		Mean [%]	
Water control	0.0	0.0	0.0	0.0	0.0	0.0
Solvent control	0.0	0.0	0.0	0.0	0.0	0.0
Test item [µg a.s./bee]						
100.0	0.0	2.0	2.0	0.0	2.0	0.0
Reference item [µg a.s./bee]						
0.10	0.0	0.0	10.0	6.0	16.0	6.0
0.15	0.0	2.0	50.0	28.0	74.0	4.0
0.20	0.0	16.0	82.0	12.0	100	0.0
0.30	0.0	44.0	94.0	6.0	100	0.0

Results are averages from 5 replicates (ten bees each) for the test item, control groups and the reference item groups
Test item = fluopyram, reference item = dimethoate, water control = tap water with 1 % Adhasit (100 g/L Marlopon nominal), solvent control = pure acetone
Behav. abnorm. = behavioural abnormalities

Oral Test

In the oral toxicity test the maximum nominal test concentration of fluopyram (100 µg/bee) corresponded to an actual intake of 102.3 µg a.s./bee. No mortality occurred at this test concentration after 48 hours. In the solvent and water control no mortality occurred. No test item related behavioural effects were observed at any time.

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Table 8.3.1.1.1- 2: Mortality and behavioural abnormalities of the bees in the oral toxicity test

Treatment group	After 4 h		After 24 h		After 48 h	
	Mortality	Behav. abnorm.	Mortality	Behav. abnorm.	Mortality	Behav. abnorm.
	Mean [%]		Mean [%]		Mean [%]	
Water control	0.0	0.0	0.0	0.0	0.0	0.0
Solvent control	0.0	0.0	0.0	0.0	0.0	0.0
Test item [µg a.s./bee]						
102.3	0.0	0.0	0.0	0.0	0.0	0.0
Reference item [µg a.s./bee]						
0.04	0.0	0.0	0.0	0.0	0.0	0.0
0.08	0.0	2.0	2.0	0.0	6.0	0.0
0.15	0.0	0.0	30.0	0	42.0	0.0
0.27	0.0	18.0	88.0	6.0	94.0	0.0

Results are mean values of 5 replicates (ten bees each) for all treatment groups
Test item = fluopyram; reference item = dimethoate, water control = tap water with sugar solution, solvent control = acetone with sugar solution
Behav. abnorm. = behavioural abnormalities

The endpoints for the contact and oral toxicity test are shown in the table below.

Table 8.3.1.1.1- 3: Contact and oral toxicity of fluopyram to honey bees

Test item	Fluopyram	
Test species	Honey bee *Apis mellifera* L.	
Exposure	**Contact**	**Oral**
Test duration	48 h	48 h
Dose rate [µg a.s./bee]	100.0	Nominal dose: 100.0 Actual dose: 102.3
LD_{50} [µg a.s./bee]	> 100.0	> 102.3

<u>Reference item</u>

The contact and oral LD_{50} (24 h) values of the reference item (dimethoate) were calculated to be 0.15 µg a.s./bee and 0.18 µg a.s./bee, respectively. These values corresponded to the expected range cited in the OECD Guideline 213 (1998) and 214 (1998) and thus demonstrated the sensitivity of the test item.

<u>Validity criteria:</u>

The contact and oral toxicity tests were considered valid as the control mortality in each case was ≤ 10 % and the LD_{50} values obtained with the reference item (dimethoate) were within the required ranges.

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Table 8.3.1.1.1- 4: Validity criteria

Validity criteria	Recommended		Obtained
Control mortality	Contact Test		
	Water control	≤ 10 %	0.0 %
	Solvent control	≤ 10 %	0.0 %
	Oral Test		
	Control	≤ 10 %	0.0 %
	Solvent control	≤ 10 %	0.0 %
LD_{50} of reference item (24 h)	Contact Test		
	Dimethoate	0.10 - 0.30 µg a.s./bee	0.15 µg a.s./bee
	Oral Test		
	Dimethoate	0.10 - 0.35 µg a.s./bee	0.18 µg a.s./bee

III. CONCLUSION

The toxicity of fluopyram was tested in an acute contact and oral toxicity test on honey bees. The LD_{50} (48 h) was determined to be > 100.0 µg a.s./bee in the contact toxicity test. The LD_{50} (48 h) was determined to be > 102.3 µg a.s./bee in the oral toxicity test.

Assessment and conclusion by applicant:

The study and its data are considered as acceptable and reliable for use in risk assessment.

The endpoints are:

LD_{50} contact (48 hours) > 100.0 µg a.s./bee

LD_{50} oral (48 hours) > 102.3 µg a.s./bee

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Metabolite fluopyram-benzamide

Data Point:	KCA 8.3.1.1.1/02
Report Author:	
Report Year:	2016
Report Title:	Fluopyram-benzamide: Effects (acute oral) on honey bees (Apis mellifera L.) in the laboratory
Report No:	103081035
Document No:	M-532472-01-2
Guideline(s) followed in study:	OECD 213 (1998) U.S. EPA OCSPP 850.SUPP
Deviations from current test guideline:	Current Guidelines OECD 213 (1998) Deviations: The use of a concentration of 5 % solvent was essential, to obtain the maximum dose rate of 100 µg/bee. This deviation to the guideline did not affect the outcome of the study. No further deviations to the current OECD Guideline 213 occurred. All validity criteria were met.
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 the metabolite Fluopyram-benzamide to the honey bee (*Apis mellifera* L.). Mortality of bees was used as the toxic endpoint. Sublethal effects, such as changes in behaviour, were also assessed.

Under laboratory conditions 30 worker bees were exposed for 48 hours to doses of 100.0, 50.0, 25.0, 12.5 and 6.25 µg p.m./bee by feeding (oral dose response test; actual doses based on the intake of the test item were 41.8, 29.9, 23.6, 13.5 and 6.6 µg p.m./bee).

The oral test comprised a water control group and a solvent control group, respectively. A toxic reference item (dimethoate) was included.

In the oral toxicity test the LD_{50} value (48 h) was identified as 22.6 µg p.m./bee. The oral NOED value (48 h) was estimated to be 13.5 µg p.m./bee.

The study fulfils all validity criteria of the current OECD Guidelines 213 (1998).

I. MATERIAL AND METHODS

Test item: Fluopyram-benzamide (Synonym: BCS-AA10014); Origin Batch No.: B26F; Batch Code: AE F148815-PU-02; LIMS No.: 1443626; purity: 99.4 % w/w

Test species: Honey bee (*Apis mellifera* L.); female worker bees from disease-free and queen-right honey bee colonies.

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Test design: Under laboratory conditions 30 worker bees (*Apis mellifera* L.) were exposed for 48 hours to doses of 100.0, 50.0, 25.0, 12.5 and 6.25 µg p.m./bee by feeding (oral dose response test; actual dose based on the actual intake of the test item was 41.8, 29.9, 23.6, 13.5 and 6.6 µg p.m./bee).

For the oral test 50 % w/v sucrose solution (water control group) and 50 % w/v sucrose solution containing 5 % acetone (solvent control group) were used as controls. As a toxic reference dimethoate (BAS 152 11 I EC 400, 400.0 g/L nominal, 420.3 g/L analytical) was applied at nominal dose levels of 0.30, 0.15, 0.08 and 0.05 µg dimethoate/bee.

In the oral toxicity test each treatment group (test item, controls, reference item) comprised 3 replicates including 10 bees each.

Application in the oral test: The test item was diluted in acetone and then applied in 50 % w/v sucrose solution. For the controls 50 % w/v sucrose solution (control) and 50 % w/v sucrose solution containing 5 % acetone (solvent control) were offered to the bees. The treated food was offered in syringes, which were weighed before and after introduction into the cages (duration of uptake was 6 hours). After a maximum of 6 hours, the uptake was complete and the syringes were removed, weighed and replaced by ones containing fresh, untreated food.

Dose levels:

Nominal doses of the test item: 100.0, 50.0, 25.0, 12.5 and 6.25 µg p.m./bee

Actual dose of the test item: 41.8, 29.9, 23.6, 13.5 and 6.6 µg p.m./bee (based on the actual food intake)
Nominal doses of the reference item: 0.30, 0.15, 0.08 and 0.05 µg dimethoate/bee

Actual doses of the reference item: 0.32, 0.16, 0.08 and 0.06 µg dimethoate/bee

Test conditions: Temperature: 25 °C; relative humidity: 50 – 68 %; photoperiod: 24 h darkness (except during observations).

Statistics: Results obtained from the bees treated with the test item and the reference item were compared to those obtained from the control in the oral test. The oral $LD_{10,\ 20,\ 50}$ values of the test item were estimated with Probit Analysis (according to Finney 1971). The oral LD_{50} value of the reference item was estimated according to moving average computations (Thompson and Weil, 1952).

It was not necessary to correct the test item and the reference item mortality, since no control mortality occurred in the oral toxicity test. The NOED was estimated using Fisher's Exact Test (pairwise comparison, one-sided greater, $\alpha = 0.05$), which is a distribution-free test and does not require testing for normality or homogeneity prior to analysis. The software used to perform the statistical analysis was ToxRat Professional, Version 2.10.05, (® ToxRat Solutions GmbH).

Dates of work: June 23rd to June 25th, 2015

II. Results and discussion

Biological results:

In the oral toxicity test, the maximum nominal dose levels of the test item (6.25, 12.5, 25, 50 and 100.0 µg p.m./bee) could not be achieved, because the bees did not ingest the full volume of treated 50 % w/v sucrose solution even when offered over a period of 6 hours. Actual oral doses of 41.8, 29.9, 23.6 and 13.5 µg p.m./bee resulted in mortalities ranging from 100 % to 10.0 % at the end of the test (after 48 hours). No mortality occurred in the 6.6 µg p.m./bee treatment as well as in the water and solvent control groups, respectively.

During the 4 hours assessment nearly all bees of the 41.8 and 29.9 µg p.m./bee treatments were apathetic, affected or moribund. Two bees of the 23.6 µg p.m./bee treatment were affected. During the 24 hours assessment one bee was affected in the 41.8 µg p.m./bee treatment. No further test item related behavioural abnormalities were observed during the test.

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The endpoints for the oral toxicity test are shown in the table below.

Table 8.3.1.1.1- 5: Mortality and behavioural abnormalities of the bees in the oral toxicity test

Treatment group	After 4 h		After 24 h		After 48 h	
	Mortality	Behav. abnorm.	Mortality	Behav. abnorm.	Mortality	Behav. abnorm.
	Mean [%]		Mean [%]		Mean [%]	
Water control	0.0	0.0	0.0	0.0	0.0	0.0
Solvent control	0.0	0.0	0.0	0.0	0.0	0.0
Test item [µg p.m./bee]						
6.6	0.0	0.0	0.0	0.0	0.0	0.0
13.5	0.0	0.0	0.0	0.0	10.0	0.0
23.6	0.0	6.7	33.3	0.0	33.3	0.0
29.9	0.0	93.3	83.3	0.0	93.3	0.0
41.8	3.3	96.7	96.7	3.3	100	0.0
Reference item [µg a.s./bee]						
0.06	0.0	0.0	0.0	0.0	0.0	0.0
0.08	0.0	0.0	13.3	0.0	26.7	0.0
0.16	6.7	0.0	90.0	3.3	100	0.0
0.32	16.7	50.0	100	0.0	100	0.0

Results are mean values of 3 replicates (ten bees each) for all treatment groups
Behav. abnorm. = behavioural abnormalities
Test item: fluopyram- benzamide; reference item: dimethoate; control = 50 % w/v sucrose solution; solvent control = 50 % w/v sucrose solution containing 5 % acetone

The endpoints for the oral toxicity test are shown in the table below.

Table 8.3.1.1.1- 6: Oral toxicity of Fluopyram-benzamide to honey bees

Test item	Fluopyram-benzamide
Test species	Honey bee *Apis mellifera* L.
Exposure	**Oral (50 % w/v sucrose solution + 5 % acetone)**
Test duration	48 h
Dose rate [µg p m./bee]	Nominal dose: 100.0, 50.0, 25.0, 12.5 and 6.25 Actual dose: 41.8, 29.9, 23.6, 13.5 and 6.6
LD_{50} [µg p m./bee] (95 % C.I.)	22.6 (0.96 - 35.8)
LD_{20} [µg p m./bee]	17.6
LD_{10} [µg p m./bee]	15.4
NOED [µg p m./bee] [A]	13.5

C.I.: Confidence interval
[A] The NOED was estimated using Fisher's Exact Test (pairwise comparison, one-sided greater, α = 0.05).

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Reference item

The oral LD_{50} (24 h) value of the reference item (dimethoate) was calculated to be 0.14 µg a.s./bee, respectively. This value corresponded to the expected range cited in the OECD Guideline 213 (1998) and thus demonstrated the sensitivity of the test item.

Validity criteria:

The oral toxicity test is considered valid as the control mortality was ≤ 10 % and the LD_{50} value obtained with the reference item (dimethoate) was within the required range.

Table 8.3.1.1.1- 7: Validity criteria

Validity criteria	Recommended		Obtained
Control mortality	Control	≤ 10 %	0 %
	Solvent control	≤ 10 %	0 %
LD_{50} of reference item (24 h)	Dimethoate	0.10 – 0.35 µg a.s./bee	0.14 µg a.s./bee

III. Conclusion

The toxicity of Fluopyram-benzamide was tested in an acute oral toxicity test on honey bees. The oral LD_{50} values (24- and 48-hours) were 25.5 and 22.6 µg p.m./bee, respectively.

Assessment and conclusion by applicant:

The study and its data are considered as acceptable and reliable for use in risk assessment.

The endpoint is: LD_{50} oral (48 hours) = 22.6 µg p.m./bee

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Metabolite Fluopyram-pyridyl-acetic acid

Data Point:	KCA 8.3.1.1.1/03
Report Author:	[redacted]
Report Year:	2015
Report Title:	Fluopyram-pyridyl-acetic acid (BCS-AA10189): Effects (acute oral) on honey bees (Apis mellifera L.) in the laboratory
Report No:	103091035
Document No:	M-532426-01-1
Guideline(s) followed in study:	OECD 213 (1998) Data Requirement: US EPA Guideline No. 850.SUPP
Deviations from current test guideline:	Current Guideline: OECD 213 (1998) Deviations: No deviations to the current OECD Guideline 213 occurred. All validity criteria were met.
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 the metabolite Fluopyram-pyridyl-acetic acid to the honey bee (*Apis mellifera* L.). Mortality of bees was used as the toxic endpoint. Sublethal effects, such as changes in behaviour, were also assessed.

Under laboratory conditions 50 worker bees were exposed for 48 hours to a single dose of 100.0 µg p.m./bee by feeding (actual dose based on the actual intake of the test item was 109.8 µg p.m./bee).

The oral test comprised a control and a toxic reference item (dimethoate).

Since no mortality occurred in the 109.8 µg p.m./bee group at test end, the oral LD_{50} can be considered as > 109.8 µg p.m./bee.

The study fulfils all validity criteria of the current OECD Guidelines 213 (1998).

I. Material and Methods

Test item: Fluopyram-pyridyl-acetic acid (Synonym: BCS-AA10189); Batch ID: BCS-AA10189-01-02; Sample Description: TOX 10869-00; LIMS No.: 1520423; purity: 97.6 % w/w.

Test species: Honey bee (*Apis mellifera* L.); female worker bees from disease-free and queen-right honey bee colonies.

Test design: Under laboratory conditions 50 worker bees (*Apis mellifera* L.) were exposed for 48 hours to a single dose of 100.0 p.m./bee by feeding (actual dose based on the actual intake of the test item was 109.8 p.m./bee).

For the oral test 50 % w/v sucrose solution (water control group) was used as control. As a toxic reference dimethoate (BAS 152 11 I EC 400, 400.0 g/L nominal, 420.3 g/L analytical) was applied at nominal dose levels of 0.05, 0.08, 0.15 and 0.30 µg dimethoate.

In oral toxicity test each treatment group (test item, control, reference item) comprised 5 replicates including 10 bees each.

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Application in the oral test: The test item and reference item were applied in 50 % w/v sucrose solution, which was used as carrier (food) in the oral test. For the control pure 50 % w/v sucrose solution was offered to the bees. The treated food was offered in syringes, which were weighed before and after introduction into the cages (duration of uptake was 2 hours 10 minutes for the test item treatments). After a maximum of 2 hours 10 minutes, the uptake was complete and the syringes containing the treated food were removed, weighed and replaced by ones containing fresh, untreated food.

Dose levels:

Nominal doses of the test item:	100.0 µg p.m./bee
Actual dose of the test item:	109.8 µg p.m./bee (based on the actual food intake)
Nominal doses of the reference item:	0.30, 0.15, 0.08 and 0.05 µg dimethoate/bee
Actual doses of the reference item:	0.31, 0.16, 0.08 and 0.06 µg dimethoate/bee

Test conditions: Temperature: 25 °C; relative humidity: 53 - 81 %; photoperiod: 24 h darkness (except during observations).

Statistics: Results obtained from the bees treated with the test item and the reference item were compared to those obtained from the control in the oral test. The oral LD_{50} value of the reference item was estimated using the binomial distribution (according to STEPHAN, 1977). The LD_{50} calculation was carried out taking into account the mortality data corrected by control mortality using Abbott's formula (1925). The NOED was estimated using Fisher Exact Test (pairwise comparison, one-sided greater, α = 0.05), which is a distribution-free test and does not require testing for normality or homogeneity prior to analysis. The software used to perform the statistical analysis was ToxRat Professional, Version 2.10.05, (® ToxRat Solutions GmbH).

Dates of work: July 27th to July 29th, 2015

II. RESULTS AND DISCUSSION

Biological results:

In the oral toxicity test the maximum nominal test level of Fluopyram-pyridyl-acetic acid (100.0 µg p.m./bee) corresponded to an actual intake of 109.8 µg p.m./bee. This dose level did not cause mortality after 48 hours. In the control group (50 % w/v sucrose solution = 500 g sucrose/L tap water), 2.0 % mortality occurred.

No test item induced behavioural effects were observed at any time in the oral toxicity test.

Since no mortality occurred in the 109.8 µg p.m./bee group at test end, the oral LD_{50} can be considered as > 109.8 µg p.m./bee.

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Table 8.3.1.1.1- 8: Mortality and behavioural abnormalities of the bees in the oral toxicity test

Treatment group	After 4 h		After 24 h		After 48 h	
	Mortality	Behav. abnorm.	Mortality	Behav. abnorm.	Mortality	Behav. abnorm.
	Mean [%]	Mean [%]	Mean [%]	Mean [%]	Mean [%]	Mean [%]
Control	0.0	0.0	0.0	0.0	2.0	0.0
Test item [µg p.m./bee]						
109.8	0.0	0.0	0.0	0.0	0.0	0.0
Reference item [µg a.s./bee]						
0.06	0.0	0.0	0.0	0.0	0.0	0.0
0.08	0.0	0.0	2.0	0.0	14.0	0.0
0.16	0.0	6.0	76.0	8.0	90.0	0.0
0.31	26.0	32.0	100	0.0	100	0.0

Results are mean values of 5 replicates (ten bees each) for all treatment groups
Behav. abnorm. = behavioural abnormalities
Test item= fluopyram-pyridyl-acetic acid , reference item= dimethoate; control = 50 % w/v sucrose solution

The endpoints for the oral toxicity test are shown in the table below.

Table 8.3.1.1.1- 9: Oral toxicity of Fluopyram-pyridyl-acetic acid to honey bees

Test item	Fluopyram-pyridyl-acetic acid
Test species	Honey bee *Apis mellifera* L.
Exposure	**Oral (50 % w/v sucrose solution)**
Test duration	48 h
Dose rate [µg p.m./bee]	Nominal dose: 100.0 Actual dose: 109.8
LD_{50} [µg p m./bee]	> 109.8
LD_{20} [µg p m./bee]	> 109.8
LD_{10} [µg p m./bee]	> 109.8
NOED [µg p m./bee] [A]	≥ 109.8

[A] The NOED was estimated using Fisher's Exact Test (pairwise comparison, one-sided greater, $\alpha = 0.05$).

Reference item

The oral LD_{50} (24 h) value of the reference item (dimethoate) was calculated to be 0.13 µg a.s./bee, respectively. This value corresponded to the expected range cited in the OECD Guideline 213 (1998) and thus demonstrated the sensitivity of the test item.

Validity criteria:

The oral toxicity test is considered valid as the control mortality was ≤ 10 % and the LD_{50} value obtained with the reference item (dimethoate) was within the required range.

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Table 8.3.1.1.1- 10: Validity criteria

Validity criteria	Recommended		Obtained
Control mortality	Control	≤ 10 %	2.0 %
LD_{50} of reference item (24 h)	Dimethoate	0.10 - 0.35 µg a.s./bee	0.13 µg a.s./bee

III. CONCLUSION

The toxicity of Fluopyram-pyridyl-acetic acid was tested in an acute oral toxicity test on honey bees. The oral LD_{50} (48 h) was determined to be > 109.8 µg p.m./bee.

Assessment and conclusion by applicant:

The study and its data are considered as acceptable and reliable for use in risk assessment.

The endpoint is: LD_{50} oral (48 hours) > 109.8 µg p.m./bee

Metabolite fluopyram-pyridyl-carboxylic acid

Data Point:	KCA 8.3.1.1.1/04
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) Deviations from OECD Guideline 213: No deviations to the current OECD Guideline 213 occurred. All validity criteria were met. Deviations from OECD Guideline 214: An application volume of 5 µL was chosen in deviation to the guideline-specified value of 1 µL to ensure reliable dispersion. This deviation is not expected to have impacted the study results. All validity criteria were met.
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 fluopyram-pyridyl-carboxylic acid to the honey bee (*Apis mellifera* L.). Mortality of bees was used as the toxic endpoint. Sublethal effects, such as changes in behaviour, were also assessed.

Under laboratory conditions 50 worker bees were exposed for 48 hours to a single dose of 100.0 µg

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p.m./bee by topical application (contact limit test) and 100.0 µg p.m./bee by feeding (oral limit test; actual dose based on the actual intake of the test item was 110.9 µg p.m./bee).

The contact test comprised a water control group and a solvent control group. In the oral test bees in the control group were exposed to 50 % w/v aqueous sucrose solution and to 50 % w/v aqueous sucrose solution with 5 % acetone. In both tests a toxic reference item (dimethoate) was included.

The contact LD_{50} value (48 h) was determined to be > 100.0 µg p.m./bee. The oral LD_{50} value (48 h) was determined to be > 110.9 µg p.m./bee.

The study fulfils all validity criteria of the current Guidelines OECD 213 (1998) and OECD 214 (1998).

I. MATERIAL AND METHODS

Test item: fluopyram-pyridyl-carboxylic acid, pure metabolite Batch-Code: AE C657188-PU-01; Origin Batch No.: SES 10250-1-1; purity: 98.5 % w/w.

Test species: Honey bee (*Apis mellifera* L.); female worker bees from a healthy and queen-right colony.

Test design: Under laboratory conditions 50 worker bees were exposed for 48 hours to a single dose of 100.0 µg p.m./bee by topical application (contact limit test) and 100.0 µg p.m./bee by feeding (oral limit test; actual dose based on the actual intake of the test item was 110.9 µg p.m./bee).

The controls used for the contact test were tap water with 0.5 % Adhäsit (improves spreading of the test droplet on the water-repellent hairs on the thorax of bees) (water control group) and pure acetone (solvent control). In the oral test bees in the control group were exposed to 50 % w/v aqueous sucrose solution (control) and 50 % w/v sucrose solution containing 5 % acetone (solvent control). As a toxic reference dimethoate (Perfekthion EC 400, 400.0 g/L nominal, 420.3 g/L analytical) was applied at nominal dose levels of 0.10, 0.15, 0.20 and 0.30 µg dimethoate/bee in the contact and at nominal dose levels of 0.05, 0.08, 0.15 and 0.30 µg dimethoate/bee in the oral test.

In the contact and oral toxicity test each treatment group (test item, controls and reference item) comprised 5 replicates including 10 bees each.

Application in the contact test: The test item was applied as one 5 µL droplet of test item dissolved in acetone, placed on the dorsal bee thorax using a calibrated pipette. The reference item was applied as one 5 µL droplet of dimethoate dissolved in tap water containing 0.5 % Adhäsit. For the two controls, one 5 µL droplet of tap water containing 0.5 % Adhäsit and pure acetone were applied. A 5 µL droplet was chosen in deviation to the guideline recommendation of a 1 µL droplet, since a higher volume ensured a more reliable dispersion of the test item.

Application in the oral test: The test item was diluted in acetone and then applied in 50 % w/v sucrose solution. For the controls 50 % w/v sucrose solution (control) and for the solvent control 50 % w/v sucrose solution containing 5 % acetone (solvent control) was offered to the bees and. The reference item was diluted in tap water and applied in 50 % w/v sucrose solution.

The treated food was offered in syringes, which were weighed before and after introduction into the cages (duration of uptake was 1 hour 35 minutes for the test item treatments). After a maximum of 1 hour 35 minutes, the uptake was complete and the syringes containing the treated food were removed, weighed and replaced by ones containing fresh, untreated food.

Dose levels:

Nominal doses of the test item:	100.0 µg p.m./bee (contact limit test)
	100.0 µg p.m./bee (oral limit test)
Actual dose of the test item (oral test):	110.9 µg p.m./bee
Nominal doses of the reference item:	0.30, 0.20, 0.15 and 0.10 µg dimethoate/bee (contact test)

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0.30, 0.15, 0.08 and 0.05 µg dimethoate/bee (oral test);

Actual doses of the reference item (oral test): 0.33, 0.17, 0.08 and 0.06 µg dimethoate/bee

Test conditions: Temperature: 25 °C; mean: 27 °C; relative humidity: 56 - 64 %; photoperiod: 24 h darkness (except during observations).

Statistics: Results obtained with the bees treated with the test item and the reference item were compared to those obtained with the control in both the contact and oral tests. The contact and oral LD_{50} values of the reference item were estimated using the binomial distribution (according to STEPHAN, 1977). The LD_{50} calculation was carried out taking into account the mortality data corrected by control mortality using Abbott's formula (1925). The NOED was estimated using Fisher Exact Test (pairwise comparison, one-sided greater, $\alpha = 0.05$), which is a distribution-free test and does not require testing for normality or homogeneity prior to analysis. The software used to perform the statistical analysis was ToxRat Professional, Version 3.2.1, (® ToxRat Solutions GmbH).

Dates of experimental work: July 05th to July 08th, 2016

II. RESULTS AND DISCUSSION

Biological findings:

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. No mortality occurred in the water control group (water with 0.5 % Adhäsit) and 2.0 % in the solvent control group (acetone), respectively.

No behavioural abnormalities were detected within 48 hours after application.

Table 8.3.1.1.1-11: Mortality and behavioural abnormalities of the bees in the contact toxicity test

Treatment group	After 4 h		After 24 h		After 48 h	
	Mortality	Behav. abnorm.	Mortality	Behav. abnorm.	Mortality	Behav. abnorm.
	Mean [%]	Mean [%]	Mean [%]	Mean [%]	Mean [%]	Mean [%]
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
Test item [µg p.m./bee]						
100.0	0.0	0.0	0.0	0.0	6.0	0.0
Reference item [µg a.s./bee]						
0.10	0.0	0.0	0.0	0.0	8.0	0.0
0.15	0.0	16.0	0.0	0.0	22.0	0.0
0.20	0.0	66.0	44.0	0.0	50.0	0.0
0.30	0.0	96.0	76.0	0.0	78.0	0.0

Results are mean values of 5 replicates (ten bees each) for all treatment groups

Behav. abnorm. = behavioural abnormalities
Test item= fluopyram-pyridyl-carboxylic acid, reference item = dimethoate; water control = tap water; solvent control = pure acetone

Oral test

In the oral toxicity test, the maximum nominal test level of fluopyram-pyridyl-carboxylic acid (*i.e.* 100 µg p.m./bee) corresponded to an actual intake of 110.9 µg p.m./bee. This dose level caused no mortality after 48 hours. At the end of the oral toxicity test (after 48 hours) 4.0 % mortality occurred in

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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.

No test item induced behavioural effects were observed at any time in the oral toxicity test.

Table 8.3.1.1.1- 12: Mortality and behavioural abnormalities of the bees in the oral toxicity test

Treatment group	After 4 h		After 24 h		After 48 h	
	Mortality	Behav. abnorm.	Mortality	Behav. abnorm.	Mortality	Behav. abnorm.
	Mean [%]	Mean [%]	Mean [%]	Mean [%]	Mean [%]	Mean [%]
Control	0.0	0.0	0.0	0.0	4.0	0.0
Solvent control	0.0	0.0	0.0	0.0	2.0	0.0
Test item [µg p.m./bee]						
110.9	0.0	0.0	0.0	0.0	0.0	0.0
Reference item [µg a.s./bee]						
0.06	0.0	0.0	0.0	0.0	0.0	0.0
0.08	0.0	4.0	0.0	4.0	0.0	0.0
0.17	0.0	70.0	68.0	8.0	74.0	0.0
0.33	36.0	56.0	98.0	2.0	98.0	0.0

Results are mean values of 5 replicates (control, test item and reference item) containing 10 bees each
Behav. abnorm. = behavioural abnormalities;
Test item= fluopyram-pyridyl-carboxylic acid; reference item= dimethoate; control = 50 % w/v sucrose solution; solvent control = 50 % w/v sucrose solution with 5 % acetone

The endpoints for the contact and oral toxicity test are shown in the table below.

Table 8.3.1.1.1- 13: Contact and oral toxicity of fluopyram-pyridyl-carboxylic acid to honey bees

Test item	Fluopyram-pyridyl-carboxylic acid	
Test species	Honey bee *Apis mellifera* L.	
Exposure	**Contact (dissolved in acetone)**	**Oral (50 % w/v sucrose solution and acetone)**
Test duration	48 h	48 h
Dose rate [µg p m./bee]	100.0	Nominal dose: 100.0 Actual dose: 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] [A]	≥ 100.0	≥ 110.9

[A] The NOED was estimated using Fisher's Exact Test (pairwise comparison, one-sided greater, α = 0.05).

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Reference item

The contact and oral LD_{50} (24 h) values of the reference item (dimethoate) were calculated to be 0.22 and 0.14 µg a.s./bee, respectively. These values corresponded to the expected range cited in the OECD Guideline 213 (1998) and 214 (1998) and thus demonstrated the sensitivity of the test item.

Validity criteria:

The contact and oral tests were considered valid as the control mortality in each case was ≤ 10 % and the LD_{50} values obtained with the reference item (dimethoate) were within the required ranges.

Table 8.3.1.1.1- 14: Validity criteria

Validity Criteria	Recommended		Obtained
Control mortality	Contact Test		
	Water control	≤ 10 %	0 %
	Solvent control	≤ 10 %	2 %
	Oral Test		
	Control	≤ 10 %	4 %
	Solvent control	≤ 10 %	2 %
LD_{50} of reference item (24 h)	Contact Test		
	Dimethoate	0.10 - 0.30 µg a.s./bee	0.22 µg a.s./bee
	Oral Test		
	Dimethoate	0.10 - 0.35 µg a.s./bee	0.14 µg a.s./bee

III. CONCLUSION

The toxicity of fluopyram-pyridyl-carboxylic acid was tested in both, an acute contact and oral toxicity test on honey bees.

The contact LD_{50} value (48 h) was determined to be > 100.0 µg p.m./bee. The oral LD_{50} value was determined to be > 110.9 µg p.m./bee.

Assessment and conclusion by applicant:

The study and its data are considered as acceptable and reliable for use in risk assessment.

The endpoints are:

LD_{50} oral (48 hours) > 110.9 µg p.m./bee

LD_{50} contact (48 hours) > 100.0 µg p.m./bee

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Metabolite fluopyram-7-hydroxy

Data Point:	KCA 8.3.1.1.1/05
Report Author:	Kling, A.
Report Year:	2020
Report Title:	BCS-AA10065: Acute oral and contact toxicity to the honey bee (*Apis mellifera* L.) under laboratory conditions
Report No:	S20-02018
Document No:	M-758325-01-1
Guideline(s) followed in study:	Regulation (EC) No. 1107/2009 (Oct. 2009) Directive 2003-01 (Canada/PMRA) US EPA OCSPP 850.3020/850.supp. OECD Guideline No. 213 (1998) OECD Guideline No. 214 (1998)
Deviations from current test guideline:	Current Guidelines: OECD 213 (1998) and OECD 214 (1998) Deviations from OECD Guideline 213: A 10 % solvent concentration was necessary to derive a maximum nominal dose rate of 50 µg/bee. This deviation to the guideline did not affect the outcome of the study. All validity criteria were met. Deviations from OECD Guideline 214: An application volume of 2 µL was chosen in deviation to the guideline-specified value of 1 µL to ensure reliable dispersion. This deviation is not expected to have impacted the study results. All validity criteria were met.
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 fluopyram-7-hydroxy to the honey bee (*Apis mellifera* L.). Mortality of bees was used as the toxic endpoint. Sublethal effects, such as changes in behaviour, were also assessed.

Under laboratory conditions 40 worker bees per treatment group were exposed for 48 hours to doses of 250, 125, 62.5, 31.3 and 15.6 µg p.m./bee by topical application (contact dose response test) and 50, 25, 12.5, 6.25 and 3.13 µg p.m./bee by feeding (oral dose response test; actual doses based on the intake of the test item were 51.3, 25.8, 12.8, 6.38 and 3.25 µg p.m./bee).

The contact test comprised a water control group and a solvent control group. In the oral test bees in the control group were exposed to 50 % w/v aqueous sucrose solution and to 50 % w/v aqueous sucrose solution with 10 % acetone. In deviation to the guideline, a solvent content of 10 % was used in order to facilitate a maximum solubility and a homogenous distribution of the test item in the oral application solution. In both tests a toxic reference item (dimethoate) was included.

The contact LD_{50} value (48 h) was determined to be > 250 µg p.m./bee. The oral LD_{50} value (48 h) was determined to be > 51.3 µg p.m./bee.

The study fulfils all validity criteria of the current Guidelines OECD 213 (1998) and OECD 214 (1998).

I. Material and Methods

Test item: Fluopyram-7-hydroxy (Synonym: BCS-AA10065); Origin Batch No.: SES12367-10-8; purity: 99.4 % w/w

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Test species: Honey bee *(Apis mellifera* L.); female worker bees from disease-free and queen-right honey bee colonies.

Test design: Under laboratory conditions 40 worker bees (*Apis mellifera* L.) were exposed for 48 hours to doses of 250, 125, 62.5, 31.3 and 15.6 µg p.m./bee by topical application (contact dose response test) and to doses of 50, 25, 12.5, 6.25 and 3.13 µg p.m./bee by feeding (oral dose response test; actual doses based on the intake of the test item were 51.3, 25.8, 12.8, 6.38 and 3.25 µg p.m./bee).

In the oral and contact toxicity tests each treatment group (test item, controls, reference item) comprised 4 replicates including 10 bees each.

The controls used for the contact test were tap water with 0.1% Triton X-100 (wetting agent) (water control group) and pure acetone (solvent control). For the oral test 50 % w/v sucrose solution (water control group) and 50 % w/v sucrose solution containing 10 % acetone (solvent control group) were used as controls. As a toxic reference dimethoate (BAS 152 65 I, 400.0 g/L nominal, 412 g/L analytical) was applied at nominal dose levels of 0.34, 0.23, 0.15, and 0.10 µg dimethoate/bee in the contact test and at nominal dose levels of 0.14, 0.11, 0.08 and 0.06 µg dimethoate/bee in the oral test.

