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Version history

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CA 8 ECOTOXICOLOGICAL STUDIES ON THE ACTIVE SUBSTANCE

Fluopicolide (AE C638206) was included in Annex I to Council Directive 91/414/EEC in 2010 (Commission Directive 2010/15/EU, Entry into Force on June 1, 2010). The expiration of approval of fluopicolide is May 31, 2023 (Commission Implementing Regulation (EU) 2017/1529). The Supplementary Dossier contains only data which were not submitted at the time of the Annex Anclusion of fluopicolide under Council Directive 91/414/EEC and which were therefore not evaluated during the first EU review. All data which were already submitted by Bayer AG (former Bayer CropScience) for the Annex I inclusion under Council Directive 91/414/EFC are contained in the Dtaft Assessment Report (DAR) and its Addenda, and are included in the Baseline Dossier provided by Bayer AG.

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

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

Fluopicolide is active against a wide range of Ormycete fungi, the causal spents of devastating plant diseases of economic importance in EU27 such as ported late bligh (*Phytophthorg infestans*) of downy mildew diseases in a broad range of gops.

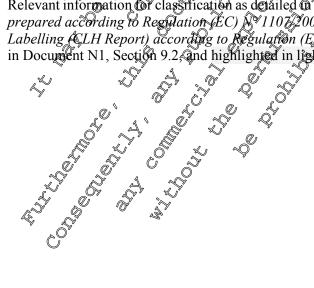
It provides effective, long lasting protection at low application rates against Oonlycetes diseases at different stage of development of the fungi, giving flexibility of use to the darmer

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

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

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

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





CA 8.1 Effects on birds and other terrestrial vertebrates

CA 8.1.1 **Effects on Birds**

CA 8.1.1 Effects on Birds Studies on quail species, mallard ducks and finch species have been conducted with the active substances fluopicolide. Detailed information on acute, short-term and long-term effects of Buopicolide on Birds is presented in the following chapters. presented in the following chapters.

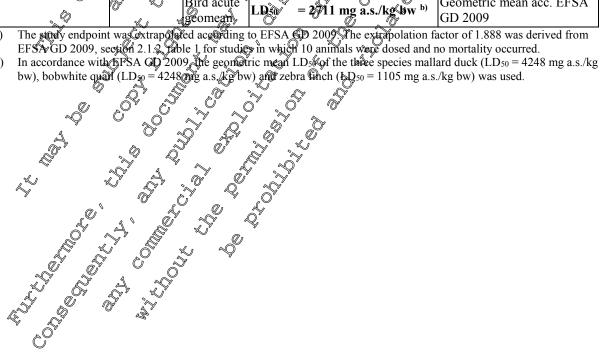
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CA 8.1.1.1 Acute oral toxicity to birds

Table 8.1.1.1- 1:	Acute oral to		<u>_</u>		
Test substance	Test design	Test species	Endpoint		Reference 2
Fluopicolide		Mallard duck bobwhite duck	LD_{50} 2250 D_{50} 4248 D_{50} 2250 D_{50} 2250 D_{50} 2250 D_{50} 2248 D_{50} 2248	mg a.s. kg bw mg a.s. kg bw mg a.s. kg bw mg a.s. kg bw	2001: M- 240576-01-1 KCA & 1.1.1/01 Extrapolated acc. to EFSA 2009 2001: M- 240577-01 KCA 8.1.1/02 Extrapolated acc. to EFSA GD 2009 2015: M- 544294-01-1
		Bird acute	$LD_{50} = 2711$	mg a.s./kg/bw ^{b)}	KCA 8.1.1.1/03 Geometric mean acc. EFSA GD 2009

a)

b)





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

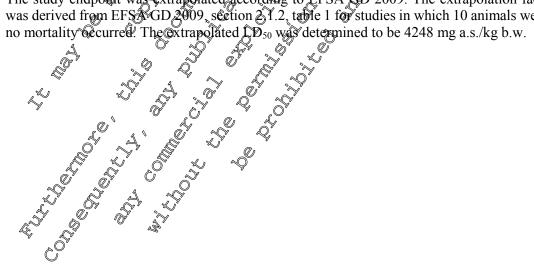
Executive Summary

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

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

Based of this study the LD walue for mallard dick exposed to fluopicolide was determined to be > 2250 mg a.s./kg b.w\$

The study endpoint was extrapolated according to EFSA (D) 2009. The extrapolation factor of 1.888 was derived from EFS&GD 2009, section & 1.2, the 1 for studies in which 10 animals were dosed and





I. MATERIAL AND METHODS:

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

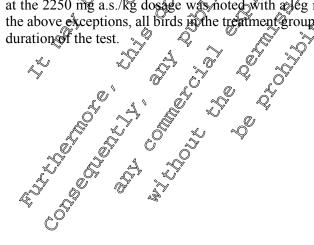
Water and feed were provided ad libitum during acclimation and during the test, except during periods of fasting prior to testing. Birds were held at an average temperature of $22.7 \pm 0.7^{\circ}C$ (SD) with any average relative humidity of $60 \pm 13\%$ (SD). The photoperiod was approximately eight/hours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

During the subsequent observation period of 140 days the birds were observed, for mortality and symptoms. Body weight was measured at test initiation and on day 8, 7 and 14 Average feed consumption was determined by pen for each dosage group and control group for days 0-3, 4-7 and 8-14.

II. RESULTS AND DISCUSSION:
II. RESULTS AND DISCUSSION: A A A
II. RESULTS AND DISCUSSION:
Mortality in the control
$\frac{1}{1000} \frac{1}{1000} \frac{1}{1000$
Acute oral toxicity of fluopicolide tecture. to birds
Acute oral toxicity of fluopicolide techn. to birds
Test substance
Test object 🔬 🖉 🔍 🏹 Matlard ducks (male, female)
LD ₅₀ [mg a.s./kg b.w.]
Lowest lethal effect dose (LLED, mg acs/kg b.w.) 2230 L
Lowest observed effect dose (EØED) [mg a.s.kg b.w.) > 2250
No observed effect dose (NOED) [ng a.s./kg b.w.]
Observations:
Observations:
Mortality and clinical observations $\mathcal{O} \times \mathcal{O} \times \mathcal{A}$

Mortality and clinical observations

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





Body weight and feed consumption

Treatment	Mean l	oodyweig	ght ± SD					0
(mg a.s./kg bw)	day 0	day 3	day 7	day 14	Δ d 0-3	Δ d 3-7	Δ d 7-14	Δ day 0.14
	-	-	-	Fem	nales	•		ž i
	986	1025	1020	1018	39	-6	-20	320 6
Control	±	±	±	±	±	±	Ê,	±¥ , Ş
	75	93	78	78	23	30	18	Q27 ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
• • •	968	1006	989	993	37	-16	3	24 0 5
292	±	±	±	±	±Č	±		+
	55	75	69	45	29	15	32 0	
486	1016	1060	1074	1067	44		-/ & ~	
480	± 45	± 42	± 38	$\begin{array}{c} \pm\\ 26\end{array}$	2 <u>4</u> 12	Qui por	\pm	
	991	1024	1027	1036	13 33	¥1 0 2.0	10 0	$\begin{array}{c} 51 \\ 29 \\ 43 \end{array}$
810	±	1024 ±	±					
010	27	35	36	38		\$5 0	215	
	991	1022	1020		31 0 4	P-2 0	-4 O ^Y	25
1350	±	±	± 🔬	1016	±	±	æ.	
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100	¥124		1154	1122	S C	-31	-29	-3
486	¥± 0	- ff - '	± 🔍	±	\downarrow^{\pm} \mathcal{S}^{\pm}	Ø. S	±	±
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010 Q	≝ 95 ,≪		96		12 .	28	± 21	$\frac{\pm}{35}$
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là.	1079	115	1164	1150	75 0	10	-15	70
2250	±		æ ^r .	¥ \$	± \$	±	±	+0 ±
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Treatment	Mean food	l consumpt			
(mg a.s./kg bw)	day 0-3	day 4-7	day 8-14		
Females	<u>.</u>			1	
Control	141	122	157	1	
292	124	112	124	1	ð
486	141	120	129	1	S
810	145	135	139	1	"O"
1350	136	118	112		\$
2250	170	97	112	¢ Å	×
Males	1			₹ _Q	, Ø
Control	129	153	149		Ő
292	137	131	142		Ô. ć
486	158	128	114		a × N
810	133	124	\$30 9		ĭ "Q
1350	161	140	114 [*] 30 170 135 2 135 2 2 2 2 2 2 2 2 2 2 2 2 2		<i>w</i>
2250	162	146	× 135 , , , , , , , , , , , , , , , , , , ,		Å.

Gross pathology

Postmortem examinations revealed no findings which were spisideted treatment relates

Ŵ HD. Conclusions: 🖇

Based on this study the LD50 value formalland duck exposed to huopic fide was determined to be A > 2250 mg as/kg b.w. 🔬 õ C

> 2250 mg as/kg b.w. The study endpoint was extrapolated according of EFSA GD 2009. The extrapolation factor of 1.888 was derived from SFSA GD 2009, section 2.1.2, table for sondies in which 10 animals were dosed and no mortality occurred. The extrapolated LD was determined to be 4248 mg a.s./kg b.w. "O

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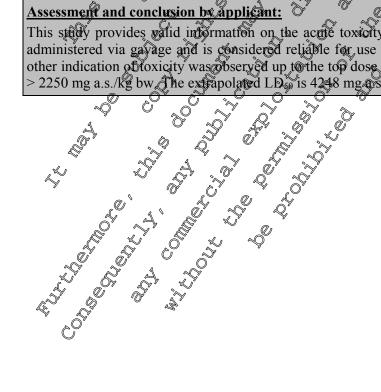
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Assessment and conclusion by applicant: Ĉ

This story provides ralid information on the acree toxicity of Oluopicolide to Mallard ducks when administered via gavage and is considered reliable for use in risk assessments. No mortality or any other indication obtoxicity was beryed up to the top dose level of 2250 mg a.s./kg bw. The LD₅₀ is > 2250 mg a.s./kg bw The expapolated LDs is 4248 mg as./kg b.w.