Application in the contact test: The test item was applied as one 2 µL droplet of test item dissolved in acetone, placed on the dorsal bee thorax using a hand-operated micro-applicator following anaesthetization of the bees with carbon dioxide. The reference item was applied as one 2 µL droplet of dimethoate dissolved in tap water containing 0.1% Triton X-100. For the two controls, one 2 µL droplet of tap water containing 0.1% Triton X-100 and pure acetone were applied. A 2 µL droplet was chosen in deviation to the guideline recommendation of a 1 µL droplet, since a higher volume ensured a more reliable dispersion of the test item.

Application in the oral test: The test item and icing sugar were first ground and mixed using a mortar. A defined volume of deionised water containing 10% acetone was then added to this test item/icing sugar mix such that the final solution contained 50 % w/v sucrose. For the preparation of the lower dose levels the stock solution was diluted by using 50 % (w/v) aqueous sucrose solution containing 10 % acetone. In deviation to the guideline a solvent content of 10 % was used in order to facilitate maximum solubility and a homogenous distribution of the test item in the oral application solution. Prior experience and the results of the current study have shown that honey bees tolerate 10 % acetone in the food and that it does not affect the bees and does not cause (increased) mortality compared to the control group. The solutions were homogenised by shaking by hand and using a vortex for about one minute. For the controls 50 % w/v sucrose solution (control) and 50 % w/v sucrose solution containing 10 % acetone (solvent control) were offered to the bees. The treated food was offered in syringes, which were weighed before and after introduction into the cages. After a maximum of 6 hours, the syringes were removed, weighed and replaced by ones containing fresh, untreated food.

Dose levels:

Nominal doses of the test item:	250, 125, 62.5, 31.3 and 15.6 µg p.m./bee (contact dose response test)
	50, 25, 12.5, 6.25 and 3.13 µg p.m./bee (oral dose response test)
Actual doses of the test item (oral test):	51.3, 25.8, 12.8, 6.38 and 3.25 µg p.m./bee µg p.m./bee
Nominal doses of the reference item:	0.34, 0.23, 0.15, and 0.10 µg dimethoate/bee (contact test)
	0.14, 0.11, 0.08 and 0.06 µg dimethoate/bee (oral test);

Actual doses of the reference item (oral test): 0.15, 0.12, 0.09 and 0.06 µg dimethoate/bee

Test conditions: Temperature: 24.2 – 26.4°C; relative humidity: 55.1 – 64.2 %; photoperiod: 24 h darkness (except during observations).

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Statistics: Due to low mortality in all test item treatment groups (oral and contact) the 24 and 48 h LD_{50} values could not be calculated. They are all assumed to be greater than the highest tested dose.

Dates of experimental work: July 06th to July 09th, 2020

II. Results and Discussion

Biological findings:

Contact test

In the control group and in the solvent control group mortalities of 2.5 % and 0.0 % were observed at the end of the 48 hour exposure period, respectively. In the highest test item dose level of 250 µg p.m./bee a mortality of 2.5 % was observed at the end of the 48 hour exposure period. The maximum mortality observed was 5.0 % at the lowest dose level at the end of the test.

No behavioural abnormalities were observed in any test item treatment group.

Table 8.3.1.1.1- 15: Mortality and behavioural abnormalities of the bees in the contact toxicity test

Treatment group	24 hour Mortality	48 hour Mortality
	Mean [%]	Mean [%]
Control	2.5	2.5
Solvent control	0.0	0.0
Test item [µg p.m./bee]		
15.6	5.0	5.0
31.3	0.0	0.0
62.5	0.0	0.0
125	2.5	2.5
250	2.5	2.5
Reference item [µg a.s./bee]		
0.10	7.5	10.0
0.15	42.5	50.0
0.23	65.0	72.5
0.34	85.0	92.5

Results are mean values of 4 replicates (ten bees each) for all treatment groups
Test item= fluopyram-7-hydroxy, reference item = dimethoate; water control = tap water; solvent control = pure acetone

Oral test

In the control group and in the solvent control group mortalities of 2.5 % and 0.0 % were observed at the end of the 48 hour exposure period, respectively. In the test item group a maximum mortality of 5.0 % was observed at the end of the 48 hour exposure period.

No behavioural abnormalities were observed in any test item treatment group.

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Table 8.3.1.1.1- 16: Mortality and behavioural abnormalities of the bees in the oral toxicity test

Treatment group		24 hour Mortality	48 hour Mortality
		Mean [%]	Mean [%]
Control		2.5	2.5
Solvent control		0.0	0.0
Test item [µg p.m./bee]			
Target	Actual*		
3.13	3.25	5.0	5.0
6.25	6.38	2.5	2.5
12.5	12.8	0.0	0.0
25.0	25.8	2.5	2.5
50.0	51.3	5.0	5.0
Reference item [µg a.s./bee]			
Target	Actual*		
0.06	0.06	7.5	10.0
0.08	0.09	20.0	27.5
0.17	0.12	65.0	82.5
0.33	0.15	82.5	90.0

Results are mean values of 4 replicates (control, test item and reference item) containing 10 bees each
Test item= fluopyram-7-hydroxy; reference item= dimethoate; control = 50 % w/v sucrose solution; solvent control = 50 % w/v sucrose solution with 10 % acetone
* Based on actual food consumption

The endpoints for the contact and oral toxicity test are shown in the table below. Since the mortalities in the test item treatment groups for both the contact and oral tests were ≤ 50 % at the end of the tests the 24 and 48 h LD_{50} values could not be calculated. They are assumed to be greater than the highest tested doses.

Table 8.3.1.1.1- 17: Contact and oral toxicity of fluopyram-7-hydroxy to honey bees

Test item	Fluopyram-7-hydroxy	
Test species	Honey bee *Apis mellifera* L.	
Exposure	**Contact (dissolved in acetone)**	**Oral (50 % w/v sucrose solution and acetone)**
Test duration	48 h	48 h
Dose rates [µg p m./bee]	250, 125, 62.5, 31.3 and 15.6	Nominal doses: 50, 25, 12.5, 6.25 and 3.13 Actual doses: 51.3, 25.8, 12.8, 6.38 and 3.25
LD_{50} [µg p m./bee]	> 250	> 51.3

Reference item

The contact and oral LD_{50} (24 h) values of the reference item (dimethoate) were calculated to be 0.19 and 0.11 µg a.s./bee, respectively. These values corresponded to the expected range cited in the OECD Guideline 213 (1998) and 214 (1998) and thus demonstrated the sensitivity of the test item.

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Validity criteria:

The contact and oral tests were considered valid as the control mortality in each case was ≤ 10 % and the LD_{50} values obtained with the reference item (dimethoate) were within the required ranges.

Table 8.3.1.1.1- 18: Validity criteria

Validity Criteria	Recommended		Obtained
Control mortality	Contact Test		
	Water control	≤ 10 %	2.5 %
	Solvent control	≤ 10 %	0.0 %
	Oral Test		
	Control	≤ 10 %	2.5 %
	Solvent control	≤ 10 %	0.0 %
LD_{50} of reference item (24 h)	Contact Test		
	Dimethoate	0.10 - 0.30 µg a.s./bee	0.19 µg a.s./bee
	Oral Test		
	Dimethoate	0.10 - 0.35 µg a.s./bee	0.11 µg a.s./bee

III. CONCLUSION

The toxicity of fluopyram-7-hydroxy was tested in both, an acute contact and oral toxicity test on honey bees.

The oral 48 hour LD_{50} for fluopyram-7-hydroxy could not be calculated but is assumed to be > 51.3 µg p.m./bee. This dose represents the maximum possible test dose based on non-GLP solubility/ homogeneity tests. The contact 48 hour LD_{50} for fluopyram-7-hydroxy could not be calculated but is assumed to be > 250 µg p.m./bee.

Assessment and conclusion by applicant:

The study and its data are considered as acceptable and reliable for use in risk assessment.

The endpoints are:

LD_{50} oral (48 hours) > 51.3 µg p.m./bee

LD_{50} contact (48 hours) > 250 µg p.m./bee

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Bumble bees

Active substance fluopyram

Data Point:	KCA 8.3.1.1.1/06
Report Author:	[redacted]
Report Year:	2015
Report Title:	Fluopyram tech.: Effects (Acute oral) on bumble bees (Bombus terrestris L.) in the laboratory
Report No:	97691105
Document No:	M-542447-01-1
Guideline(s) followed in study:	No specific guidelines available; study design based on OECD 213 (1998) Van der Steen (2001) and ICPPR non-apis group (2014) US EPA OCSPP Guideline No. 850.SUPP
Deviations from current test guideline:	Current Guideline: OECD 247 (2017) Deviations from OECD Guideline 247: The test treatment solution and the stock solution were not analysed as the study was conducted before the OECD Guideline 247 was published. These deviations are not expected to have impacted the study results. All validity criteria of the current guideline were met.
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 fluopyram technical to the bumble bee (*Bombus terrestris* L.). Mortality of bumble bees was used as the toxic endpoint. Sublethal effects, such as changes in behaviour, were also assessed.

Under laboratory conditions 50 worker bumble bees were exposed for 48 hours to a single dose of 100 µg a.s./bumble bee by feeding (oral limit test; actual dose based on the on the intake of the test item was 92.5 µg a.s./bumble bee).

The test comprised a water control group, a solvent control group and a toxic reference item (dimethoate).

In the oral toxicity test the LD_{50} value (48 h) was estimated to be > 92.5 µg a.s./bumble bee. The NOED value (48 h) was determined to be ≥ 92.5 µg a.s./bumble bee.

The study fulfils all validity criteria of the current Guideline OECD 247 (2017). As this OECD Guideline was not published at the time of study conduct, the study design was based on the Guidelines OECD 213 (1998) and OECD 214 (1998), Van der Steen (2001) and recommendations of the ICPPR non-*Apis* group (2014).

I. MATERIAL AND METHODS

Test item: Fluopyram tech.; Specification No.: 102000029510; Origin Batch No.: PFV14BO001; Sample description: TOX20009-00; purity: 97.3 % w/w.

Test species: Adult bumble bees (*Bombus terrestris* L.); adult female worker bumble bees from healthy and queen-right bumble bee colonies obtained from a commercial bumble bee breeding company.

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Test design: Under laboratory conditions 50 worker bumble bees were exposed for 48 hours to a single dose of 100 µg a.s./bumble bee by oral appliaction (oral limit test; actual value based on the intake of the test item was 92.5 µg a.s./bumble bee) in 50 % w/v sucrose solution containing maximum 5 % acetone and 1 % Tween80.

The controls used were water containing 50 % w/v sucrose solution (water control group) and solvent (5 % acetone) containing 1% Tween80 (solvent control group). As a toxic reference dimethoate (BAS 152 11 I, 400.0 g/L nominal, 420.3 g/L analytical) was applied at nominal 4 µg dimethoate/bumble bee in the oral test.

After collection from the hive the bumble bees were kept individually in cylindrical, latticed plastic cages. Middle sized bumble bees were selected visually and randomly distributed to the treatment groups. Each bumble bee was weighed individually after anesthetisation with CO_2 to prove a consistent distribution among the treatment groups. Bumblebees were acclimatised to test conditions over night (20 hours and 50 minutes) with *ad libitum* access to untreated 50% w/v sucrose solution.

Each treatment group (test item, water controls, solvent controls and reference item) comprised 50 bumble bees.

Application in the oral test: The test item treatment dose was diluted in 50 % w/v sucrose solution containing max. 5 % acetone and 1 % w/w Tween80. The reference item was applied in 50 % w/v sucrose solution. For the water control 50 % w/v sucrose solution and for the solvent control 50 % w/v sucrose solution containing 5 % acetone and 1 % w/w Tween80 were offered to the bumble bees.

Food was provided in syringes which were weighed before and after introduction into the cages. Empty syringes were removed, weighed and replaced by syringes containing fresh, untreated food (50 % w/v sucrose solution). After a maximum of 5 hours all syringes containing remaining food were removed, weighed and replaced by syringes containing fresh, untreated food (50 % w/v sucrose solution). The calculation of the target dose was based on 40 mg food uptake. The ingested consumed oral doses were calculated based on the measured consumption. The nominal target dose levels of 100 µg a.s./bumble bee would have been obtained if exactly 40 mg/bumble bee of the treated food were consumed. In practice, uptake of the treated sugar solutions differed from the nominal 40 mg/bumble bee.

Dose levels:

Nominal doses of the test item: 100.0 µg a.s./bumble bee

Actual dose of the test item: 92.5 µg a.s./bumble bee

Nominal doses of the reference item: 4 µg dimethoate/bumble

Actual dose of the reference item: 3.9 µg dimethoate/bumble bee

Test conditions: Temperature: 24 - 25 °C; relative humidity: 61.3 - 61.9 %; photoperiod: 24 h darkness (except handling procedures, including treatment and observations).

Statistics: Results obtained from the bumble bees treated with the test item were compared to those obtained from the solvent control. The NOED of the test item was estimated using the Fisher's Exact Test (pairwise comparison, one-sided greater, $\alpha = 0.05$). The software used to perform the statistical analysis was ToxRat Professional, Version 2.10 (® ToxRat Solutions GmbH).

Dates of work: October 29th to October 31st, 2015

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II. Results and Discussion

Biological findings:

Oral Test

After 48 hours there was no mortality in the 92.5 µg a.s./bumble bee test item group, in the control group (50 % w/v sucrose solution) and in the solvent control group (5 % acetone and 1 % Tween80). No test item related behavioural abnormalities or sublethal effects occurred at any time during the test.

Table 8.3.1.1.1- 19: Mortality and behavioural abnormalities of the bumble bees in the oral toxicity test

Dose	After 4 h		After 24 h		After 48 h	
	Mortality	Behav. abnorm.	Mortality	Behav. abnorm.	Mortality	Behav. abnorm.
	Mean [%]	Mean [%]	Mean [%]	Mean [%]	Mean [%]	Mean [%]
Water control	0.0	0.0	0.0	0.0	0.0	0.0
Solvent control	0.0	0.0	0.0	0.0	0.0	0.0
Test item [µg a.s./bumble bee]						
92.5	0.0	0.0	0.0	0.0	0.0	0.0
Reference item [µg a.s./bumble bee]						
3.9	98.0	0.0	98.0	0.0	98.0	0.0

Results are mean values of 50 individuals per treatment group (control, test item and reference item)
Behav. abnorm. = behavioural abnormalities
Test item = fluopyram tech., reference item = dimethoate, water control = 50 % w/v sucrose solution, solvent control = 50 % w/v sucrose solution containing 5 % w/w acetone and 1 % w/w Tween80

The endpoints for the contact and oral toxicity test are shown in the table below.

Table 8.3.1.1.1- 20: Oral toxicity of fluopyram to bumble bees

Test item	Fluopyram tech.
Test species	*Bombus terrestris* L.
Exposure	**Oral test** (50 % w/v sucrose solution containing 5 % w/w acetone and 1 % w/w Tween80)
Test duration	48 h
Target (nominal) dose rate [µg a.s./bumble bee]	100.0
Actual dose rate [µg a.s./bumble bee]	92.5
LD_{50} [µg a.s./bumble bee] [1]	> 92.5
NOED [µg a.s./bumble bee] [2,3]	≥ 92.5

[1] As the test item treatment groups did not show mortality above 50.0 %, no statistical evaluation on the LD_{50}, LD_{20} and LD_{10} was carried out.
[2] The NOED was determined using Fisher's Exact Test (pairwise comparison, one-sided greater, α = 0.05).
[3] Results obtained from test item treated group were compared to those obtained from the solvent control treated group.

Reference item

The mortality in the reference item treatment group was 98.0 % at the end of the test (48 hours after application).

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Validity criteria:

The oral toxicity test is considered valid as the control mortality in each case was ≤ 10 % and ≥ 50 % in the reference item.

The study fulfils all validity criteria of the current Guideline OECD 247 (2017). As this OECD Guideline was not published at the time of study conduct, the study design was based on the Guidelines OECD 213 (1998) and OECD 214 (1998), Van der Steen (2001) and recommendations of the ICPPR non-*Apis* group (2014).

Table 8.3.1.1.1- 21: Validity criteria

Validity criteria	Recommended		Obtained
Control mortality	Water control	≤ 10 %	0.0 %
	Solvent control	≤ 10 %	0.0 %
Reference item mortality	Dimethoate	≥ 50 %	98.0 %

III. Conclusion

The toxicity of Fluopyram tech. was tested in an acute oral toxicity test on bumble bees.

The oral NOED value was calculated to be ≥ 92.5 µg a.s./bumble bee. The oral LD_{50} value was > 92.5 µg a.s./bumble bee.

The concentration tested in this study was limited by the maximum solubility of the test item. Since this concentration caused no mortality, the generated endpoints are limited by solubility of the test item.

Assessment and conclusion by applicant:

The study and its data are considered as acceptable and reliable for use in risk assessment.

The endpoint is: LD_{50} oral (48 hours) > 92.5 µg a.s./bumble bee

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Data Point:	KCA 8.3.1.1.1/07
Report Author:	[redacted]
Report Year:	2021
Report Title:	Acute toxicity of fluopyram tech to the bumblebee Bombus terrestris L. under laboratory conditions
Report No:	21 48 BBA 0003
Document No:	M-763123-01-1
Guideline(s) followed in study:	Regulation (EC) No 1107/2009 (2009) Directive 2003-01 (CANADA/PMRA) US EPA OCSPP 850.SUPP OECD Test Guidelines No. 246 and No. 247 (2017)
Deviations from current test guideline:	Current Guidelines: OECD 246 and 247 (2017) Deviations from OECD Guideline 246: The nominal reference item test dose was 1.51 µg dimethoate/bumble bee instead of 4 µg dimethoate/bumble bee. These deviations are not expected to have impacted the study results. All validity criteria of the current guideline were met. Deviations from OECD Guideline 247: None
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 fluopyram tech. to the bumble bee (*Bombus terrestris* L.). Mortality of bumble bees was used as the toxic endpoint. Sublethal effects, such as changes in behaviour, were also assessed.

Under laboratory conditions 50 worker bumble bees were exposed for 48 hours to a single dose of 100 µg a.s./bumble bee by topical application (contact limit test) and 100.2 µg a.s./bumble bee by oral application (oral limit test; actual dose based on the on the intake of the test item was 90.5 µg a.s./bumble bee).

The contact test comprised a water control group, a surfactant control group and a solvent control group. In the oral test bees in the control groups were exposed to 50 % w/v aqueous sucrose solution (control group) and to 50 % w/v aqueous sucrose solution with 5 % acetone and 1 % Tween (solvent control group). In both tests a toxic reference item (dimethoate) was included.

In the contact toxicity test the LD_{50} value (48 h) was estimated to be > 100 µg a.s./bumble bee. The NOED value (48 h) was calculated to be ≥ 100 µg a.s./bumble bee. In the oral toxicity test the LD_{50} value (48 h) was estimated to be > 90.5 µg a.s./bumble bee. The NOED value (48 h) was calculated to be ≥ 90.5 µg a.s./bumble bee.

The study fulfils all validity criteria of the current OECD Guideline 246 (2017) and OECD Guideline 247 (2017).

I. Material and Methods

Test item: Fluopyram tech., Specification No.: 102000017196; Origin Batch No.: PFV187P078; Sample description: TOX 20941-01; purity: 99.0 % w/w.

Test species: Adult bumble bees (*Bombus terrestris* L.); adult female worker bumble bees from healthy and queen-right bumble bee colonies obtained from a commercial bumble bee breeding company.

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Test design: Under laboratory conditions 50 worker bumblebees (*Bombus terrestris* L.) were exposed for 48 hours to a single dose of 100.0 µg a.s./bumblebee by topical application (contact limit test) and to 100.2 µg a.s./bumblebee by feeding (oral limit test; actual dose based on the intake of the test item was 90.5 µg a.s./bumblebee).

The controls used in the oral test were water containing 50 % w/v sucrose solution (water control group) and 50 % w/v sucrose solution containing 5 % acetone and 1 % Tween80 (solvent control group). The controls used for the contact test were tap water (water control group), 0.5 % TritonX solution (surfactant control group) and acetone (solvent control group). As a toxic reference dimethoate (Dimethoate EC 400, 400.0 g/L nominal, 411.2 g/L analytical) was applied at nominal 1.51 µg dimethoate/bumble bee in the oral test and at nominal 10 µg dimethoate/bumble bee in the contact test.

After collection from the hive the bumble bees were kept individually in cylindrical plastic cages. Middle sized bumble bees were selected visually and randomly distributed to the treatment groups. Bumblebees were acclimatised to test conditions (17 hours) with *ad libitum* access to untreated 50 % w/v sucrose solution.

Each treatment group (test item, water controls, surfactant control group and solvent controls) comprised 50 replicates of 1 bumble bee each. The reference item comprised 30 replicates of 1 bumble bee each.

Application in the contact test: The test item treatment dose was dissolved in acetone and applied in one 2 µL droplet onto the dorsal thorax of bumble bees using a calibrated pipette. For the controls, one 2 µL droplet of water, one 2 µL droplet containing 0.5 % (v/v) TritonX and one 2 µL droplet of pure acetone were used. The reference item was applied as one 2 µL droplet of dimethoate dissolved in tap water containing 0.5 % (v/v) TritonX. Bumble bees were anaesthetized with CO_2 until they were completely immobilized immediately before application.

Application in the oral test: The test item treatment dose was diluted in 50 % w/v sucrose solution containing max. 5 % acetone and 1 % w/w Tween80. The reference item was applied in 50 % w/v sucrose solution. For the water control 50 % w/v sucrose solution and for the solvent control 50 % w/v sucrose solution containing 5 % acetone and 1 % w/w Tween80 were offered to the bumble bees.

Food was provided in syringes which were weighed before and after introduction into the cages. Empty syringes were removed, weighed and replaced by syringes containing fresh, untreated food (50 % w/v sucrose solution). After 2 hours all syringes containing remaining food were removed, weighed and replaced by syringes containing fresh, untreated food (50 % w/v sucrose solution). The calculation of the target dose was based on 40 mg food uptake. The ingested consumed oral doses were calculated based on the measured consumption. The nominal target dose levels of 100.2 µg a.s./bumble bee would have been obtained if exactly 40 mg/bumble bee of the treated food were consumed. In practice, uptake of the treated sugar solutions differed from the nominal 40 mg/bumble bee.

Dose levels:

Nominal doses of the test item:	100.0 µg a.s./bumble bee (contact limit test),
	100.2 µg a.s./bumble bee (oral limit test)
Actual dose of the test item (oral test):	90.5 µg a.s./bumble bee (based on actual food intake)
Nominal doses of the reference item:	10 µg dimethoate/bumble bee (contact test),
	1.51 µg dimethoate/bumble bee (oral test),
Actual doses of the reference item (oral test):	1.39 µg dimethoate/bumble bee

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Test conditions: Temperature: 23.5 – 23.8 °C; relative humidity: 47 - 72 %; photoperiod: 24 h darkness (except during observation).

Statistics: No statistical analysis was necessary since no test item mortality occurred during the contact and oral toxicity test.

Analytics: For verification of the exposure concentration, the test item solution as well as the respective solvent control solutions were sampled in duplicate directly after preparation. Samples were analysed by using high performance liquid chromatography (HPLC) with mass-spectrometric (MS-MS) detection.

Dates of work: November 04th to November 06th, 2020

II. Results and Discussion

Analytical results:
In the contact test item solution, the recovery of fluopyram was 99 %. In the control samples, no residues of fluopyram were detected. In the oral test item feeding solution, the recovery of fluopyram was 94 %. In the control samples, no residues of fluopyram were detected. Thus, the concentrations in the samples of the biological part of the study were verified.

Table 8.3.1.1.1- 22: Analytical results for contact test

Treatment	Nominal [A] concentration of fluopyram [mg a.s./kg]	Analysed concentration of fluopyram [mg a.s./kg]	Recovery [%]
Solvent control	0.00	<30 % LOQ	-
100.0 µg a.s./bumble bee	63205	62609	99

LOQ: Limit of quantification= 29457 mg a.s./kg, corresponding to 116.50 µg a.s./L in diluted samples
solvent control = acetone
[A] Based on analysed purity of fluopyram of 99 % w/w.

Table 8.3.1.1.1- 23: Analytical results for oral test

Treatment	Nominal [A] concentration of fluopyram [mg a.s./kg]	Analysed concentration of fluopyram [mg a.s./kg]	Recovery [%]
Solvent control	0.00	<30 % LOQ	-
100.2 µg a.s./bumble bee	2123	1994	94

LOQ: Limit of quantification= 1009 mg a.s./kg, corresponding to 100.94 µg a.s./L in diluted extracts.
solvent control = 50 % w/v sucrose solution containing 5 % w/w acetone and 1 % w/w Tween80
[A] Based on analysed purity of fluopyram of 99 % w/w.

Biological findings:

Contact Test:

After 48 hours there was no mortality in the 100 µg a.s./bumble bee test item group, in the water control group (tap water) and in the surfactant control group (0.5 % v/v TritonX). In the solvent control group (5 % acetone) 2 % mortality occurred. No test item related behavioural abnormalities occurred at any time during the test.

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Table 8.3.1.1.1- 24: Mortality and behavioural abnormalities of the bumble bees in the contact toxicity test

Dose	After 4 h		After 24 h		After 48 h	
	Mortality	Behav. abnorm.	Mortality	Behav. abnorm.	Mortality	Behav. abnorm.
	Mean [%]	Mean [%]	Mean [%]	Mean [%]	Mean [%]	Mean [%]
Water control	0.0	0.0	0.0	0.0	0.0	0.0
Surfactant control	0.0	0.0	0.0	0.0	0.0	0.0
Solvent control	2.0	0.0	2.0	0.0	2.0	0.0
Test item [µg a.s./bumble bee]						
100	0.0	0.0	0.0	0.0	0.0	0.0
Reference item [µg a.s./bumble bee]						
10	33.3	0.0	93.0	0.0	100.0	-

Mortality results are averages based on 50 (30 for reference item) replicates consisting of 1 bumblebee each
Behav. abnorm. = behavioural abnormalities
Test item = fluopyram tech.; reference item = dimethoate; water control = tap water; solvent control = acetone; surfactant control = 0.5 % (v/v) TritonX

Oral Test:

After 48 hours there was no mortality in the 90.5 µg a.s./bumble bee test item group, in the water control group (50 % w/v sucrose solution) and in the solvent control group (5 % acetone and 1 % Tween80). No test item related behavioural abnormalities occurred at any time during the test.

Table 8.3.1.1.1- 25: Mortality and behavioural abnormalities of the bumble bees in the oral toxicity test

Dose	After 4 h		After 24 h		After 48 h	
	Mortality	Behav. abnorm.	Mortality	Behav. abnorm.	Mortality	Behav. abnorm.
	Mean [%]	Mean [%]	Mean [%]	Mean [%]	Mean [%]	Mean [%]
Water control	0.0	0.0	0.0	0.0	0.0	0.0
Solvent control	0.0	0.0	0.0	0.0	0.0	0.0
Test item [µg a.s./bumble bee]						
90.5	0.0	0.0	0.0	0.0	0.0	0.0
Reference item [µg a.s./bumble bee]						
1.39	0.0	0.0	60.0	0.0	90.0	0.0

Mortality results are averages based on 50 (48 for AT and 30 for reference item) replicates consisting of 1 bumblebee each
Behav. abnorm. = behavioural abnormalities
Test item = fluopyram tech.; reference item = dimethoate; water control = 50 % w/v sucrose solution, solvent control = 50 % w/v sucrose solution containing 5 % w/w acetone and 1 % w/w Tween80

The endpoints for the contact and oral toxicity test are shown in the table below.

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Table 8.3.1.1.1- 26: Contact and oral toxicity of fluopyram tech. to bumble bees

Test item	Fluopyram tech.	
Test species	Bumble bee *Bombus terrestris* L.	
Exposure	**Contact** (dissolved in acetone)	**Oral** (50 % w/v sucrose solution containing 5 % w/w acetone and 1 % w/w Tween80)
Test duration	48 h	48 h
Dose rate [µg a.s./bumble bee]	100.0	Nominal dose: 100.2 Actual dose: 90.5
LD_{50} [µg a.s./bumble bee]	> 100.0	> 90.5
NOED [µg a.s./bumble bee]	≥ 100.0	≥ 90.5

Reference item

The bumble bees of the reference item group were treated with 10 µg dimethoate a.s./bumble bee in the contact test and with 1.39 µg dimethoate a.s./bumblebee in the oral test. The mortality in the contact and oral reference item treatment groups was 100 % and 90 %, respectively, at the end of the test (48 hours after application).

Validity criteria:

The contact and oral toxicity test is considered valid as the control mortality in each case was ≤ 10 % and ≥ 50 % in the reference item.

Table 8.3.1.1.1- 27: Validity criteria

Validity criteria		**Recommended**	**Obtained**
Control mortality		Contact test	
	Water control	≤ 10 %	0.0 %
	Surfactant control	≤ 10 %	0.0 %
	Solvent control	≤ 10 %	2.0 %
		Oral test	
	Water control	≤ 10 %	0.0 %
	Solvent control	≤ 10 %	0.0 %
Reference item mortality		Contact test	
	Dimethoate	≥ 50 %	100.0 %
		Oral test	
	Dimethoate	≥ 50 %	90.0 %

III. CONCLUSION

The toxicity of fluopyram tech. was tested in an acute contact and oral toxicity test on bumble bees. The contact NOED value was calculated to be ≥ 100 µg a.s./bumble bee. The contact LD_{50} value was estimated to be > 100 µg a.s./bumble bee.

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The oral NOED value was calculated to be ≥ 90.5 µg a.s./bumble bee. The oral LD_{50} value was estimated to be > 90.5 µg a.s./bumble bee.

Assessment and conclusion by applicant:

The study and its data are considered as acceptable and reliable for use in risk assessment.

The endpoints are:

LD_{50} contact (48 hours) > 100 µg a.s./bumble bee

LD_{50} oral (48 hours) > 90.5 µg a.s./bumble bee

CA 8.3.1.1.2 Acute contact toxicity

Honeybees

Active substance fluopyram

Data Point:	KCA 8.3.1.1.2/01
Report Author:	[redacted]
Report Year:	2005
Report Title:	Effects of AE C656948 (acute contact and oral) on honey bees (Apis mellifera L.) in the laboratory
Report No:	24851035
Document No:	M-261594-01-1
Guideline(s) followed in study:	OECD Guideline 213, 21.09.1998; OECD Guideline 214, 21.09.1998; U.S. EPA OPPTS Guideline No. 850.3020
Deviations from current test guideline:	Current Guidelines: OECD 213 (1998) and OECD 214 (1998) Deviations from OECD Guideline 213: The use of a concentration of 5 % solvent was essential, to obtain the maximum dose rate of 100 µg/bee. This deviation to the guideline did not affect the outcome of the study. No further deviations to the current OECD Guideline 213 occurred. All validity criteria were met. Deviations from OECD Guideline 214: An application volume of 5 µL was chosen in deviation to the guideline-specified value of 1 µL to ensure reliable dispersion This deviation is not expected to have impacted the study results. All validity criteria were met.
Previous evaluation:	yes, evaluated and accepted in DAR (2011)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

For study summary on acute contact and oral toxicity of fluopyram to the honey bee, please refer to section CA 8.3.1.1.1/01.

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Metabolite fluopyram-pyridyl-carboxylic acid

Data Point:	KCA 8.3.1.1.2/02
Report Author:	Schabio, S.
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) Deviations from OECD Guideline 213: No deviations to the current OECD Guideline 213 occurred. All validity criteria were met. Deviations from OECD Guideline 214: An application volume of 5 µL was chosen in deviation to the guideline-specified value of 1 µL to ensure reliable dispersion. This deviation is not expected to have impacted the study results. All validity criteria were met.
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

For study summary on acute contact and oral toxicity of fluopyram-pyridyl-carboxylic acid to the honey bee, please refer to section CA 8.3.1.1.1/04.

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Metabolite fluopyram-7-hydroxy

Data Point:	KCA 8.3.1.1.2/03
Report Author:	Kling, A.
Report Year:	2020
Report Title:	BCS-AA10065: Acute oral and contact toxicity to the honey bee (Apis mellifera L.) under laboratory conditions
Report No:	S20-02018
Document No:	M-758325-01-1
Guideline(s) followed in study:	Regulation (EC) No. 1107/2009 (Oct. 2009) Directive 2003-01 (Canada/PMRA) US EPA OCSPP 850.3020/850.supp. OECD Guideline No. 213 (1998) OECD Guideline No. 214 (1998)
Deviations from current test guideline:	Current Guidelines: OECD 213 (1998) and OECD 214 (1998) Deviations from OECD Guideline 213: A 10 % solvent concentration was necessary to derive a maximum nominal dose rate of 50 µg/bee. This deviation to the guideline did not affect the outcome of the study. All validity criteria were met. Deviations from OECD Guideline 214: An application volume of 2 µL was chosen in deviation to the guideline-specified value of 1 µL to ensure reliable dispersion. This deviation is not expected to have impacted the study results. All validity criteria were met.
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

For study summary on acute contact and oral toxicity of fluopyram-7-hydroxy to the honey bee, please refer to section CA 8.3.1.1.1/05.

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Bumble bees

Active substance fluopyram

Data Point:	KCA 8.3.1.1.2/04
Report Author:	Haupt, S.
Report Year:	2015
Report Title:	Effects of fluopyram tech. (acute contact) on bumblebees (Bombus terrestris L.) in the laboratory
Report No:	88581105
Document No:	M-510849-01-1
Guideline(s) followed in study:	No specific guidelines available; study design based on OECD 214 (1998) Van der Steen (2001) and ICPPR non-apis group (2014) US EPA OCSPP Guideline No. 850.SUPP
Deviations from current test guideline:	Current Guideline: OECD 246 (2017) Deviations from OECD Guideline 246: No analytical verification of test item and control solutions was carried out as the study was conducted prior to guideline publication. No information to bumble bee colonies concerning size, brood stages and number of bumble bees are reported. An application volume of 5 µL was chosen in deviation to the guideline-specified value of 2 µL to facilitate a higher test dose. Temperatures fell below the guideline-specified range for more than two hours due to an inappropriate position of the climate data recorder. These deviations are not expected to have impacted the study results. All validity criteria of the current guideline were met.
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 of fluopyram tech. to the bumble bee (*Bombus terrestris* L.). Mortality of bumble bees was used as the toxic endpoint. Sublethal effects, such as changes in behaviour, were also assessed.

Under laboratory conditions 50 worker bumble bees were exposed for 48 hours to a single dose of 100 µg a.s./bumble bee by topical application (contact limit test).

The contact test comprised a water control group, a solvent control group and a toxic reference item (dimethoate).

In the contact toxicity test the LD_{50} value (48 h) was estimated to be > 100 µg a.s./bumble bee. The NOED value (48 h) was calculated to be ≥ 100 µg a.s./bumble bee.

The study fulfils all validity criteria of the current OECD Guideline 246 (2017). As this OECD Guideline was not published at the time of study conduct, the study design was based on the Guideline OECD 214 (1998), Van der Steen (2001) and recommendations of the ICPPR non-*Apis* group (2014).