Ø





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

Executive Summary

Fluopicolide technical was administered orally to 10 adult boowhite quails 5 males and 5 females) at dose levels of 0, 292, 486, 810, 1050 and 2250 mg a.s. Kg b.v. Birdowere held at an average temperature of 23.6 °C with an average relative humidity of 64% and approximately 8 hours light per day. Birds were observed for 14 days for mortality and cublethal symptoms. Body weight and average feed consumption were measured for each dosage and control group.

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

Based on this study the LB $_{10}$ value for both the quait exposed to floopicolide was determined to be > 2250 mg $\frac{1}{38}$ kg b.w.

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

C 2 2 1. Marerian ANP Methods:

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

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



During the subsequent observation period of 14 days the birds were observed for mortality and symptoms. Body weight was measured at test initiation and on day 3, 7 and 14. Average feed consumption was determined by pen for each dosage group and control group for days 0-3, 4-7 and 8-14. The light source was fluorescent light with eight hours with eight hours light and 16 hours dock at an average illumination of approximately 171 lux. Average temperature was $236^{\circ}C \pm 0.56^{\circ}C$ (SD) and average relative humidity was $64\% \pm 11\%$ (SD).

II. RESULTS AND DISC	cussion:	Obtained of the
	CUSSION: C, Y	Obtained of the second
Validity criteria (according to OECD 223, 2016)	Required	Öbtained ö ^ş k
Mortality in the control	≤ 1000	
Acute oral toxicity of fluopicolide techn. to birds		fetile)
Test substance	Agech. as	
Test object	Bobwhite quail (male,	
LD ₅₀ [mg a.s./kg b.w.]	× ~ >2250 ,	
Lowest lethal effect dose (LLED) [mg a.s./kg b.s.]	D2250	
Lowest observed effect dose (LOED) wg a.s./ g b.w.	@ 2250	
No observed effect dose (NOED) [mg a.s./kg b.w.]	~ <u>© 2250</u>	
Observations:		
increase, and connect way to be way to be	× 0′ 4	

No compound related mortalities occurred at any of the dose levels in the test. All birds in the treatment

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



Body weight and feed consumption

Treatment					Mean bodyv	veight [g]		0
(mg a.s./kg bw)	day 0	day 3	day	day	Δ day 0-3	Δ day 3-7	Δ day 7-14	Δ day 0-14
				Fe	males		\$	
	189	194	197	200	5	3	<u></u>	OTI S
Control	±	±	±	±	± 2	±	÷ ±	
	6	5	6	8	2	3	3	
	194	200	202	208	6		6	× 13 ~~
292	$\frac{\pm}{6}$	$\frac{\pm}{8}$	$\frac{\pm}{9}$	$\frac{\pm}{9}$	A			
				-	<u> </u>	Ő¥	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	
	188	194	196	199	<u> </u>	$\mathcal{Q}^{\vee 2}$		
486	± 5	$\frac{\pm}{3}$	$\frac{\pm}{4}$	$\frac{\pm}{5}$	* ± 3			
				v .		r in a		
010	202 ±	206 ±	208 ±	2% Q				
810	14	13	12	A. 14. 0	r "I	\mathcal{Q}^{+} \mathcal{Z}^{+} \mathcal{Z}^{+}		
	184	191					Ô ^V s u	$ \begin{array}{c} \begin{array}{c} \begin{array}{c} \begin{array}{c} \begin{array}{c} \begin{array}{c} \end{array} \\ \end{array} \\ \end{array} \\ \begin{array}{c} \end{array} \\ \end{array} \\ \end{array} \\ \end{array} \\ \end{array} \\ \begin{array}{c} \end{array} \\ \end{array} \\$
1350	104 ±	191 ±	193	\$ <u>9</u>		$\mathcal{O}_{+}^{\mathbf{z}}$		
1550	10	10	۵ <u>۵</u> ۶	×10 .	\$ <u>2</u> \$	$ \overline{\mathcal{O}} 1 \mathcal{O} $		
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292		10	\mathbb{Z}^{+}	\sim			$\frac{\pm}{3}$	$\frac{\pm}{2}$
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	202 202	207	0208 \$	× 2116			3	9
486	$\mathscr{O}_{\pm}^{\circ 2}$		±		₩±		5 ±	±
	185	10	10	Ŕ	~~~ 2 _~ ~	$\sqrt[6]{2}$	2	2
K.	<u>, 19</u> 4	ر (19 <b>%</b>	⁽ ¶99	\$202 C	°¥v°,	©°2	3	8
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Ő	<u>79</u>	1 IQV	12 %	10gr 1806		1 4	3	4 12
1350 @			Ô±	°€± Ć		+ ±	5 ±	±
~Ç [®]	010 ×	0 10 ~	≥ 10	10~	≥2	0	1	1
A	192 [©]	199	200	2003	J	3	3	12
2250	, ±O	Đ,	₩ 		× ±	± 3	±	±
× .	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~			<u> </u>	2	3	2	2
4			' Q	age i				
1350 2250 2250 250 250 250 250 250			5 ~~	Q.	$\begin{array}{c} 2 \\ 3 \\ 4 \\ 2 \\ 2 \\ 2 \\ 2 \\ 2 \\ 2 \\ 2 \\ 2 \\ 2$			



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

#### Gross pathology

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

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Based on this study the LD50 value for boowhite quail exposed to the picelide was determined to be Č, . Ç > 2250 mg a.s./kg b.w.

The study endpoint was extrapolated according to EFSA GD 2609. The extrapolation factor of 1.888 was derived from EPSA GD 2009 section 2.1.2, table 1 for studies in which 10 animals were dosed and no mortality occurred. The extrapolated LD50 was determined to be 4248 neg a.s./kg b.w.

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### Assessment and conclusion by applicant:

S. This study provides valid information the acute toxicity of fluppicolide to Bobwhite quails when administered via gavage and can be used for risk assessment. No mortality or any other indication of toxicity was observed up to the top dose devel of 2250 mg a.s./kg bw. The LD₅₀ is > 2250 mg a.s./kg

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Data Point:	KCA 8.1.1.1/03
Report Author:	
Report Year:	2015
Report Title:	Fluopicolide: An acute oral toxicity study with the zebra finch
Report No:	263-177
Document No:	<u>M-544294-01-1</u>
Guideline(s) followed in	U.S. Environmental Protection Agency
study:	Series 850 - Ecological Effects Test Guidelines OCSPP Number 850 200 (202)
Deviations from current	Current Guideline: OECD 223 (2016)
test guideline:	No deviations
Previous evaluation:	No, not previously submitted 🕅 🖉 🖉 🖉
GLP/Officially	Yes, conducted under GLP/Officially recognised testing facilities
recognised testing	
facilities:	
Acceptability/Reliability:	Yes & & V & V V

#### **Executive Summary**

Fluopicolide technical was administered wally to 10 three to six months old zebra tinches (5 mates and 5 females) at dose levels of 0, 125, 250, 500, 1000 and 2000 mg a.s./kg b.w. Birds were held at an average temperature of 22.4°C with an average relative humidity of 79% and approximately 8 hours light per day. Birds were observed for 14 days for mortality and sublethal symptoms. Body weight and average feed consumption were measured foreach dosage and control group. Gross accropsies were performed on all mortalities and on three birds from the control group and from each treatment group at test termination, if available.

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

# J I. MATERIAL AND METHODS:

The acute oral toxicity of fluopicolide technical (AF C638206-01-25, purity 100.5%) was investigated in a test with nominal dosages of 0, 125, 250, 500, 1000 and 2000 mg a.s./kg bw. Zebra finches (*Taeniopygia guttata*) approximately three to an months of age and weighing 12.8-17.5 g with a mean of 14.6  $\pm$  0.9 grams at dest initiation were used as test organisms. Test birds were housed indoors by dosage groups in batteries of pens. Each per contained one male and female, randomly assigned to pens. Each dosage group was assigned five pens. Each pen had floor space that measured approximately 58 × 26 cm with a ceiling of 91 cm Birds were maintained at 22.4  $\pm$  0.2°C (SD) and an average relative humidity of 70  $\pm$  3% (SD). The photoperiod was approximately eight hours of light per day during acclimation and throughout the test. The birds were exposed to an average of approximately 388 of lux. The test substance was dosed using capsules. A single dose of the test substance in a capsule was orally

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



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

#### **II. RESULTS AND DISCUSSION:**

		<hr/>		
Validity criteria (according to OECD 223, 2016)	Č V	Required	Obtained ^	Y ô ^s o
Mortality in the control	L	<u>_</u> 010%		
	AU'	b°		

There were no mortalities in the control group, or the 125 and 250 mga.s./kg treatment groups. There was 30% mortality at the 500 mg a.s./kg dosage level and 60% mortality at the 1000 and 2000 mg a.s./kg dosage levels. Signs of toxicity noted at 2500mg a Wkg bw and above wcluded depression, reduced reaction to external stimuli, prostrate posture, raified appearance and lethargy. No indications of regurgitation were noted for any of the birds in the control group or for the birds from the 125, 256 and 500 mg a.s./kg treatment groups. There was 10% regugitation seen at the 1000 mg a.s./kg/dosage level and 30% regurgitation at the 2000 mg Ri./kg dosage level All the birds that were noted as regurgitating resulted in mortality so regurgitation did nor affective calculation of an LDs value.