I. Material and Methods

Test item: Fluopyram tech., specification No.: 102000017196; Origin Batch No.: PFV127P012; TOX. No.: 09909-00; purity: 97.7 % w/w.

Test species: Adult bumble bees (*Bombus terrestris* L.); adult female worker bumble bees from healthy and queen-right bumble bee colonies obtained from a commercial bumble bee breeding company.

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Test design: Under laboratory conditions 50 worker bumble bees were exposed for 48 hours to a single dose of 100 µg a.s./bumble bee by topical application (contact limit test).

The controls used for the contact test were tap water containing 0.5 % Tween80 (water control group) and acetone (solvent control group). As a toxic reference dimethoate (BAS 152 11 I EC 400, 400.0 g/L nominal, 400.9 g/L analytical) was applied at nominal 12 µg dimethoate/bumble bee.

After collection from the hive the bumble bees were kept individually in cylindrical, latticed plastic cages. Middle sized bumble bees were selected visually and randomly distributed to the treatment groups. Each bumble bee was weighed individually after anesthetisation with CO_2 to prove a consistent distribution among the treatment groups. Bumblebees were acclimatised to test conditions (19 hours 40 minutes) with *ad libitum* access to untreated 50 % w/v sucrose solution.

Each treatment group (test item, water controls, solvent controls and reference item) comprised 50 replicates including 1 bumble bees each.

Application: The test item treatment dose was dissolved in acetone and applied in one 5 µL droplet onto the dorsal thorax of bumble bees using a calibrated pipette. For the controls, one 5 µL droplet of water containing 0.5 % Tween80 and one 5 µL droplet of pure acetone were used. The reference item was applied as one 5 µL droplet of dimethoate dissolved in tap water containing 0.5 % Tween80. Bumble bees were anaesthetized with CO_2 until they were completely immobilized immediately before application.

Dose levels:

Nominal doses of the test item: 100 µg a.s./bumble bee

Nominal doses of the reference item: 12 µg dimethoate/bumble bee

Test conditions: Temperature: 22 - 25 °C; relative humidity: 53 - 75 %; photoperiod: 24 h darkness (except during observation).

Statistics: Results obtained from the bumblebees treated with the test item and the reference item were compared to those obtained from the control in the contact test. As the test item treatment group did not show mortality above 2 %, no statistical evaluation on the LD_{50}, LD_{20} and LD_{10} was carried out. The NOED was estimated using Fisher Exact Test (pairwise comparison, one-sided greater, $\alpha = 0.05$), which is a distribution-free test and does not require testing for normality or homogeneity prior to analysis. The software used to perform the statistical analysis was ToxRat Professional, Version 2.10 (® ToxRat Solutions GmbH).

Dates of work: September 23rd to September 25th, 2014

II. RESULTS AND DISCUSSION

Biological findings:

Contact Test:

At the end of the contact toxicity test (48 hours after application) there was 2.0 % mortality at 100 µg a.s./bumblebee. 2.0 % mortality occurred in the water control group (water with 0.5 % Tween80) and there was no mortality in the solvent control group (acetone).

No test item related behavioural abnormalities occurred at any time during the test.

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Table 8.3.1.1.2- 1: Mortality and behavioural abnormalities of the bumble bees in the contact toxicity test

Dose	After 4 h		After 24 h		After 48 h	
	Mortality	Behav. abnorm.	Mortality	Behav. abnorm.	Mortality	Behav. abnorm.
	Mean [%]	Mean [%]	Mean [%]	Mean [%]	Mean [%]	Mean [%]
Water control	0.0	0.0	0.0	0.0	2.0	0.0
Solvent control	0.0	0.0	0.0	0.0	0.0	0.0
Test item [µg a.s./bumble bee]						
100	0.0	0.0	0.0	0.0	2.0	0.0
Reference item [µg a.s./bumble bee]						
12	6.0	2.0	84.0	12.0	96.0	4.0

Results are mean values of 50 individuals per treatment group (control, test item and reference item)
Behav. abnorm. = behavioural abnormalities
Test item = fluopyram tech.; reference item = dimethoate; water control = tap water containing 0.5 % Tween80; solvent control = acetone

The endpoints for the contact toxicity test are shown in the table below.

Table 8.3.1.1.2- 2: Contact toxicity of fluopyram tech. to bumble bees

Test item	Fluopyram tech.
Test species	*Bombus terrestris* L.
Exposure	**Contact test** (solution in acetone)
Test duration	48 h
Target (nominal) dose rate [µg a.s./bumble bee]	100
LD_{50} [µg a.s./bumble bee]	> 100
NOED [µg a.s./bumble bee] [A]	≥ 100

[A] The NOED was estimated using Fisher's Exact Test (pairwise comparison, one-sided greater, α = 0.05)

Reference item

The bumblebees of the reference item group were treated with 12 µg dimethoate a.s./bumblebee in the contact test. The reference item mortality of 96.0% in the end of the test (48 hours after application) was within the required range.

Validity criteria:

The contact toxicity test is considered valid as the control mortality in each case was ≤ 10 % and ≥ 50 % in the reference item.

Table 8.3.1.1.2- 3: Validity criteria

Validity criteria	Recommended		Obtained
Control mortality	Water control	≤ 10 %	2.0 %
	Solvent control	≤ 10 %	0.0 %
Reference item mortality	Dimethoate	≥ 50 %	96.0 %

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III. Conclusion

The toxicity of fluopyram tech. was tested in an acute contact toxicity test on bumblebees.

The contact NOED value was calculated to be ≥ 100 µg a.s./bumblebee. The contact LD_{50} value was estimated to be > 100 µg a.s./bumblebee.

Assessment and conclusion by applicant:
The study and its data are considered as acceptable and reliable for use in risk assessment.
The endpoint is: LD_{50} contact (48 hours) > 100 µg a.s./bumble bee.

****

Data Point:	KCA 8.3.1.1.2/05
Report Author:	[redacted]
Report Year:	2021
Report Title:	Acute toxicity of fluopyram tech to the bumblebee Bombus terrestris L. under laboratory conditions
Report No:	21 48 BBA 0003
Document No:	M-763123-01-1
Guideline(s) followed in study:	Regulation (EC) No 1107/2009 (2009) Directive 2003-01 (CANADA/PMRA) US EPA OCSPP 850.SUPP OECD Test Guidelines No. 246 and No. 247 (2017)
Deviations from current test guideline:	Current Guidelines: OECD 246 and 247 (2017) Deviations from OECD Guideline 246: The nominal reference item test dose was 1.51 µg dimethoate/bumble bee instead of 4 µg dimethoate/bumble bee. These deviations are not expected to have impacted the study results. All validity criteria of the current guideline were met. Deviations from OECD Guideline 247: None
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

For study summary on acute contact and oral toxicity of fluopyram to bumble bee, please refer to section CA 8.3.1.1.1/07.

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CA 8.3.1.2 Chronic toxicity to bees

Active substance fluopyram (FLU SC 500 tested as surrogate formulation)

Data Point:	KCA 8.3.1.2/01
Report Author:	[redacted]
Report Year:	2015
Report Title:	Chronic oral toxicity test of fluopyram SC 500B G on the honey bee (Apis mellifera L.) in the laboratory
Report No:	87481136
Document No:	M-540072-01-1
Guideline(s) followed in study:	GLP compliant study based on OECD 213 (1998) and CEB No. 230 with modifications and current recommendations of the ring test group (2014) US EPA OCSPP Guideline No. 850.SUPP
Deviations from current test guideline:	Current Guideline: OECD 245 (2017) The test solution was not checked for possible evaporation from the feeders. The measured humidity (40-90 %) exceeded the recommended range of 50-70 %. These deviations are not expected to have impacted the study results. No further deviations to the current OECD Guideline 245 occurred. All validity criteria were met.
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 oral toxicity (LDD_{50}/LC_{50} and NOEDD/NOEC) of FLU SC 500 applied on 10 consecutive days to young adults of the honey bee (*Apis mellifera* L.). Mortality of bees was used as the toxic endpoint. Sublethal effects, such as changes in behaviour, were also assessed.

Worker honey bees (*Apis mellifera* L.) aged two days or less were orally exposed to a daily application of FLU SC 500 diluted in the bee food (50 % w/v aqueous sucrose solution) at nominal concentrations of 208, 417, 833, 1667 and 3333 mg a.s./kg feeding solution for ten consecutive days. An untreated control (50 % w/v aqueous sucrose solution) and a reference item (dimethoate) were included in this study.

Mortality and behavioural abnormalities were assessed daily. The concentration of the test item in the feeding solutions was verified analytically.

The LDD_{50} was determined to be > 81.4 µg a.s./bee/day and the LC_{50} to be > 3333 mg a.s./kg feeding solution, respectively. The NOEDD was determined to be 81.4 µg a.s./bee/day and the NOEC to be 3333 mg a.s./kg feeding solution, respectively.

The study fulfils all validity criteria of the current Guideline OECD 245 (2017). As the OECD Guideline 245 (2017) was not published at the time of study conduct, the study design was based on OECD 213 (1998) and CEB No. 230 with current recommendations of the ring test group (2014).

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I. Material and methods

Test item: FLU SC 500, Specification No.: 102000018148 - 01; Batch No.: EM4L011550; Sample Description: TOX10112-00; analysed content of active substance: 42.2 % w/w, (501.4 g/L); Density 1.188 g/mL (20 °C).

Test species: Honey bees *(Apis mellifera carnica* L.); freshly emerged young female worker bees (max. 2 days old) from healthy, disease-free and queen-right honey bee colonies.

Test concentrations and dose levels:

Test item concentrations: 3333, 1667, 833, 417, and 208 mg a.s./kg feeding solution

Nominal test item doses (calculated based on mean expected uptake of feeding solution of 30 mg/bee/day): 100.0, 50.0, 25.0, 12.5 and 6.25 µg a.s./bee/day

Actual test item doses: 81.4, 46.6, 24.2, 12.7 and 6.24 µg a.s./bee/day

Reference item concentration: 1 mg dimethoate/kg feeding solution

Nominal reference item dose (calculated based on mean expected uptake of feeding solution of 30 mg/bee/day): 0.03 µg a.s./bee/day

Actual reference item dose: 0.021 µg a.s./bee/day

Control group: 50 % w/v sucrose solution

Each group (test item, controls and reference item) comprised 3 replicates containing 10 bees each.

Test design: Worker honey bees (*Apis mellifera* L., about 2 days old) were orally exposed to a daily application of FLU SC 500 diluted in the bee food (50 % w/v aqueous sucrose solution) for 10 consecutive days.

For the collection of honey bees, brood frames from one colony with capped cells which are expected to hatch on the same day were kept without nurse bees in an excluder box in the hive until hatch. This comb contained pollen which was used as a first feeding source for the freshly hatched bees. Bees were acclimatized to test conditions for one day under test conditions. Any dead bees occurring during the acclimatisation phase were replaced prior to the start of the test of the same age and acclimatisation conditions. The following day (application day), the bees were 2 days old and the test was started.

Daily dose rates were based on a theoretical food consumption of 30 mg/bee/day. Test item solutions were prepared freshly every day. The reference item feeding solutions was prepared at least every 4 days and stored in the refrigerator at about 4 ± 4 °C. The respective feeding solutions (test item, control and reference item) were provided *ad libitum* in a plastic syringe, which had been weighed before application. The feeders remained in the cages for about 24 h. The actual consumption was determined by reweighing the syringe containing the remaining test solution each day after removal from the test units. Any unconsumed food was discarded. The food consumption per bee was calculated based on the number of surviving bees per assessment and the amount of food consumed on the following assessment day.

Mortality and behavioural abnormalities were assessed daily.

Test conditions: Temperature: 32 - 34 °C, Relative humidity: 40 - 90 % (average relative humidity was 84 %); Photoperiod: 24 h darkness (except during observation).

Statistics: Results obtained from the bees treated with the test item and the reference item were compared to those obtained from the control. Since mortality in any of the test item treated dose levels was < 50 % the oral LC_{50} and LDD_{50} can be considered as > 3333 mg a.s./kg and > 81.4 µg a.s./bee/day. The NOEC/NOEDD of the test item was estimated using Fisher's Exact Test (pairwise comparison, one-sided greater, $\alpha = 0.05$), which is a distribution-free test and does not require testing for normality or homogeneity prior to analysis. The statistical calculations were performed with the computer program ToxRat Professional 2.10.05 (® ToxRat Solutions GmbH.).

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Analytics: Samples (2-3 ml) for analysis were taken in triplicates on day 0 to day 9 from the treated feeding solutions per concentration and from the control feeding solution. The chemical analysis was performed by using reversed phase High Performance Liquid Chromatograph (HPLC) – MS/MS.

Dates of work: June 10th to June 20th, 2014

II. Results and Discussion

Analytical results:

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 SANTE/2020/12830, Rev.1.

The mean recoveries of fluopyram in the test item spiked feeding solutions ranged between 83 % and 100 %. No residues of fluopyram above the Limit of Detection (LOD: 0.003 mg a.s./kg) were found in any of the control feeding solutions.

Table 8.3.1.2- 1: Analytical results

Treatment group	Target concentration of fluopyram		Mean measured concentrations of fluopyram	Mean recovery from target
	[µg a.s./bee/day] A	[mg a.s./kg diet] B	[mg a.s./kg diet]	[%] C
Control	-	-	< LOD	-
Test item	6.25	208	172	83
	12.5	417	362	87
	25.0	833	773	93
	50.0	1667	1659	100
	100	3333	3311	99

LOD (Limit of Detection) for fluopyram = 0.003 mg/kg (= 3 µg/kg = 3 ppb)

A Taking into account a mean uptake of feeding solution of 30 mg/bee/day

B Taking into consideration the nominal concentration of 501.4 g/L a.s. and a density of 1.188 g/mL

C For the calculation of the mean measured fluopyram concentration and the "Recovery from Target [%]" as it appears in the result table above, unrounded values were used. Therefore, minor deviations may occur between the values shown above and when the values given in the residue result column are used for calculation

Recovery from target fluopyram [%] = Measured concentration [mg/kg]/Target concentration [mg/kg]*100

Biological results:

Summary of mean mortality and toxicity of FLU SC 500 to adult honey bees after 10 days of chronic exposure:

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Table 8.3.1.2- 2: 10-day chronic oral toxicity test with FLU SC 500 to young honey bees

Treatment group	Daily dose		Concen-tration	At day 10		
	Nominal	Consumed [A]		Mean mortality	Number of bees with behavioural abnormalities	Mean behavioural abnormalities
	[µg a.s./bee/day]		[mg a.s./kg diet]	[%]	[No.]	[%]
Control	-	-	-	0.0	0 out of 30	0.0
Test item	6.25	6.24	208	0.0	0 out of 30	0.0
	12.5	12.7	417	6.7	0 out of 28	0.0
	25.0	24.2	833	3.3	0 out of 1	0.0
	50.0	46.6	1667	3.3	0 out of 1	0.0
	100	81.4	3333	10.0	0 out of 27	0.0
Reference Item	0.03	0.021	1.0	100.0*	0 out of 0	0.0

Endpoints		10 d
Test item doses	LDD_{50} [µg a.s./bee/day] [B]	> 81.4
	NOEDD [µg a.s./bee/day] [C]	81.4
Test item concentrations	LC_{50} [mg a.s./kg feeding solution] [B]	> 3333
	NOEC [mg a.s./kg feeding solution] [C]	3333

Results are mean values of 3 replicates, containing 10 bees each

A mean dose per bee per day; dose measured based on consumed feeding solution

B Lethal dietary dose/concentration

C No observed effect dietary dose/concentration (NOEC/NOEDD) was calculated using Fisher's Exact Test (pairwise comparison, one-sided greater, $\alpha = 0.05$).

* Statistically significant different compared to the control ($\alpha = 0.05$)

No test item related behavioural abnormalities occurred at any time of the test.

Taking into account the actual food uptake the bees consumed doses of 81.4, 46.6, 24.2, 12.7 and 6.24 µg a.s./bee/day, which caused mortalities of 10, 3.3, 3.3, 6.7 and 0 % respectively, after 10 days. No mortality occurred in the control group. Mortalities in the test item treatment groups were not statistically significantly different compared control group. No concentration – mortality relationship was indicated in this study for the test item and mortality did not exceed 10 % at the highest test concentration. Thus, both the LC_{50} and LDD_{50} were estimated to be greater than the highest test item concentration/dose assessed.

Reference item

The reference item (dimethoate) was administered in one dosage of 0.03 µg a.s./bee/day (actual average intake based on food consumption was 0.021 µg a.s./bee/day) which caused a continuously increasing mortality leading to 100 % mortality at day 6.

Validity criteria

The study fulfils all validity criteria of the current OECD Guideline 245 (2017). As this OECD Guideline was not published at the time of study conduct, the study design was based on the Guideline OECD 213 (1998) and CEB No. 230 with current recommendations of the ring test group (2014).

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Table 8.3.1.2- 3: Validity criteria

Validity criteria according to OECD GD 245 (2017)	Recommended		Obtained
Mortality after 10 days of exposure	Control	≤ 15 %	0.0 %
Mortality after 10 days of exposure	Dimethoate	≥ 50 %	100 % (reached at day 6)

III. CONCLUSION

The chronic oral toxicity of FLU SC 500 was tested on young adult honey bees (*Apis mellifera* L.) in a 10-day feeding study under laboratory conditions.

The LDD_{50} was determined to be > 81.4 µg a.s./bee/day and the LC_{50} to be > 3333 mg a.s./kg feeding solution, respectively.

The NOEDD was determined to be 81.4 µg a.s./bee/day and the NOEC to be 3333 mg a.s./kg feeding solution, respectively.

Assessment and conclusion by applicant:

The study and its data are considered as acceptable and reliable for use in risk assessment.

The endpoints are:

LDD_{50} oral (10 days) > 81.4 µg a.s./bee/day

LC_{50} oral (10 days) > 3333 mg a.s./kg diet

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Metabolite fluopyram-benzamide

Data Point:	KCA 8.3.1.2/02
Report Author:	[redacted]
Report Year:	2020
Report Title:	AE C656948-benzamide: Chronic toxicity to the honey bee Apis mellifera L. under laboratory conditions
Report No:	19 48 BAC 0045
Document No:	M-688788-01-1
Guideline(s) followed in study:	EU Directive 91/414/EEC Regulation (EC) No 1107/2009 (2009) US EPA OCSPP 850.SUPP OECD 245 (adopted 9 October 2017)
Deviations from current test guideline:	Current Guideline: OECD 245 (2017) Deviations: None. All validity criteria were met.
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 oral toxicity ($LDD_{50/20/10}$/$LC_{50/20/10}$ and NOEDD/NOEC) of Fluopyram-benzamide applied on ten consecutive days to young adults of the honey bee (*Apis mellifera* L.). Mortality of bees was used as the toxic endpoint. Sublethal effects, such as changes in behaviour, were also assessed.

In this 10-day chronic toxicity feeding test worker honey bees (*Apis mellifera* L.) aged two days or less were orally exposed to a daily application of Fluopyram-benzamide diluted in the bee food (50 % w/v aqueous sucrose solution with 5 % v/v acetone) at nominal concentrations of 320, 128, 51.2, 20.5, 8.19 and 3.28 mg p.m./kg feeding solution.

The study included an untreated control (50 % w/v aqueous sucrose solution), a solvent control (acetone) and a reference item (dimethoate).

Mortality and behavioural abnormalities were assessed daily. The concentration of the test item in the feeding solutions was verified analytically.

The LDD_{50} (95 % CI) was determined to be 8.58 µg p.m./bee/day (7.14 - 10.1 µg p.m./bee/day) and the LC_{50} (95 % CI) to be 199 mg p.m./kg feeding solution (160 - 241 mg p.m./kg food), respectively.

The NOEDD was determined to be 1.17 µg p.m./bee/day and the NOEC to be 20.5 mg p.m./kg feeding solution, respectively.

The study fulfils all validity criteria of the current Guideline OECD 245 (2017).

I. Material and Methods

Test item: Fluopyram-benzamide (BCS-AA10014); Origin batch no.: B26F, Batch code: AE F148815-PU-02; purity: 99.4 % w/w

Test species: Honey bees (*Apis mellifera* L. subspecies *iberiensi*); young female worker bees (max. 2 days old) from healthy, disease-free and queen-right honey bee colonies.

Test concentrations and dose levels:

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Test item concentrations: 320, 128, 51.2, 20.5, 8.19 and 3.28 mg p.m./kg feeding solution

Nominal test item doses (calculated based on mean expected uptake of feeding solution of 33 µL/bee/day): 12.5, 4.99, 1.99, 0.798, 0319 and 0.128 µg p.m./bee/day

Actual test item doses: 12.5, 6.34, 2.50, 1.17, 0.396 and 0.160 µg p.m./bee/day

Reference item concentration: 0.694 mg dimethoate/kg feeding solution

Nominal reference item dose (calculated based on mean expected uptake of feeding solution of 33 µL/bee/day): 0.0273 µg a.s./bee/day

Actual reference item dose: 0.0172 µg a.s./bee/day

Control group: 50 % w/v sucrose solution (Control) and 50 % (w/v) aqueous sucrose solution with 5 % (v/v) acetone (Solvent control).

Each group (test item, controls and reference item) comprised three replicates containing 10 bees each.

Test design: In a 10-day chronic toxicity feeding test max. two days old worker honey bees (*Apis mellifera* L.) were orally exposed to a daily application of Fluopyram-benzamide diluted in the bee food (50 % w/v aqueous sucrose solution with 5 % v/v acetone).

For the collection of honey bees, brood combs with capped cells were taken from outside hives and different colonies. Sufficient food supply was ensured by pollen which was on the same brood comb. These frames were placed without adult worker bees in a "five comb hive body" and incubated under controlled environmental conditions in a climatic chamber at 33 ± 2 °C in darkness. The next day, the newly hatched worker bees were assigned into the test cages in groups of per cage. The following day the test was initiated (Day 0, first dose administration).

Daily dose rates were based on a theoretical food consumption of 33 µL/bee/day. Test item solutions were prepared freshly every day. The reference item stock solution was prepared once for the whole feeding period and the respective feeding solutions was prepared at least every 4 days and stored in the refrigerator at about 6 °C. The respective feeding solutions (test item, control and reference item) were provided *ad libitum* in a plastic syringe, which had been weighed before application. The feeders remained in the cages for about 24 h (± 2 h). The actual consumption was determined by reweighing the syringe containing the remaining test solution each day after removal from the test units. Any unconsumed food was rejected.

Assessments of mortality and behavioural abnormalities were done daily. The daily food consumption was corrected by subtracting the mean evaporation figure of each day of application.

Test conditions: Temperature: 33.1 - 33.9 °C; Relative humidity: 57.2 - 69.2 %; Photoperiod: 24 h darkness (diffuse artificial light only during handling and assessments).

Statistics: Results obtained from bees treated with the test item were compared to those obtained from the solvent control group. The LC_x and LDD_x values of the test item were determined by Weibull analysis using linear maximum likelihood regression. For each concentration the corrected mortality was calculated according to ABBOTT (1925) modified by SCHNEIDER-ORELLI (1947). The NOEC/NOEDD of the test item was estimated using Step-down Cochran-Armittage Test Procedure, one-sided greater, $\alpha = 0.05$. The statistical calculations were performed with the computer program ToxRat Professional 3.2.1 (2015).

Analytics: Samples for analysis were taken in duplicates on day 0 to day 9 from the treated feeding solutions per concentration and from the control feeding solution. The chemical analysis was performed by using High Performance Liquid Chromatograph (HPLC) – MS/MS detection.

Dates of work: March 3rd to March 13th, 2020, April 22nd, 2020 (analytical phase completion)

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II. RESULTS AND DISCUSSION

Analytical results:

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 SANTE/2020/12830, Rev.1.

The concentrations of fluopyram-benzamide were determined in feeding solutions of all treatment groups. The mean recoveries were between 99.8 % and 105 %. No residues of fluopyram-benzamide was detected in the control sample above 30 % of the Limit of Quantification (LOQ: 0.00990 mg p.m./kg).

Table 8.3.1.2- 4: Analytical results

Treatment group	Target concentration of Fluopyram-benzamide		Measured concentration of Fluopyram-benzamide	Mean recovery from target
	Daily dose [µg p m./bee/day] [A]	Concentration [mg p.m./kg diet]	[mg p.m./kg diet]	[%]
Control	-	-	< 30 % of LOQ	-
Test item	0.128	3.28	3.43	105
	0.319	8.19	9.78	101
	0.798	20.5	25.1	104
	1.99	51.2	60.3	99.8
	4.99	128	158	104
	12.5	320	389	103

LOQ (Limit of Quantification) for Fluopyram-benzamide = 0.00990 mg/kg
[A] Taking into account a mean uptake of feeding solution of 33 µL/bee/day

Biological results:

Summary of mean mortality and toxicity of Fluopyram-benzamide to adult honey bees after 10 days of chronic exposure:

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Table 8.3.1.2- 5: 10-day chronic oral toxicity test with Fluopyram-benzamide to young honey bees

Treatment group	Daily dose		Concen-tration	At day 10		
	Nominal	Consumed		Mean mortality	Number of bees with behavioural abnormalities [A]	Mean behavioural abnormalities
	[µg p.m./bee/day]		[mg p m./kg diet]	[%]	[No.]	[%]
Control	-	-	-	0.0	0 out of 30	0
Solvent control	-	-	-	0.0	0 out of 30	-
Test item	0.128	0.160	3.28	0.0	0 out of 30	0.0
	0.319	0.396	8.19	0.0	0 out of 30	0.0
	0.798	1.17	20.5	0.0	0 out of 30	0.0
	1.99	2.50	51.2	10.0*	0 out of 27	0.0
	4.99	6.34	128	20.0*	1 out of 24	4.2
	12.5	12.5	320	83.3*	0 out of 5	0
Reference Item	0.0273	0.0172	0.694	100	0 out of 0	0.0

	Endpoints	10 d
Test item doses	LDD_{50} [µg p m./bee/day] [B] (95 % C.I.)	8.58 (7.14 - 10.1)
	LDD_{20} [µg p.m./bee/day] [B] (95 % C.I.)	5.21 (3.62 - 6.40)
	LDD_{10} [µg p m./bee/day] [B] (95 % C.I.)	3.74 (2.23 - 4.91)
	NOEDD [µg p m./bee/day] [C]	1.17
Test item concentrations	LC_{50} [mg p m./kg feeding solution] [B] (95 % C.I.)	199 (160 - 241)
	LC_{20} [mg p m./kg feeding solution] [B] (95 % C.I.)	110 (73.9 - 140)
	LC_{10} [mg p m./kg feeding solution] [B] (95 % C.I.)	74.2 (42.5 - 102)
	NOEC [mg p.m./kg feeding solution] [C] (95 % C.I.)	20.5

Results are mean values of 3 replicates, containing 10 bees each; Calculations are performed with non-rounded values and

C.I.: Confidence interval

A Number of bees with behavioural abnormalities referring to number of remaining bees

B Lethal dietary dose/concentration (95%-cl lower/upper) were calculated by Weibull analysis using linear max. likelihood regression

C No observed effect dietary dose/concentration (NOEC/NOEDD) was calculated using Step-down Cochran-Armitage Test Procedure; α = 0.05; one sided greater

* Statistically significant difference in pairwise comparison between test item treatment and untreated solvent control group (Step-down Cochran-Armitage Test Procedure; α = 0.05; one-sided greater)

Behavioural abnormalities were observed at the two highest test levels (4.99 and 12.5 µg p.m./bee/day). Single bees were described as being affected in terms of uncoordinated movements on D7, D9 and D10. In the final assessment on the last day of the test, one out of 24 remaining bees was described as affected at the second highest test level (4.99 µg p.m./bee/day).

The daily food consumption was corrected by subtracting the mean evaporation figure of each day of application. The mean daily amount of evaporated feeding solution of the control and solvent control

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ranged between 50.0 - 65.3 mg per day per feeding tube and 82.3 - 109.7 mg per day per feeding tube, respectively. In the test item group bees effectively consumed doses of 12.5, 6.34, 2.50, 1.17, 0.396 and 0.160 µg p.m./bee/day which caused mortalities of 83.3, 20.0, 10.0, 0.0, 0.0 and 0.0 % respectively after 10 days. The obtained mortalities in the three highest test levels (12.5, 6.34 and 2.50 µg p.m./bee/day) were statistically significantly increased compared to the solvent control group. No mortality occurred in the control and solvent control group.

In the test item group the food consumption ranged between 39.1 and 56.9 mg solution per bee per day.

Reference item:

The reference item (dimethoate) was administered in one dosage of 0.0273 µg a.s./bee/day (actual average intake based on food consumption was 0.0172 µg a.s./bee/day) which caused a continuously increasing mortality leading to 100.0 % mortality at day 8.

Validity criteria:

All validity criteria of the current OECD Guideline 245 (2017) were met in this study.

Table 8.3.1.2- 6: Validity criteria

Validity criteria according to OECD GD 245 (2017)	Recommended		Obtained
Mortality after 10 days of exposure	Control	≤ 15 %	0.0 %
	Solvent control	≤ 15 %	0.0 %
Mortality after 10 days of exposure	Dimethoate	≥ 50 %	100 %

III. CONCLUSION

The chronic oral toxicity of Fluopyram-benzamide was tested on young adult honey bees (*Apis mellifera* L.) in a 10-day feeding study under laboratory conditions.

The LDD$_{50}$ and LC$_{50}$ were determined to be 8.58 µg p.m./bee/day and 199 mg p.m./kg feeding solution, respectively. The LDD$_{20}$ and LC$_{20}$ were determined to be 5.21 µg p.m./bee/day and 110 mg p.m./kg feeding solution, respectively. The LDD$_{10}$ and LC$_{10}$ were determined to be 3.74 µg p.m./bee/day and 74.2 mg p.m./kg feeding solution, respectively.

The NOEDD and NOEC were determined to be 1.17 µg p.m./bee/day and 20.5 mg p.m./kg food, respectively.

Assessment and conclusion by applicant:

The study and its data are considered as acceptable and reliable for use in risk assessment.

The endpoints are:

LDD$_{50}$ oral (10 days) = 8.58 µg p.m./bee/day

LC$_{50}$ oral (10 days) = 199 mg p.m./kg diet

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CA 8.3.1.3 Effects on honeybee development and other honeybee life stages

Laboratory studies

Active substance fluopyram

Data Point:	KCA 8.3.1.3/01
Report Author:	Oberrauch, S.
Report Year:	2018
Report Title:	Fluopyram - Honey bee (Apis mellifera L.) 22 day larval toxicity test (repeated exposure) - Final report -
Report No:	S17-03384
Document No:	M-617279-01-1
Guideline(s) followed in study:	OECD (2016): Series on Testing and Assessment Number 239: Guidance Document on Honey Bee (Apis mellifera) Larval Toxicity Test, Repeated Exposure Regulation (EC) No 1107/2009 (2009) Directive 2003-01 (Canada/PMRA) US EPA OCSPP 850.SUPP
Deviations from current test guideline:	Current Guidance Document: OECD 239 (2016) Deviations (≥ 2 hours) from the recommended humidity range of 50-80 % occurred on days 19 and 21. This deviation is not expected to have impacted the study results. All validity criteria were met.
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 ($ED_{50/20/10}$, $EC_{50/20/10}$, NOED/NOEC for adult emergence at day 22) of fluopyram applied to honey bee, *Apis mellifera carnica* P., larvae in an *in vitro* test after repeated exposure.

First instar honey bee larvae of *Apis mellifera carnica* P. were transferred from brood combs of 3 hives to polystyrene grafting cells in 48-well cell culture plates 2 days before the start of the exposure period (D1, grafting). Larvae were exposed to 5 concentrations of fluopyram concentrations (nominal 32.5, 65.0, 130, 260 and 520 mg a.s./kg diet; cumulative doses (nominal): 5.01, 10.0, 20.0, 40.0, 80.1 µg a.s./larva) via the larval diet on 4 consecutive days (D3 to D6). No additional feeding of the larvae took place after D6.

A blank control group (larval diet), a solvent control (larval diet with acetone) and a reference item group (dimethoate) were included in the experimental design.

The larval mortality was assessed daily from day 4 to day 8. Additionally, other observations such as small body size or unconsumed diet on D8 were noted. Pupal mortality was evaluated at day 15 and the adult emergence rate was assessed on day 22.

The purpose of the analytical part of this study was to verify the concentration of fluopyram in the larval diets.

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The cumulative NOEC for adult emergence on day 22 was determined as ≥ 520 mg a.s./kg diet, equivalent to a NOED of ≥ 80.1 µg a.s./larva per developmental period. The ED_{50} (based on adult emergence) was determined to be > 80.1 µg a.s./larva and the EC_{50} > 520 mg a.s./kg diet, respectively.

The study fulfils all validity criteria of the current OECD Guidance Document 239 (2016).

I. MATERIAL AND METHODS

Test item: Fluopyram; Specification No.: 102000017196; Batch No.: EDTE017337; purity: 99.72 %w/w.

Test species: Honey bee (*Apis mellifera carnica* P.), synchronized first instar (L1, one day old) larvae originating from three adequately fed, healthy, as far as possible parasite-free and queen-right colonies. The larvae were taken from hives that had not received treatments with chemical substances for at least one month.

Test concentrations and dose levels:

5 test item groups; concentrations (nominal): 520, 260, 130, 65.0 and 32.5 mg a.s./kg

Cumulative doses (nominal): 80.1, 40.0, 20.0, 10.0 and 5.01 µg a.s./larvae

One reference item group exposed to a cumulative dose of 7.39 µg dimethoate/larva (concentration of dimethoate: 48.0 mg a.s./kg diet).

One blank control group (untreated feeding diet) and solvent control (untreated feeding diet with 0.5 % acetone) was also assessed.

Each treatment group (test item, control, reference item) comprised 3 replicates, with 16 larvae each (each colony represented a replicate).

Test design: First instar honey bee larvae of *Apis mellifera carnica* P. were transferred from brood combs of 3 hives to polystyrene grafting cells in 48-well cell culture plates 2 days before the start of the exposure period (D1, grafting). From day 3 until day 6 of the test, 5 different concentrations of fluopyram mixed into the larval diet (aqueous yeast/sugar solution mixed with royal jelly 1:1 (w/w)) were fed to larvae of the test item groups. One single concentration of the reference item dimethoate mixed into the larval diet was fed to the larvae of the reference item group. A blank control (larval diet with water) and a solvent control (larval diet with 0.5 % acetone) were included in the experimental design. The volumes and contents of diets are presented in the table below.

Table 8.3.1.3- 1: Feeding scheme

Test day	1 [1]	2	3 [2]	4 [2]	5 [2]	6 [2]
Artificial diet	A	-	B	C	C	C
Volume of diet per larva	20 µL	-	20 µL	30 µL	40 µL	50 µL
Composition of diets:						
Royal jelly	50 % w/w	-	50 % w/w	50 % w/w		
Sugar solution	50 % w/w		50 % w/w	50 % w/w		
Composition of sugar solution:						
Glucose	12 % w/v	-	15 % w/v	18 % w/v		
Fructose	12 % w/v		15 % w/v	18 % w/v		
Yeast extract	2 % w/v		3 % w/v	4 % w/v		

[1] Day of grafting
[2] Days of exposure

The daily feeding volume increased from 20 µL to 50 µL diet per larva over the application period.