When compared to the control group, there were no apparent related effects on body weight among the surviving males and remales in the 125, 250 and 500 mg a.s./kg treatment groups. The slight loss in body weight from Day-1 to Day 30 as statistically significant at 100 and 2000 mg a.s./kg. At the end of the observation period Patest, all surviving birds had recovered from the initial body weight loss.

MIL CONCLUSIONS

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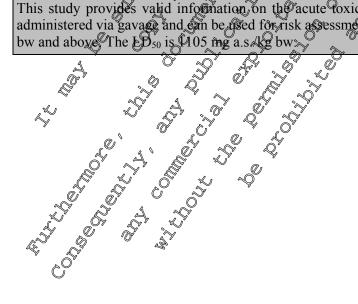
The acute oral  $LD_{50}$  value for zebra tinch exposed to fluopicolide was calculated to be 1105 mg a.s./kg. The no-mortany lever was 200 mg Q.s./kg, The no-observed- effect level was 125 mg a.s./kg, based on signs of toxicity observed at the 250 mg/a.s./kg dosage level,

### Assessment and conclusion by applicant:

This study provides valid information on the acute Poxicity of fluopicolide to Zebra finches when administered via gavage and can be used for risk assessment. Toxicity was observed at 250 mg a.s./kg

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#### Short-term dietary toxicity to birds CA 8.1.1.2

Test substance	Test de	sign	Test species	Endpoint	Reference of the
Fluopicolide	dietary toxicity (short-to		Bobwhite quail	$LC_{50} > 5620 \text{ ppm}$ $LDD_{50} > 1744 \text{ mg a.s./kg for/}{}$	day <u>M_24071391-1</u> KCA 8_51.2/01 S
	dietary toxicity (short-te		Mallard duck	LC ₅₀ > 5620 ppm	Say <u>2009: M-240714-</u> KC 8.1.1.2702 &
M-01 (2,6-dichloro- benzamide)	dietary toxicity (short-te		Bobwerte quaiD	$\begin{array}{c} 4 \\ 4 \\ 4 \\ 5 \\ 5 \\ 4 \\ 2 \\ 5 \\ 6 \\ 6 \\ 6 \\ 6 \\ 6 \\ 6 \\ 6 \\ 6 \\ 6$	$\frac{1}{2003}$
Data Point:		KCA	8.1 ¥.2/01		× .Q
Leport Author:	°/	\$			
Report Year:		2002			<u> </u>
Report Title:	<u> </u>			nical: A dietary, LC50 Study with t	he Northern Bobwhite
Report No:		B003			с. Гл
Document No:		⊅ <u>M-24</u>	<u>0713-01-4</u>	a) Protection Agency: Series 8564	× ×
Guideline(s) follo	wed in	0.8.1	Environment	al Protection Agency: Series 850-1	cological Effects Test
tudy: 🏷	ñ			Number 850 2200 (1996)	
, Q	4		Guidenne	on E Section $71-2$ (1982)	
Deviations from c				ss from Grrent gridelin@SANCO/	2020/00 row 4
est guideline:		Proci	sion data con	Id not be obtained directly as recov	veries were determined at
st guidenne.	\$9 °			centrations without replicates. How	
(	d A			nt concentration demonstrate very	
<i>a</i>	i R	preels	sion calculate	form drese data accounts for 3.1	and 0.6%, respectively, for
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	Õ	both 1	ime points.	The method can therefore be regard	led as fit for purpose.
1	Ĭ	Study	Qurrent	nidebne: OECD 205 (1984)	
Ĩ,	ر في ا			f the brooding compartment was 4	
1 de la companya de l				mmended by the guideline. The re	
L.	S,	belov	the range	f $50 - 75\%$ recommended by the g	udeline. These deviations are
¥	<u> </u>			pacythe study results.	
revious evaluatio	n: A	in Brin	valuated and AR (2005)	Q, -	
GLP/Officiatly	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	Pes,	conducted	wher GLP/Officially recognised test	ing facilities
ecognised	Š P		$\sim \sim$		
acilities 0	/ <u> </u>	0	-		

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



per test concentration; unknown sex. Birds were held at an average temperature of 27.3°C with an average relative humidity of 24% and approximately 16 hours light per day.

Birds were observed at least once daily for mortality, signs of toxicity and abnormal behaviour. Individual body weights were measured on day 0 at test initiation, on day 5 and at termination of the stability and homogeneity of the test substance in the diets.

There was a single incidental mortality in the control group. There were no mortalities or overt signs of toxicity in the 562, 1000, 1780, 3160 and 5620 ppm groups. A single bird at 1000 ppm was noted with a swollen leg from the afternoon of day 4 until test termination. Additionally, one bird at 5620 ppm was euthanized on day 4 of the test due to a severe leg injury. All other birds at all test concentrations were normal in appearance and behaviour throughout the test. There were no treatment related effects on body weight at 562, 1000 and 1780 ppm. Reductions in body weight gain at 3160 and 5620 ppm were statistically significant during the exposure period and for the entire test period. Analysis of diet samples verified the test concentrations administered and confirmed the stability and honogeneity of the test substance in the diets.

The subacute dietary LC₅₀ of fluopicolide to 10 day of Bobwbite quail chicks is determined to be > 5620 ppm, corresponding with an LDD₅₀ of > 2064.2 mg a O kg by d.

MATERIAL AND METHODS:

Fluopicolide techn., purity: 97.1 %, Specification (Batch No. 2050190/P241024/2, *Bobwhite quail* (*Colinus virginianus*) chicks (10-days old) were exposed for 5 days to nominal dietary concentrations (techn. a.s.) of 562, 1000, 1780, 3160 and 5620 mg as/kg feed (bpm). 90 chicks per control; 10 chicks per test concentration; unknown 6x. Dietary test concentrations were corrected for purity of the test substance. Water and feed were provided ad libitum during acclimation and during the test. During the test average temperature in the brooding compartment of the pens was $40 \pm 2^{\circ}$ C (8D). Average ambient room temperature for this study was 27.3 \pm 0.6 % (SD) with an average relative humidity of $24 \pm 5\%$ (SD). The photoperiod was sixteen hours of light per day during acclimation and throughout the test. The birds were observed at least once daily, mortality, soms of toxicity and abnormal behaviour were assessed. Individual body weights were measured on day 0 at test initiation, on day 5 and at termination of the test on day 8. Following the exposure period, birds were namination on untreated diet for a post observation period of 2 days.

Samples of the test diets were collected to verify the test concentrations administered and to confirm the stability and homoveneity of the test substance in the diets. Homogeneity of the test substance in the diet was evaluated by collecting six samples from the top middle and bottom from the 562 and 5620 ppm a.s. test diets at preparation on Day 0. The homogeneity samples also served as verification samples for those concentrations. One verification sample was collected from the control diet and two verifications samples were collected from each remaining treatment group at preparation on Day 0. At the end of the exposure period Day 5), one sample was collected from the control diet and two samples were collected from all treatment groups to determine stability of the test substance in the diet. The samples were collected from feedremanning in the feeders.

Validity Priteria (according to DECD 205, 1984)	Required	Obtained
Mortality in the control	$\leq 10\%$	3.3% *
Fest concentration maintained	\geq 80% of nominal over the 5 day exposure	Fulfilled
Effects in the lowest treatment level	No effects should occur	Fulfilled

YI. RESULTS AND DISCUSSION:

* Incidental mortality (one bird)



Short-term dietary toxicity of fluopicolide to Bobwhite quails.

Test substance			Techr	
Test object			Chicks (10 days)
Exposure			ndiet 🔊	ary 🔊
		[1	mg a.s./kg/feed]	[mg/kg bw/d
LC50 [mg a.s./kg feed]			> 5620	≥ 2064.25
Lowest lethal effect concentration (LLEC)			\$ 620	0≥206€2
Lowest observed effect concentration (LOEC)	Ĉs		£3160 *	× 14 18/8*
No observed effect concentration (NOEC)	- T		<u></u> 1780	837.7 J
based on body weight	L.	Ő	\$ \$	
	4 ⁰	á v		
		. A	\$ A	4

Observations:

Mortality and clinical observations

Mortality and clinical observations There was a single incidental mortality in the control group. There were no treatment related prortalities or overt signs of toxicity in the 562, 1000, 1780, 3160 and 5620 ppm/treatment groups. A single bird at 1000 ppm was noted with a swollen leg from the afternoon of day 4 antil test termination. Additionally, one bird in the 5620ppm group was withanized on day 4 of the test due to a secter legshjury. All other birds at all test concentrations were normal in appearance and behaviour throughout the test L.

Body	weight	and	feed	consumption

Jouy weight un		<u>isumption</u>	, 0.	N V		× 6
Treatment	Mean box	lyweight ±	SD¢g &	× 4. ~		
(ppm)	day 0 🔬	day	gday 8	Δ aday 0-50	Aday 5-8 ^Å	A day 0-8 ^A
Chicks				5 5		4
Control	20 2	(31 ± 30)	4 0 ⊊4	~11±2° _(20 ± 3
562	19 ± 1	30 ≭2	38 ± 2	10,£M 🔊	8 2 5	19 ± 2
1000	$\tilde{Q}20\pm 20$	29 ± 2	¥37 * 90°	10 [±] 2	\$£ 2 ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	18 ± 3
1780	20 1	≪3/1 ± 4	39 ± 4	¥1 ± 25	98 ± 2	19 ± 3
3160 🔊	20 ± 2 🔬	29 ±3	<i>2</i> 77 ± 4 0	9*±2 @	8,±2	17*±3
5620	20 ± 2^{0}	29 4	36 ± 5	88¥≇2 √	80 € 2	$16* \pm 4$

* Statistically different from the control group at p 0.05

^A Mean change in weight were calculated from individual changes in bodyweight

T	Mean food consumptio	n (g) in the second sec
Treatment (ppm)	ExposurePerio	Post Exposure Period
10	day 0-5	day 6-8
Chicks		
Control	7 * * *	Q11 ~ ~
56Ž	7. ¹⁰ C. a	11
1000		12
1780	127	218
3160		13
5620		13



(ppm) Chicks	bw day 0 [g]	bw day 5 [g]	mean bw day 0-5 [g]	mean FC day 0-5 [g/bird/day]	FC/bw* [g/kg bw/day]	Dose* [mg/kg bw/d]
Control	20	31	25.5	7	274.5	0.0
562	19	30	24.5	7	285.7	0.0 5 160.6 5 1440 0 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5
1000	20	20	24.5	11	119.0	
1780	20	31	25.5	12	470.6	8357 5
3160	20	29	24.5	11 &	449.0	8357 1#18.85 20640
5620	20	29	24.5	9 1	2367 3 Č	2064
ances not pre				11 12 11 9 % 4 9 % 10 11 9 % 10 11 9 % 10 10 10 10 10 10 10 10 10 10		



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Analytical results

None of the control samples showed any indication of the presence of the test substance fluopicolide. Diet samples were collected from the 562 and 5620 ppm a.s. test concentrations and were analysed to evaluate the homogeneity of the test substance in the diet. Means and standard deviations for the two test concentrations were 558 ± 14.4 ppm a.s. and 5790 ± 101 ppm a.s., respectively. Samples collected during the test to verify test substance concentrations for the 1000, 1780 and 2060 ppm a.s. Wets had means of 1020 ppm a.s., 1780 ppm and 3300 ppm a.s., respectively. These values represented 102, 400 and 104% of nominal concentrations. Analysis of diet samples collected from feeders after being held at ambient temperature for 5 days averaged 103, 102, 103, 105 and 102% of the day ovalues for the 562, 1000, 1780, 3160 and 5620 ppm a.s. test concentrations.

Full details and acceptable validation data to support this method are presented within document 4, which comply with the EU regulatory requirements outlined within SoNCO 29/99 rev 4. J.

III. Conclusio

chicks quai is determined to be The subacute dietary LC50 of fluopicolide, to 10-day old Bobwhite > 5620 ppm, corresponding with an LDD $_{10}$ of > 2064in the second se 2 mag a /kǧ

Contraction of the second seco This study provides valid information on the short-term dietary toxicity of fluopicolide to Bobwhite quail chicks when administered over & days in the diet and can be used for risk assessment. Toxicity was observed at 3160 ppm (1418.8 mg a kg bw) and above The Isc 50 is > 56200ppm (LDD50 >



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

Executive Summary

0 No Mallard duck chicks (70 days old) were exposed for 5 days to rominal dietary concentrations of 562, 1000, 1780, 3160 and 5620 mg for picoude technical free feet (ppm); 30 chicks per control; 10 chicks per test concentration; unknown sex. Birds were held at an average temperature of 22.7°C with an average relative numidary of 50% and approximately 16 hours light per day. Birds were observed at least once daily for mortality, signs of toxicity and abnormal behavious Individual body weights were measured of day 0 at test initiation, on day S and at termination of the test on day 8. Samples were collected to verify the test concentrations administered and to confirm the stability and homogeneity of the test substance in the diets, L,

There were no mortalities in the control and test item groups. All birds in the control and in the treatment, groups were normal in appearance and behaviour throughout the test. No overt signs of toxicity were observed in all treatment groups. There were no treatment related effects on feed consumption at any of the concentrations tested. When compared to the control group, there were no apparent treatment related effects on body weight among bods at \$62, 1000, 1780 and 3160 ppm during exposure period and for the entire rest period. Astatistically significant reduction of weight gain compared to the control was observed at 5620 pproduring the exposure and post exposure period. None of the control sample showed any indication of the presence of the test substance. Analysis of diet samples verified the test concentrations administered and confirmed the stability and homogeneity of the test substance in the Ą \sim diets.

diets. The subacut dietary LC_{50} of fluopicolide to 10-day old mallard duck chicks is determined to be > 5620

ppm, corresponding with an LDD₅₀ of \$2946.4 mg a.s./kg bw/d.



I. MATERIAL AND METHODS:

Fluopicolide techn., purity: 97.1 %, Batch No: 2050190/PP241024/2, Mallard duck (Angs *platyrhynchos*) (10-days-old) were exposed for 5 days to nominal dietary concentrations (techn. a.s.) of 562, 1000, 1780, 3160 and 5620 mg a.s./kg feed (ppm); 30 chicks per control; 10 chicks per test concentration; unknown sex. Dietary test concentrations were corrected for purity of the test substance. During the test the average temperature in the brooding compartment of the period was $30 \pm 4\%$ C (SP). Average ambient room temperature for this study was 22.7 ± 0.5 °C (SD) with an average relative humidity of $50 \pm 6\%$ (SD). The photoperiod was sixteen hours of light per day during a limit on and throughout the test. From test initiation until termination all birds were observed at least once daily, mortality, signs of toxicity and abnormal behaviour were assessed individual body weights were measured on day 0 at test initiation, on day 5 and at termination of the test on day 8. Following the exposure period, birds were maintained on untreated diet for a postobservation period of 3 days. Samples of the test diets were collected to verify the test concentrations administered and to confirm the stability and homogeneity of the test substance in the diets. Homogeneity of the pest substance in the diet was evaluated by collecting six samples from the top, middle and bottom from the 562 and 5620 ppm a.s. test diets at preparation on Day 0. The homogeneity samples also served as ver fication samples for those concentrations. One verification sample was collected from the control dist and two verifications samples were collected from each remaining treatment group at preparation on Day 0. At the end of the exposure period (Day 5) Qone sample was collected from the control diet and two samples were collected from all treatment groups to determine stability of the test substance in the diet. The samples were collected from feed temaining in the feeders.

II. RESPLTS AND DISCUSSIO		0 O
Validity criteria (accordingto OFOD 20501984)	d J A	Obtained
Mortality in the control		0%
Test concentration montained	f nominal over the s	5 Fulfilled
Effects in the lowest treatment level Street Street	ssure Should occur	Fulfilled
lost sabetanco	Techr	
Test subject	Chicks (
Exposure of the second	diet [mg a.s./kg feed]	ary [mg/kg bw/d]
	> 5620	> 2946.4
Lowest lethal effect concentration (LLE6)	> 5620	> 2946.4
Lowest observed effect concentration (EOEC)	5620*	2946.4
No observed effect concentration (NOEC)	3160	1394.8
Test object Test object Exposure LDD ₅₀ Lowest lethal effect concentration (LLEG) Lowest observed effect concentration (NOEC) * based on body weight * based b		



Observations:

Mortality and clinical observations

There were no mortalities in the control group and all control birds were normal in appearance and behaviour throughout the test. In addition, there were no mortalities or overt signs of toxicity in the 562,000 1000, 1780, 2160 and 5620 ppm treatment groups. All birds in all treatment groups were dermalon appearance and behaviour throughout the test.

opearance an ody weight a	d behaviou and feed co	ar througho	out the test.	ě		J. J.	
Treatment		*	Mean bod	yweight <u>±</u> SD	[g]	Ĵ ^Y	
(ppm)	day 0	day 5	day 8	∆ day20-5	∆ day 5 ≁8	Δ day 0-8	
Chicks							
Control	156 ± 17	287 ± 34	383 ± 43	132 ± 20	96 ± 13~	228 ± 30	
562	157 ± 19	288 ± 36	384 ± 50	\$√131 ± 22 ,	$396 \pm 22^{\circ}$	227 ± 41	
1000	157 ± 19	291 ± 41	390 ± 47	134 # 26	100@9	234 ± 30	of the se
1780	156 ± 20	275 ± 43	367 🛫 🕉 🌶	\$19 ± 24	_22¥18	212 39	
3160	156 ± 20	279 ± 36	365 🐓 53 🗸	122 = 20	86 ± 20	208 ± 36	
5620	153 ± 16	259 ± 32	3⊕ ± 43%	106 ± 23	¥ 86 ±€15	<u>192* ± 4</u>	ý O
Statistically dif	ferent from th	ne control gro	wpat p ≤0.0			y st	
		- Ç		, 'A			
Traatmont		Mean fo	od «o nsum	ption [g]			

T	Mean food consumption [2]
Treatment (ppm)	Exposure Period Rost-Exposure Period C &
(ppm)	Exposure Period day 0-5 965 20 10 20 10 10 10 10 10 10 10 10 10 1
Chicks	
Control	
562	
1000	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$
6	<u> </u>
3160	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$
5620	
K, V	

~ 7	×~		~~ °			
Treatment	bw day 0	bw day 5	mean bw	mean FC day 0-5	FC/bw*	Dose*
(ppm)	[g]∾ ≟	<u>[g]</u>	∦g	[g/bird/day	[g/kg bw/day]	[mg/kg bw/d]
Chicks						
Control 🔌	Q156 C	287	2275	»96 🏷	433.4	0.0
562	157	288	# #2.5 . Q	110	494.4	277.8
1000 &	157	291	224.0	106	473.2	473.2
1780	156 🖉	275 . 0	21	×ðn	417.6	743.4
3160		^م کر 79	217.5	96	441.3	1394.8
5620	153 4 1	259	206.®	108	524.7	2946.4

* Values not presented my study port. Calculated on the basis of results for FC, bw and treatment rate given in study report \sim

* values not presented in study report. Calcu bw = body weight, FC = Food Shsumption



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Analytical results

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

Full details and acceptable validation data to support this method are presented within documen 4, which comply with the EU regulatory requirements outlined within SoNCO 29/29 rev 4.