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After the applications, no additional feeding of the larvae took place.

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/mL) were considered for the calculation of the cumulative doses per larva.

Assessment of larval mortality was performed during the larval phase from day 4 until day 8. Pupal mortality was assessed at day 15 and emergence of adults was evaluated at day 22. The presence of unconsumed food was qualitatively recorded on day 8. Other observations and any other adverse effects were qualitatively recorded to aid in the interpretation of mortality in comparison to the solvent control group.

Test conditions: Temperature: 33.1 - 35.3 °C; relative humidity: day 1 to 8: 70.4 - 100 %, day 8 to 15: 59.1 - 88.9 %, day 15 to 22: 38.1 - 73.6 %; photoperiod: 24 h darkness (except during handling and assessments).

Statistics: For each concentration the cumulative mortalities were corrected for control mortality according to the formula of ABBOTT (1925), modified by SCHNEIDER-ORELLI (1947). Multiple Chi²-test with Bonferroni-Holm adjustment (one-sided greater, $\alpha = 0.05$) was used to evaluate whether there was a significant difference between the test item groups and the solvent control for larval mortality on day 8, larval and pupal mortality on day 15, pupal mortality from day 8 through 22 and of adult emergence on day 22. The EC_{10} and EC_{20} with 95 % confidence limits were calculated by probit analysis using linear max. likelihood regression. The calculation was performed using solvent control corrected percentage of non-emerged bees. The EC_{50} with 95 % confidence limits could not be calculated due to the lack of inhibition in emergence above 50 % but can be regarded as above the highest concentration tested. The statistical calculations were performed with the computer program ToxRat Professional 3.2.1.

Analytics: All final diets of the control and test item treatment group were sampled in duplicate as analysis and retain samples directly from the prepared diet. The chemical analysis was performed by using Reversed Phase High Performance Liquid Chromatograph (RP-HPLC) with MS/MS detection.

Dates of work: July 3rd to December 8th, 2017

II. RESULTS AND DISCUSSION

Analytical results:

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 SANTE/2020/12830, Rev.1.

The analytical dose verification of the larval diet from day 3 until day 6 resulted in mean measured recoveries of 93 - 115 %. In the control samples, the concentration of fluopyram was below 30 % of the Limit of Quantification (LOQ: 0.90 mg/kg).

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.

Table 8.3.1.3- 2: Analytical results

Treatment group	Nominal conc. of fluopyram* [mg a.s./kg diet]	Sampling Time	Measured conc. of fluopyram [mg a.s./kg diet]	Recovery from target [%]	Mean recovery from target [%][A]
Control	0.0	D 3	< LOD	-	-
		D 4	< LOD	-	
		D 5	< LOD	-	
		D 6	< LOD	-	
Solvent control	0.0	D 3	< LOD	-	-
		D 4	< LOD	-	
		D 5	< LOD	-	
		D 6	< LOD	-	
Test item	32.5	D 3	34.1	105	115
		D 4	36.3	112	
		D 5	36.2	111	
		D 6	42.4	130	
	65.0	D 3	68.2	105	104
		D 4	71.8	110	
		D 5	67.0	103	
		D 6	63.4	98	
	130	D 3	135	104	105
		D 4	138	106	
		D 5	142	109	
		D 6	133	102	
	260	D 3	289	111	101
		D 4	260	100	
		D 5	246	95	
		D 6	256	98	
	520	D 3	501	96	93
		D 4	567	109	
		D 5	411	79	
		D 6	451	87	

Limit of Detection (LOD) = 30 % of Limit of Quantification (LOQ: 0.900 mg a.s./kg diet)

A Based on the analysed purity of the test item according to the certificate of analysis (99.72 % w/w).

Biological results:

On day 8, larval mortality was 14.6 % in the control group, 14.6 % in the solvent control group and 95.8 % in the reference item group. The larval mortality was 0.0, 4.2, 4.2, 12.5 and 22.9 % in the test item groups of 32.5, 65.0, 130, 260 and 520 mg fluopyram/kg diet, respectively.

On day 22, the adult emergence rate was 75.0 % in the control group and 70.8 % in the solvent control group. The adult emergence rates were 85.4, 79.2, 85.4, 68.8 and 56.3 % in the test item groups of 32.5, 65.0, 130, 260 and 520 mg a.s./kg diet, respectively.

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 group, in the test item groups of 32.5, 130, 260 and 520 mg fluopyram/kg diet, as well as in the reference item group.

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Table 8.3.1.3- 3: Mortality of larvae and adult emergence in the repeated exposure toxicity test

Treatment group	Cumulative dose (nominal)	Concentration (nominal)	Day 8		Day 22	
			Larval mortality Day 3 - 8		Adult Emergence rate	
			abs.	corr.	actual [A]	Inhibition [B]
	[µg a.s./ larva] [C, D]	[mg a.s./ kg diet] [C]	[%]		[%]	
Control	-	-	14.6	-	75.0	-
Solvent control	-	-	14.6	-	70.8	-
Test item (Fluopyram)	5.01	32.5	0.0	- 17.1	85.4	- 20.6
	10.0	65.0	4.2	- 12.2	79.2	- 11.9
	20.0	130	4.2	- 12.2	85.4	- 20.6
	40.0	260	12.5	- 2.5	68.8	2.8
	80.1	520	22.9	9.7	56.3	20.5
Reference Item (Dimethoate)	7.39	48.0	95.8	95.1		-

Results are mean values of 3 replicates (hives), containing 16 larvae each.

corr.: corrected mortality (according to ABBOTT (1925), MODIFIED BY SCHNEIDER-ORELLI (1947)): mortality in test and reference item treated groups were corrected by the mortality of the control

[A] statistical evaluation for non-emergence

[B] Compared to solvent control. 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

Table 8.3.1.3- 4: Calculated endpoints of the repeated exposure larvae toxicity test

Treatment	Endpoint: Adult emergence at day 22	
Test item cumulative doses [µg a.s./larva per development period] [CD]	ED_{50} (95 % C.I.) [A]	> 80.1
	ED_{20} (95 % C.I.)	78.7 (63.3 - 123)
	ED_{10} (95 % C.I.)	60.1 (41.0 - 75.5)
	LOED [B]	n.d.
	NOED	≥ 80.1
Test item concentrations [mg a.s./kg diet] [C]	EC_{50} (95 % C.I.) [A]	> 520
	EC_{20} (95 % C.I.)	511 (411 - 800)
	EC_{10} (95 % C.I.)	390 (266 - 490)
	LOEC [B]	n.d.
	NOEC	≥ 520

C.I. = Confidence interval

[A] The EC_{50}/ED_{50} could not be determined (n.d.) due to the lack of inhibition in emergence above 50 %, but can be regarded as above the highest concentration/dose tested[1]

[B] The LOEC/LOED could not be determined due to the lack of statistically significant differences compared to the solvent control (Multiple Chi²-test with Bonferroni-Holm adjustment, one-sided greater, $\alpha = 0.05$)

[C] Based on the analysed purity

[D] Based on the cumulative feeding volume from day 3 until day 6 of 140 µL

Validity criteria:

All validity criteria of the OECD Guidance Document 239 (2016) were met.

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Table 8.3.1.3- 5: Validity criteria

Validity criteria acc. to OECD TG 239 (2016)	Recommended		Obtained
Larval mortality between day 3 and day 8 in the control group (across all replicates)	Control	≤ 15 %	14.6 %
	Solvent control	≤ 15 %	14.6 %
Adult emergence rate until day 22 in the control group (across all replicates)	Control	≥ 70 %	75.0 %
	Solvent control	≥ 70 %	70.8 %
Larval mortality between day 3 and day 8 in the reference group (across all replicates)	Dimethoate	≥ 50 %	95.8 %

III. CONCLUSION

In a repeated exposure larval toxicity study performed in a dose-response design with fluopyram and a duration of 22 days, the NOEC for adult emergence on day 22 was determined as ≥ 520 mg a.s./kg diet, equivalent to a NOED of ≥ 80.1 µg a.s./larva per developmental period.

The ED_{50}, ED_{20} and ED_{10} values (based on adult emergence) were determined to be > 80.1 µg, 78.7 µg and 60.1 µg a.s./larva, respectively. The EC_{50}, EC_{20} and EC_{10} values were determined to be > 520 mg, 511 mg and 390 mg a.s./kg diet, respectively.

Assessment and conclusion by applicant:

The study and its data are considered as acceptable and reliable for use in risk assessment.

The endpoints are:

NOED - emergence (22 days) ≥ 80.1 µg a.s./larva

NOEC - emergence (22 days) ≥ 520 mg a.s./kg diet

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Metabolite fluopyram-benzamide

Data Point:	KCA 8.3.1.3/02
Report Author:	
Report Year:	2020
Report Title:	AE C656948-benzamide - Repeated exposure to honey bee larvae (Apis mellifera L.) under laboratory conditions
Report No:	19 48 BLC 0049
Document No:	M-704606-01-1
Guideline(s) followed in study:	Regulation (EC) No 1107/2009 (2009) Directive 2003-01 (CANADA/PMRA) US EPA OCSPP 850.SUPP OECD Guidance Document 239 (2016)
Deviations from current test guideline:	Current Guidance Document: OECD 239 (2016) Deviations: None. All validity criteria were met.
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 ($ED_{50/20/10}$, $EC_{50/20/10}$, NOED/NOEC for adult emergence at day 22) of fluopyram-benzamide applied to honey bee, *Apis mellifera* L., larvae in an *in vitro* test after repeated exposure.

First instar honey bee larvae of *Apis mellifera L.* were transferred from brood combs of 3 hives to polystyrene grafting cells in 48-well cell culture plates 2 days before the start of the exposure period (D1, grafting). Larvae were exposed to 6 concentrations of fluopyram-benzamide (nominal 79.3, 31.7, 12.7, 5.1, 2.0 and 0.8 mg p.m./kg diet; corresponding to nominal cumulative doses of 12.6, 5.0, 2.0, 0.80, 0.32 and 0.13 µg p.m./larva, respectively) via the larval diet on 4 consecutive days (D3 to D6). No additional feeding of the larvae took place after D6.

A blank control group (larval diet), a solvent control (larval diet with 0.5 % v/v acetone) and a reference item group (dimethoate) were included in the experimental design.

The larval mortality was assessed daily from day 4 to day 8. Additionally, other observations such as small body size or unconsumed diet on D8 were noted. Pupal mortality was evaluated at day 15 and the adult emergence rate was assessed on day 22.

The purpose of the analytical part of this study was to verify the concentration of fluopyram-benzamide in the larval diets. The mean recovery for fluopyram-benzamide ranged between 84.4 and 90.7 % in the final diets.

The NOED and LOED were determined to be 2.0 µg and 5.0 µg p.m./larva (based on adult emergence), respectively. The NOEC and LOEC were 12.7 mg and 31.7 mg p.m./kg diet, respectively. The ED_{50}, ED_{20} and ED_{10} values (based on adult emergence) were determined to be >12.6 µg, 6.5 µg and 2.1 µg p.m./larva, respectively. The EC_{50}, EC_{20} and EC_{10} values were determined to be >79.3 mg, 41.1 mg and 13.0 mg p.m./kg diet, respectively.

The study fulfils all validity criteria of the current OECD Guidance Document 239 (2016).

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I. MATERIAL AND METHODS

Test item: Fluopyram-benzamide (AE C656948-benzamide); Origin batch no.: B26F, Batch code: AE F148815-PU-02; purity: 99.4 % w/w

Test species: Honey bee (*Apis mellifera* L., subspecies *iberiensis*), synchronized first instar (L1, one day old) larvae originating from three adequately fed, healthy, as far as possible parasite-free and queen-right colonies. The larvae were taken from hives that had not received treatments with chemical substances for at least one month.

Test concentrations and dose levels:

5 test item groups; concentrations (nominal): 79.3, 31.7, 12.7, 5.1, 2.0 and 0.8 mg p.m./kg diet

Cumulative doses (nominal): 12.6, 5.0, 2.0, 0.80, 0.32 and 0.13 µg p.m./larva

One reference item group exposed to a cumulative dose of 7.6 µg dimethoate/larva (concentration of dimethoate: 48.0 mg a.s./kg diet).

One blank control group (untreated feeding diet) and solvent control (untreated feeding diet with 0.5% v/v acetone) was also assessed.

Each treatment group (test item, control, reference item) comprised 3 replicates, with 12 larvae each (each colony represented a replicate).

Test design: First instar honey bee larvae of *Apis mellifera* L. were transferred from brood combs of 3 hives to polystyrene grafting cells in 48-well cell culture plates 2 days before the start of the exposure period (D1, grafting). From day 3 until day 6 of the test, 5 different concentrations of fluopyram-benzamide mixed into the larval diet (aqueous yeast/sugar solution mixed with royal jelly 1:1 (w/w)) were fed to larvae of the test item groups. One single concentration of the reference item dimethoate mixed into the larval diet was fed to the larvae of the reference item group. A blank control (larval diet with water) and a solvent control (larval diet with 0.5% v/v acetone) were included in the experimental design. The volumes and contents of diets are presented in the table below.

Table 8.3.1.3- 6: Feeding scheme

Test day	**1** [1]	**2**	**3** [2]	**4** [2]	**5** [2]	**6** [2]
Artificial diet	A	-	B	C	C	C
Volume of diet per larva	20 µL	-	20 µL	30 µL	40 µL	50 µL
Composition of diets: **Royal jelly** **Sugar solution**	 50 % w/w 50 % w/w	-	 50 % w/w 50 % w/w	 50 % w/w 50 % w/w		
Composition of sugar solution: **Glucose** **Fructose** **Yeast extract**	 12 % w/v 12 % w/v 2 % w/v	-	 15 % w/v 15 % w/v 3 % w/v	 18 % w/v 18 % w/v 4 % w/v		

[1] day of grafting
[2] days of exposure

The daily feeding volume increased from 20 µL to 50 µL diet per larva over the application period. After the applications, no additional feeding of the larvae took place.

The cumulative feeding volume from day 3 until day 6 of 140 µL diet per larva and the density of the diet (1.13 g/mL) were considered for the calculation of the cumulative doses per larva.

Assessment of larval mortality was performed during the larval phase from day 4 until day 8. Pupal

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mortality was assessed at day 15 and emergence of adults was evaluated at day 22. The presence of unconsumed food was qualitatively recorded on day 8. Other observations and any other adverse effects were qualitatively recorded to aid in the interpretation of mortality in comparison to the solvent control group.

Test conditions: Temperature: 34.1 – 34.9°C; relative humidity: day 1 to 8: 90 - 100 %. day 8 to 15: 77 - 85 %, day 15 to 22: 62 - 69 %; photoperiod: 24 h darkness (except during handling and assessments).

Statistics: For each concentration the cumulative mortalities were corrected for control mortality according to the formula of ABBOTT (1925), modified by SCHNEIDER-ORELLI (1947). The Step-down Cochran-Armitage Test was used for statistical analysis of the adult emergence data and the estimation of the NOEC/NOED and LOEC/LOED. The accepted significance level was $\alpha = 0.05$ (one-sided greater). The $ED/EC_{10/20/50}$ values were determined with the Weibull analyses using linear maximum likelihood regression. The statistical calculations were performed with the computer program ToxRat Professional 3.3.0.

Analytics: All final diets of the solvent control and test item treatment group were sampled in duplicate as analysis and retain samples directly from the prepared diet on day 3 to day 6. The chemical analysis was performed by using Reversed Phase High Performance Liquid Chromatograph (RP-HPLC) with MS detection.

Dates of work: November 18th to December 9th, 2019; April 08th 2020 (analytical phase completion)

II. RESULTS AND DISCUSSION

Analytical results:

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 SANTE/2020/12830, Rev.1.

The analytical dose verification of the larval diet from day 3 until day 6 resulted in mean measured recoveries of 84.8 – 90.7 %. In the control samples, the concentration of fluopyram- benzamide was below 30 % of the Limit of Quantification (LOQ: 0.01 mg a.s./kg).

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Table 8.3.1.3- 7: Analytical results

Treatment group	Nominal conc. of fluopyram-benzamide [mg p m./kg diet]	Sampling time	measured conc. of fluopyram-benzamide [mg p m./kg diet]	Mean measured conc. of fluopyram-benzamide [mg p m./kg diet]	Mean recovery from target [%]
Solvent control	0.000	D 3	< 30 % of LOQ	< 30 % of LOQ	-
		D 4	< 30 % of LOQ		
		D 5	< 30 % of LOQ		
		D 6	< 30 % of LOQ		
Test item	79.3	D 3	68.0	67.9	85.6
		D 4	67.6		
		D 5	69.4		
		D 6	66.6		
	31.7	D 3	27.8	27.6	87.0
		D 4	27.1		
		D 5	27.1		
		D 6	28.5		
	12.7	D 3	10.6	11.3	89.3
		D 4	11.2		
		D 5	11.4		
		D 6	12.1		
	5.08	D 3	4.39	4.31	84.8
		D 4	4.30		
		D 5	4.16		
		D 6	4.38		
	2.03	D 3	2.00	1.83	90.2
		D 4	1.92		
		D 5	1.67		
		D 6	1.74		
	0.812	D 3	0.800	0.737	90.7
		D 4	0.792		
		D 5	0.688		
		D 6	0.688		

LOQ: Limit of Quantification (LOQ: 0.01 mg a.s./kg)

Biological results:

On day 8, larval mortality was 2.8 % in the control group, 5.6 % in the solvent control group and 55.6 % in the reference item group. In the test item treatment groups, no larval mortality was observed on day 8 following a treatment with 12.6, 5.0, 2.0, 0.80, 0.32 and 0.13 mg p.m./kg larva, respectively.

On day 22, the adult emergence rate was determined to be 77.8% and 75.0 % in the control and solvent control group, respectively. In the test item treated group the adult honey bees emerged at rates of 55.6 %, 61.1 %, 66.7 %, 66.7 %, 75.0 % and 77.8% exposed to a cumulative dose 12.6, 5.0, 2.0, 0.80, 0.32 and 0.13 µg p.m./larva, respectively, during the larval stages. On day 22, larvae treated with 12.6 or 5.0 µg p.m./larva, showed an emergence rate, which was statistically significantly different compared to the control.

On day 8, none of the remaining larvae treated with the test item were observed to have food left and/or a smaller body size.

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Table 8.3.1.3- 8: Mortality of larvae and adult emergence in the repeated exposure toxicity test

Treatment group	Cumulative dose (nominal)	Concen-tration (nominal)	Day 8			Day 22		
			Larval mortality Day 3 - 8		Mean OO	Total mortality Day 3- 22		Adult emergence rate
			abs.	corr.		abs.	corr.	abs.
	[µg p m./larva]	[mg p m./ kg diet]	[%]		[%]	[%]		[%]
Control	-	-	2.8	0.0	0.0	22.2	0.0	77.8
Solvent control	-	-	5.6	0.0	0.0	25.0	0.0	75.0
Test item (Fluopyram-benzamide)	12.6	79.3	0.0	0.0	0.0	44.4	25.9	55.6 *
	5.0	31.7	0.0	0.0	0.0	38.9	18.5	61.1 *
	2.0	12.7	0.0	0.0	0.0	33.3	11.1	66.7
	0.80	5.1	0.0	0.0	0.0	33.3	11.1	66.7
	0.32	2.0	0.0	0.0	0.0	25.0	0.0	75.0
	0.13	0.8	0.0	0.0	0.0	22.2	0.0	77.8
Reference Item (Dimethoate)	[µg a.s./larva]	[mg a.s./ kg diet]	[%]		[%]	[%]		[%]
	7.6 [A]	48.0 [B]	55.6	52.9	0.0	88.9	85.2	11.1

Results are mean values of 3 replicates (hives), containing 16 larvae each.
corr.: corrected mortality (according to SCHNEIDER-ORELLI (1947)): mortality in test and reference item treated groups were corrected by the mortality of the solvent control
abs.: absolute mortality as counted from the results; calculation were performed with non-rounded values
OO: Other observations (e.g. remaining food); negative values were set to "0"
[A] For the reference item, the value indicates the amount of active ingredient (dimethoate) in µg/larvae.
[B] For the reference item, the value indicates the amount of active ingredient (dimethoate) in mg/kg diet.
* Statistically significant difference compared to control (Step-down Cochran-Armitage Test; p≤ 0.05; one sided greater)

Table 8.3.1.3- 9: Calculated endpoints of the repeated exposure larvae toxicity test

Treatment	Endpoint: Adult emergence at day 22	
Test item cumulative doses [µg p m./larva]	ED_{50} (95 % C.I.)	> 12.6 (n.d.)
	ED_{20} (95 % C.I.) [B]	6.5 (3.4 – 12.4)
	ED_{10} (95 % C.I.) [B]	2.1 (1.0 – 4.3)
	LOED [A]	5.0
	NOED [A]	2.0
Test item concentrations [mg p.m./kg diet]	EC_{50} (95 % C.I.)	> 79.3
	EC_{20} (95 % C.I.) [B]	41.1 (21.5 – 78.4)
	EC_{10} (95 % C.I.) [B]	13.0 (6.2 – 27.0)
	LOEC [A]	31.7
	NOEC [A]	12.7

C.I.: Confidence interval
n.d.: Not determined
[A] Step-down Cochran-Armitage Test; α=0.05; one-sided greater
[B] Weibull analyses using linear maximum likelihood regression

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Validity criteria:

All validity criteria of the OECD Guidance Document 239 (2016) were met.

Table 8.3.1.3- 10: Validity criteria

Validity criteria acc. to OECD TG 239 (2016)	Recommended		Obtained
Larval mortality between day 3 and day 8 in the control group (across all replicates)	Control	≤ 15 %	2.8 %
	Solvent control	≤ 15 %	5.6 %
Adult emergence rate until day 22 in the control group (across all replicates)	Control	≥ 70 %	77.8 %
	Solvent control	≥ 70 %	75.0 %
Larval mortality between day 3 and day 8 in the reference group (across all replicates)	Dimethoate	≥ 50 %	55.6 %

III. CONCLUSION

In a repeated exposure larval toxicity study performed in a dose-response design with fluopyram-benzamide, the NOED and LOED were determined to be 2.0 µg and 5.0 µg p.m./larva (based on adult emergence), respectively. The NOEC and LOEC were 12.7 mg and 31.7 mg p.m./kg food, respectively.

The ED_{50}, ED_{20} and ED_{10} values (based on adult emergence) were determined to be > 12.6 µg, 6.5 µg and 2.1 µg p.m./larva, respectively. The EC_{50}, EC_{20} and EC_{10} values were determined to be > 79.3 mg, 41.1 mg and 13.0 mg p.m./kg food, respectively.

Assessment and conclusion by applicant:

The study and its data are considered as acceptable and reliable for use in risk assessment.

The endpoints are:

NOED - emergence (22 days) = 2.0 µg p.m./larva

NOEC - emergence (22 days) = 12.7 mg p.m./kg diet

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Higher-tier studies

Data Point:	KCA 8.3.1.3/03
Report Author:	[redacted]
Report Year:	2016
Report Title:	Honey bee colony feeding study, evaluating the effects of fluopyram-fortified sugar- and pollen diet on the development of honey bee colonies under confined semi-field conditions, followed by a post-exposure field observation period
Report No:	E 319 4525-9
Document No:	M-549350-01-2
Guideline(s) followed in study:	US EPA OCSPP Guideline No. 850.SUPP Special design study (internal testing method). There is no guideline available
Deviations from current test guideline:	Current Guidance Document: No Guidance Document available; Special study design; Deviations 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

This study was designed to determine potential adverse acute, short-term and long-term effects on honey bees (*Apis mellifera* L.) and honey bee colonies, during and after the continuous exposure to fluopyram-fortified carbohydrate and protein diet.

The exposure part of the study was conducted by exposing 10 honey bee colonies in gauze tunnels exclusively to fluopyram-fortified diet or untreated control diet for a period of two complete honey bee brood cycles (6 weeks, 42 days), after the colonies were allowed to acclimate to the confined conditions and the feeding regime for a period of 1 week (7 days); during this 7-weeks lasting confinement period, mortality, foraging activity, behaviour, colony- and brood development, colony health as well as food consumption and food storage were assessed regularly.

Thereafter, the honey bee colonies were released from confinement and allowed to forage freely (post-exposure field monitoring period); during this period, the colonies were maintained according to Good Apicultural Practice and the development of the colonies as well as their overall health status were assessed in regular intervals throughout the remainder of the season until overwintering. The study continued until the assessment of the overwintering performance in the following spring.

The experiment comprised five blocks, each consisting of two differently treated plots - one untreated control (C) and one test item treatment group (T; 10000 µg fluopyram/kg diet), resulting in total in 10 plots. Each plot consisted of a pre-installed gauze tunnel, which harboured one randomly assigned honey bee colony at the beginning of the confinement period, resulting in 5 replicates per exposure group. The gauze tunnels were erected on perennial ryegrass, regularly cut short inside the tunnels, which effectively prevented the presence of any flowering weeds. Colonies were provided with both pollen and sugar diet to mediate exposure. The pollen dough was provided inside the respective hives, whereas the solid sugar diet was offered in Petri dishes outside the hive, placed about 1 m above the ground, via three separate feeders.

No test-item related adverse effects on the mortality of worker bees, drones and immature honey bee life stages were found. No test-item related adverse effects on foraging activity and on behaviour were observed during the entire course of the study. There were no observable effects on overall colony development, development of brood and colony strength.

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The purpose of the analytical part of this study was to determine the residues of fluopyram in sugar syrup diet and pollen diet. Chemical analysis of the different batches of control and fluopyram-fortified diets revealed that the actual test item concentration was well in accordance to the nominal test item concentration. No detectable residues of fluopyram were found in any of the control diets.

Based on the results of the study, it can be concluded that a continuous exposure of honey bee colonies under confined conditions to a fluopyram concentration of 10000 µg a.s./kg diet has no adverse acute, short-term and long-term effects on mortality, colony strength and -development, brood development, food storage, honey bee behaviour, queen survival, overall hive vitality and colony health, as well as on overwintering performance.

As this was a special design study (internal testing method), no guideline was available.

I. MATERIAL AND METHODS

Test item: Fluopyram tech., Specification No.: 102000017196, Batch ID: PFV127P012, Sample description: TOX09909-00; purity: 97.7 % w/w.

Test species: Honey bees (*Apis mellifera* L.); bee colonies, maintained according to normal beekeeping practice. The preliminary brood check indicated healthy, queen-right colonies, with all brood stages present and a sufficient amount of pollen and honey to guarantee colony viability. The mean strength of the colonies of the test item treatment group and the control group, one day before confined exposure, was similar and ranged between 5175 ± 983.7 and 5700 ± 442.8 adult bees per colony.

Treatment groups:

- Control: five tunnels (with one colony per tunnel) with untreated sugar- and pollen diet (serving as controls)
- Test item treatment group: five tunnels (with one colony per tunnel) treated with fluopyram-fortified solution in ethanol sugar- and pollen diet (10000 µg fluopyram/kg diet).

Test design: This study was designed to determine potential adverse acute, short-term and long-term effects on honey bees (*Apis mellifera* L.) and honey bee colonies, during (exposure part) and after the continuous exposure (post-exposure field monitoring period) to fluopyram-fortified carbohydrate and protein diet.

The exposure part of the study was conducted by exposing 10 honey bee colonies in gauze tunnels exclusively to fluopyram-fortified diet or untreated control diet for a period of two complete honey bee brood cycles (6 weeks or 42 days), after the colonies were allowed to acclimate to the confined conditions and the feeding regime for a period of 1 week (7 days).

The confined exposure part of the study was conducted by following a full factorial randomized block design. The experiment comprised 10 plots (five blocks, each consisting of two plots - one untreated control (C) and one test item treatment group (T, 10000 µg fluopyram/kg diet). Each plot consisted of a pre-installed gauze tunnel (20 m x 5 m x 3 m), which harboured one randomly assigned honey bee colony at the beginning of the confinement period, resulting in 5 replicates per exposure group. The gauze tunnels were erected on perennial ryegrass, regularly cut short inside the tunnels, which effectively prevented the presence of any flowering weeds.

All honey bee test colonies were prepared at the same time and equalised for adult worker bees, brood and food stores as reasonably possible. At the beginning of the test, each colony contained one laying sister-queen and five occupied frames (4 brood frames, 1 bee-bread (pollen) and honey frame, just enough, to maintain colony vitality throughout the acclimation period) with about 3750 honey bees, in a hive with one brood chamber. Additionally three empty frames were added to each hive in order to permit colony growth.

Throughout the entire confined acclimation (1 week) and subsequent exposure period (6 weeks), the

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honey bee colonies received *ad libitum* solid "sugar diet" (commercial sucrose with commercial syrup (73 % w/w sugar; 1:1:1 sucrose, glucose and fructose), for carbohydrate supply) and a "pollen diet" (fresh multi-flora pollen with the commercial syrup, for protein supply) and had further *ad libitum* access to tap water. Sugar diet and pollen diet were replenished three times a week and the remainder of the respective diet was re-weighed to determine the actual food consumption of the colonies. The pollen dough was provided inside the respective hives, whereas the solid sugar diet was offered in Petri dishes outside the hive, placed about 1 m above the ground, via three separate feeders. During periods where honey bees did not sufficiently forage on the sugar diet and/or during periods of elevated carbohydrate demand (e.g. spells of cold or rainy weather, etc.) - treatment-specific supplemental sugar syrup (250 – 500 mL/colony) was placed inside the colonies.

The following parameters were assessed daily during the confined exposure period: mortality (assessed on each of the 7 days of acclimation and over the entire 42 day exposure period) and foraging activity (assessed twice daily for a period of 3 minutes, with a 5 minute break between counts at each of the 3 feeders/tunnel).. Mortality was assessed in front of the hive as well as on the sheets along the tunnel walls. Dead honey bees were separated in dead worker bees, drones and immature stages (i.e. larvae and pupae).

In addition, colony strength, brood development, colony health (visual symptoms of parasites/diseases) as well as food storage were assessed weekly. During each colony assessment, the absolute number of adult honey bees, the area-percentage of eggs, larvae and pupae covering the comb cell area per comb side, and the absolute number of food (nectar/pollen) cells was estimated. Food consumption was measured thrice a week, i.e. whenever fresh diet was provided. Whenever observed, behavioural anomalies were recorded. Colony weight was assessed at the start of the confined exposure period and immediately following the end of confinement.

In the post-exposure field monitoring period, the honey bee colonies were released from confinement and allowed to forage freely. The colonies were maintained according to Good Apicultural Practice and the development of the colonies (colony strength, brood development, food storage) as well as their overall health status were assessed at three-week intervals throughout the remainder of the season until overwintering. The study continued until the assessment of the overwintering performance in the following spring.

Test conditions: Natural field conditions. Climatic conditions were obtained from the permanently installed weather station (< 1 km distance to the tunnels) throughout the confined exposure period of the study. In addition, the actual temperature inside the gauze tunnels was recorded by a calibrated data logger during the entire exposure period. From the beginning of the post-observation field monitoring period (including the overwintering period) to the end of the study climatic data were obtained from the weather station (approx. 5 km distance to the post-exposure field monitoring location).

Statistics: The effects of fluopyram were assessed by comparing the data from the test item treatment group with the data of the control group. Linear mixed effects models were used to evaluate the potential effect of test item, the effect of the different honey bee colonies (hives) was added as an error term. Count data (i.e. mortality, colony parameter, estimates of bees at the tunnel/feeder) was log (decadic logarithm) transformed (log x + 1) in order to achieve normal distribution of residuals (homoscedasticity). ANOVA was performed for the fitted model in order to detect the influence of factors and interaction on the encountered variance. A significance level of $\alpha = 0.05$ was selected.

All statistical analyses were performed with the statistical software package R 3.1.3 (R Development Core Team, 2015).

Analytics: In order to determine the homogeneity of the individual treatment levels and to determine the actual exposure concentration, samples of about 2.5 g of sugar diet were taken out of eight arbitrarily selected plastic containers per batch from each of the 6 test item treatment and 10 control batches. The samples were immediately placed in a deep-freezer until analysis. Similarly, samples of about 2.5 g pollen diet were taken out of eight arbitrarily selected portions per batch in both treatments and control

and stored deep-frozen until chemical residue analysis. In addition, samples of about 2.5 g of sugar syrup diet were taken out of four arbitrarily selected plastic containers per batch from each of the 6 test item treatment and 5 control batches. Analysis of fluopyram and its metabolites (fluopyram-benzamide and fluopyram-pyridyl-acetic acid) in the respective honey bee diets (i.e. sugar syrup diet, sugar diet and pollen diet) was performed by using High Performance Liquid Chromatography (HPLC), coupled with electrospray and tandem mass spectrometry (MS/MS) detection.

Dates of work: May 03rd, 2013 to March 10th, 2014.

II. Results and Discussion

Analytical results:

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 SANTE/2020/12830, Rev.1.

No detectable residues of fluopyram and its metabolites (fluopyram-benzamide and fluopyram-pyridyl-acetic acid) were found in any of the batches of the control sugar syrup-, control sugar- and the control pollen diet, respectively (i.e. residues were always below the Limit of Detection, i.e. < 3 µg/kg). Thus, all employed batches of control honey bee diets were free of residues.

The average actual fluopyram concentration (in terms of % of nominal) in individual batches of spiked sugar syrup diet ranged from 84 – 103 %, the average actual fluopyram-concentration in individual batches of spiked sugar diet ranged from 86 – 103 % and from 95 – 99 % in the spiked pollen diet, respectively. The relative standard deviation (RSD) values were always below 20 %. The overall average actual concentrations of fluopyram (in terms of % of nominal) in spiked sugar syrup, sugar- and pollen diet were 95 %, 97 % and 96 %, respectively. Thus, the actual fluopyram concentration in fortified, freshly prepared honey bee diets (i.e. in the sugar syrup-, sugar- and pollen-diet, respectively) was well in-line with the nominal treatment level. Moreover, the analysis indicated that fluopyram was homogenously distributed within the respective honey bee diets.

Biological results:

Consumption of sugar- and pollen diet during confinement

The amount of consumed sugar- and pollen diet was calculated from the weight-difference of the Petri dishes or provided pollen-patties, respectively, in the respective exposure groups (C, T) between two subsequent feeding events (“feeding interval”).

On several instances (days) the consumption of sugar diet was low in both the control and the test item treatment groups due to rainy and/or cold weather which prevented honey bees from leaving the hive and foraging for food. However, additional in-hive feeding with sugar syrup assured sufficient carbohydrate supply of the colonies in C and T and also continuous exposure to the test item (in the treatment group T). Statistical analysis (linear mixed effects model) revealed no significant effect of the test-item treatment on the consumption of sugar diets ($p = 0.789$). A significant effect of the date was found ($p = < 0.001$), the interaction of date and treatment was not significant ($p = 0.240$), indicating that the consumption of sugar diets changed over time, but in the same manner for treatment and control, respectively.

The consumption of pollen diet was very similar for control and treatment throughout the confinement period, indicating that the confined colonies readily accepted the spiked pollen as a protein source. For both groups the consumption of pollen increased over time, as the growing colonies had an increased demand for pollen as food for larvae. Statistical analysis (linear mixed effects model) revealed no significant effect of the treatment on consumption of pollen diet ($p = 0.433$). A statistically significant

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effect of the date was found (p = < 0.001), the interaction of date and treatment was not significant (p = 0.572), indicating that the consumption of pollen diets changed over time, but in the same manner for T and C, respectively.