III. CONCLUSION

is determined to be The subacute dietary LC50 of fluopicolide to 10-day old martard dick ppm, corresponding with an LDD₅₀ of > 29746.4 mg a.s. /kg bw/d.

redictor of the second of the This study provides valid information on the short-term dietary toxicity of flappicohie to Mallard duck chicks when administered over 5 days in the diet and can be used for risk assessment. Toxicity was observed at 5620 ppm (2946.4 mg a.s./kg bw) and above. The Jac 50 is 5620 ppm (DDD50 > 2946.4



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

Executive Summary

Bobwhite quail chicks (10 days ob) were exposed for 5 days to nominal dietary concentrations of 562, 1000, 1780, 3160 and 5620 mg M-01 (2,6-dictrorobenzamide (code AE 6653711))/kg feed (ppm); 30 chicks per control; 10 chicks per test concentration; unknown sex. Birds were held at an average temperature of 29.0 % with an average relative hamidity of 59% and approximately 16 hours light per day.

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

The subacute dietary LC_{50} of M-04 (2,6-dQ hlorobenzamide) to 10-day old Bobwhite quail chicks is determined to be 386 ppm corresponding with an LDD₅₀ of 1171 mg p.m./kg bw/d.



I. MATERIAL AND METHODS:

M-01 (2,6-dichlorobenzamide (AE C653711)); purity: 98%, Lot number: I8499A, CAS Number: 2008-58-4. Bobwhite quail (*Colinus virginianus*) chicks (10-days-old) were exposed for 5 days to nondietary concentrations of 562, 1000, 1780, 3160 and 5620 mg p.m./kg feed (ppm); 30 chicks per control: 10 chicks per test concentration; unknown sex. Dietary test concentrations were corrected for parity of the test substance. Water and feed were provided ad libitum during acclimation and during the test. During the test average temperature in the brooding compartment of the pens was 38.4 ± 4.4 °C (SD). Average ambient room temperature for this study was $29 \pm 1.1^{\circ}$ C (SD) with an average relative hamidity of $59 \pm 4\%$ (SD). The photoperiod was sixteen hours of light per day during acclimation and through sit the test. The birds were exposed to an average of approximately 172 hix. With the exception of day 0 and day 8 of the test, all birds were observed twice daily. Birds were observed on four ocasions on day 0 and once prior to test termination on day 8. Bollowing the Q-day exposure period, birds were maintained on untreated diet for a post observation period of 3 days. Q, Samples of the test diets were collected to verify the test concentrations administered and to confirm the stability and homogeneity of the test substance in the diets. Homogeneity of the test substance in the diet was evaluated by collecting six samples from the top middle and bottom from the 562 and 5620 ppm test diets at preparation on day 0. The homogeneity samples also served as verification samples for those concentrations. One verification sample was collected from the control diet and two verifications samples were collected from each remaining treatment group at preparation on day 0. At the end of the exposure period (day 5), one sample was collected from the control diet and two samples were collected from all treatment groups to determine stability of the test substance in the diet. The samples were collected from feed remaining in the feeders.

OII. RESULTS AND DISCUSSION:	L.
Validity criteria (according to OECD 205, 1984)	Obtained
Mortality in the control $\sqrt{3}$ $\sqrt{3}$ $\sqrt{3}$ $\sqrt{3}$ $\sqrt{3}$	0%
Test concentration maintained 280% of nominal over the 5 day	Fulfilled (except at
rest concentrations management of expassine	562 ppm)
	reduced bw gain over
Effects in the lowest treatment level A Deffects should occur	5-d treatment phase
	in all test levels

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	ΟŤΙ.	RESU	LTSÅN	ID DIŠC	USSION	$\sim \bigcirc$	M	
	÷	6	20			"Kn"	A Y	

	• 1
Short-term dietary toxicity of Mell (2.6-dichlorobenzamide) to Bobwhite	0119116
Short-term dictary to here of 1200-dicty of 00012 diffice with Doow inte	quans.

Test substance	M-01 (2,6-
	dichlorobenzamide)
	Chicks (10 days)
Expositive in the second secon	dietary
LC ₅₄ [mg p.m./kg feed]	3867
Lowest lethal effect concentration (LLEC) [mg p.m./kg feed]	3160
Lowest observed effect concentration (LOEC) [mg p.m./kg feed] No observed effect concentration (NOEC) [mg p.m./kg feed]	562 *
No observed effect concentration (NOEC) [mg p.m./kg feed]	Not determined
* based on body weight ' ' ' ' ' ' ' ' ' ' ' ' ' ' ' ' ' ' '	



Observations:

Mortality and clinical observations

There were no mortalities in the control group. There were no treatment-related mortalities in the \$62, \$1000 and 1780 ppm groups. A single incidental mortality occurred at 562 ppm after a chick had scaped from the brooding compartment; this was not considered treatment-related.

<u>C</u>	umulative m	ortalitie	<u>s</u>			۵.		S.			2
	Treatment	Exposu	ire perio	d			č	Post expo	sureperiod	Y , OY	. O
	(ppm)	day 0	day 1	day 2	day 3	day 4	day 5	⁄day 6	dary7 🔊	day	Ő
	Chicks					4Ú ^Y		0	¢ ×		1
	Control	0/30	0/30	0/30	0/30	A3 30	0/30	Ø30 Q	0/30	0/30	
	562	0/10	0/10	0/10	1 ^A /10	₽1/10 。	1/0 3	1/10		1/100	
	1000	0/10	0/10	0/10	0/10	0/100	10	0/10/	10 ×	0/10	
	1780	0/10	0/10	0/10	0/10		0/100	010	0/10	• 10 °	
	3160	0/10	0/10	0/10	0/10 >>	2/10	5/10 4	5/10	5/10	5/10 Ø	
	5620	0/10	0/10	0/10	1/10	6/10		7/10	J/10 🔬	7/1	

A Mortality determined to be incidental, not included in the calculation of the LGS0 value

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

	\$		Ô Á	× 4	\wedge \checkmark \sim	i W
Treatment	Mean bod	lyweight ± :	SD [g] 🚿	<u> </u>	\$. \$	
(ppm)	day 🔊	daay 5 ≪	day 8	A day 0,5*	Dday 5 8 A	A day 0-8 ^A
Chicks	<u> </u>	6	~Q*	N N (
Control	20 ± 2 0	30 ± 3⁄	40 ± 4	11 \$ 2	10 2 ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	21 ± 3
562	$020 \pm 20^{\circ}$	23 ± 3	√33±±∞	4, 4/2		14 ± 4
1000 ©	19 📩 2	$\sqrt{20} \pm 4$	30±7	\$\P ± 3 \	010 ± 40	11 ± 6
1780 🧳	20 ± 2 🔬	19 ±3	27 ± 5 🖒	$7 - 1 \pm 20^{\circ}$	8±2	7 ± 4
3160	$19 \pm 20^{\circ}$	15 2	22 ± 2	-4-\$3	7 1	3 ± 3
5620	20 ±2	365±3 √	23 ± Ø	2 = 2	°~∳±3	1 ± 5

Body weight and feed consumption

^A Mean changes in weight were calculated from individual changes in bodyweight

T	Mean food consumption (gobird/day)
Treatment (ppm)	Exposure Period
20"	day 0-5, , , , , , , , , , , , , , , , , , ,
Chicks	
Control	8 ± 2 $\sqrt{7}$ $\sqrt{10} \pm \sqrt{7}$
562	
1000	
1780	
3160	
5620	

Dose calculation was reported as presented below, note that the calculated dose at 1780 ppm is higher than at 3500 ppm, suggesting that the reduced feed consumption and body weight effects impair a proper assessment of the achieved doses. The study director proposed a dose conversion of the LC_{50} into a dietary LD_{50} of 1171 mg a.i./kg body weight/day as reasonable.



Treatment	e e	Mean BW day 0-5 [g]	Dose [mg/kg bw/d]
Control	8	25	0
562	14	21	376
1000	10	20	528
1780	9	19	876
3160	4	17	715
5620	5	18	1468
BW = body weightstressed	ght, FC = Food consum	nption	

None of the control samples showed any indication of the presence of the test item $M-\theta'$ dichlorobenzamide).