Thus, there was no evidence that the test item treatment had any adverse effects on the pollen- and sugar diet consumption of the honey bee colonies and as such, the treated diets provided a continuous and exhaustive exposure to fluopyram.

Mortality of the adult bees

Worker bees

No increase in mortality in the test item treatment group was recorded after the switch from acclimation to exposure. Mortality increased towards the end of the study in both the control and the test item treatment groups. This increase in total number of dead bees is to be expected and a direct consequence of the increased colony strength. Overall, no statistically significant differences in worker bee mortality between control and test item treatment groups were observed either in front of the hive (p = 0.282) or at the tunnel walls (p = 0.369) were observed. A significant effect of the date was found (p < 0.001), the interaction of date and treatment was not significant (p= 1.000 for mortality in front of the hive; p = 0.969 for mortality at the tunnel walls), indicating that mortality changed over time, but in the same manner for treatment and control, respectively. Thus

Drones

Throughout the entire confined exposure period, when combining the number of dead drones in front of the hive and at the tunnel walls, there were no distinct differences in drone mortality between control and test item treatment throughout the entire confined exposure period.

Immature life stages

Throughout the entire confined exposure period, when combining the number of dead immature life stages in front of the hive and at the tunnel walls, there were no distinct differences in mortality of immature life stages between control and test item treatment throughout the entire confined exposure period.

Thus, no test-item related adverse effects on the mortality of worker bees, drones and immature honey bee life stages were found.

Foraging Activity

The same pattern in foraging activity was observed in both the test item treatment group and the control group throughout the entire course of the confinement period. Statistical analysis (linear mixed effects model) revealed no significant effect of the test-item treatment on foraging activity (p= 0.646) or on the number of bees on the tunnel walls (p = 0.654). A significant effect of the date was found (p = < 0.001), however, the interaction of date and treatment was not significant (p = 0.852 for foraging activity; p = 0.977 for bees on tunnel walls), indicating that both foraging activity and the number of bees on the tunnel wall changed over time, but in the same manner for T and C, respectively.

Thus, no test-item related adverse effects on foraging activity were found; moreover, there was also no evidence that the test item had any repellent or disorientating effect, which would have resulted in increased numbers of bees at the tunnel walls.

Behavioural abnormalities

In both, test-item treatment and control, respectively, no aggressive or any other abnormal behaviour of the bees was noted either during the confinement or during the monitoring period.

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Thus, no test-item related adverse effects on behaviour were observed during the entire course of the study.

Colony Strength

Colony strength increased from an initial level of 3750 ± 0 in both control (C) and treatment (T) groups to 5700 ± 442.8 in C and 5175 ± 983.7 in T by the end of the acclimation period. Thus, the two groups entered the 6 week confined exposure period on a comparable level. During confinement, colony strength increased to 6786 ±2728 in C and 8923 ± 2575 in T. Following the placement of colonies at the monitoring site, colony strength continued to increase in both C and T as a result of access to ample foraging resources. Colonies were strong and healthy when entering the overwinter period. At the last colony assessment following overwintering colony strength had decreased in both C and T, which is a typical and natural apidological phenomenon. Overall, the development of the colonies was very homogenous throughout the entire study period with no distinct differences in colony strength between treatment and T. Statistical analysis (linear mixed effects model) revealed no significant effect of the test item treatment on the number of worker bees (p= 0.404). A significant effect of the date was found (p = < 0.001), the interaction of date and treatment was not significant (p = 0.347), indicating that the number of worker bees changed over time, but in the same manner for treatment and control, respectively.

Thus, no test-item related adverse effects on colony strength (measured as number of worker bees) were found.

Table 8.3.1.3- 11: Colony strength as the average number of honey bees

Treatment Group	Initial	Beginning of the confined exposure	Beginning of the post-exposure	Beginning overwintering period	After overwintering Period
Control	3750 ± 0	5700 ± 442.8	6786 ± 2728	14375 ± 1784.8	5667.5 ± 1438.4
Test Item	3750 ± 0	5175 ± 983.7	8923 ± 2575	15600 ± 3336.8	5896.5 ± 1835.2

Brood Development

The average number of brood cells in both the control (C) and the treatment group (T) was very similar during the initial colony assessment. At the third colony assessment, at the beginning of the confined exposure period brood development had increased in both C and T and the two groups entered the exposure period on a comparable level. Average number of brood cells remained steady over the course of the confined exposure period in both C and T and following the release of colonies at the monitoring site, numbers of brood cells continued to increase as more resources became available. Thereafter, as typical for brood development of honey bee colonies through late summer and autumn in Central Europe, the number of cells with brood decreased. The number of brood cells had increased again at the last assessment in spring 2014, following the overwintering period, which indicated the presence of healthy queens which had continued to lay eggs. Overall, the brood development of the colonies as assessed by the mean number of cells filled with brood was very homogenous throughout the entire study period with no distinct differences in total brood between control and test item treatment. Statistical analysis (linear mixed effects model) revealed no significant effect of the test item treatment on the number of cells with brood (total brood) present in the colonies (p = 0.338). A significant effect of the date was found (p = < 0.001), the interaction of date and treatment was not significant (p = 0.909), indicating that the number of brood cells (= total brood) changed over time, but in the same manner for treatment and control, respectively.

Thus, no test-item related adverse effects on honey bee brood development were found.

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Table 8.3.1.3- 12: Brood development of the colonies as assessed by the mean number of cells filled with brood throughout the entire study period

Treatment Group	Initial	Beginning of the confined exposure	Beginning of the post-exposure	Peak in summer	Beginning overwintering period	After overwintering Period
Control	7520 ± 334.7	12104 ± 2254	10576 ± 2818.9	22408 ± 6664.6	2280 ± 1527.1	5520 ± 2563.6
Test Item	7600 ± 282.8	10768 ± 1730	11400 ± 3870.4	21040 ± 5773	2024 ± 3058.3	6640 ± 1779.9

Development of Food Storage

The average number of honey/nectar cells in both the control (C) and the treatment group (T) was very similar during the initial colony assessment. At the third colony assessment, at the beginning of the confined exposure period the amount of honey/nectar had increased in both C and T and the two groups entered the 6 week confined exposure period on a comparable, low carbohydrate supply level. At the end of the confined exposure period, i.e. just before the colonies were released out of the tunnels and therefore just before the beginning of the postexposure observation period, the average number of cells with nectar/honey per exposure group had increased in a similar manner in both C and T, indicating that the colonies in both exposure groups readily and equally accepted the offered carbohydrate diets during their confinement period, and that the food supply of the colonies in T provided a continuous and exhaustive exposure to fluopyram. During the course of the post-exposure observation period, the colonies were fed with additional sugar syrup as preparation for overwintering, which was reflected in an increase in honey stores.

Statistical analysis (linear mixed effects model) revealed no significant effect of the test-item treatment on the nectar/honey storage behaviour of the colonies under investigation (p = 0.478). A significant effect of the date was found (p = < 0.001), the interaction of date and treatment was not significant (p = 0.827), indicating that the number of cells with nectar/honey changed over time, but in the same manner for treatment and control, respectively.

Table 8.3.1.3- 13: Nectar/Honey stores in study colonies as assessed by the mean number of cells filled with nectar/honey throughout the entire study period

Treatment Group	Initial	Beginning of the confined exposure	Beginning of the post-exposure	Beginning overwintering period
Control	3200 ± 282.8	1536 ± 967.7	5680 ± 3920	50760 ± 5824.8
Test Item	3520 ± 657.3	2176 ± 1592.1	5640 ± 2654.8	51800 ± 9138

The average number of pollen cells in both the control (C) and the treatment group (T) was very similar during the initial colony assessment. At the third colony assessment, at the beginning of the confined exposure period the average number of cells with pollen had decreased in both C and T. Thus, the two groups entered the 6 week confined exposure period with a negligible pollen supply level. Throughout the following six colony assessments, i.e. throughout the entire confined exposure period, none of the cells within the colonies in both C and T contained any pollen/bee bread, although pollen (protein) was provided to the colonies in form of a pollen dough. At the first colony assessment after confinement, i.e. at the first colony assessment of the post-exposure observation period, after the honey bees were again

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allowed to forage freely, the average number of cells containing pollen/bee bread in both C and T had increased again indicating that colonies had already re-adapted under field conditions to their routine flower/pollen foraging and pollen storage. Pollen storage in C and T peaked around middle of July until beginning of August and declined thereafter.

Statistical analysis (linear mixed effects model) revealed no significant effect of the test-item treatment on the pollen storage behaviour of the colonies under investigation ($p = 0.285$). A significant effect of the date was found ($p = < 0.001$), the interaction of date and treatment was not significant ($p = 0.225$), indicating that the number of pollen cells changed over time, but in the same manner for treatment and control, respectively.

Table 8.3.1.3- 14: Pollen stores in study colonies as assessed by the mean number of cells filled with pollen throughout the entire study period

Treatment Group	Initial	Beginning of the confined exposure	Beginning of the post-exposure
Control	2040 ± 606.6	104 ± 232.6	2680 ± 1603.7
Test Item	1920 ± 228	112 ± 163.5	4080 ± 2335

Thus, no test-item related adverse effects on nectar/honey or pollen stores were found.

Colony Health

The pathogens *Malpighamoeba mellificae*, *Paenibacillus larvae* spores, *Melissococcus plutonius* as well as the viruses acute bee paralysis virus, chronic bee paralysis virus, Kashmir bee virus and Israel acute paralysis virus were not detected in any of the samples analysed. The occurrence of the pathogens *Nosema* sp. and *Varroa destructor* as well as of the viruses deformed wing virus, sacbrood virus and black queen cell virus occurred in both exposure groups to a similar extent, and their occurrence was therefore not linked to the absence or presence of the test item. Thus, no test-item related adverse effects on colony health in terms of pathogen/bee disease and virus infestation were observed during the entire course of the study.

Validity criteria:

As this was a special design study (internal testing method), no guideline and thus no validity criteria were available.

III. CONCLUSION

In a full-factorial randomized block design, honey bee colonies were exposed to nominally 10000 µg a.s./kg diet under confined conditions in gauze tunnels by *ad libitum* feeding on treatment-specific sugar- and pollen diet for a period of 6 consecutive weeks during springtime/early summer. During this confined exposure period, the colonies received additionally to the *ad libitum* feeding on treatment specific sugar diet, particularly during periods of elevated carbohydrate demand (e.g. spells of cold or rainy weather, etc.) identical quantities of treatment-specific supplemental sugar syrup placed inside each hive. These amounts of supplemental sugar syrup were always completely taken up by the colonies within a short period of time and guaranteed on the one hand that all colonies were always sufficiently supplied with carbohydrates, and, on the other hand that all colonies in the test item treatment group were continuously and exhaustively exposed to fluopyram via carbohydrate-diet.

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Thereafter, the colonies were released from confinement to be repeatedly monitored under field conditions for the remainder of the season until overwintering and were assessed for a final time after overwintering in the next spring.

Chemical analysis of the different batches of control and fluopyram-fortified diets revealed that the actual test item concentration was well in accordance to the nominal test item concentration. No detectable residues of fluopyram were found in any of the control diets.

The continuous exposure of honey bee colonies under confined conditions to a fluopyram-concentration of 10000 µg a.s./kg diet has not resulted in adverse acute, short-term and long-term effects on mortality, colony strength and colony development, brood development, food storage, honey bee behaviour, queen survival, overall hive vitality and colony health, as well as on overwintering performance.

Assessment and conclusion by applicant:

The study and its data are considered as acceptable and reliable for use in risk assessment.

The results are:

Overall, no adverse acute, short-term and long-term effects on mortality, colony strength and colony development, brood development, food storage, honey bee behaviour, queen survival, overall hive vitality and colony health, as well as on overwintering performance after continuous exposure of honey bee colonies under confined conditions to a fluopyram-concentration of 10000 µg a.s./kg diet for a period of 6 consecutive weeks during springtime/early summer.

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.

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CA 8.3.2 Effects on non-target arthropods other than bees

Studies on non-target arthropods have been performed with the representative formulations and are presented in the respective Document MCP, Section 10.3.2.

Data Point:	KCA 8.3.2/01
Report Author:	[redacted]
Report Year:	2007
Report Title:	Chronic dose-response toxicity (ER50) of AE C656948 SC 500 to the rove beetle Aleochara bilineata GYLL. under extended laboratory conditions
Report No:	07 10 48 018 A
Document No:	M-295187-01-1
Guideline(s) followed in study:	IOBC Guideline (GRIMM et al. 2000); Equivalent to US OPPTS Guideline No. 850.SUPP
Deviations from current test guideline:	Current Guideline: Grimm et al. (2000) Deviations: not applicable
Previous evaluation:	yes, evaluated and accepted in DAR (2011)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

The study above was performed with an outdated formulation. It is only shown for transparency reasons since it was part of the first listing process.

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CA 8.3.2.1 Effects on Aphidius rhopalosiphi

Studies on non-target arthropods have been performed with the representative formulations and are presented in the respective Document MCP, Section 10.3.2.

Data Point:	KCA 8.3.2.1/01
Report Author:	[redacted]
Report Year:	2007
Report Title:	Dose-response toxicity (LR50) of AE C656948 SC 500 to the parasitic wasp Aphidius rhopalosiphi (Destefani-Perez) under laboratory conditions
Report No:	06 10 48 187
Document No:	M-283320-01-1
Guideline(s) followed in study:	IOBC (MEAD-BRIGGS et al. 2000) Equivalent to US EPA OPPTS Guideline 850 (SUPP)
Deviations from current test guideline:	Current Guideline: IOBC - Mead-Briggs et al. (2000) Deviations: not applicable
Previous evaluation:	yes, evaluated and accepted in DAR (2011)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

The study above was performed with an outdated formulation. It is only shown for transparency reasons since it was part of the first listing process. New data has been generated with the representative formulation for the active substance renewal process, which is presented in the corresponding section of the MCP.

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CA 8.3.2.2 Effects on Typhlodromus pyri

Studies on non-target arthropods have been performed with the representative formulations and are presented in the respective Document MCP, Section 10.3.2.

Data Point:	KCA 8.3.2.2/01
Report Author:	[redacted]
Report Year:	2007
Report Title:	Dose-response toxicity (LR50) of AE C656948 SC 500 to the predatory mite Typhlodromus pyri (Scheuten) under laboratory conditions
Report No:	06 10 48 188
Document No:	M-283517-01-1
Guideline(s) followed in study:	IOBC (Bluemel et al. 2000); Equivalent to US EPA OPPTS Guideline No. 850.3400 (SUPP)
Deviations from current test guideline:	Current Guideline: BLÜMEL ET AL. (2000) Deviations: not applicable
Previous evaluation:	yes, evaluated and accepted in DAR (2011)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

The study above was performed with an outdated formulation. It is only shown for transparency reasons since it was part of the first listing process. New data has been generated with the representative formulation for the active substance renewal process, which is presented in the corresponding section of the MCP.

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CA 8.4 Effects on non-target soil mesoand macrofauna

Studies describing the toxicity of fluopyram to earthworms, springtails and soil mites have been performed with the representative formulations and are presented in the respective Document MCP.

Data Point:	KCA 8.4/01
Report Author:	[redacted]
Report Year:	2005
Report Title:	AE C656948: Acute toxicity to earthworms (Eisenia fetida) tested in artificial soil with 5 percent peat
Report No:	LKC/RG-A-57/05
Document No:	M-258932-01-1
Guideline(s) followed in study:	OECD 207, "OECD-Guideline for Testing of Chemicals,""Earthworm, Acute Toxicity Tests" (1984); Equivalent to US EPA OPPTS Guideline No. 850.6200
Deviations from current test guideline:	Current Guideline: OECD 207 (1984) Deviations: not applicable since test system is no longer a data requirement in EU
Previous evaluation:	yes, evaluated and accepted in DAR (2011)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

This study type is no longer a data requirement in the EU. It is only shown for transparency reasons since it was part of the first listing process.

Data Point:	KCA 8.4/02
Report Author:	[redacted]
Report Year:	2007
Report Title:	AE C656948 SC 500A G: Influence on the reproduction of the collembola species Folsomia candida tested in artificial soil with 5 % peat
Report No:	FRM-COLL-50/07
Document No:	M-288904-01-1
Guideline(s) followed in study:	ISO 11267 (1999) Equivalent to US EPA OPPTS Guideline No. 850.SUPP
Deviations from current test guideline:	Current Guideline: OECD 232 (2016) Deviation: not applicable
Previous evaluation:	yes, evaluated and accepted in DAR (2011)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

The study above was performed with an outdated formulation. It is only shown for transparency reasons since it was part of the first listing process. New data has been generated with the representative

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formulation for the active substance renewal process, which is presented in the corresponding section of the MCP.

Data Point:	KCA 8.4/03
Report Author:	[redacted]
Report Year:	2007
Report Title:	Fluopyram SC 500: Influence on mortality and reproduction on the soil mite species Hypoaspis aculeifer tested in artificial soil with 5 % peat
Report No:	KRA-HR-3/07
Document No:	M-287030-01-1
Guideline(s) followed in study:	Recommendations of the Hypoaspis Ring-test group (HASTE), Final Meeting on January 15, 2007 in Utrecht; Equivalent to US EPA OPPTS Guideline No. 850.SUPP
Deviations from current test guideline:	Current Guideline: OECD 226 (2016) Deviations: not applicable
Previous evaluation:	yes, evaluated and accepted in DAR (2011)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

The study above was performed with an outdated formulation. It is only shown for transparency reasons since it was part of the first listing process. New data has been generated with the representative formulation for the active substance renewal process, which is presented in the corresponding section of the MCP.

Data Point:	KCA 8.4/04
Report Author:	[redacted]
Report Year:	2007
Report Title:	AE C656948 SC 500: Effects on soil litter degradation
Report No:	LRA-SLD-30/07
Document No:	M-290278-01-1
Guideline(s) followed in study:	Effects of Plant Protection Products on Functional Endpoints in Soil (EPFES, Lisbon 2002), Guidance Document, Joerg Roembke et al.; Equivalent to US EPA OPPTS Guideline No. 850.SUPP
Deviations from current test guideline:	Current Guideline: not applicable Deviations: not applicable
Previous evaluation:	yes, evaluated and accepted in DAR (2011)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

The study above was performed with an outdated formulation and the study type is no longer a data requirement in the EU. However, it is shown for transparency reasons since it was part of the first listing process.

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CA 8.4.1 Earthworm, sub-lethal effects

Study describing the toxicity of fluopyram to earthworms have been performed with the representative formulations and are presented in the respective Document MCP, Section 10.4.1.1.

Table 8.4.1- 1: Ecotoxicological endpoints for earthworms and Fluopyram and its metabolites

Test item	Test species, test design	Ecotoxicological endpoint	Reference
FLU SC 500	*Eisenia fetida* reproduction 56 d, mixed	NOEC = 316 mg prod./kg dws NOEC$_{corr}$ = 158 mg prod./kg dws [A] NOEC = 134 mg a.s./kg dws [B] NOEC$_{corr}$ = 67 mg a.s./kg dws [A, B] EC$_{10}$ = 277 mg prod./kg dws EC$_{10,corr}$ = 138.5 mg prod./kg dws [A] EC$_{10}$ = 117.5 mg a.s./kg dws [A] EC$_{10,corr}$ = 59 mg a.s./kg dws [A, B]	(2020) M-680776-01-1 KCA 8.4.1/02 KCP 10.4.1.1/02 [C]
Fluopyram-7-hydroxy	*Eisenia fetida* reproduction 56 d, mixed	NOEC = 18 mg p.m./kg dws NOEC$_{corr}$ = 9 mg p.m./kg dws [A] EC$_{10}$ = calculation not possible	(2021) M-762139-01-1 KCA 8.4.1/03
Trifluoroacetic acid (TFA)	*Eisenia fetida* reproduction 56 d, mixed	NOEC = 320 mg p.m./kg dws [B]	(2005) M-251328-01-1 KCA 8.4.1/04
FLU SC 400	Field study, 1 year, surface and in furrow application	No unacceptable ecologically adverse effects on the population at surface application rates of 500 g a.s./ha and 1000 g a.s./ha, and as in-furrow application at a rate of 500 g a.s./ha.	(2014) M-497763-01-1 KCA 8.4.1/05

dws = dry weight soil, a.s. = active substance; p m. = pure metabolite, prod. = product

[A] Endpoint corrected by a factor of 2 due to lipophilic substance (log Pow > 2)

[B] NOEC of 320 mg/kg dws is based on effects on the body weight in the concentration 1000 mg/kg dws.

[C] Full details on this study are described in the corresponding MCP for the formulation FLU SC 500.

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Fluopyram SC 500

Data Point:	KCA 8.4.1/01
Report Author:	
Report Year:	2006
Report Title:	AE C656948 SC 500: Effects on survival, growth and reproduction of the earthworm Eisenia fetida tested in artificial soil with 5 percent peat
Report No:	LKC-RG-R-20/06
Document No:	M-268821-01-1
Guideline(s) followed in study:	ISO 11268-2: 1998 (E) and OECD 222: April 13, 2004; Equivalent to US EPA OPPTS Guideline No. 850.6200 (SETPP)
Deviations from current test guideline:	Current Guideline: OECD 222 (2016) Deviations: not applicable
Previous evaluation:	yes, evaluated and accepted in DAR (2011)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

The study above was performed with an outdated formulation. It is only shown for transparency reasons since it was part of the first listing process. New data has been generated with the representative formulation for the active substance renewal process, which is presented in the corresponding section of the MCP.

Data Point:	KCA 8.4.1/02
Report Author:	
Report Year:	2020
Report Title:	Fluopyram SC 500 (500 g/L): Effects on survival, growth and reproduction of the earthworm Eisenia fetida tested in artificial soil
Report No:	M-680776-01-1
Document No:	M-680776-01-1
Guideline(s) followed in study:	EU Directive 91/414/EEC, Regulation (EC) No. 1107/2009, US EPA OCSPP Not Applicable, ISO 11268-2: 1998 (E), OECD 222: July 29, 2016
Deviations from current test guideline:	Current Guideline: OECD 222 (2016) Deviations: None. All validity criteria were met.
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

For the detailed study summary please refer to the Fluopyram SC 500 MCP Section 10.4.1.1/02.

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Metabolite fluopyram-7-hydroxy

Data Point:	KCA 8.4.1/03
Report Author:	[redacted]
Report Year:	2021
Report Title:	AE C656948-7-hydroxy (BCS-AA10065): Effects on survival, growth and reproduction of the earthworm Eisenia fetida tested in artificial soil
Report No:	E 312 05558-9
Document No:	M-762139-01-1
Guideline(s) followed in study:	EU Directive 91/414/EEC (1991) Regulation (EC) No. 1107/2009 (2009) US EPA OCSPP Not Applicable ISO 11268-2: 1998 (E) OECD 222: July 29, 2016
Deviations from current test guideline:	Current Guideline: OECD 222 (2016) Deviations: None. All validity criteria were met.
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

In a laboratory study the effects of fluopyram-7-hydroxy on survival and reproduction of adult earthworms *Eisenia fetida* was tested during an exposure of 4 weeks (first part) in artificial soil by comparing control and treatment. After this period, the adult earthworms were removed from the test vessels and the cocoons and juvenile earthworms remained in the test vessels for additional 4 weeks (second part). The total duration of the study was 8 weeks. The test was conducted in two runs since in the 1st test run all tested concentrations showed statistically significant differences in weight change of adult earthworms after 28 days, a 2nd run was prepared. In the 1st run five test item rates from 56 to 562 mg p.m./kg dry weight soil were tested. In the 2nd run eight test item rates from 3.2 to 140 mg p.m./kg dry weight soil were tested. Per test item rate 4 replicates and for the control group 8 replicates with 10 earthworms each were exposed to fluopyram-7-hydroxy mixed into artificial soil.

After a period of 4 weeks the survivors were counted, and their fresh weight was measured. From these data mortality and biomass effects were determined. After an additional four weeks exposure of the cocoons and juvenile earthworms the reproduction was determined by counting the number of off-spring hatched from the cocoons per test vessel.

The study fulfilled all validity criteria of OECD 222 guideline.

The overall endpoints were: $NOEC_{mortality} \geq 562$ mg p.m./kg dry weight artificial soil, $LOEC_{mortality} > 562$ mg p.m./kg dry weight artificial soil, $NOEC_{growth} \geq 140$ mg p.m./kg dry weight artificial soil, $LOEC_{growth} > 140$ mg p.m./kg dry weight artificial soil, $NOEC_{reproduction} = 18$ mg p.m./kg dry weight artificial soil, $LOEC_{reproduction} = 32$ mg p.m./kg dry weight artificial soil.

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I. MATERIALS AND METHODS

Test item: Fluopyram-7-hydroxy, Batch No.: BCS-AA10065-01-01; TOX21541-00, purity: 99.4 % w/w.

Test design: Since in the 1st test run all tested concentrations showed statistically significant differences in weight change of adult earthworms after 28 days, a 2nd test run was performed.

1st run: Adult earthworm *Eisenia fetida* 9-10 months old, 8 x 10 earthworms for the control group and 4 x 10 animals per test concentration of the treatment groups with weight between 0.35 and 0.55 g, were exposed to control and treatment. Nominal test concentrations of 56, 100, 178, 316 and 562 mg p.m./kg dry weight artificial soil were mixed into the artificial soil.

2nd run: Adult earthworm *Eisenia fetida* 10-11 months old, 8 x 10 earthworms for the control group and 4 x 10 animals per test concentration of the treatment groups with weight between 0.30 and 0.50 g, were exposed to control and treatment. Nominal test concentrations of 3.2, 5.6, 10, 18, 32, 45, 80 and 140 mg p.m./kg dry weight artificial soil were mixed into the artificial soil.

Each of the test runs consisted of 2 parts: Adult earthworms were exposed to the test item for a period of 4 weeks (first part). After this period, the adult earthworms were removed from the test vessels and the cocoons and juvenile earthworms remained in the test vessels for additional 4 weeks (second part). The total duration of each test run was 8 weeks.

During the test the adult earthworms were fed once per week with approximately 5 g food/vessel (animal manure). The offspring were fed only once at the start of the second 4 weeks exposure period by mixing the food into the soil. The artificial soil was prepared according to the guideline 222 with the following constituents: 70 % fine quartz sand, 10% Sphagnum peat (air dried and finely ground), 20 % Kaolin clay.

After four weeks, the adult worms were removed from the test vessels. The number of surviving worms (adult mortality) and their biomass change were determined and behaviour (including feeding activity) were recorded. The adult worms were discarded after counting and weighing.

At the end of the test after 8 weeks, the number of surviving juveniles per test vessel was determined and emerging earthworms were removed and counted.

Statistics: Mortality data were analysed for significance by using the Fisher`s exact binominal test (one-sided greater, $\alpha = 0.05$). Body weight change and reproduction data were tested for normal distribution and homogeneity of variance using Shapiro-Wilk's-Test and Leven's -Test ($\alpha = 0.05$) respectively. Data of growth were normally distributed and homogeneity of variances was given. Therefore Dunnett`s Multiple t-test, two-sided, $\alpha = 0.05$ was used to determine NOEC and LOEC values in the 1st run. In the 2nd run Multiple sequentially-rejective Welsh-t-test after Bonferroni-Holm, two-sided, $\alpha = 0.05$ was used for NOEC and LOEC determination. Data of reproduction were normally distributed, and homogeneity of variances was given. Therefore, Multiple Sequentially-rejective Welsh-t-test After Bonferroni-Holm, one-sided-smaller, $\alpha = 0.05$ was used to determine NOEC and LOEC values in both test runs. The calculation of EC_{10}/EC_{20} and their 95% confidence limits was based on the model which provides the best fit out of different suitable regression models. The software used to perform the statistical analysis was ToxRatPro, version 3.2.1.

The climatic conditions were in the temperature range 19.8 – 22.2 °C (1st run) and 19.7 – 20.5 (2nd run) with a photoperiod of 16 hours light and a light intensity of 400 - 800 lux.

Dates of work: June 29, 2020 – December 09, 2020

II. Results and discussion

Table 8.4.1- 2: Effects of fluopyram-7-hydroxy on *Eisenia fetida* in the 1st run

Parameter	Treatment [mg p m./kg dry weight artificial soil dws]					
	Control	56	100	178	316	562
Mortality of adult earthworms [%] after 28 days	0	2.5	0	0	0	0
Significance (Mortality*)	---	-	-	-	-	-
Mean change of body weight of the adults from day 0 to day 28 [%]	20.4	32.8	34.3	32.1	29.5	27.2
Standard deviation	6.3	8.2	5.2	5.5	3.8	10.0
Significance (body fresh weight)**	---	+	+	+	-	-
Mean number of off-spring per test vessel after 56 days	343.3	387.5	379.0	339.0	358.0	208.5
Standard deviation	27.0	63.2	31.4	46.4	36.6	8.6
Coefficient of variance (%)	7.9	16.3	8.3	13.7	10.2	4.1
% of control	---	112.9	110.4	98.8	104.3	60.7
Significance (reproduction)***	---	-	-	-	-	+

	Adult Mortality	Growth	Reproduction
NOEC [mg p m./kg dry weight soil]	≥562	Not calculable	316
LOEC [mg p m./kg dry weight soil]	>562	Not calculable	562

* (Fisher`s Exact Binominal Test, one-sided greater, $\alpha = 0.05$), + significant, - not significant
** (Dunnett´s multiple t-test procedure, two-sided, $\alpha = 0.05$), + = significant, - = not significant
*** (Welsh-t-test after Bonferroni-Holm, one-sided smaller, $\alpha = 0.05$), + significant, - not significant
--- control was used for the statistical evaluation

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Table 8.4.1- 3: Effects of fluopyram-7-hydroxy on *Eisenia fetida* in the 2nd run

Parameter	Treatment [mg p.m./kg dry weight artificial soil dws]								
	Control	3.2	5.6	10.0	18.0 ▲	32.0	45.0	80.0	140.0
Mortality of adult earthworms [%] after 28 days	0	0	2.5	0	0	0	0	0	0
Significance (Mortality*)	---	-	-	-	-	-	-	-	-
Mean change of body weight of the adults from day 0 to day 28 [%]	50.8	46.6	48.9	52.0	44.3	54.5	49.8	55.1	53.5
Standard deviation	5.8	3.8	7.6	4.4	8.3	3.2	4.4	14.6	8.2
Significance (body fresh weight)**	---	-	-	-	-	-	-	-	-
Mean number of off-spring per test vessel after 56 days	260.9	233.3	242.3	236.3	241.8	189.8	219.0	186.0	174.5
Standard deviation	29.2	15.2	24.5	28.1	42.5	27.8	48.7	22.0	20.4
% of control	---	89.4	92.9	90.6	92.7	72.7	83.9	71.3	66.9
Significance (reproduction)***	---	-	-	-	-	+	-	+	+

	Adult Mortality	Growth	Reproduction
NOEC [mg p m./kg dry weight soil]	≥140	≥140	18
LOEC [mg p m./kg dry weight soil]	>140	>140	32

▲ Original values in consideration of 11 adult worms in one replicate. This replicate was not considered in the statistical calculations on body fresh weight and number of offspring.
* (Fisher`s Exact Binominal Test, one-sided greater, α = 0.05), + significant, - not significant
** (Welsh-t-test after Bonferroni-Holm, two-sided, α = 0.05), + = significant, - = not significant
*** (Welsh-t-test after Bonferroni-Holm, one-sided smaller, α = 0.05), + significant, - not significant
--- control was used for the statistical evaluation

Mortality:

After 28 days of exposure, no mortality in the control group was observed in both test runs. In the 1st run no statistically significant effects up to and including 562 mg p.m./kg dws were observed (Fisher`s exact binomial test, one-sided greater, α = 0.05). In the 2nd run no statistically significant effects up to and including 140 mg p.m./kg dws were observed (Fisher`s exact binomial test, one-sided greater, α = 0.05).

Effects on growth:

1st run: Statistically significant effects for the growth relative to the control were observed in the test item concentrations 56, 100 and 178 mg p.m./kg dry weight artificial soil. No statistically significant effects for growth relative to the control were observed in the test item concentrations 316 and 562 mg p.m./kg dws (Dunnett`s Multiple t-test, two-sided, α = 0.05).

2nd run: No statistically significant effects for the growth relative to the control were observed in the test item concentrations up to and including 140 mg p.m./kg dws (Multiple sequentially-rejective Welsh-t-test after Bonferroni-Holm, two-sided, α = 0.05).

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Effects on reproduction:

1st run: Statistically significant differences concerning the number of juveniles relative to the control were observed in the test item concentration 562 mg p.m./kg dws (Multiple sequentially-rejective Welsh-t-test after Bonferroni-Holm, one-sided smaller, $\alpha = 0.05$).

2nd run: Statistically significant differences concerning the number of juveniles relative to the control were observed in the test item concentration 32 mg p.m./kg dws (Multiple sequentially-rejective Welsh-t-test after Bonferroni-Holm, one-sided smaller, $\alpha = 0.05$).

Validity criteria:

All validity criteria of the OECD 222 guideline were met.

Table 8.4.1- 4: Validity criteria

Validity criteria acc. to OECD 222 (adopted 2016)	Required	Obtained 1st run	Obtained 2nd run
Mortality of the adults in the control	≤ 10 %	0 %	0 %
Number of juveniles (earthworms per control vessel)	≥ 30	295 - 387	225 - 312
Coefficient of variance of reproduction in the control	≤ 30 %	7.9 %	11.2 %

Reference test:

The corresponding toxic standard reference test, with the reference test item mixed into the artificial soil, was performed from 2020-01-23 to 2020-03-25 (Rg-R-Ref 34/20; NON-GLP). Effects on mortality and growth of the adults after an exposure period of 28 days and the number of offspring after 56 days were determined. No mortality of the adult earthworms was observed 28 days after application. The change of body weight of the adult earthworms of the test concentration of 5.0 mg a.s./kg dry weight soil was statistically significant reduced in comparison to the control (results of a Dunnett’s multiple t-test, two-sided, $\alpha = 0.05$). The number of juveniles per test vessel of the test concentrations 2.5 and 5.0 mg a.s./kg dry weight soil were statistically significant reduced in comparison to the control (results of a William´s multiple t-test, one-sided smaller, $\alpha = 0.05$). According to the guideline significant effects should be observed between 1 and 5 mg a.s./kg dry weight artificial soil. Thus, the results of this reference test indicated that the test system was sensitive to the reference test item.

III. Conclusion

All validity criteria were met. The endpoints were:

$NOEC_{mortality}$: ≥ 562 mg p.m./kg d.w. artificial soil.

$LOEC_{mortality}$: > 562 mg p.m./kg d.w. artificial soil

$NOEC_{growth}$: ≥ 140 mg p.m./kg d.w. artificial soil

$LOEC_{growth}$: > 140 mg p.m./kg d.w. artificial soil

$NOEC_{reproduction}$: = 18 mg p.m./kg d.w. artificial soil

$LOEC_{reproduction}$: = 32 mg p.m./kg d.w. artificial soil

Due to the lack of a clear concentration-response relationship no reliable EC_x-calculation was possible.

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Assessment and conclusion by applicant:

The study and its data are considered as acceptable and reliable for use in risk assessment.