Diet samples were collected from the 562 and 5620 ppm M&01 (2, 9 dich probenzamide) test concentrations and were analysed to evaluate the homogeneity of the test substance in the diet. Means and standard deviations for the two test concentrations were 500 ± 8.38 ppm, and 5080 ± 050 ppm, respectively. Samples collected during the test to verify test substance concerdations, for the 1000,0780 and 3160 ppm diets had means of 1000 ppm/1850 ppm and 3260 ppm, respectively. These values represented 100, 104 and 103% of nominal concentrations. Analysis of diet sample collected from feeders after being held at ambient temperature for 5 days averaged 78, 85, 84, 90 and 90% of the day 0 values for the 562, 1000, 1980, 3160 and 5020 ppm Me91 (2,0-dichlorobenzamide) test concentrations concentrations.

Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory equirements outlined within SANCO/302999 rev 4.

III. Conceusions: 0

The subacute dietar LCs of M (2, chichlorobenzamide) to 10-day old Bobwhite quail chicks is determined to be 3867 ppm, corresponding with an LDD50 of 171 mg p. pc/kg bw/d.

The assessment of the Jethal rexicity endpoints is hampered by reduced feed consumption and body weight offects, so that calculation of the LDD₅₀ iouncertain. No treatment-related mortality occurred up to 528 mg/kg byod.

the second state of the se



CA 8.1.1.3 Sub-chronic and reproductive toxicity to birds

		Test		
Test substance	Test design	species	Endpoint	Reference 4
Fluopicolide	21 weeks feeding chronic, reproduction	Bobwhite quail	NOAEC 1000 ppm NOAEL $2 \le 88.9 \text{ mg a.s.}/kg \text{ bw/d}$ EC ₁₀ $46.7 (29.7 - 89.7)$ mg a.s./kg bw/d 3 SOEC $2 \ge 1000 \text{ ppm}$ NOEL $240.8 \text{ sp a.s.}/kg$	EC ₁₀ calculation 20127M-660212-01-1 KCA 8.16 ³ /03
	21 weeks feeding chronic, reproduction	Mallard G	32.2(31.1-33.4) 32.2(31.1-33.4) 32.2(31.1-33.4) 32.2(31.1-33.4)	$\begin{array}{c} 2003 M - 225404 - 01 - 2 \\ KCA & 1.1.3/02 \\ EC_{10} calconation \\ \hline 2019 M - 663971 - 01 - 1 \\ KC & 8.1.1.3/04 \end{array}$
			$ \begin{array}{c} \begin{array}{c} \begin{array}{c} \begin{array}{c} \begin{array}{c} \end{array} \\ \end{array} \end{array} \\ $	



Data Point:	KCA 8.1.1.3/01
Report Author:	
Report Year:	
Report Title:	AE C638206 technical: A reproduction study with the Northern Bobwhite
Report No:	M-225403-01-2
Document No:	<u>M-225403-01-2</u>
Guideline(s) followed in	U.S. Environmental Protection Agency: Series 850-Ecological Effects Test
study:	Guidelines OPPTS Number 850.2300 (1996)
	U.S. Environmental Protection Agency: Series 850-Ecological Effects Test Guidelines OPPTS Number 850.2300 (1996) FIFRA Subdivision E, Section 71-4 (1982) OECD Guideline 206 (1984)
	OECD Guideline 206 (1984)
Deviations from current	Method: Deviations from current guideline SANGO/3029/99 reg4:
test guideline:	Recoveries were determined at five different concentrations in triplicate. However,
	the obtained data demonstrate very good recoveries and the precision. The prethod
	can therefore be regarded as ht for purpose.
	Study: Current Guidelin@OECD 206 (1984)
	The birds were 28 weeks of age at the beginning of the study slightly older than
	the 20 - 24 weeks recommended by the guideline. The floor area per pair was
	0.1377 m2, below the 0.25 m2 recommended. The hatchlings were kept and temperature of 380°C during their second week, higher than the 28 - 32°C recommended. The humidity during egg storage was 87%, higher than the recommended 55 - 75%. These deviations are not expected to have impacted the study results.
	temperature of 380°C during their second week, angher man the 28 - 32 °C
	recommended the mundally during egg storage was \$7%, higher that the
	study results.
Previous evaluation:	
Trevious evaluation.	in DAR (2005)
GLP/Officially	Yes conducted under GLP/Officially recognised testing facilities
recognised testing	rest conducted under OLI / arrientary recognised testing identities
facilities:	
Acceptability/Reliability:	Wes A S A S A S

Executive Summary

The aim of the study was to determine effects on reproduction of floppicolide (AE C638206) to Bobwhite quai (Colinus virginiant). Full picolide technical was administered in the diet to three groups of sexually mature Bobyhite quails. Each group consisted of 16 breeding pairs and received nominal dierary concentrations of 0, 160, 400 or 1000 mg a 2/kg feed (ppm) over a period of 21 weeks. Birds were held at an overage remperature of 21.35° with an average relative humidity of 30.7% and approximately 8 hours light per day in the first 7 weeks. The photoperiod was increased to 17 hours of light per day during week 8. From onset of egg production eggs were collected daily. Eggs were selected randomly for eggshell thickness measurement and remaining eggs were candled to detect egg shell cracks. Crack@and aonormal eggs Were discarde@ All offer eggs were placed in an incubator, candled again to determine embry viability on day 11 and embryo survival on day 21. On day 21 eggs were placed into a hatcher. Hatchlings were weighed and kept on untreated diet until 14 days of age when they were weighed again and sacrificed. Addits were observed daily for mortality, abnormal behaviour and signs of toxicity. Adult body weight was measured at test initiation, on weeks 2, 4, 6, 8 and at adult sacrifice. Feed consumption was measured weekly for each pen for a 7-day period. Necropsies were performed on all adults surviving until adult sacrifice and on all adults that died during the test. In addition, effects upon egg production and quality, embryo development, hatchlings and 14-d chicks were examined. Endpoints were statistically evaluated for possible treatment related effects. According to OECD 206 guideline the test results can be considered as valid. Samples were collected to verify the test concentrations administered and to confirm the stability and homogeneity of the test substance in the diets.



There were no treatment-related mortalities, overt signs of toxicity or treatment-related effects upon adult body weight or feed consumption at any of the concentrations tested. Additionally, there were no treatment-related effects upon any of the reproductive parameters measured at the 160-test concentration. At the 400 and 1000 ppm a.s. test concentrations there were significant reductions in hatchling body weights that were considered treatment related. Analysis of diet samples verified the test concentrations administered and confirmed the stability and homogeneity of the test substance in the diets.

Based upon the statistically significant effects on hatchling weight observed at 400 and 500 ppm the no-observed-effect concentration (NOEC) for northern bobwhite quail exposed to fluopicolide technication in the diet during this study was 160 ppm a.s., which corresponds to a SOEL of 13.0 mg a S kg by d. However, the effects were considered not biologically relevant, so that the NOAEC from this study is considered to be 1000 ppm, corresponding to a NOAEC of 88.9 mg a.s./kg bw/d.

I. MATERIAL AND METHODS:

Fluopicolide (AE C638206) techn., purity: 901%, batch OP 2050046. Europicoride was administered in the diet to three groups of sexually mature Boowhite quail (18 weeks old at test initiation) which approached their first breeding season. Each group consisted ob16 breeding pairs and received nominal dietary concentrations of either 0, 160, 400 or 1000 ppm a.s. over a period of 21 weeks (mean measured concentrations during the test: 162, 464 and 992 ppm a.s.)

Each pen was equipped with feed and water troughs. Weekly, sufficient feed for the beding period was presented to the birds. Additional feed was weighed and added to the troughs as beeded. The average temperature in the adult bobwhite qual study room during the course of the test was $24.3 \pm 3^{\circ}$ C with an average relative humidity of 30.7 ± 10.6 % (SD).

For the first 7 weeks of the set the birds were held under a photoperiod of 8 hours of light per day. The photoperiod was increased to 17 hours of light per day during week 8 to induce egg laying until adult sacrifice. Eggs were collected daily from the onset of egg-production for 11 weeks and stored in a cold room. All eggs laid in a weekly interval were considered as one lot. At the end of the weekly interval, eggs were selected by indescriminate draw for egg shell thickness measurement. The remaining eggs were candled to detect egg shell cracks. Cracked eggs and abnormal eggs were discarded. All eggs that were not discarded or used for egg shell thickness measurements were placed in an incubator at 37.4°C. Eggs were candled again on day 11 of incubation to determine embryo viability and on day 21 for determination of embryo survival. On the 21 of incubation, eggs were placed into a hatcher and allowed to hatch. Hatchlings were weighed and kept on intreated diet until 14 days of age when they were weighed again and sacrificed.

The adults were observed daily for mortality, obnormal behaviour, and signs of toxicity. Adult body weight was measured actest intriation on weeks 2, 4, 6, 8 and at adult sacrifice. Feed consumption was measured weekly for each pen for a 7-day period. Necropsies were performed on all adults surviving until adult sacrifice and op all adults that died daring the test.

Statistical evaluation for possible treathent refated effects was conducted for the following endpoints: adult body weight, adult feed consumption eggs cracked of eggs laid, eggshell thickness, viable 11-d embryos eggs per eggs set. Ave 3-week embryos per viable 11-d embryos, hatchlings per eggs set or per live 3-week embryos, 14-d survivor per eggs set or per hatchling, hatchling bodyweight and 14-d survivor bodyweight. The parameters eggs faid per maximum laid", "hatchlings of maximum set" and "14-d survivor of maximum set" are not included in this summary since they lack any biological meaning.