The endpoint is: NOEC = 18 mg p.m./kg dws (based on reproduction)

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Metabolite trifluoroacetic acid (TFA)

Data Point:	KCA 8.4.1/04
Report Author:	[redacted]
Report Year:	2005
Report Title:	Effects of AE C502988 00 1B99 0001 on reproduction and growth of earthworms Eisenia fetida in artificial soil
Report No:	C048065
Document No:	M-251328-01-1
Guideline(s) followed in study:	BBA: Part VI, No. 2-2, (1994); ISO: 11268-2; (1998)
Deviations from current test guideline:	Current guideline: OECD 222 (2016) deviation: Deviation from recommended replicates in the control (4 instead of 8). Concentrations spaced by a higher factor than recommended. All validity criteria were met. The deviations listed above had no influence on the reliability of the study and endpoints.
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

In a laboratory study the effects of trifluoroacetic acid (TFA) on survival and reproduction of adult earthworms *Eisenia fetida* was tested during an exposure of 4 weeks (first part) in artificial soil by comparing control and treatment. After this period, the adult earthworms were removed from the test vessels and the cocoons and juvenile earthworms remained in the test vessels for additional 4 weeks (second part). The total duration of the study was 8 weeks. Five test item rates from 10 to 1000 mg p.m./kg dry weight soil were tested. Per test item rate 4 replicates and for the control group 4 replicates with 10 earthworms each were exposed to TFA mixed into artificial soil.

After a period of 4 weeks the survivors were counted, and their fresh weight was measured. From these data mortality and biomass effects were determined. After an additional four weeks exposure of the cocoons and juvenile earthworms the reproduction was determined by counting the number of off-spring hatched from the cocoons per test vessel.

The study fulfilled all validity criteria of OECD 222 guideline.

The endpoints were: $NOEC_{mortality}$ ≥1000 mg p.m./kg dry weight artificial soil, $LOEC_{mortality}$ >1000 mg p.m./kg dry weight artificial soil, $NOEC_{growth}$ = 320 mg p.m./kg dry weight artificial soil, $LOEC_{growth}$ = 1000 mg p.m./kg dry weight artificial soil, $NOEC_{reproduction}$ ≥1000 mg p.m./kg dry weight artificial soil, $LOEC_{reproduction}$ >1000 mg p.m./kg dry weight artificial soil.

I. MATERIALS AND METHODS

Test item: Trifluoroacetic acid (TFA), Batch No.: 18921; TOX08523-00, purity: 98.8 % w/w.

Test design: Ten adult earthworms (*Eisenia fetida*) per replicate (4 replicates for the control group and 4 replicates for each treatment group) were exposed to control and treatments in an artificial soil (with 10 % peat content). The study consisted of 2 parts: Adult earthworms were exposed to the test item for a period of 4 weeks (first part). After this period, the adult earthworms were removed from the test

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vessels and the cocoons and juvenile earthworms remained in the test vessels for additional 4 weeks (second part). The total duration of the study was 8 weeks.

During the test the adult earthworms were fed once per week with approximately 5 g food/vessel (animal manure). The offspring were fed only once at the start of the second 4 weeks exposure period by mixing the food into the soil. The artificial soil was prepared according to the guideline 207 with the following constituents: 69.5 % fine quartz sand, 10% Sphagnum peat (air dried and finely ground), 20 % Kaolin clay.

After four weeks, the adult worms were removed from the test vessels. The number of surviving worms (adult mortality) and their biomass change were determined and behaviour (including feeding activity) were recorded. The adult worms were discarded after counting and weighing.

At the end of the test after 8 weeks, the number of surviving juveniles per test vessel was determined and emerging earthworms were removed and counted.

Statistics: Mortality data were analysed for significance by using the Fisher-exact test (two-sided, a = 0.05). Body weight change and reproduction data were tested for normal distribution and homogeneity of variance using the Kolmogoroff-Smirnov test and the Cochran test. As data of body weight changes and reproduction were normally distributed and homogeneous, the Dunnett test was used (multiple comparison, two-sided for weight and one sided smaller for reproduction, a = 0.05). The software used to perform the statistical analysis was ToxRatPro, version 2.09.

The climatic conditions were in the temperature range 19 - 21.0 °C with a photoperiod of 16 hours light and a light intensity of 480 - 790 lux.

Dates of work: February 15, 2005 – April 14, 2005

II. Results and Discussion

Table 8.4.1- 5: Effects of trifluoroacetic acid on *Eisenia fetida*

Parameter	Treatment [mg p m./kg dry weight artificial soil dws]					
	Control	10	32	100	320	1000
Mortality of adult earthworms [%] after 28 days	5	0	2.5	5	0	0
Standard Deviation	± 5.8	± 0	± 5.0	± 5.8	± 0	± 0
Statistical comparison to the control *	—	n.s.	n.s.	n.s.	n.s.	n.s.
Mean change of body weight of the adults from day 0 to day 28 [%]	+ 42.7	+ 36.3	+ 39.0	+ 34.2	+ 35.9	+ 28.4
Standard deviation	± 4.5	± 6.0	± 3.3	± 8.1	± 8.5	± 5.7
Statistical comparison to the control **	—	n.s.	n.s.	n.s.	n.s.	s.
Mean number of off-spring per test vessel after 56 days	291	307	377	304	322	309
Standard deviation	± 58	± 89	± 31	± 97	± 28	± 20
Statistical comparison to the control ***	—	n.s.	n.s.	n.s.	n.s.	n.s.
	Adult Mortality			**Growth**	**Reproduction**	
NOEC [mg p m./kg dry weight soil]	**≥ 1000**			**320**	**≥ 1000**	
LOEC [mg p m./kg dry weight soil]	**> 1000**			**1000**	**> 1000**	

Values in table are rounded

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* Result of a Fisher exact test, two-sided, $\alpha = 0.05$
** Result of a Dunnett test, two-sided, $\alpha = 0.05$
*** Result of a Dunnett test, one sided smaller, $\alpha = 0.05$
n.s.: mean value not statistically significant different compared to the control ($p \geq 0.05$)
s.: mean value statistically significant different compared to the control ($p < 0.05$)

Mortality:

A mortality of 5% was observed in the control and at the concentration of 100 mg p.m./kg soil and 2.5% of mortality were observed at 32 mg p.m./kg soil. The mortality in the test item treated group was not significantly different compared to the control (Fisher exact test, $\alpha = 0.05$) and is not considered to be treatment related since at the two highest concentrations no mortality was observed.

Effects on growth:

The body weight changes of the test item treated groups were not significantly different compared to the control up to and including the concentration of 320 mg p.m./kg soil (Dunnett test, $\alpha = 0.05$, two sided). At 1000 mg p.m./kg soil the body weights showed a weight increase of 28.4% which, however, was statistically significantly lower compared to the control (Dunnett test, a = 0.05, two sided).

Effects on reproduction:

The reproduction rates were not significantly different compared to the control in any test item treated groups (Dunnett test, $\alpha = 0.05$, one sided smaller).

Effects on feeding activity and behavioural abnormalities:

The results show that the turnover of biomass of those earthworms exposed to the five different rates of the test item was comparable to the control. No behavioural abnormalities were observed and all worms did burrow into the soil within 15 min after introduction.

Validity criteria:

All validity criteria of the OECD 222 guideline were met.

Table 8.4.1- 6: Validity criteria

Validity criteria acc. to OECD 222 (adopted 2016)	Required	Obtained
Mortality of the adults in the control	≤ 10 %	5 %
Number of juveniles (earthworms per control vessel)	≥ 30	246 - 375
Coefficient of variance of reproduction in the control	≤ 30 %	19.8 %

Reference test:

The reference item Brabant Carbendazim Flowable (500 g/L) was tested in a separate study from October 2004 to December 2004 under the IBACON Project No. 21341022. The test was performed with concentrations of 1.20 and 6.00 mg product/kg soil dry weight.

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The reference item showed statistically significant effects on reproduction at a concentration of 1.1 mg carbendazim/kg artificial soil (dry weight); the EC_{50} for reproduction was calculated as 1.25 mg carbendazim/kg soil dry weight.

III. CONCLUSION

All validity criteria were met. The endpoints were:

$NOEC_{mortality}$: ≥ 1000 mg p.m./kg d.w. artificial soil.

$LOEC_{mortality}$: > 1000 mg p.m./kg d.w. artificial soil

$NOEC_{growth}$: = 320 mg p.m./kg d.w. artificial soil

$LOEC_{growth}$: = 1000 mg p.m./kg d.w. artificial soil

$NOEC_{reproduction}$: ≥ 1000 mg p.m./kg d.w. artificial soil

$LOEC_{reproduction}$: > 1000 mg p.m./kg d.w. artificial soil

Due to the lack of a clear concentration-response relationship no reliable EC_x-calculation was possible.

Assessment and conclusion by applicant:
The study and its data are considered as acceptable and reliable for use in risk assessment.
The endpoint is: NOEC = 320 mg p.m./kg dws (based on growth)

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Field study with Fluopyram SC 400

Data Point:	KCA 8.4.1/05
Report Author:	[redacted]
Report Year:	2014
Report Title:	Fluopyram SC 400 G: Effect on the earthworm fauna of a field within one year
Report No:	MNU/Rg-F-13/14
Document No:	M-497763-01-1
Guideline(s) followed in study:	BBA (Federal Biological Research Centre for Agriculture and Forestry, Germany): Guidelines for the Testing of Plant Protection Products within Registration, Part VI, 2 - 3 (January 1994): Effects of Plant Protection Products on Earthworms in the Field ISO (International Standard Organization): Draft Guideline CD 11268-3 (E): Soil Quality - Effects of Pollutants on Earthworms, Part 3: Guidance on the determination of effects in field situations (1999) US EPA OCSPP: Not available
Deviations from current test guideline:	Current Guideline: BBA: Guidelines for the Testing of Plant Protection Products within Registration, Part VI, 2 - 3 (January 1994): Effects of Plant Protection Products on Earthworms in the Field ISO: Draft Guideline CD 11268-3 (E): Soil Quality - Effects of Pollutants on Earthworms, Part 3: Guidance on the determination of effects in field situations (1999) deviation: 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

The study investigated the effects of Fluopyram SC 400 on earthworm populations under field conditions. The parameters abundance and biomass of the total earthworm population on the control plots were investigated on a field site on which seed potatoes were dibbled. Two different application scenarios were prepared. On June 11, 2013, FLU SC 400 was applied as surface application (spray application) at rates of 500 g a.s./ha and 1000 g a.s./ha, respectively, on four plots of 10 x 10 m for each concentration. For the second application scenario FLU SC 400 was applied as in furrow application during sowing of potatoes on June 12, 2013 at rates of 500 g a.s./ha (in furrow application) on further four plots (10 x 10 m). Four untreated plots served as controls, four plots were used as positive controls and were treated with Carbendazim SC 500 with an application rate of 10 kg a.s./ha (toxic reference).

FLU SC 400 applied as surface application at rates of 500 g a.s./ha and 1000 g a.s./ha, and as in furrow application at a rate of 500 g a.s./ha had no unacceptable ecologically adverse effects on the population of earthworms 4-5 weeks, 4-5 months and 11-12 months after the application.

I. MATERIALS AND METHODS

Test item: FLU SC 400, TOX09950-00, specification no.: 102000026892 - 02; Batch No.: 2012-004623; analytical findings: 35.2 % w/w fluopyram equivalent to 408.0 g/L; density: 1.160 g/mL.

Test design: The effects of FLU SC 400 on earthworm populations under field conditions were studied. To ensure an abundant earthworm population, an area was selected which was used as cropland for a few years, located on Bayer Experimental Farm Höfchen/Burscheid, Germany. Between May 21 and

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June 5, 2013, a pre-sampling of earthworms was conducted (non-GLP) to ensure a sufficient abundance of earthworms being present at the test site.

On June 11th, 2013, FLU SC 400 was applied at a rate of 615 g a.s./ha to all 12 treatment plots (10 x 10 m) in order to achieve a concentration of fluopyram in soil simulating a long-term plateau concentration after several years of use.

Two different application scenarios were prepared. On June 11th, 2013, FLU SC 400 was applied as surface application (spray application) at rates of 500 g a.s./ha and 1000 g a.s./ha, respectively, on 4 plots of 10 x 10 m for each concentration. For the second application scenario FLU SC 400 was applied as in furrow application during sowing of potatoes on June 12th, 2013 at rates of 500 g a.s./ha (in furrow application) on further 4 plots (10 x 10 m). Four untreated plots served as controls, 4 plots were used as positive controls and were treated with Carbendazim SC 500 with an application rate of 10 kg a.s./ha (toxic reference).

Within 10 days after application 59 mm of precipitation was measured.

One, two and three days after each application, all plots were visually inspected for alive and dead earthworms on the soil surface. The earthworm abundance and biomass were sampled 4-5 weeks (July 8th - 16th, 2013), 4-5 months (October 8th – 24th, 2013) and 11-12 months (May 12th - June 13th, 2014) after the application, by “hand-sorting”. At each sampling occasion 4 earthworm samples were collected per plot.

Analytics: For analytical verification of the test item application rate, soil samples from the treated plots and from the non-treated control plots were taken on June 11th and 12th, 2013. Soil samples were analysed by LC-MS/MS.

Statistics: The results of the sampling were statistically evaluated by Student t-test (probability level P = 0.05, one-sided smaller). The statistic software used was ToxRatPro Version 2.10. No statistical comparisons between treatment groups were made in case taxa occurred at a level of less than 10 ind./m^2, according to the recommendation of Kula et al.

Climatic conditions: The monthly climatic conditions were in the temperature range 5.26 – 20.28 °C, a cumulative precipitation range 25.5 – 134.3 mm and sunshine hours form 16.76 – 270.01 h.

Dates of work: May 21, 2013 – June 13, 2004

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II. RESULTS AND DISCUSSION

Analytical results:

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 SANTE/2020/12830, Rev.1.

Table 8.4.1- 7: Analytical results (mean values) for application rates, as given by recovery of Fluopyram

Sample description	Concentration [g a.s./L]	Number of plots	Fluopyram residue % (recovery of applied) Range of recovery	Fluopyram residue % (recovery of applied) Mean of recovery[1]
Control (plateau)	0	Mix of 4 plots	< LOQ	–
Plateau Fluopyram SC 400 G (surface application on 2013-06-11)	615	12	69.7 – 104.2	90.94
Control (surface application)	0	Mix of 4 plots	< LOQ	–
Surface application Fluopyram SC 400 G (application on 2013-06-12)	500	4	101.6 – 113.3	107.1
	1000	4	88.6 – 115.4	104.38
Control (in-furrow application)	0	Mix of 4 plots	< LOQ	–
In-furrow application (application on 2013-06-12)	500	4	73.91 – 99.16	89.12

[1] Not given in the report calculations based on individual recoveries

Biological results:

Total earthworm and total single earthworm species abundance and biomass were not affected throughout the whole study by treatment with FLU SC 400.

4 – 5 weeks after application, the total abundance of the adult earthworms was transiently reduced significantly as compared to the control at the dose of 500 g a.s./ha when applied as in furrow application and in the dose of 1000 g a.s./ha when applied as spray application.

After 4 – 5 month there were no significant effects anymore and at the end of the study (11-12 months after the application) a full recovery was observed and in the plots treated with FLU SC 400 the control level was reached.

Considering the biomass of adult earthworms, only in the samples taken 4 – 5 months after application in the 500 g a.s./ha in furrow application there was a significant reduction as compared to the control. In the first sampling, 4-5 weeks after application and notably at the end of the study (11-12 months after application) there were no adverse effects observed.

At all rates of FLU SC 400, the abundances of adult earthworms were significantly reduced compared to the control in the first sampling (4-5 weeks after the application). At the rate of 1000 g a.s./ha (spray application), also the biomass of the adult earthworms was significantly reduced initially. For these

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effects there was a recovery already at the second sampling time (4 – 5 months after application). At the end of the study (11-12 months after application) there were also no differences to the control anymore.

11-12 months after the application, a statistically significant reduction of adult anecic earthworms of 28.6 % for the abundance and 35.8% for the biomass was observed at the high dose rate of 1000 g a.s./ha (spray application).

The total number and biomass of anecic earthworms and the number and biomass of juvenile anecic earthworms were not affected during the whole study period. Additionally, the number of juvenile anecic earthworms was even slightly higher in the 1000 g a.s./ha application rate compared to the control at all sampling dates. Thus, after growing of these juveniles no long-term effects are expected anymore.

The total numbers of anecic earthworms (including the juveniles) observed in the studied field was low. Considering the magnitude of effects (only about 30%) and the low absolute numbers of adult earthworms, the statistically significant results for the adult anecic earthworms are based on very low abundance numbers (10.5 adult anecic earthworms per m^2 in the control and 7.5 adult anecic earthworms per m2 in the 1000 g a.s./ha application rate). Due to this low abundance, a small difference in numbers can result in the statistical significance of a difference which might not be a real biological effect. For comparison, the number of 8 adult earthworms in the reference group (Carbendazim treatment) was statistically not significantly different from control.

Considering the fact that the total abundance of anecic earthworms was not affected at any time during the study and that the total numbers of adult earthworms and total numbers of juvenile anecic earthworms was higher at the end of the study than at the beginning, the statistically significant findings for adult anecic earthworms are considered to be not ecologically relevant. Thus, the rate of 1000 g a.s./ha is considered as the ecological no effect concentration.

Table 8.4.1- 8: Changes in abundance for total earthworms, total juveniles & total adults and the dominant species *L. terrestris* and *A. chlorotica*

	Mean abundance ± SD [individuals/m²] (relative abundance [% of control])			
Treatment group	**pre-sampling**	**1st Sampling**	**2nd Sampling**	**3rd Sampling**
		Total		
Control	498.25 ± 141.01	258.00 ± 39.62 (100)	522.50 ± 92.07 (100)	622.75 ± 155.21 (100)
Test item (500 g a.s./ha, furrow application)	565.50 ± 125.59	322.00 ± 90.24 (125)	564.75 ± 121.31 (108)	723.25 ± 159.01 (116)
Test item (500 g a.s./ha, spray application)	566.50 ± 83.63	362.25 ± 136.34 (140)	647.25 ± 172.06 (124)	711.75 ± 229.70 (114)
Test item (1000 g a.s./ha, spray application)	538.75 ± 96.56	335.50 ± 77.28 (130)	579.25 ± 111.50 (111)	629.00 ± 214.86 (101)
Reference item	584.00 ± 172.39	144.25 ± 28.85* (56)	345.00 ± 122.08 * (66)	378.75 ± 166.90 * (61)
		Total adults		
Control	50.50 ± 16.44	22.50 ± 3.00 (100)	81.50 ± 21.79 (100)	79.75 ± 13.05 (100)
Test item (500 g a.s./ha, furrow application)	59.75 ± 14.64	15.00 ± 4.55 * (67)	65.00 ± 33.52 (80)	77.75 ± 19.70 (97)

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Treatment group	Mean abundance ± SD [individuals/m²] (relative abundance [% of control]) pre-sampling	1st Sampling	2nd Sampling	3rd Sampling
Test item (500 g a.s./ha, spray application)	55.50 ± 7.19	18.25 ± 3.59 (81)	60.75 ± 16.78 (75)	80.50 ± 17.14 (101)
Test item (1000 g a.s./ha, spray application)	58.25 ± 9.91	10.75 ± 2.63 * (48)	85.00 ± 25.70 (104)	80.25 ± 17.46 (101)
Reference item	56.00 ± 21.32	9.25 ± 1.50 * (41)	50.75 ± 11.18 * (62)	67.25 ± 12.63 (84)
Total juveniles				
Control	447.75 ± 126.62	235.50 ± 40.09 (100)	441.00 ± 110.79 (100)	543.00 ± 143.45 (100)
Test item (500 g a.s./ha, furrow application)	505.75 ± 119.03	307.00 ± 90.42 (130)	499.75 ± 107.20 (113)	645.50 ± 168.65 (119)
Test item (500 g a.s./ha, spray application)	511.00 ± 79.80	344.00 ± 133.36 (146)	586.50 ± 170.03 (133)	631.25 ± 228.66 (116)
Test item (1000 g a.s./ha, spray application)	480.50 ± 86.97	324.75 ± 76.92 (138)	494.25 ± 115.10 (112)	548.75 ± 197.68 (101)
Reference item	528.00 ± 157.89	135.00 ± 28.58 * (57)	294.25 ± 112.56 (67)	311.50 ± 158.37 * (57)
Lumbricus terrestris				
Control	22.00 ± 4.69	13.00 ± 2.58 (100)	23.25 ± 7.27 (100)	22.75 ± 9.71 (100)
Test item (500 g a.s./ha, furrow application)	27.25 ± 7.14	11.00 ± 5.48 (85)	18.75 ± 15.63 (81)	21.00 ± 14.94 (92)
Test item (500 g a.s./ha, spray application)	30.75 ± 3.20	12.50 ± 5.45 (96)	18.50 ± 9.11 (80)	22.75 ± 7.37 (100)
Test item (1000 g a.s./ha, spray application)	32.50 ± 4.43	16.75 ± 4.65 (129)	23.75 ± 9.91 (102)	22.75 ± 10.14 (100)
Reference item	25.75 ± 10.50	2.75 ± 1.71 * (21)	11.25 ± 6.95 * (48)	14.75 ± 7.41 (65)
Apporectodea Caliginosa/rosea				
Control	269.75 ± 66.51	148.25 ± 21.20 (100)	282.00 ± 50.82 (100)	327.50 ± 53.04 (100)
Test item (500 g a.s./ha, furrow application)	303.50 ± 58.27	190.25 ± 52.53 (128)	299.75 ± 59.49 (106)	400.75 ± 93.56 (122)
Test item (500 g a.s./ha, spray application)	300.25 ± 56.06	203.50 ± 81.82 (137)	359.50 ± 122.26 (127)	381.75 ± 155.50 (117)
Test item (1000 g a.s./ha, spray application)	278.50 ± 57.18	188.50 ± 47.56 (127)	336.25 ± 74.91 (119)	334.75 ± 92.24 (102)

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Treatment group	Mean abundance ± SD [individuals/m²] (relative abundance [% of control]) pre-sampling	1st Sampling	2nd Sampling	3rd Sampling
Reference item	329.75 ± 48.55	84.50 ± 22.07 * (57)	227.75 ± 70.76 (81)	236.75 ± 95.56 (72)

SD: Standard deviation
* Significant difference from control according to the Student-t test one-sided smaller at the significance level alpha = 0.05.
Pre-sampling on May 21- June 5, 2013 (6-21 days before the first application)
1st sampling on July 8-16, 2013 (4-5 weeks after the application)
2nd sampling on October 8-24, 2013 (4-5 months after the application)
3rd sampling on May 12 - June 13, 2014 (11-12 months after the application)

Table 8.4.1- 9: Changes in biomass for total earthworms, total juveniles and total adults and separated out for the dominant species *L. terrestris* and *A. chlorotica*

Treatment group	Mean biomass ± SD [g/m²] (relative biomass [% of control]) pre-sampling	1st Sampling	2nd Sampling	3rd Sampling
Total				
Control	51.25 ± 15.25	25.61 ± 3.79 (100)	83.96 ± 9.47 (100)	112.62 ± 30.99 (100)
Test item (500 g a.s./ha, furrow application)	57.57 ± 10.34	24.71 ± 2.57 (96)	70.89 ± 29.39 (84)	108.01 ± 23.93 (96)
Test item (500 g a.s./ha, spray application)	58.92 ± 12.13	26.75 ± 9.09 (104)	78.69 ± 17.27 (94)	106.48 ± 25.22 (95)
Test item (1000 g a.s./ha, spray application)	59.50 ± 13.82	32.23 ± 8.39 (126)	85.42 ± 17.80 (102)	103.78 ± 26.86 (92)
Reference item	54.94 ± 13.91	13.05 ± 2.81 * (51)	63.76 ± 18.53 (76)	86.72 ± 25.59 (77)
Total adults				
Control	18.82 ± 8.12	8.12 ± 2.81 (100)	39.17 ± 10.12 (100)	49.40 ± 8.13 (100)
Test item (500 g a.s./ha, furrow application)	24.22 ± 3.79	7.41 ± 2.49 (91)	23.37 ± 14.89 * (60)	43.97 ± 11.04 (89)
Test item (500 g a.s./ha, spray application)	24.67 ± 8.18	6.98 ± 1.86 (86)	30.39 ± 8.79 (78)	44.39 ± 9.39 (90)
Test item (1000 g a.s./ha, spray application)	23.29 ± 6.78	7.75 ± 3.21 (95)	35.92 ± 7.55 (92)	39.73 ± 4.47 (80)
Reference item	22.92 ± 7.99	5.02 ± 2.43 (62)	30.78 ± 7.62 (79)	43.89 ± 8.60 (89)
Total juveniles				
Control	32.44 ± 8.07	17.49 ± 2.76 (100)	44.79 ± 6.49 (100)	63.22 ± 22.93 (100)
Test item (500 g a.s./ha, furrow application)	33.35 ± 7.71	17.37 ± 4.65 (99)	47.51 ± 15.98 (106)	64.04 ± 17.65 (101)
Test item	34.25 ± 5.46	19.77 ± 8.15	48.30 ± 14.98	62.09 ± 24.12

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Treatment group	Mean biomass ± SD [g/m²] (relative biomass [% of control]) pre-sampling	1st Sampling	2nd Sampling	3rd Sampling
(500 g a.s./ha, spray application)		(113)	(108)	(98)
Test item (1000 g a.s./ha, spray application)	36.21 ± 8.00	24.50 ± 7.16 (140)	49.50 ± 14.36 (111)	64.05 ± 23.74 (101)
Reference item	32.02 ± 7.45	8.04 ± 1.60 * (46)	32.98 ± 12.51 (74)	42.84 ± 22.04 (68)
Lumbricus terrestris				
Control	8.07 ± 3.62	4.88 ± 1.03 (100)	20.09 ± 7.39 (100)	40.78 ± 14.73 (100)
Test item (500 g a.s./ha, furrow application)	12.77 ± 2.24	5.10 ± 1.59 (105)	11.52 ± 8.88 (57)	33.24 ± 17.86 (82)
Test item (500 g a.s./ha, spray application)	16.71 ± 7.04	6.35 ± 3.57 (130)	15.86 ± 7.90 (79)	37.18 ± 9.19 (91)
Test item (1000 g a.s./ha, spray application)	11.75 ± 3.13	9.84 ± 5.25 (202)	17.35 ± 8.49 (86)	31.61 ± 11.87 (78)
Reference item	10.16 ± 2.14	2.20 ± 0.46 * (45)	10.52 ± 6.46 * (52)	26.88 ± 10.24 (66)
Apporectodea Caiginosa/rosea				
Control	18.51 ± 2.08	12.77 ± 2.00 (100)	32.40 ± 3.49 (100)	32.56 ± 7.71 (100)
Test item (500 g a.s./ha, furrow application)	21.11 ± 5.67	10.89 ± 2.13 (85)	30.62 ± 6.16 (95)	34.58 ± 0.39 (106)
Test item (500 g a.s./ha, spray application)	19.31 ± 3.67	11.39 ± 3.51 (89)	32.78 ± 7.56 (101)	31.38 ± 10.44 (96)
Test item (1000 g a.s./ha, spray application)	21.53 ± 5.77	12.41 ± 2.03 (97)	36.43 ± 7.36 (112)	33.80 ± 8.05 (104)
Reference item	20.93 ± 6.58	6.50 ± 2.30 * (51)	36.05 ± 9.60 (111)	31.35 ± 8.98 (96)

SD: Standard deviation

* Significant difference from control according to the Student-t test one-sided smaller at the significance level alpha = 0.05

Pre-sampling on May 21- June 5, 2013 (6-21 days before the first application)

1st sampling on July 8-16, 2013 (4-5 weeks after the application)

2nd sampling on October 8-24, 2013 (4-5 months after the application)

3rd sampling on May 12 - June 13, 2014 (11-12 months after the application)

III. CONCLUSION

The present earthworm field study shows that FLU SC 400, applied as surface application at rates of 500 g a.s./ha and 1000 g a.s./ha, and as in furrow application at a rate of 500 g a.s./ha had no unacceptable ecologically adverse effects on the population of earthworms 4-5 weeks, 4-5 months and 11-12 months after the application.

Assessment and conclusion by applicant:

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The study and its data are considered as supplementary data with no use in risk assessment.

CA 8.4.2 Effects on non-target soil meso and macrofauna (other than earthworms)

Studies describing the toxicity of fluopyram to non-target soil meso and macrofauna (other than earthworms) have been performed with the representative formulations and are presented here and in the respective Document MCP, Section 10.4.2.

Table 8.4.2- 1: Springtail and soil mite reproduction studies with fluopyram metabolites

Test substance	Test species, test design	Ecotoxicological Endpoint	Reference
Springtails, reproduction			
FLU SC 500	*Folsomia candida* reproduction 28 d, mixed	NOEC = 178 mg prod./kg dws $NOEC_{corr}$ = 89 mg prod./kg dws[A] NOEC = 75.5 mg a.s./kg dws[B] $NOEC_{corr}$ = 37.8 mg a.s./kg dws[A, B] EC_{10} = 240 mg prod./kg dws $EC_{10,corr}$ = 120 mg prod./kg dws[A] EC_{10} = 102 mg a.s./kg dws[B] $EC_{10,corr}$ = 51 mg a.s./kg dws[A, B]	[redacted] (2019) M-675002-01-1 KCA 8.4.2.1/01 KCP 10.4.2.1/03 [C]
Fluopyram-7-hydroxy	*Folsomia candida* reproduction 28 d, mixed	NOEC = 562 mg p.m./kg dws $NOEC_{corr}$ = 281 mg p.m./kg dws[A] EC_{10} = 611 mg p.m./kg dws	[redacted] (2020) M-755397-01-1 KCA 8.4.2.1/03
Trifluoroacetic acid (TFA)	*Folsomia candida* reproduction 28 d, mixed	NOEC ≥ 100 mg p.m./kg dws (Na-TFA) ≥ 84 mg p m./kg dws (TFA) [B] EC_{10} calculation not possible	[redacted] (2012) M-436127-01-1 KCA 8.4.2.1/05
Soil mites, reproduction			
FLU SC 500	*Hypoaspis aculeifer* reproduction 14 d, mixed	NOEC ≥ 1000 mg prod./kg dws $NOEC_{corr}$ ≥ 500 mg prod./kg dws[A] NOEC ≥ 424 mg a.s./kg dws[B] $NOEC_{corr}$ ≥ 212 mg a.s./kg dws[A, B] EC_{10} calculation not possible	[redacted] (2020) M-678468-01-1 KCA 8.4.2.1/02 KCP 10.4.2.1/04 [C]
Fluopyram-7-hydroxy	*Hypoaspis aculeifer* reproduction 14 d, mixed	NOEC ≥ 1000 mg p.m./kg dws $NOEC_{corr}$ ≥ 500 mg p.m./kg dws[A] EC_{10} calculation not possible	[redacted] (2020) M-754291-01-1 KCA 8.4.2.1/04
Trifluoroacetic acid (TFA)	*Hypoaspis aculeifer* reproduction 14 d, mixed	NOEC ≥ 100 mg p.m./kg dws (Na-TFA) ≥ 84 mg p m./kg dws (TFA) [B] EC_{10} calculation not possible	[redacted] (2012) M-436326-01-1 KCA 8.4.2.1/05

dws = dry weight soil, a.s. = active substance; p m. = pure metabolite, prod. = product

A Endpoint corrected by a factor of 2 due to lipophilic substance (log P_{OW} > 2)

B As the study was conducted with sodium trifluoroacetate which is the sodium salt of trifluoroacetic acid, the endpoint was converted to trifluoroacetic acid with factor 0.84.

C Full details on this study are described in the corresponding MCP for the formulation FLU SC 500.

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CA 8.4.2.1 Species level testing

Fluopyram SC 500

Data Point:	KCA 8.4.2.1/01
Report Author:	
Report Year:	2019
Report Title:	Fluopyram SC 500 g/L: Influence on mortality and reproduction of the collembolan species Folsomia candida tested in artificial soil
Report No:	E 314 05458-0
Document No:	M-675002-01-1
Guideline(s) followed in study:	EU Directive 91/414/EEC Regulation (EC) No. 1107/2009 OECD Guideline 232 US EPA OCSPP Not Applicable
Deviations from current test guideline:	Current Guideline: OECD 232 (2016) Deviations: None. All validity criteria were met.
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

For the detailed study summary please refer to the Fluopyram SC 500 MCP Section 10.4.2.1/03.

Data Point:	KCA 8.4.2.1/02
Report Author:	
Report Year:	2020
Report Title:	Fluopyram SC 500 g/L: Influence on mortality and reproduction of the soil mite species Hypoaspis aculeifer tested in artificial soil
Report No:	E 428 05448-5
Document No:	M-678468-01-1
Guideline(s) followed in study:	EU Directive 91/414/EEC Regulation (EC) No. 1107/2009 OECD Guideline 226 (2016) US EPA OCSPP Not Applicable
Deviations from current test guideline:	Current Guideline: OECD 226 (2016) Deviations: None. All validity criteria were met.
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

For the detailed study summary please refer to the Fluopyram SC 500 MCP Section 10.4.2.1/04.

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Metabolite fluopyram-7-hydroxy

Data Point:	KCA 8.4.2.1/03
Report Author:	████
Report Year:	2020
Report Title:	AE C656948-7-hydroxy (BCS-AA10065): Influence on mortality and reproduction of the collembolan species Folsomia candida tested in artificial soil
Report No:	E 314 05535-6
Document No:	M-755397-01-1
Guideline(s) followed in study:	EU Directive 91/414/EEC Regulation (EC) No. 1107/2009 OECD Guideline 232 US EPA OCSPP Not Applicable
Deviations from current test guideline:	Current Guideline: OECD 232 (2016) Deviation: none. All validity criteria were met.
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

In a laboratory study the effects of fluopyram-7-hydroxy on survival and reproduction of the collembolan species *Folsomia candida* was tested during an exposure of 28 days in artificial soil by comparing control and treatment. Eight test item rates from 100 to 1000 mg pure metabolite/kg dry weight soil were tested. Per test item rate 4 replicates and for the control group 8 replicates with 10 collembolans each were exposed to fluopyram-7-hydroxy mixed into artificial soil. Mortality and reproduction of the collembolans was assessed after 28 days.

The study fulfilled all validity criteria of OECD 232 guideline.

Concerning mortality no statistically significant difference between control and any treatment group up to and including ≥1000 mg p.m./kg dry weight artificial soil. Therefore, the No-Observed-Effect-Concentration (NOEC) for mortality is ≥1000 mg p.m./kg dry weight artificial soil. The Lowest-Observed-Effect-Concentration (LOEC) for mortality is >1000 mg p.m./kg dry weight artificial soil. Due to the lack of a concentration-response relationship no reliable LCx-calculation was possible. Therefore, no LC_{10}/LC_{20} value can be reported.

Concerning the number of juveniles statistical analysis revealed no significant difference between control and any treatment group up to and including 562 mg p.m./kg dry weight artificial soil. Therefore, the No-Observed-Effect-Concentration (NOEC) for reproduction is 562 mg p.m./kg dry weight artificial soil. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction is 1000 mg p.m./kg dry weight artificial soil. The EC_{10} and EC_{20} values for reproduction were calculated to be 611 mg p.m./kg soil dry weight (95% confidence limits: 598-625) and 825 mg p.m./kg soil dry weight (95% confidence limits: 815-835), respectively (Probit analysis).