Statistical evaluation: ANOVAS followed by Dunnett's multiple comparison procedure was used to determine statistically significant differences between the control group and each of the treatment groups. Except for adult bodyweight, the sample units were the individual pens within each experimental group. Percentage data were examined using Dunnett's method following an arcsine transformation. The pens in which adult mortality occurred were not used in statistical comparisons of the reproductive data.



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

II. RESULTS AND DISCUSSION: Validity Criteria (according to OECD 206, 1984) Adult mortality in control: ≤ 10 % Mean number of 14-day old survivors in the controls:
Validity Criteria (according to OECD 206, 1984)
Adult mortality in control: $\leq 10\%$ 0% 2% 0% 2% 0%
\geq 12 per hen
Eggshell thickness in control: $\geq 0.19 \text{ mm}$ \downarrow
Concentration of the test item in the feed 80% of the nominal concentrations.
Concentration of the test item in the feed 80 % of 101-108 % @ the nominal concentrations 5
Test object
NOAEC for parental toxicity [ppm] ϕ δ $2 \ge 1000$ 4
NOAEL for parental $\delta xicity (mg a s, kg bw/d) = \frac{3}{2} = \frac{388.9}{2} = \frac{3}{2} = $
NOEC for reproduction $[pm]$ $\sqrt{2}$ $\sqrt{2}$ $\sqrt{2}$ $\sqrt{2}$
NOEC for reproduction [rpm] 2 2 4060 4 0 NOEL for reproduction [mg a.s/kg bw/d] 13.9 4 2
NOAEC for α production [point] \mathcal{O} (α) \mathcal{O} (\mathcal{O}) (\mathcal{O}
NOAEL for reproduction [mg a s@kg bw/d]
Parentar Toxicity

No mortalities occupied in the coordinate of the 160 of 1000 ppm treatment groups. No overt signs of toxicity were observed at any of the concentrations tested. Four incidental mortalities occurred at 400 ppm. Due to the nature of the lesions observed at necessary, all mortalities were considered to be incidental to be atment. Incidental glinical observations goted during the test included those that normally are associated with

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

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

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

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



Test Interval	Test Concentration	Mean Body Weight	Mean Feed Consumption	Daily Dietary Dose
	[ppm a.s.]	[g]	[g/bird/day]	[mg a.s /kg bw/day]
	0	203	17	0
Weeks 1-21	160	201	17	3 .9
	400	202	18	35.6
	1000	200	18	88.9
Reproduction To	•	Ŭ V		

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

	<u>_</u>	\sim	, 0 ³	Q°, or	
Reproductive Performance per hen (absolute dat	a)	<u>. 0</u>	NY W	<u></u>	U A
Parameter 👋	Ø	Control	460 ppm	400 ppm	1000
1			0 8		ppm 🗸
Number of replicates	×,	<u>)</u> 6	16 0	^{ور} 12	16 705
# of eggs laid	4	592	<u>683</u>	599	707
# of eggs laid / hen		379 .	¥43 .~	\$0 0.5 0.5	A4
# of eggs laid / hen / day	* %	0.38 5 ⁴ 7 15 0	O MAY C	0.5	[©] 0.46
# of eggs cracked	A.	× 15	1004 <u>c</u>	40 (L.	12
# of eggs set	"0"	507	604 6	533	615
# of viable 11-d embryos	Ŷ	483	584 2	⁹ 50 <i>8©</i>	591
# of live 3-week embryos 4		¥ 479 🔊	584	50 ⁴	585
# of normal hatchlings $\sqrt{2}$	Ĩ	458	¥ 563≰	A68	559
# of 14-day survivor	Ş ^r	, 324	51 🖗 🕺	418	495
# of 14-day survivors	2° 2	27 Q 0.234	\$2 ~	35	31
	, ^e	0.234	× 0.233	0.236	0.233
Hatchling weight [g]		2007.I O	5, Ø	5.7*	5.5**
		⁰ 25 0	5, C O5	25	24
$\frac{14 - d \operatorname{surv}(\operatorname{\texttt{Wor}} \operatorname{weight} [g]_{\mathcal{U}} \mathcal{U} \mathcal{V} \mathcal{V}}{* p < 0.05} ** p < 0.00 \mathcal{U} \mathcal{V} $	Ő		» >		
	ý				
	í () »			
	~				

Reproductive Performance (celative data)		ý ý		
Parameter V O A Y	Control	160 ppm	400 ppm	1000 ppm
% cracked eggs of eggs laid		0	1	2
% of vieble 11-d embryos of eggs set	95°~>	97	95	95
% of live 3-week empryos of viable mbry of	199 199	100	99	99
% of hatchlings of live 3-week embryos	95	96	93	95
% of hatchling @ f eggs set	90	93	88	90
% of 14 d survivors at agrees at	84	86	78	80
% of 14-dGurvivers of ha@hlings	93	92	89	89
% of 14-d survivals of hathlings				



Reproductive Performance in %	ó of control		
Parameter	160 ppm	400 ppm	1000 ppm
# of eggs laid	115%	101%	119% Q° 119% X
# of eggs laid / hen	116%	135%	119%
# of eggs laid / hen/day	116%	134%	→ 121% → → → → → → → → → → → → → → → → → → →
# of eggs cracked	13%	27%	80%
# of eggs set	119%	105%	121% 🖓 🗇
# of viable 11-d embryos	121%	105%	122%
# of live 3-week embryos	121%	105%	
# of normal hatchlings	123%	1020	¥122% \$ \$
# of 14-day survivors	121% 🕰	99 % ~ ^	
# of 14 -ay survivors / hen	11920	A30% 0 4	195% @
Eggshell thickness [mm]	1 Q 0% @°	\$101%	Q100% V
Hatchling weight [g]	9% v 6	93%	0 90% A
14-d survivor weight [g]	100%	100% 3	100%

Analytical results

None of the control samples showed any indication of the presence of the test substance fluopicolide. Diet samples were collected from the 160, 400 and 1000 ppm a.s. test concentrations and were analysed to evaluate the homogeneity of the test substance in the diet. Means and standard deviations for the three test concentrations were $156 \oplus 0.753$, 392 ± 18.0 and 992 ± 57.7 ppm a.s., respectively. Samples collected during the test to verify test substance concentrations for the 160, 400 and 1000 ppm a.s., respectively. Samples had means and standard deviations of 162 ± 56.9 , 404 ± 13.9 and 1020 ± 44.3 ppm a.s., respectively. These values represented 101, 401 and 102% of normal concentrations, respectively. Analysis of diet samples collected from feeders after being held at ambient temperature for seven days averaged 108% of the day 0 values for each of the 560, 409 and 5000 ppm a.s. test concentrations, respectively. Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4. III. CONCLUSIONS: Based on these finding the NOEC of parental toxicity is 27000 ppm a.s. (88.9 mg/kg bw/d). Based on slightly but statistically significantly reduced hatching weight at 400 and 1000 ppm a.s., the NOEC for reproductive toxicity was set in the original study report at 160 ppm a.s., which corresponds to a NOEL

of 13.9 mg a.s./kg/body weight

A statistical difference from control is observed in the hatchling weight at the two highest doses when estimated to one decimal place. However, reduction in hatchling weight is small (<10% compared to control) and could also reflect increased egg and hatchling numbers at the higher doses rather than being of adverse biological significance in terms of reproductive performance. There was essentially no treatment-related impact on 14d hatchling survivor numbers/female and 14d body weight and other reproductive parameters were unaffected

However, the effects on hatchling weight were small, transient and considered not biologically relevant in the original DAR. Therefore, the NOAEC was assigned at 1000 ppm (NOAEL = 88.9 mg a.s./kg bw/d).

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



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

Executive Summar

S The aim of the study was to determine effects on reproduction of flappicobiae (AE C638206) to Mallard duck (Anas playrhynchos). Fluopicolide technical was administered in the diet to three groups of sexually mature malfard ducks. Each group consisted of to breeding pairs and received nominal dietary concentrations of 0, 160, 400 or 1000 mg a.s./kg feed (pm) over a period of 20 weeks. Birds were held at an average temperature of 19.4°C with an average relative hunvidity of 47.0% and approximately 8 hours light per day in the first 8 weeks. The photoperiod was increased to 17 hours of light per day during week 8. From onset of egg production eggs were collected daily for 12 weeks. Eggs were selected randomly for egg shell thickness measurement and remaining eggs were candled to detect egg shell cracks. Cracked and abnormal eggs were discarded All other eggs were placed in an incubator, candled again to detective embry viability on day 14 and embryo survival on day 21. On day 24 eggs were placed into a hatcher. Hatchling were weighed and kept on untreated diet until 14 days of age when they were weighed again and sacrificed. Adults were observed daily for mortality, abnormal behaviour and signs of toxicity Adult body weight was measured at test initiation, on weeks 2, 4, 6, 8 and at adult sacrifice. Feed consumption was measured weekly for each pen for a 7-day period. Necropsies were performed on all adults surviving until adult sacrifice and on all adults that died during the test. In addition, effects upon egg production and quality, embryo development and chick weight and survivability Overe examined. Endpoints, were statistically evaluated for possible treatment related effects. According to OFOD 206 guideline, the test results can be considered as valid. Samples were collected to verify the test concentrations administered and to confirm the stability and homogeneity of the test substance in the diets?