I. Materials and Methods

Test item: Fluopyram-7-hydroxy; Batch No.: BCS-AA 10065-01-01; TOX21541-00; purity: 99.4 % w/w.

Test design: Ten collembolans (10 - 12 days old) per replicate (8 replicates for the control group and 4 replicates for each treatment group) were exposed to control and treatment. Concentrations of 100, 178,

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316, 562 and 1000 mg p. m./kg dry weight artificial soil (equivalent to 100.6, 179.1, 317.9, 565.4 and 1006.0 mg test item/kg dry weight artificial soil) were mixed into the artificial soil. During the study the collembolans were fed with granulated dry yeast. 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 and 20 % Kaolin clay. Mortality and reproduction were determined after 28 days.

The climatic conditions were in the temperature range 20.0 ± 2 °C with a photoperiod of 16 hours light and a light intensity of 400 - 800 lux.

Statistics: For statistical analysis Fisher's exact Binominal Test (Bonferroni Correction, one-sided greater, α = 0.05) was applied to mortality data and William's t-test (one-sided smaller, α = 0.05) was applied to reproduction data.

Dates of work: July 21, 2020 – August 19, 2020

II. RESULTS AND DISCUSSION

Table 8.4.2.1- 1: Effects on mortality and reproduction of *Folsomia candida* after treatment with fluopyram-7-hydroxy

Test concentration [mg p m./kg dry weight artificial soil]	Adult mortality [%]	Significance (*)	Mean number of juveniles per test vessel ± SD	Reproduction [% of control]	Significance (**)
Control	6.3	-	409.9 ± 55.1	-	--
100	2.5	-	474.8 ± 81.2	115.8	-
178	15.0	-	497.0 ± 80.2	121.3	-
316	17.5	-	406.0 ± 55.3	99.1	-
562	5.0	-	376.5 ± 64.0	91.9	-
1000	15.0	-	292.0 ± 41.1	71.2	+
Endpoints				**Adult mortality**	**Reproduction**
NOEC [mg p m./kg dry weight artificial soil]				≥1000	562
LOEC [mg p.m./kg dry weight artificial soil]				>1000	>1000
EC_{10} (C.I.) [mg p m./kg dry weight artificial soil]				-	611 (598-625)
EC_{20} (C.I.) [mg p.m./kg dry weight artificial soil]				-	825 (815-835)

The calculations were performed with un-rounded values.
Results are expressed as mg test item/kg dry weight artificial soil.
(*) (Fisher's Exact Binominal Test with Bonferroni Correction, one-sided-greater, α = 0.05, "+" = significant, "-" = not significant)
(**) (William's t-test one-sided-smaller, α = 0.05; "+" = significant, "-" = not significant)
1) Probit analysis
n.d. Could not be determined (see observations)

Mortality:

In the control group 6.3 % of the adult *Folsomia candida* died which is below the allowed maximum of ≤ 20% mortality.

Concerning the mortality of the adult test organisms statistical analysis (Fisher's Exact Binominal Test with Bonferroni Correction, one-sided-greater, α = 0.05) revealed no significant difference between control and any treatment group up to and including ≥ 1000 mg p.m./kg dry weight artificial soil.

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Therefore, the NOEC for mortality is ≥1000 mg p.m./kg dry weight artificial soil. The LOEC for mortality is > 1000 mg p.m./kg dry weight artificial soil.
Due to the lack of a concentration-response relationship no reliable LCx-calculation was possible. Therefore, no LC_{10}/LC_{20}-value can be reported.

Reproduction:

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 up to and including 562 mg p.m./kg dry weight artificial soil.

Therefore, the NOEC for reproduction is 562 mg p.m./kg dry weight artificial soil. The LOEC for reproduction is 1000 mg p.m./kg dry weight artificial soil.

The EC_{10} and EC_{20} values for reproduction were calculated to be 611 mg p.m./kg soil dry weight (95% confidence limits: 598-625) and 825 mg p. metabolite/kg soil dry weight (95% confidence limits: 815-835), respectively (Probit analysis).

Validity criteria:

Validity criteria for the untreated control of the study according OECD 232 from July 29, 2016 were used.

Table 8.4.2.1- 2: Validity criteria

Validity criteria acc. to OECD 232 (adopted 2016)	Required	Obtained
Mean adult mortality in control	≤ 20 %	6.3 %
Mean number of juveniles per replicate (with 10 collembolans introduced)	≥ 100	410
Coefficient of variation calculated for the number of juveniles per replicate	≤ 30 %	13.4 %

All validity criteria of the OECD 232 guideline were fulfilled.

Reference test:

The most recent non-GLP-test (Coll-Ref-37/20, February 2020) with the reference item Boric acid was performed at test concentrations 44, 67, 100, 150 and 225 mg Boric acid/kg dry weight artificial soil.
The $NOEC_{reproduction}$ was calculated to be 67 mg Boric acid/kg dry weight artificial soil and accordingly the $LOEC_{reproduction}$ is 100 mg Boric acid/kg dry weight artificial soil according Williams t-test, $\alpha = 0.05$, one-sided smaller.
Boric acid showed an EC_{50} of 127 mg test item/kg dry weight artificial soil (95% confidence limits from 114 mg to 141 mg Boric acid/kg dry weight artificial soil) for reproduction according Probit analysis using linear maximum likelihood regression.
The result is in the recommended range of the guideline (about 100 mg Boric acid/kg artificial soil dry weight).

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III. CONCLUSION

All validity criteria were met. The endpoints were:

$NOEC_{adult\ mortality}$: ≥1000 mg p.m./kg dry weight artificial soil
$LOEC_{adult\ mortality}$: >1000 mg p.m./kg dry weight artificial soil

$NOEC_{reproduction}$: 562 mg p.m./kg dry weight artificial soil
$LOEC_{reproduction}$: >1000 mg p.m./kg dry weight artificial soil

$EC_{10reproduction}$: = 611 mg p.m./kg dry weight artificial soil
$EC_{20reproduction}$: = 825 mg p.m./kg dry weight artificial soil

According to EFSA (2015) the level of protection for the EC_{10} is classified as "high". The normalised width of confidence interval (NW) rating for the EC_{10} is "excellent".

<u>Assessment and conclusion by applicant:</u>
The study and its data are considered as acceptable and reliable for use in risk assessment.
The endpoint is: NOEC = 562 mg p.m./kg dws

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Data Point:	KCA 8.4.2.1/04
Report Author:	[redacted]
Report Year:	2020
Report Title:	AE C656948-7-hydroxy (BCS-AA10065): Influence on mortality and reproduction of the soil mite species Hypoaspis aculeifer tested in artificial soil
Report No:	M-754291-01-1
Document No:	M-754291-01-1
Guideline(s) followed in study:	EU Directive 91/414/EEC Regulation (EC) No. 1107/2009 OECD Guideline 226 (2016) US EPA OCSPP Not Applicable
Deviations from current test guideline:	Current Guideline: OECD 226 (2016) Deviation: none. All validity criteria were met.
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

In a laboratory study the effects of fluopyram-7-hydroxy on survival and reproduction of the soil mite species *Hypoaspis aculeifer* was tested during an exposure of 14 days in artificial soil by comparing control and treatment. Eight test item rates from 100 to 1000 mg pure metabolite/kg dry weight soil were tested. Per test item rate 4 replicates and for the control group 8 replicates with 10 soil mites each were exposed to fluopyram-7-hydroxy mixed into artificial soil. Mortality of the soil mites was assessed after 14 days.
The study fulfilled all validity criteria of OECD 226 guideline.

No statistically significant differences in mortality compared to the control occurred. Therefore, the No-Observed-Effect-Concentration (NOEC) for mortality is $\geq$1000 mg p.m./kg dws. The Lowest-Observed-Effect-Concentration (LOEC) for mortality is >1000 mg p.m./kg dws. Due to the lack of a concentration-response relationship, no LC_x values could be calculated.

The reproduction rate of the soil mites was assessed after 14 days. No statistically significant differences compared to the control occurred. Therefore, the No-Observed-Effect-Concentration (NOEC) for reproduction is $\geq$1000 mg p.m./kg dws. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction is >1000 mg p.m./kg dws. Due to the lack of a concentration-response relationship, no EC_x values could be calculated.

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I. MATERIALS AND METHODS

Test item: Fluopyram-7-hydroxy; Batch No.: BCS-AA 10065-01-01; TOX21541-00; purity: 99.4 % w/w.

Test design: Ten adult, 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 (synchronised culture at an age of 28 days after start of egg laying). Concentrations of 100, 178, 316, 562 and 1000 mg p.m./kg dry weight artificial soil (equivalent to 100.6, 179.1, 317.9, 565.4 and 1006.0 mg test item/kg dry weight artificial soil) were mixed into the artificial soil. During the test, the *Hypoaspis aculeifer* were fed with nematodes bred on watered oat flakes 2, 6 and 8 days after test start. 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.

The climatic conditions were in the temperature range 20.0 ± 2 °C with a photoperiod of 16 hours light and a light intensity of 400 - 800 lux.

Statistics: For statistical analysis Fisher's exact Binominal Test (Bonferroni Correction, one-sided greater, α = 0.05) was applied to mortality data and Williams t-test (one-sided smaller, α = 0.05) was applied to reproduction data.

Dates of work: July 06, 2020 – July 24, 2020

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II. RESULTS AND DISCUSSION

Table 8.4.2.1- 3: Effects on mortality and reproduction of *Hypoaspis aculeifer* after treatment with fluopyram-7-hydroxy

Test concentration [mg p m./kg dry weight artificial soil]	Adult mortality [%]	Significance (*)	Mean number of juveniles per test vessel ± SD	Reproduction [% of control]	Significance (**)
Control	3.8	N/A	409.4 ± 28.6	N/A	N/A
100	10.0	-	410.5 ± 9.5	100.3	-
178	7.5	-	423.5 ± 20.8	103.5	-
316	5.0	-	415.5 ± 37.0	101.5	-
562	0.0	-	421.3 ± 12.6	102.9	-
1000	7.5	-	401.3 ± 31.9	98.0	-

Endpoints	Adult mortality	Reproduction
NOEC [mg p m./kg dry weight artificial soil]	≥1000	≥1000
LOEC [mg p.m./kg dry weight artificial soil]	>1000	>1000
EC_{10} [mg p m./kg dry weight artificial soil]	-	n.d.
EC_{20} [mg p m./kg dry weight artificial soil]	-	n.d.

Calculations were done with unrounded values.
Results are expressed as mg pure metabolite/kg dry weight artificial soil.
(*) = Fisher´s exact Binomial Test with Bonferroni Correction, one-sided greater, α=0.05, "-": non-significant; "+": significant
(**) = Williams t-test, one sided smaller; α=0.05; "-": non-significant; "+": significant
n.d. = not determined (see observations)
N/A = not applicable

Mortality:

In the control group 3.8% of the adult *Hypoaspis aculeifer* died which is below the allowed maximum of ≤ 20% mortality.

Concerning the mortality of the adult test organisms statistical analysis (Fisher´s Exact Binomial Test with Bonferroni Correction, one-sided greater, α = 0,05) revealed no significant difference between control group and any treatment group up to and including 1000 mg p.m./kg dry weight artificial soil.

Therefore, the NOEC for mortality is ≥1000 mg p.m./kg dry weight artificial soil. The LOEC for mortality is >1000 mg p.m./kg dry weight artificial soil.

Reproduction:

Concerning the number of juveniles statistical analysis (Williams t-test, one-sided smaller, α = 0.05) revealed no significant difference between control group and any treatment group up to and including 1000 mg pure metabolite/kg dry weight artificial soil.

Therefore, the NOEC for reproduction is ≥1000 mg p.m./kg dry weight artificial soil. The LOEC for reproduction is >1000 mg p.m./kg dry weight artificial soil.

Due to the lack of a concentration-response relationship no reliable ECx-calculation is possible. Therefore no EC_{10}/EC_{20}-value can be reported.

Validity criteria:

Validity criteria for the untreated control of the study according OECD 226 from July 29, 2016 were used.

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All validity criteria of the OECD 226 guideline were fulfilled.

Table 8.4.2.1- 4: Validity criteria

Validity criteria acc. to OECD 226 (adopted 2016)	Required	Obtained
Mean adult mortality	≤ 20 %	3.8 %
Mean number of juveniles per replicate (with 10 mites introduced)	≥ 50	409.4
Coefficient of variation calculated for the number of juveniles per replicate	≤ 30 %	7.0 %

Reference test:

The corresponding non-GLP-test (HR-Ref-29/20, February 12, 2020) with the reference item dimethoate was performed at test concentrations of 1.0, 1.8, 3.2, 5.6 and 10.0 mg dimethoate/kg dry weight artificial soil.

Dimethoate EC 400 G showed a LC_{50} of 3.1 mg a.s./kg for mortality of the adult mites according Weibull analysis using maximum likelihood regression (confidence limits from 2.2 mg a.s./kg to 4.5 mg a.s./kg).

The reproduction of the soil mites was not significantly reduced in comparison to the control up to and including 3.2 mg a.s./kg dry weight artificial soil. Therefore the NOEC is calculated to be 3.2 mg a.s./kg dry weight artificial soil and accordingly the LOEC is 5.6 mg a.s./kg dry weight artificial soil. 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 6.1 mg a.s./kg dry weight artificial soil (95% confidence limits from 6.1 mg a.s./kg to 6.2 mg a.s./kg) for reproduction according Probit analysis using maximum likelihood regression.

This is in the recommended range of the guideline, indicating that an EC_{50} based on the number of juveniles of 3.0 – 7.0 mg a.s./kg dry weight artificial soil shows that the test organisms are sufficiently sensitive.

III. Conclusion

All validity criteria were met. The endpoints were:

$NOEC_{adult\ mortality}$: ≥1000 mg p.m./kg dry weight artificial soil
$LOEC_{adult\ mortality}$: >1000 mg p.m./kg dry weight artificial soil

$NOEC_{reproduction}$: ≥1000 mg p.m./kg dry weight artificial soil
$LOEC_{reproduction}$: >1000 mg p.m./kg dry weight artificial soil

Due to the lack of a concentration-response relationship no reliable EC_x-calculation was possible.

Assessment and conclusion by applicant:

The study and its data are considered as acceptable and reliable for use in risk assessment.

The endpoint is: NOEC ≥1000 mg p.m./kg dws

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Metabolite trifluoroacetic acid (TFA)

Data Point:	KCA 8.4.2.1/05
Report Author:	████
Report Year:	2012
Report Title:	Trifluoroacetic acid Na-salt (BCS-AZ56567): Influence on the reproduction of the collembolan species Folsomia candida tested in artificial soil
Report No:	FRM-COLL-132/12
Document No:	M-436127-01-1
Guideline(s) followed in study:	OECD 232 adopted, September 07, 2009: OECD Guidelines for Testing Chemicals - Collembolan Reproduction Test in Soil
Deviations from current test guideline:	Current Guideline: OECD 232 (2016) Deviation: none. All validity criteria were met.
Previous evaluation:	yes, evaluated and accepted in flurtamone RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

In a laboratory study the effects of sodium trifluoroacetate (Trifluoroacetic acid (TFA) Na-salt) on survival and reproduction of the collembolan species *Folsomia candida* was tested during an exposure of 28 days in artificial soil by comparing control and treatment. A limit concentration of 100 mg pure metabolite/kg dry weight soil was tested. Per test item rate 8 replicates and for the control group 8 replicates with 10 collembolans each were exposed to TFA Na-salt mixed into artificial soil. Mortality and reproduction of the collembolans was assessed after 28 days.

The study fulfilled all validity criteria of OECD 232 guideline.

No statistically significant differences in mortality compared to the control occurred. Therefore, the No-Observed-Effect-Concentration (NOEC) for mortality is ≥100 mg p.m./kg dws. The Lowest-Observed-Effect-Concentration (LOEC) for mortality is >100 mg p.m./kg dws. Due to the lack of a concentration-response relationship, no LC_x values could be calculated.

Concerning the number of juveniles there was no significant difference between the control and the treatment group.

The endpoints based on nominal concentrations of sodium trifluoroacetate were: The No-Observed-Effect-Concentration (NOEC) for reproduction ≥ 100 mg p.m./kg artificial soil dry weight and the Lowest-Observed-Effect-Concentration (LOEC) for reproduction > 100 mg p.m./kg artificial soil dry weight. Due to the lack of a concentration-response relationship, no EC_x values could be calculated.

The converted endpoints based on nominal concentrations of trifluoroacetic acid were: The NOEC for reproduction ≥ 84 mg p.m./kg artificial soil dry weight and LOEC for reproduction > 84 mg p.m./kg artificial soil dry weight. Due to the lack of a concentration-response relationship, no EC_x values could be calculated.

I. MATERIALS AND METHODS

Test item: Sodium trifluoroacetate (Trifluoroacetic acid (TFA) Na-salt); Batch code: AE 1046319-01-01; Origin Batch No: SES 11755-1-1; TOX 09476-01; purity: 95.1 % w/w. Due to its pka-value < 2 trifluoroacetic acid is deprotonated under environmental conditions and hence the deprotonated form, trifluoroacetate (CF_3COO^-) is used to test the toxicological properties of this metabolite.

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Test design: Ten collembolans (10 - 12 days old) per replicate (8 replicates for the control group and 8 replicates for each treatment group) were exposed to control and treatment. A limit concentration of 100 mg p.m./kg dry weight artificial soil were mixed into the artificial soil. During the study the collembolans were fed with granulated dry yeast. 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 and 20 % Kaolin clay. Mortality and reproduction were determined after 28 days.
The climatic conditions were in the temperature range 20.0 ± 2 °C with a photoperiod of 16 hours light and a light intensity of 400 - 800 lux.

Statistics: The software used to perform the statistical analysis was ToxRat Professional 2.10 released February 20, 2010, (Ratte, 2010). Data of reproduction were tested for normal distribution and homogeneity of variance using Kolmogorov – Smirnov -Test and Cochran's -Test ($\alpha = 0.05$) respectively. Data of reproduction were normally distributed and homogeneity of variances was given. Therefore Student-t test (one-sided-smaller, $\alpha = 0.05$) was used to determine NOEC and LOEC values.

Dates of work: March 16, 2012 – April 24, 2012

II. Results and Discussion

Table 8.4.2.1- 5: Effects on mortality and reproduction of *Folsomia candida* after treatment with TFA

Test concentration [mg p.m./kg dry weight artificial soil]	% mortality (Adults)	Mean number of juveniles per test vessel ± standard dev.	Reproduction (% of control)
Control	16.3	1132.6 ± 110.4	-
100	10.0	1051.9 ± 133.4	92.9 n s
$NOEC_{reproduction}$ (mg p.m./kg soil dry weight)			≥ 100
$LOEC_{reproduction}$ (mg p.m./kg soil dry weight)			> 100

The calculations were performed with un-rounded values
SD = standard deviation
n.s. = statistically not significant (Student-t test one-sided-smaller, $\alpha = 0.05$)

Mortality:

In the control group 16.3 % of the adult *Folsomia candida* died which is below the allowed maximum of ≤ 20 % mortality.

Reproduction:

Concerning the number of juveniles statistical analysis (Student-t test, one-sided smaller, $\alpha = 0.05$) revealed no significant difference between control and the treatment group.

Therefore the No-Observed-Effect-Concentration (NOEC) for reproduction is ≥ 100 mg p.m./kg artificial soil dry weight. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction is > 100 mg p.m./kg artificial soil dry weight.

Validity criteria:

Validity criteria for the untreated control of the study according OECD 232 from July 29, 2016 were used.

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Table 8.4.2.1- 6: Validity criteria

Validity criteria acc. to OECD 232 (adopted 2016)	Required	Obtained
Mean adult mortality in control	≤ 20 %	16.3 %
Mean number of juveniles per replicate (with 10 collembolans introduced)	≥ 100	1132.6
Coefficient of variation calculated for the number of juveniles per replicate	≤ 30 %	9.7 %

All validity criteria of the OECD 232 guideline were fulfilled.

Reference test:

The most recent non-GLP-test (FRM-Coll-Ref-19/12) with the reference item Boric acid was performed at test concentrations 44 – 67 – 100 – 150 and 225 mg Boric acid/kg artificial soil dry weight.

Boric acid showed an EC_{50} of 116 mg test item/kg artificial soil dry weight (95 % confidence limits from 98 mg to 137 mg Boric acid/kg artificial soil dry weight) for reproduction according Probit analysis using maximum likelihood regression.

The result is in the recommended range of the guideline (about 100 mg Boric acid/kg artificial soil dry weight).

The $NOEC_{reproduction}$ was calculated to be 67 mg Boric acid/kg artificial soil dry weight and accordingly the $LOEC_{reproduction}$ is 100 mg Boric acid/kg artificial soil dry weight according Williams-Test multiple t-test procedure, $\alpha = 0.05$, one-sided smaller.

This shows that the test organisms are sufficiently sensitive.

III. CONCLUSION

All validity criteria were met. The endpoints based on nominal concentrations of sodium trifluoroacetate were:

$NOEC_{adult\ mortality}$: ≥100 mg p.m./kg dry weight artificial soil

$LOEC_{adult\ mortality}$: >100 mg p.m./kg dry weight artificial soil

$NOEC_{reproduction}$: ≥100 mg p.m./kg dry weight artificial soil

$LOEC_{reproduction}$: >100 mg p.m./kg dry weight artificial soil

Due to the lack of a concentration-response relationship no reliable EC_x-calculation was possible.

As the study was conducted with sodium trifluoroacetate which is the sodium salt of trifluoroacetic acid, the endpoint was converted to trifluoroacetic acid with factor 0.84. The converted endpoints based on nominal concentrations of trifluoroacetic acid were:

$NOEC_{adult\ mortality}$: ≥84 mg p.m./kg dry weight artificial soil

$LOEC_{adult\ mortality}$: >84 mg p.m./kg dry weight artificial soil

$NOEC_{reproduction}$: ≥84 mg p.m./kg dry weight artificial soil

$LOEC_{reproduction}$: >84 mg p.m./kg dry weight artificial soil

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Assessment and conclusion by applicant:
The study and its data are considered as acceptable and reliable for use in risk assessment.
The endpoint is: NOEC ≥100 mg p.m./kg dws (sodium trifluoroacetate) corresponding to ≥84 mg p.m./kg dws (trifluoroacetic acid)

Data Point:	KCA 8.4.2.1/06
Report Author:	[redacted]
Report Year:	2012
Report Title:	Trifluoroacetic acid Na-salt (BCS-AZ56567): Influence on mortality and reproduction on the soil mite species *Hypoaspis aculeifer* tested in artificial soil
Report No:	KRA-HR-58/12
Document No:	M-436326-01-1
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) Deviation: none. All validity criteria were met.
Previous evaluation:	yes, evaluated and accepted in flurtamone RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

In a laboratory study the effects of sodium trifluoroacetate (Trifluoroacetic acid (TFA) Na-salt) on survival and reproduction of the soil mite species *Hypoaspis aculeifer* was tested during an exposure of 14 days in artificial soil by comparing control and treatment. A limit concentration of 100 mg pure metabolite/kg dry weight soil was tested. Per test item rate and the control 8 replicates with 10 soil mites each were exposed to l mixed into artificial soil. Mortality of the soil mites was assessed after 14 days.

The study fulfilled all validity criteria of OECD 226 guideline.

No statistically significant differences in mortality compared to the control occurred. The reproduction rate of the soil mites was assessed after 14 days. No statistically significant differences compared to the control occurred.

The endpoints based on nominal concentrations of sodium trifluoroacetate were:

The No-Observed-Effect-Concentration (NOEC) for mortality ≥ 100 mg p.m./kg dws and the Lowest-Observed-Effect-Concentration (LOEC) for mortality > 100 mg p.m./kg dws. Due to the lack of a concentration-response relationship, no LC_x values could be calculated.

The No-Observed-Effect-Concentration (NOEC) for reproduction ≥100 mg p.m./kg dws and the Lowest-Observed-Effect-Concentration (LOEC) for reproduction >100 mg p.m./kg dws. Due to the lack of a concentration-response relationship, no EC_x values could be calculated.

The converted endpoints based on nominal concentrations of trifluoroacetic acid were:

The NOEC for mortality ≥ 84 mg p.m./kg dws and the LOEC for mortality > 84 mg p.m./kg dws. Due to the lack of a concentration-response relationship, no LC_x values could be calculated.

The NOEC for reproduction ≥ 84 mg p.m./kg dws and the LOEC for reproduction > 84 mg p.m./kg dws. Due to the lack of a concentration-response relationship, no EC_x values could be calculated.

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I. MATERIALS AND METHODS

Test item: Sodium trifluoroacetate (Trifluoroacetic acid (TFA) Na-salt); Batch code: AE 1046319-01-01; Origin Batch No: SES 11755-1-1; TOX 09476-01; purity: 95.1 %w/w. Due to its pka-value < 2 trifluoroacetic acid is deprotonated under environmental conditions and hence the deprotonated form, trifluoroacetate (CF_3COO^-) is used to test the toxicological properties of this metabolite.

Test design: Ten adult, fertilized female *Hypoaspis aculeifer* per replicate (8 replicates for the control group and 8 replicates for each treatment group) were exposed to control and treatments (synchronised culture at an age of 28 days after start of egg laying). A limit concentration of 100 mg p. m./kg dry weight artificial soil were mixed into the artificial soil. During the test, the *Hypoaspis aculeifer* were fed with cheese mites bred on brewer's yeast 3, 7 and 10 days after test start. 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.

The climatic conditions were in the temperature range 20.0 ± 2 °C with a photoperiod of 16 hours light and a light intensity of 400 - 800 lux.

Statistics: The software used to perform the statistical analysis was ToxRat Pro 2.10 (released February 20, 2010); (Ratte, 2001-2010). For normal distribution and homogeneity of variance using Kolmogorroff-Smirnov Test and Cochran-Test (α = 0.05), respectively were used. Data of reproduction were normally distributed but homogeneity of variances was not given. Therefore Student t-test for homogeneous variances (one-sided smaller, α = 0.05) was used to determine NOEC and LOEC values.

Dates of work: March 16, 2012 – April 10, 2012

II. RESULTS AND DISCUSSION

Table 8.4.2.1- 7: Effects on mortality and reproduction of *Hypoaspis aculeifer* after treatment with TFA

Test concentration [mg p.m./kg dry weight artificial soil]	% mortality (Adults)	Mean number of juveniles per test vessel ± standard dev.	Reproduction (% of control)
Control	2.5	346.5 ± 23.5	---
100	0.0	372.1 ± 19.1	107.4
NOEC (mg p.m./kg dry weight artificial soil)			≥ 100
LOEC (mg p.m./kg dry weight artificial soil)			> 100

No statistical significance (Student t-test for homogeneous variances, one-sided smaller, α = 0.05) was found.

Mortality:

In the control group 0 % of the adult *Hypoaspis aculeifer* died which is below the allowed maximum of ≤ 20 % mortality. The LC_{50} could not be calculated and is considered to be > 100 mg p.m./kg dry weight artificial soil.

Reproduction:

Concerning the number of juveniles statistical analysis (Student t-test for homogeneous variances, one-sided smaller, α = 0.05) revealed no significant differences between control and the concentration tested.

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Therefore, the No-Observed-Effect-Concentration (NOEC) for reproduction is ≥ 100 mg p.m./kg dry weight artificial soil. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction is > 100 mg p.m./kg dry weight artificial soil.

Validity criteria:

Validity criteria for the untreated control of the study according OECD 226 from July 29, 2016 were used.

All validity criteria of the OECD 226 guideline were fulfilled.

Table 8.4.2.1- 8: Validity criteria

Validity criteria acc. to OECD 226 (adopted 2016)	Required	Obtained
Mean adult mortality	≤ 20 %	2.5 %
Mean number of juveniles per replicate (with 10 mites introduced)	≥ 50	346.5
Coefficient of variation calculated for the number of juveniles per replicate	≤ 30 %	6.8 %

Reference test:

The most recent non-GLP-test (kra/HR-O-11/12) with the reference item dimethoate was performed at test concentrations 1.0, 1.8, 3.2, 5.6 and 10.0 mg dimethoate/kg dry weight artificial soil.

Dimethoate showed a LC_{50} of 3.894 mg a.s./kg for mortality of the adult mites according Probit analysis using maximum likelihood regression. Confidence limits could not be determined due to mathematical reasons.

The $NOEC_{reproduction}$ was calculated to be 3.2 mg a.s./kg dry weight artificial soil and accordingly the $LOEC_{reproduction}$ is 5.6 mg a.s./kg dry weight artificial soil. Since variances of the data were even after transformation not homogenous Welch-t-test for Inhomogeneous Variances with Bonferroni-Holm Adjustment, α = 0.05, one-sided was used. Dimethoate showed an EC_{50} of 6.62 mg a.s./kg dry weight artificial soil (95 % confidence limits from 6.02 to 2469.54 mg a.s./kg dry weight artificial soil) for reproduction according Probit analysis using maximum likelihood regression.

This is in the recommended range of the guideline of 3.0 – 7.0 mg a.s./kg dry weight artificial soil.

This shows that the test organisms are sufficiently sensitive.

III. CONCLUSION

All validity criteria were met. The endpoints based on nominal concentrations of sodium trifluoroacetate were:

$NOEC_{adult\ mortality}$: ≥100 mg p.m./kg dry weight artificial soil

$LOEC_{adult\ mortality}$: >100 mg p.m./kg dry weight artificial soil

$NOEC_{reproduction}$: ≥100 mg p.m./kg dry weight artificial soil

$LOEC_{reproduction}$: >100 mg p.m./kg dry weight artificial soil

Due to the lack of a concentration-response relationship no reliable EC_x-calculation was possible.

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As the study was conducted with sodium trifluoroacetate which is the sodium salt of trifluoroacetic acid, the endpoint was converted to trifluoroacetic acid with factor 0.84. The converted endpoints based on nominal concentrations of trifluoroacetic acid were:

$NOEC_{adult\ mortality}$: ≥84 mg p.m./kg dry weight artificial soil

$LOEC_{adult\ mortality}$: >84 mg p.m./kg dry weight artificial soil

$NOEC_{reproduction}$: ≥84 mg p.m./kg dry weight artificial soil

$LOEC_{reproduction}$: >84 mg p.m./kg dry weight artificial soil

Due to the lack of a concentration-response relationship no reliable EC_x-calculation was possible.

Assessment and conclusion by applicant:

The study and its data are considered as acceptable and reliable for use in risk assessment.

The endpoint is: NOEC ≥100 mg p.m./kg dws (trifluoroacetate) corresponding to ≥84 mg p.m./kg dws (trifluoroacetic acid)

CA 8.5 Effects on nitrogen transformation

Study describing the effects of Fluopyram on nitrogen transformation have been performed with the representative formulations and are presented here and in the respective Document MCP, Section 10.5.

Table 8.5- 1: Studies on nitrogen transformation with fluopyram and its metabolites

Test substance	Test species, test design	Ecotoxicological endpoint		Reference
N-transformation				
Fluopyram	Study duration 28 d	No unacceptable effects at an appl. rate of:	3.33 mg a.s./kg dws	███ (2006) M-281177-01-1 KCA 8.5/01
Fluopyram-7-hydroxy	Study duration 28 d	No unacceptable effects at an appl. rate of:	10 mg p.m./kg dws	███ (2020) M-754927-01-1 KCA 8.5/03
Trifluoroacetic acid (TFA)	Study duration 28 d	No unacceptable effects at an appl. rate of:	1.60 mg p m./kg dws (Na-TFA) 1.344 mg p m./kg dws (TFA) [A]	███ (2013) M-444423-01-1 KCA 8.5/04

dws = dry weight soil, a.s. = active substance; p m. = pure metabolite

[A] As the study was conducted with sodium trifluoroacetate which is the sodium salt of trifluoroacetic acid, the endpoint was converted to trifluoroacetic acid with factor 0.84.

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Active substance fluopyram

Data Point:	KCA 8.5/01
Report Author:	[redacted]
Report Year:	2006
Report Title:	AE C656948 tech.: Determination of effects on nitrogen transformation in soil
Report No:	LRT-N-78/06
Document No:	M-281177-01-1
Guideline(s) followed in study:	OECD/OECD No. 216; adopted: 21st January 2000, OECD Guideline for the Testing of Chemicals, Soil Microorganisms: Nitrogen Transformation Test; Equivalent to US EPA OPPTS Guideline No. 850 SUPP
Deviations from current test guideline:	Current Guideline: OECD 216 (2000) Deviation: none
Previous evaluation:	yes, evaluated and accepted in DAR (2011)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

In a laboratory study the effect of was used in the test. A silty sand soil was exposed for 28 days to 0.33 mg and 3.33 mg fluopyram /kg dry weight soil. Application rates were equivalent to 0.25 kg and 2.5 kg fluopyram /ha. Per treatment there were 3 replicates. The soil was enriched by 0.5 % lucerne meal and a water content of 41 - 44 % of the water holding capacity was maintained during the test.

Lucerne-grass-green meal was added to the soil (5 g/kg dry weight soil) to stimulate nitrogen transformation.
The study fulfilled all validity criteria of OECD 216 guideline.

Nitrogen transformation was determined after 0 hours, 7, 14 and 28 days. No adverse effects of fluopyram on nitrogen transformation in soil (in terms of Nitrate-N in mg/kg soil dry weight/time interval/day) were observed at both test concentrations at the end of the test (time interval 14 - 28 days after application).

I. MATERIALS AND METHODS

Test item: Fluopyram (AE C656948), specification no.: 102000012455; development No.: 3000314431, batch No.: 08528/0002, TOX-No.: 06970-01; purity: 94.7 % w/w.

Test design: The test soil was a biologically active agricultural soil, a silty sand soil with pH 6.6, 0.83 % C_{org}, and with the water holding capacity of 42.76 g/100 g dry soil. 300 g soil dry weight (soil d.w.) per test vessel was weighed and enriched with lucerne meal (concentration in soil 0.5 %). The nitrate values thus result as the difference from the subtraction: (nitrate values plus nitrite values) minus nitrite values. The nitrite contents were determined without nitrate reduction.
The soil of each treatment was tested as a series of 3 replicates. The endpoint was expressed as effect on NO_3-nitrogen production after 28 days of exposure. Non-treated soil was used as control. Soil samples (10 g soil d.w. per replicate) were taken at intervals of 0 hours, 7, 14 and 28 days after application and the NH_4-N, NO_3-N and NO_2-N content were determined. NH_4-nitrogen, NO_3- and NO_2-nitrogen were determined by using the Autoanalyzer III (BRAN+LUEBBE).

The test concentrations were 0.33 mg a.s./kg dry soil (corresponding to an application rate of 0.25 kg a.s./ha) and 3.33 mg a.s./kg dry soil (corresponding to an application rate of 2.5 kg a.s./ha). For

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calculation of the product requirements (mg/kg soil d.w.) a soil depth of 5 cm and a soil density of 1.5 g dry weight/cm^3 were assumed for conversion of soil volume to soil dry weight.

Statistics: Homogeneity of variances was determined by F-test (significance level 5 %). Depending on the results of the F-Tests, the appropriate T-tests were performed. In the T-tests, the mean values of nitrate-N/kg dry weight soil/time interval/day from control soils and treated soils were compared.

Climatic conditions: The test vessels were kept in darkness in a climatic room with a temperature of 20 ± 2 °C during the test. The soil moisture was between 14.40 - 15.90 fluopyram /kg dry weight soil and a maximum water holding capacity between 40.7 - 44.1.

Dates of work: October 19, 2006 – November 17, 2006

II. Results and Discussion

Validity criteria:

According to OECD guideline 216 (2000), the variation of the nitrate-concentrations between control replicates should be less than ± 15 %. In this study, a maximum coefficient of variation of 13 % was obtained. Therefore, the results of the study were considered valid.

Observations:

Table 8.5- 2: Rates of nitrogen transformation/ time interval/ day in soil after treatment with fluopyram

Time interval [days]	Control		0.33 mg a.s./kg dws			3.33 mg a.s./kg dws		
	Sample	Nitrate-N[1]	Sample	Nitrate-N[1]	% difference to control	Sample	Nitrate-N[1]	% difference to control
0-7	1	-1.94	1	-1.73		1	-1.95	
	2	-1.76	2	-1.84		2	-1.42	
	3	-1.63	3	-1.76		3	-1.40	
	MV	**-1.77**	**MV**	**-1.78**	**0**	**MV**	**-1.59**	**-10**
	±SD	0.16	±SD	0.06		±SD	0.31	
7-14	1	1.07	1	1.09		1	1.06	
	2	0.74	2	0.86		2	0.57	
	3	0.82	3	1.00		3	0.67	
	MV	**0.87**	**MV**	**0.98**	**13**	**MV**	**0.77**	**-12**
	±SD	0.17	±SD	0.11		±SD	0.26	
14-28	1	1.51	1	1.48		1	1.77	
	2	1.50	2	1.79		2	1.59	
	3	1.38	3	1.53		3	1.48	
	MV	**1.47**	**MV**	**1.60**	**9**	**MV**	**1.61**	**10**
	±SD	0.07	±SD	0.16		±SD	0.15	

dws: Dry weight soil
MV: Mean Value
SD: Standard Deviation
[1] Rate: Nitrate-N in mg/kg soil dry weight/time interval/day, mean of 3 replicates and standard deviation

During the 28-day test, the one-fold dose of fluopyram and the 10-fold dose of the compound had no influence on nitrogen transformation in a silty sand soil supplemented with Lucerne-grass-green meal.

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Reference test:

Sodium chloride was used as a reference standard in the tests. In tests (non-GLP) with the agricultural soil described above, 16 g NaCl/kg dry weight soil had a distinct and long-term (> 28 days) influence on microbial mineralization of nitrogen.

III. CONCLUSION

Fluopyram of caused no adverse effects (deviation from control < 25 %, OECD 216) on the soil nitrogen transformation (measured as NO_3-N production) at the end of the 28-day incubation period after application of 3.33 mg a.s./kg dws.

Assessment and conclusion by applicant:
The study and its data are considered as acceptable and reliable for use in risk assessment.
The endpoint is: 3.33 mg a.s./kg dws

Data Point:	KCA 8.5/02
Report Author:	[redacted]
Report Year:	2006
Report Title:	AE C656948 tech.: Determination of effects on carbon transformation in soil
Report No:	LRT-C-67/06
Document No:	M-281213-01-1
Guideline(s) followed in study:	OECD/OECD No. 217, Adopted: 21st January 2000, OECD Guideline for the Testing of Chemicals, Soil Microorganisms: Carbon Transformation Test; Equivalent to US EPA OPPTS Guideline No. 850.SUPP
Deviations from current test guideline:	Current Guideline: OECD 217 (2000); Deviations: not applicable since test system is no longer a data requirement in EU
Previous evaluation:	yes, evaluated and accepted in DAR (2011)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

This study type is no longer a data requirement in the EU. It is only shown for transparency reasons since it was part of the first listing process.

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Metabolite fluopyram-7-hydroxy

Data Point:	KCA 8.5/03
Report Author:	[redacted]
Report Year:	2020
Report Title:	AE C656948-7-hydroxy (BCS-AA10065): Effects on the activity of soil microflora (nitrogen transformation test)
Report No:	20 48 SMN 0026
Document No:	M-754927-01-1
Guideline(s) followed in study:	EU Directive 91/414/EEC Regulation (EC) No 1107/2009 (2009) US EPA OCSPP Not Applicable OECD 216 (2000)
Deviations from current test guideline:	Current Guideline: OECD 216 (2000) Deviation: none
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

In a laboratory study the effect of fluopyram-7-hydroxy on the activity of soil microflora with regard to nitrogen transformation was tested during an exposure of 28 days in a loamy sand soil by comparing control and treatment. Two test item rates of 2 and 10 mg pure metabolite/kg dry weight soil were tested. Per treatment there were 3 replicates. The soil was enriched by 0.5 % lucerne meal and a water content of 48.68 and 49.97 % of the water holding capacity was maintained during the test.

The study fulfilled all validity criteria of OECD 216 guideline.

Nitrogen transformation was determined after 3 hours, 7, 14 and 28 days. No adverse effects of fluopyram-7-hydroxy on nitrogen transformation in soil (in terms of Nitrate-N in mg/kg soil dry weight/time interval/day) were observed at both test concentrations at the end of the test.

I. MATERIALS AND METHODS

Test item: Fluopyram-7-hydroxy (BCS-AA10065-01-01), Origin Batch No.: SES12367-10-8, LIMS No.: 2014109, purity: 99.4 % w/w.

Test design: A loamy sand soil (DIN 4220; agriculturally utilised soil: pH 6.5, 1.37 % C_{org}, Water holding capacity: 40.94 g/100 g dry soil) was exposed for 28 days to 2 mg p.m./kg soil dry weight and 10 mg p.m./kg soil dry weight. The soil was mixed with 0.5 % (i.e. 1.0 g/200 g soil d.w.) lucerne meal. One additional soil sample (without Lucerne meal) was used for determination of the initial NO_3-N-content. The initial NO_3-N-content was 1.48 mg/100 g soil d.w.

The soil of each treatment was tested as a series of 3 replicates. 200 g soil dry weight (= one sub-sample) per test vessel was weighted. 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. Soil samples (10 g soil d.w. per replicate) were taken at intervals of 3 hours, 7, 14 and 28 days after application and the NH_4-N-, NO_3-N- and NO_2-N-contents were determined.

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Statistics: 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). The statistical analysis was performed with the software ToxRat Professional 3.3.0.

Climatic conditions: The test vessels were kept in darkness in a climatic room and the temperature ranged between 18.7 to 22.0 °C during the test. The water content of the soil ranged between 48.68 and 49.97 % of WHC. The pH value of the soil ranged between 6.3 and 6.4.

Dates of work: July 14, 2020 – August 11, 2020

II. RESULTS AND DISCUSSION

Validity criteria:

The coefficients of variation in the control for NO_3-N were maximum 8.4 % and thus fulfilled the demanded range (≤ 15 %). In the most recent test with the toxic standard (conducted from 2020-01-07 to 2020-02-04), Dinoterb caused an effect of +59.9 %, +216.3 % and +238.5 % (required ≥ 25 %) on the nitrogen transformation in a field soil at the tested concentrations of 6.80, 13.60 and 27.20 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.

Observations:

Table 8.5- 3: Effects on nitrogen transformation in soil after treatment with fluopyram-7-hydroxy

Time interval [days]	Control			2 mg pure metabolite/kg soil dry weight				10 mg pure metabolite/kg soil dry weight			
	Nitrate-N[1]			Nitrate-N[1]			% difference to control	Nitrate-N[1]			% difference to control
0-7	1.98	±	0.36	2.39	±	0.55	+20.7 ns	2.82	±	0.04	+42.3 *s
7-14	1.17	±	0.44	1.42	±	0.41	+21.1 ns	2.00	±	0.30	+70.7 ns
14-28	1.32	±	0.08	1.18	±	0.06	-10.6 ns	1.17	±	0.19	-11.4 ns

The calculations were performed with unrounded values
1) Rate: Nitrate-N in mg/kg soil dry weight/time interval/day, mean of 3 replicates and standard deviation
ns No statistically significant difference to the control (Student-t-test for homogeneous variances, 2-sided, p ≤ 0.05)
*s Statistically significantly different to control (Student-t-test for homogeneous variances, 2-sided, p ≤ 0.05)

The test item fluopyram-7-hydroxy caused a temporary stimulation of the daily nitrate rate at the tested concentration of 10 mg p.m./kg soil dry weight up to time interval 7-14 days after application.

However, no adverse effects of fluopyram-7-hydroxy 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). Differences from the control of -10.6 % (test concentration 2 mg p.m./kg soil dry weight) and -11.4 % (test concentration test concentration 10 mg p.m./kg soil dry weight) were measured at the end of the 28-day incubation period (time interval 14-28).

Reference test:

In a separate study the reference item Dinoterb caused stimulations of nitrogen transformation of +59.9 %, +216.3 % and +238.5 % at 6.80, 13.60 and 27.20 mg Dinoterb per kg soil dry weight, respectively, determined 28 days after application (time interval 14-28).

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III. CONCLUSION

Fluopyram-7-hydroxy caused no adverse effects (difference to control < 25 %, OECD 216) on the soil nitrogen transformation (expressed as NO_3-N-production) at the end of the 28-day incubation period. The study was performed in a field soil at concentrations up to 10 mg pure metabolite/kg soil dry weight.

Assessment and conclusion by applicant:

The study and its data are considered as acceptable and reliable for use in risk assessment.

The endpoint is: 10 mg p.m./kg dws

Metabolite trifluoroacetic acid (TFA)

Data Point:	KCA 8.5/04
Report Author:	[redacted]
Report Year:	2013
Report Title:	Trifluoroacetic acid Na-salt (BCS-AZ56567): Effects on the activity of soil microflora (nitrogen transformation test)
Report No:	12 10 48 080 N
Document No:	M-444423-01-1
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) Deviation: none
Previous evaluation:	yes, evaluated and accepted in flurtamone RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

In a laboratory study the effect of sodium trifluoroacetate (trifluoroacetic acid (TFA) Na-salt) on the activity of soil microflora with regard to nitrogen transformation was tested during an exposure of 28 days in a loamy sand soil by comparing control and treatment. Two test item rates of 0.32 and 1.60 mg p.m./kg dry weight soil (equivalent to 0.24 and 1.20 kg p.m./ha) were tested. Per treatment there were 3 replicates. The soil was enriched by 0.5 % lucerne meal and a water content of 45.83 – 48.03 % of the water holding capacity was maintained during the test.

The study fulfilled all validity criteria of OECD 216 guideline.

Nitrogen transformation was determined after 3 hours, 7, 14 and 28 days. No adverse effects of sodium trifluoroacetate on nitrogen transformation in soil could be observed up to a concentration of sodium trifluoroacetate at 1.60 mg p.m./kg dry weight soil, which is equivalent to application rates up to 1.20 kg p.m./ha. The converted endpoint based on nominal concentrations of trifluoroacetic acid is 1.344 mg p.m./kg dry weight soil.

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I. MATERIALS AND METHODS

Test item: Sodium trifluoroacetate (Trifluoroacetic acid (TFA) Na-salt), Substance code: AE 1046319, BCS-code: BCS-AZ56567, Batch code: AE 1046319-01-01, Origin Batch No.: SES 11755-1-1, LIMS No.: 1226556, Customer order No.: TOX 09476-02, purity: 95.1 % w/w sodium trifluoroacetate.

Test design: A loamy sand soil (DIN 4220) with pH 6.5, 1.45 % C_{org} and with the water holding capacity of 15.33 - 16.07 g/100 g dry weight soil was exposed for 28 days to 0.32 and 1.60 mg p.m./kg dry weight soil. Application rates were equivalent to 0.24 and 1.20 kg p.m./ha. For calculation of the test concentrations (mg/kg soil d.w.) a soil depth of 5 cm and a soil bulk density of 1.5 g dry weight/cm^3 were assumed for conversion of soil volume to soil dry weight. The nitrogen transformation was determined in soil enriched with lucerne meal (concentration in soil 0.5 %). One additional soil sample (without Lucerne meal) was used for determination of the initial NO_3-N-content. The initial NO_3-N-content was 2.15 mg/100 g soil d.w.

The soil of each treatment was tested as a series of 3 replicates. 200 g soil dry weight (= one sub-sample) per test vessel was weighted. 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).

Soil samples (10 g soil d.w. per replicate) were taken at intervals of 3 hours, 7, 14, 28 and 42 days after application and the NH_4-N-, NO_3-N- and NO_2-N-contents were determined.

Statistics: Statistical evaluation of the test results (2-sided Student-t-test for homogeneous variances at 5 % significance level) was performed.

Climatic conditions: The test vessels were kept in darkness in a climatic room and the temperature ranged between 19.2 -21.0 °C during the test. The water content of the soil ranged between 45.83 – 48.03 % of WHC. The pH value of the soil ranged between 5.9 and 6.3.

Dates of work: September 12, 2012 - October 10, 2012

II. RESULTS AND DISCUSSION

Validity criteria:

According to OECD guideline 216 (2000), the variation of the nitrate-concentrations between control replicates should be less than ± 15 %. In this study, a maximum coefficient of variation of 2.4 % was obtained. Therefore, the results of the study are considered valid.

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Observations:

Table 8.5- 4: Effects on nitrogen transformation/ time interval/ day in soil after treatment with TFA

Time Interval (days)	Control			0.32 mg p m./kg dry weight soil equivalent to 0.24 kg p m./ha				1.60 mg p m./kg dry weight soil equivalent to 1.20 kg p m./ha			
	Nitrate-N[1)]			Nitrate-N[1)]			% difference to control	Nitrate-N[1)]			% difference to control
0-7	1.79	±	0.10	1.62	±	0.06	**-9.1** [n s]	1.76	±	0.48	**-1.6** [n s]
7-14	0.80	±	0.11	0.85	±	0.02	**+5.3** [n s]	0.70	±	0.35	**-13.0** [n s]
14-28	0.61	±	0.08	0.63	±	0.15	**+3.1** [n s]	0.76	±	0.04	**+24.2** [*s]

The calculations were performed with unrounded values
1) Rate: Nitrate-N in mg/kg soil dry weight/time interval/day, mean of 3 replicates and standard deviation
n s = No statistically significant difference to the control (Student-t-test for homogeneous variances, 2-sided, $p \leq 0.05$)
*s = statistically significantly different to control (Student-t-test for homogeneous variances, 2-sided, $p \geq 0.05$)

No adverse effects of TFA Na-salt on nitrogen transformation in soil could be observed in both test concentrations (0.32 mg/kg dry weight soil and 1.60 mg/kg dry weight soil) after 28 days. Differences from the control of +3.1 % (test concentration 0.32 mg/kg dry soil) and +24.2 % (test concentration 1.60 mg/kg dry weight soil) were measured at the end of the 28-day incubation period (time interval 14-28).

Reference test:

In a separate study the reference item Dinoterb caused a stimulation of nitrogen transformation of +40.4 %, +68.1 % and +83.5 % at 6.80 mg, 16.00 mg and 27.00 mg Dinoterb per kg soil dry weight, respectively, 28 days after application.

III. Conclusion

Sodium trifluoroacetate (Trifluoroacetic acid (TFA) Na-salt) caused no adverse effects (difference to control < 25 %, OECD 216) on the soil nitrogen transformation (measured as NO_3-N production) at the end of the 28-day incubation period. The study was performed in a field soil at concentrations of sodium trifluoroacetate up to 1.60 mg p.m./kg dry weight soil, which are equivalent to application rates up to 1.20 kg p.m./ha.

As the study was conducted with sodium trifluoroacetate which is the sodium salt of trifluoroacetic acid, the endpoint was converted to trifluoroacetic acid with factor 0.84. The converted endpoint based on nominal concentrations of trifluoroacetic acid is 1.344 mg p.m./kg dry weight soil.

Assessment and conclusion by applicant:

The study and its data are considered as acceptable and reliable for use in risk assessment.

The endpoint is: 1.60 mg p.m./kg dws (sodium trifluoroacetate) corresponding to 1.344 mg p.m./kg dws (trifluoroacetic acid)

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CA 8.6 Effects on terrestrial non-target higher plants

CA 8.6.1 Summary of screening data

Such studies are not necessary for the Fluopyram active substance renewal process, since guideline GLP studies for terrestrial non-target plants are available that were conducted with the representative formulations (see Point KCP 10.6.2).

Data Point:	KCA 8.6.1/01
Report Author:	[redacted]
Report Year:	2007
Report Title:	Evaluation of the pre-emergence (PPI) biological activity of AE C656948 SC 500
Report No:	PPI-07002
Document No:	M-297136-01-1
Guideline(s) followed in study:	Equivalent to US EPA OPPTS Guideline No. 850.SUPP
Deviations from current test guideline:	Current Guideline: not applicable Deviations: not applicable
Previous evaluation:	yes, evaluated and accepted in DAR (2011)
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

The study above was performed with an outdated formulation. It is only shown for transparency reasons since it was part of the first listing process. New data has been generated with the representative formulation for the active substance renewal process, which is presented in the corresponding section of the MCP.

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CA 8.6.2 Testing on non-target plants

Studies on terrestrial non-target plants (seedling emergence and vegetative vigour) conducted with the representative formulations for fluopyram are presented under Point KCP 10.6.2.

Data Point:	KCA 8.6.2/01
Report Author:	[redacted]
Report Year:	2007
Report Title:	AE C656948 SC 500A G - Effect on the vegetative vigour of ten species of non-target terrestrial plants (Tier 1)
Report No:	VV07/038
Document No:	M-295544-01-1
Guideline(s) followed in study:	US EPA 123-1, described by Horst and Ellwanger (1982) and OECD 227 (July 2006, adopted); Equivalent to US EPA OPPTS Guideline No. 850.4150
Deviations from current test guideline:	Current Guideline: not applicable
Previous evaluation:	yes, evaluated and accepted in DAR (2011)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

The study above was performed with an outdated formulation. It is only shown for transparency reasons since it was part of the first listing process. New data has been generated with the representative formulation for the active substance renewal process, which is presented in the corresponding section of the MCP.

Data Point:	KCA 8.6.2/02
Report Author:	[redacted]
Report Year:	2007
Report Title:	AE C656948 SC 500A G effect on seedling emergence and seedling growth test of ten species of non-target terrestrial plants (Tier 1 and 2)
Report No:	SE07/037
Document No:	M-295406-01-1
Guideline(s) followed in study:	US EPA 123-1, described by Horst and Ellwanger (1982) and OECD 208 (July 2006, adopted); Equivalent to US EPA OPPTS Guideline No. 850.4100
Deviations from current test guideline:	Current Guideline: not applicable
Previous evaluation:	yes, evaluated and accepted in DAR (2011)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

The study above was performed with an outdated formulation. It is only shown for transparency reasons since it was part of the first listing process. New data has been generated with the representative

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formulation for the active substance renewal process, which is presented in the corresponding section of the MCP.

Data Point:	KCA 8.6.2/03
Report Author:	████
Report Year:	2012
Report Title:	FLUOPYRAM SC 500B G - Effects on the seedling emergence and growth of one species of non-target terrestrial plants (Tier 2)
Report No:	SE12/006
Document No:	M-428356-01-1
Guideline(s) followed in study:	OPPTS 850.4225, US EPA Ecological Effect Test Guideline, April 1996 Seedling emergence, Tier II and OECD 208 Guidelines for the testing of chemicals, Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test
Deviations from current test guideline:	Current Guideline: OECD 208 (2006) Deviations: Temporary deviation from climate condition (light). The range of light intensity was not reported. However, natural daylight was supplemented by artificial lighting, when light intensities was < 15000 Lux (referring to day light spectrum 15000 lux result in 345 µmol/s/m²). Deviation from recommended plant density. All validity criteria were met. The deviation listed above had no influence on the reliability of the study and endpoints.
Previous evaluation:	yes, evaluated and accepted in Addendum 2 to the DAR (2012)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Details on the study above are presented in the MCP for the formulation FLU SC 500, please refer to KCP 10.6.2/03.

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CA 8.7 Effects on other terrestrial organisms (flora and fauna)

In view of the results presented in the Summary MCP Section 10, Points CP 10.1 to CP 10.6, no further studies on flora and fauna are deemed necessary.

However, information on biological activity testing is provided at the end of this section.

Data Point:	KCA 8.7/01
Report Author:	[redacted]
Report Year:	2008
Report Title:	AE C656948 tech.: Determination of effects on growth of pure cultures of a soil fungus, Mucor circinelloides var. griseocyanus, on a soil-nutrient medium
Report No:	LRT-SF 01/07
Document No:	M-297676-01-1
Guideline(s) followed in study:	Principles of Good Laboratory Practice (Chemicals Law (ChemG) of June 20, 2002, Current Version of Annex 1 and OECD Principles of Good Laboratory Practice (GLP) of November 26, 1997 [C(97)186/Final]); US EPA OPPTS Guideline: not applicable
Deviations from current test guideline:	Current Guideline: not applicable, no test guideline available
Previous evaluation:	yes, evaluated and accepted in DAR (2011)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

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Data Point:	KCA 8.7/02
Report Author:	[redacted]
Report Year:	2008
Report Title:	AE C656948 tech.: Determination of effects on growth of pure cultures of a soil fungus, Phytophthora nicotianae, on a soil-nutrient medium
Report No:	LRT-SF 02/07
Document No:	M-297693-01-1
Guideline(s) followed in study:	Principles of Good Laboratory Practice (Chemicals Law (ChemG) of June 20, 2002, Current Version of Annex 1 and OECD Principles of Good Laboratory Practice (GLP) of November 26, 1997 [C(97) 186/Final]). US EPA OPPTS Guideline No. 850.SUPP
Deviations from current test guideline:	Current Guideline: not applicable, no test guideline available
Previous evaluation:	yes, evaluated and accepted in DAR (2011)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

This study was evaluated in the last EU evaluation process and are listed here for transparency reasons.

Data Point:	KCA 8.7/03
Report Author:	[redacted]
Report Year:	2008
Report Title:	AE C656948 tech.: Determination of effects on growth of pure cultures of a soil fungus, Agrocybe aegerita, on a soil-nutrient medium
Report No:	LRT-SF 03/07
Document No:	M-297700-01-1
Guideline(s) followed in study:	Principles of Good Laboratory Practice (Chemicals Law (ChemG) of June 20, 2002, Current Version of Annex 1 and OECD Principles of Good Laboratory Practice (GLP) of November 26, 1997 [C(97) 186/Final]); US EPA OPPTS Guideline: Not Appliable
Deviations from current test guideline:	Current Guideline: not applicable, no test guideline available
Previous evaluation:	yes, evaluated and accepted in DAR (2011)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

This study was evaluated in the last EU evaluation process and are listed here for transparency reasons.

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Data Point:	KCA 8.7/04
Report Author:	
Report Year:	2008
Report Title:	AE C656948 tech.: Determination of effects on growth of pure cultures of a soil fungus, Cladorrhinum foecundissimum, on a soil-nutrient medium
Report No:	LRT-SF 04/07
Document No:	M-297702-01-1
Guideline(s) followed in study:	Principles of Good Laboratory Practice (Chemicals Law (ChemG) of June 20, 2002, Current Version of Annex 1 and OECD Principles of Good Laboratory Practice (GLP) of November 26, 1997 [C(97) 186/Final]); UUS EPA OPPTS Guideline No. 850.SUPP
Deviations from current test guideline:	Current Guideline: not applicable, no test guideline available
Previous evaluation:	yes, evaluated and accepted in DAR (2011)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

This study was evaluated in the last EU evaluation process and are listed here for transparency reasons.

Data Point:	KCA 8.7/05
Report Author:	
Report Year:	2008
Report Title:	AE C656948 tech.: Determination of effects on growth of pure cultures of a soil fungus, Penicillium simplicissimum, on a soil-nutrient medium
Report No:	LRT-SF 05/07
Document No:	M-297703-01-1
Guideline(s) followed in study:	Principles of Good Laboratory Practice (Chemicals Law (ChemG) of June 20, 2002, Current Version of Annex 1 and OECD Principles of Good Laboratory Practice (GLP) of November 26, 1997 [C(97) 186/Final]); US EPA OPPTS Guideline No.: 850.SUPP
Deviations from current test guideline:	Current Guideline: not applicable, no test guideline available
Previous evaluation:	yes, evaluated and accepted in DAR (2011)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

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Biological activity testing

Data Point:	KCA 8.7/06
Report Author:	[redacted]
Report Year:	2020
Report Title:	Determination of the inhibition of fungal growth by the metabolite BCS-AL85845 as compared to the parent compound fluopyram
Report No:	DPO 2020-02
Document No:	M-758875-01-1
Guideline(s) followed in study:	None
Deviations from current test guideline:	Current 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

The biological activity of fluopyram and the metabolite Trifluoroacetic acid were compared in greenhouse tests performed with the plant – fungal pathogen combinations of tomato - *Alternaria solani*, beans - *Botrytis cinereal*, barley - *Pyrenophora teres* and wheat - *Septoria tritici*. The parent compound fluopyram efficiently inhibited the disease development at appropriate concentrations while the metabolite showed no fungicidal activity against the tested fungi at the rates tested.

I. MATERIALS AND METHODS

Test items: Fluopyram (AE C656948; BCS-AR83685) and Trifluoroacetic acid (AE C502988; BCS-AL85845)

Test design: The biological activity of fluopyram and the metabolite Trifluoroacetic acid were compared. Preventive *in vivo* plant tests were performed in a greenhouse with the metabolite Trifluoroacetic acid and fluopyram on relevant fungal species. The tested pathogens were *Alternaria solani*, *Botrytis cinereal*, *Pyrenophora teres* and *Septoria tritici*. Test plants were tomato, beans, barley and wheat.

To produce a suitable preparation of active compound, 1 part by weight of active compound was mixed with 24.5 parts by weight of acetone, 24.5 parts by weight of dimethylsulfoxide and 1 part by weight emulsifier (polyethylene glycol sorbitan monooleate). The concentrate was diluted with water to the desired concentration.

To test for preventive activity, young plants were sprayed with the preparation of active compound at the stated rate of application. After the spray coating has dried on, the plants are inoculated with an aqueous spore suspension. Afterwards plants were incubated under specific climatic conditions favorable for infection by the respective pathogen.

The test was evaluated 2 days (for *B. cinereal*), 3 days (for *A. solani*), 8 days (for *P. teres*) and 21 days (for *S. tritici*) after the inoculation. 0% means an efficacy which corresponds to that of the untreated control, while an efficacy of 100% means that no disease is observed.

Climatic conditions: Tomato plants were placed in an incubation cabinets with temperature of approximately 20°C and relative atmospheric humidity of 100%.

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Beans plants were placed in a darkened chamber with a temperature of approximately 20°C and relative atmospheric humidity of 100%.

Barley plants remained for 48 hours in an incubation cabinet at approximately 20°C and a relative atmospheric humidity of approximately 100% before they were placed in the greenhouse at a temperature of approximately 20°C and a relative atmospheric humidity of approximately 80%.

Wheat plants remained for 48 hours in an incubation cabinet at approximately 20°C and a relative atmospheric humidity of approximately 100% and afterwards for 60 hours at approximately 15°C in a translucent incubation cabinet at a relative atmospheric humidity of approximately 100%. Then the plants were placed in the greenhouse at a temperature of approximately 15°C and a relative atmospheric humidity of approximately 80%.

II. Results and Discussion

In the conducted plant tests the metabolite Trifluoroacetic acid showed no fungicidal activity against the tested organisms while fluopyram showed high activity against these pathogens. The two tables below show the test rates used to investigate dicot and monocot diseases with the corresponding percentage of efficiency or disease control. 0% means an efficacy which corresponds to that of the untreated control, while an efficacy of 100% means that no disease is observed.

Table 8.7- 1: *In planta* activity of fluopyram and the metabolite Trifluoroacetic acid against dicot pathogens

		% efficacy / disease control	
BCS Code / Compound name	Dose rate [g/ha]	ALTESO	BOTRCI
Trifluoroacetic acid (BCS-AL85845)	60 30 15 6 3 0.6	7 -- -- 10 10 17	0 5 14 0 -- --
Fluopyram (BCS-AR83685)	100 50 25 10 5 1	92 -- -- 77 53 50	95 76 24 16 -- --

ALTESO (*Alternaria solani*), BOTRCI (*Botrytis cinerea*)

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Table 8.7- 2: *In planta* activity of fluopyram and the metabolite Trifluoroacetic acid against monocot pathogens

BCS Code / Compound name	Dose rate [g/ha]	% efficacy / disease control	
		PYRNTE	SEPTTR
Trifluoroacetic acid (BCS-AL85845)	250	14	0
	100	14	14
	50	14	14
	20	14	5
	10	0	0
	5	0	5
Fluopyram (BCS-AR83685)	250	100	100
	100	100	100
	50	100	100
	20	57	62
	10	43	52
	5	43	43

PYRNTE (*Pyrenophora teres*), SEPTTR (*Septoria tritici*)

III. Conclusion

It could be demonstrated that compared to the parent compound fluopyram, the metabolite Trifluoroacetic acid did not show fungicidal activity on the pathogens *Alternaria solani*, *Botrytis cinereal*, *Pyrenophora teres* and *Septoria tritici* in the corresponding plant tests performed with tomato, beans, barley and wheat, whereas the parent compound showed very good efficacy in all tested cases.

Assessment and conclusion by applicant:

The study and its data are considered as acceptable and reliable for use in risk assessment.

The conclusion is: Compared to the active substance Fluopyram, the metabolite Trifluoroacetic acid does not exhibit fungicidal activity up to and including 250 g/ha.

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CA 8.8 Effects on biological methods for sewage treatment

Data Point:	KCA 8.8/01
Report Author:	[redacted]
Report Year:	2006
Report Title:	AE C656948- Toxicity to bacteria
Report No:	2006/0031/01
Document No:	M-269401-01-1
Guideline(s) followed in study:	Directive 88/302/EEC, EG L133 Part C (1988); Equivalent to US EPA OPPTS Guideline No. 850.SUPP
Deviations from current test guideline:	Current Guideline: OECD 209 (2010) Deviations: The number of replicates followed the guideline of 1988 and not the current guideline OECD 209. There were two replicates for the control and one replicate per test concentration instead of 6 replicates for the control and 5 for each test concentration as recommended by the guideline. Furthermore, the test comprised 3 treatment concentrations instead of five treatment concentrations. Since all current validity criteria were met, these deviations were not considered to have a negative impact on the study results.
Previous evaluation:	yes, evaluated and accepted in DAR (2011)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

A study was performed to assess the toxicity of fluopyram to bacteria by measuring the respiration rate under defined conditions in the presence of different concentrations of the test item. The activated sludge was exposed to fluopyram at concentrations of 100, 1000 and 10000 mg a.s./L. Two controls were included in the test design, one at the start and the other at the end of the test series. In addition, the reference substance dichlorophenol was tested. The respiration rate of each mixture was determined after aeration periods of 3 hours. The biological results were based on nominal concentration, since no analytical monitoring was performed.

The study fulfils all validity criteria of OECD 209 guideline.

Fluopyram showed no respiration inhibition of activated sludge at 10000 mg a.s./L. The EC_{50} (3 hours) was > 10000 mg a.s./L.

I. Materials and Methods

Test material	Fluopyram (AE C656948) Specification No.: 102000012455 Batch No.: 08528/0002 Purity: 94.7 % w/w
Guideline(s) adaptation	The study was conducted according to the Commission Directive 88/302/EEC; Official Journal of the EG L 133 Part C.I 1 (1988). This test method is in most parts identical with OECD Guideline 209.
Test species	Activated sludge of a predominantly domestic sewage
Details on inoculum:	Origin: aeration tank of a domestic wastewater treatment plant (Municipal STP Cologne-Stammheim)
Pretreatment	Aeration of the activated sludge Daily feeding with synthetic medium.
Analytical monitoring	No

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Test concentrations:	Nominal concentrations: 100 – 1000 – 10000 mg a.s./L Concentration in physico-chemical oxygen consumption control: 10000 mg a.s./L Controls: Water Reference substance concentrations (3.5 Dichlorophenol): 5 – 10 – 20 mg/L Evidence of undissolved material: not reported
Replication:	No. of vessels per concentration (replicates): 1 No. of vessels per control (replicates): 2 No. of vessels for reference item (replicates): 1
Test loading of sludge:	960 mg/L suspended solids
Exposure	Static, 3 hours The test item was added to about 130 mL deionized water and stirred overnight (17 hours) before start testing (equilibration phase).
Test conditions	Temperature: 21.5 - 21.8 °C (Physicochemical oxygen consumption control: 21.8 °C) pH: 7.0 (Physicochemical oxygen consumption control: 7.2) Dissolved oxygen: 2.6 - 4.9 mg O_2/L (Physicochemical oxygen consumption control: 7.4 mg O_2/ L) Aeration: permanent aeration
Parameters Measured / Observations	The respiration rate for each control and concentration was determined after aeration periods of 3 hours. Oxygen content, temperature and pH values were measured during the exposure phase.
Data analysis	The EC values are determined by probit analysis.

II. Results and Discussion

Table 8.8- 1: Validity criteria

Criteria according to OECD 209	Required	Obtained in study
EC_{50} of reference substance (3.5-dichlorophenol)	Total respiration: 2–25 mg/L Heterotrophic respiration 5–40 mg/L Nitrification respiration: 0.1–10 mg/L	7.4 mg a.s./L
Coefficient of variation of oxygen uptake rate in control replicates at the end of the test	≤ 30 %	4.3 % [A]
Oxygen uptake rate of blanc controls (without the test substance or reference substance)	≥ 20 mg O_2/(g sludge ×h)	34.4 mg O_2/ (g sludge ×h) [A]

[A] Not given in report. Calculation based on control data for respiratory rate.

Analytical results:

No chemical analysis was performed. Therefore, the biological results were based on nominal concentrations.

Biological results:

Fluopyram showed no respiration inhibition of activated sludge at the highest test concentration of 10000 mg a.s./L

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Table 8.8- 2: Results for fluopyram

Nominal test concentration [mg a.s./L]	Respiratory rate test item [mg/L × h]	Phys.-chem. O_2 consumption [mg/L × h]	Respiratory rate phys.-chem. O_2 consumption [mg O_2/L × h]	Inhibition [%]
Control 1	32.0	-	-	-
Control 2	34.0	-	-	-
Control, mean (CV)	**33.0 (4.3)**	-	-	-
100	30.0	0.0 *	30.0	9.1
1000	32.0	0.0 *	32.0	3.0
10000	33.0	0.0	33.0	0.0

* The physico-chemical oxygen consumption has been determined at 10000 mg/L test substance concentration. As no physico-chemical oxygen consumption was observed at that test item concentration this observation also holds true for the lower test item concentrations.

Reference item:

Table 8.8- 3: Results for the reference item 3.5-Dichlorophenol

Nominal test concentration of reference item [mg/L]	Respiratory rate [mg O_2/L × h]	Inhibition [%]
Control 1	32.0	-
Control 2	34.0	-
Control, mean	**33.0**	-
5	22.5	31.8
10	12	63.6
20	3.6	89.1

The toxicity of the reference substance 3.5-Dichlorophenol to bacteria was: EC_{50} = 7.4 mg/L

III. CONCLUSION

The study meets the validity criteria and the endpoint based on nominal concentrations was:

EC_{50} – 3 hours (95 % C.I.):	**> 10000 mg a.s./L** (Not applicable)

Assessment and conclusion by applicant:

The study and its data are considered as acceptable and reliable without use in risk assessment.

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