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



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

I. MATERIAL AND METHODS:

Fluopicolide (AE C638206) technical, purity: 95.9%, batch OP 2050046. Fluopicolide was administered in the diet to three groups of sexually mature Mallard duck (25 weeks old at test initiation) which approached their first breeding season. Each group consisted of 16 breeding pairs and received moninal dietary concentrations of either 0, 160, 400 or 1000 mg a.s./kg feed (ppm) over a period of 20 weeks (mean measured concentrations during the test: 162, 404 and 1020 ppm?a.s.). Ø Each pen was equipped with a bin feeder. Weekly, sufficient feed for the feeding period was presented to the birds. Additional feed was weighed and added to the troughs as needed. The average temperature

in the adult mallard duck study room during the course of the test was 8.4 ± 2.7 °C with an average L) relative humidity of 47 ± 15 % (SD).

For the first 8 weeks of the test the birds were held under a photoperiod of 8 hours of light per day. The photoperiod was increased to 17 hours of light per day during weak 8 to induce agg laying until adult sacrifice. Eggs were collected daily from the onsetof egg@roduction for 12 weeks and store@in a cold room. All eggs laid in a weekly interval were considered as one lot. At the end of the weekly interval, eggs were selected by indiscriminate draw for egg shell thickness measurement. The remaining eggs were candled to detect egg shell cracked eggs and abnormal eggs were discarded. All eggs that were not discarded or used for egg shell thickness measurements were placed in an invubator at 37.4°C. Eggs were candled again on day 14 of ancubation to determine embryo Pability and on day 21 for determination of embryo survival. On day 24 of incubation eggs were placed into a hatcher and allowed to hatch. Hatchlings were weighed and kept on untreated diet until 14 days of age when they were weighed again and sacrificed.

The adults were observed daily for mortality abnormal behaviour, and signs of roxicity. Adult body weight was measured statest institution on weeks 2, 0, 6, 8 and at adult sacrifice. Feed consumption was measured weekly for each pen for 7-da@period? Necropsies were performed on all adults surviving until adult sacrifice and on all adults that died during the tests

Statistical evaluation for possible treatment related effects was conducted for the following endpoints: adult body weight, adult feed consomption, eggs laid, eggs cracked of eggs laid, eggshell thickness, viable 14-d embryo beggs per eggs set, live 3-week enbryos per viable 14-d embryos, hatchlings per eggs set or per live 3-week emptyos, 14-d survivor per eggs set or per hatchling, hatchling bodyweight and 14-d survivor bodyweight. The parameters "eggs laid per maximum laid", "hatchlings of maximum set" and "14-d survivor of maximum set" are not included in this summary since they lack any biological meaning.

Statistical evaluation: ANOVAS, followed by Dunnett's multiple comparison procedure was used to determine statesticalles significants differences between the control group and each of the treatment groups. Except for adult body weight the sample anits were the individual pens within each experimental group. Percentage data were examined using Dunnett's method following an arcsine transformation.

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



II. RESULTS AND DISCUSSION:

Validity Criteria (according to OECD 206, 1984)	Obtained in this study
Adult mortality in control: $\leq 10 \%$	0%
Mean number of 14-day old survivors in the controls: \geq 14 per hen	40 per hen
Eggshell thickness in control: ≥ 0.34 mm	0.395 mm
Concentration of the test item in the feed: ≥ 80 % of the nominal concentrations.	99©104 % of the rominal concentrations
Findings:	990104 % of the rominal concentrations
Subchronic and reproduction toxicity to Mallard dock	
Test object	Mallard duck 1 2 2 2 2 2 2
NOEC for parental toxicity [ppm]	
NOEL for parental toxicity [mg a.s./kg bw/et]	$ \begin{array}{c} 1000 \\ \hline > 140.8 \\ \hline >$
NOEC for reproduction [ppm]	
NOEL for reproduction [mg a.s./kg bw 4]	

Parental Toxicity

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

Incidental clinical observations noted during the test included those that hormally are associated with injuries and pen wear such as foot lesions. Except for incidental findings, all birds appeared normal throughout the study The gross pathological examination of adult birds at termination of the study did

not reveal findings that wore considered freatment retated. . K O of the concentrations dested, O

of the concentrations tested O V V For the pre-egg laying phase (weeks 1-10), for the egglaying phase (weeks 11,221), and for the total test duration (weeks 1-21). For use in this summary the overall dose over the total study duration is considered relevant.

0 1060 158 0 Weeks -20 169 1966 155 23.3 400 1057 154 58.3	Test Interval		Mean Feed Consumption [g/bird/day]	Daily Dietary Dose [mg a.s /kg bw/day]
Week 2.0 160 2.3 155 23.3 100 2.5 1057 154 58.3			10 17	0
	Washer	169 2 2 2066	155	23.3
1000 1000 1000 1000 1000 1000 1000	weeks -20	400 10579	154	58.3
	- A		151	140.8

Reproduction Toxicity

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



Reproductive Performance per hen (absolute data)					
Parameter	Control	160 ppm	400 ppm	1000 ppm	
Number of replicates	16	16	16	16 🖉 🕺	
# of eggs laid	808	818	774	773	
# of eggs laid / hen	51	51	48	48 0 6	
# of eggs laid / hen/day	0.60	0.61	0.58	0.58	
# of eggs cracked	3	7	14	67 57 0	
# of eggs set	733	729	682	¢689 ~ √	
# of viable 14-d embryos	706	694	297 C	6090	
# of live 3-week embryos	705	A683	D 3594 🔊	M S K	
# of normal hatchlings	646	595 Q	548° 🔏	ر 505 Č 🛒 🖉	
# of 14-day survivors	635	588	83 5 ^(C)	499%	
# of 14-day survivors / hen	40 🔬	637 5 *	33 2 2	3V V	
Eggshell thickness [mm]	0.395	0.380	0 97 * 0	C.385 °	
Hatchling weight [g]	32	32	×31 ×	32	
14-d survivor weight [g]	£92 . V	302	296 ~	299	
p < 0.05					

Reproductive Performation	nce (relative data)				Z°
Parameter		Control	260 ppm	000 ppm	1000 ppm
% cracked eggs of eggs la	iid 🖉 🖉 🖉				1
% of viable 14-d embryos		Ø Ø	95 ~~	852 852	90
% of live 3-week embryos	s of viable embryos	100	98	299 <u>2</u> 5	98
% of hatchlings of live		^O 92 [°] , [™]	86 ~ &	, 92 🔊	82
% of hatchlings of eggs se	et a a	88 N	84, 0	78	72
% of 14-d survivers of eg	gs set N	×87 ×	\$0 ²	T.	71
% of 14-d survivors of that	tchlings	99	99 🖓 🎽	^y 97	99
0			Ő "Ø		

Reproductive Performance in % of control 1 Cor

'araneter	160 ppm	_{\$} 400 ppm	1000 ppm
	101% &	\$96%	96%
of eggs laid / her	100%	94%	94%
of eggs laid / hen/day	102% 5	97%	97%
$U \cup U \cup$	233%	467%	200%
of eggs set	\$9 9 %	93%	94%
of vighte 11-d embryos	999% 98% ~~ 07%	85%	86%
of live 3-week empryos A	9/70%	84%	85%
of normal hatchlings	90%	85%	78%
	<u>0</u> 93%	84%	79%
of 14-day Orvivors hen	[*] 93%	83%	78%
of 14-day survivors	98%	95%	97%
latchling weight [g]	97%	93%	90%
4-dişurviyo weight [g]	100%	100%	100%
4-d'survivo@weight[jg]			



Analytical results

None of the control samples showed any indication of the presence of the test substance fluopicolide. Diet samples were collected from the 160, 400 and 1000 ppm a.s. test concentrations, and were analysed to evaluate the homogeneity of the test substance in the diet. Means and standard deviations for the three test concentrations were 156 ± 0.753 , 392 ± 18.0 and 992 ± 37.7 ppm a.s., respectively. Samples collected during the test to verify test substance concentrations for the 160, 400 and 1000 ppped.s. diets had means and standard deviations of 162 ± 5.49 , 404 ± 13.9 and $1020 \pm 440^{\circ}$ ppm a.s., respectively. These values represented 101, 101 and 102% of nominal concentrations, respectively. Addivise of diet samples collected from feeders after being held at ambient temperature for seven days averaged 99, 104 and 104% of the day 0 values for each of the 160, 400 and 100 ppm a.s. test concentrations, respectively.

Full details and acceptable validation data to support this method are presented within document 4, which comply with the EU regulatory requirements outlined within SoNCO 29/29 rev 4.

III. CONCLUSIONS:

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

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Assessment and conclusion by applicant:



Data Point:	KCA 8.1.1.3/03
Report Author:	Kleinmann, J.; Wang, M.
Report Year:	2019
Report Title:	Calculation of EC10 and EC20 for fluopicolide for reproduction endpoint of one of the second
Report No:	19016-BAY-1
Document No:	<u>M-660212-01-1</u>
Guideline(s) followed in study:	None O V V V V
Deviations from current test guideline:	Not applicable
Previous evaluation:	No, not previously submitted
GLP/Officially	No, not conducted under GLPOfficially recognised testing facilities
recognised testing	
facilities:	
Acceptability/Reliability:	Yes $\sqrt[4]{\gamma}$ $\sqrt[4]{\gamma}$ $\sqrt[4]{\gamma}$ $\sqrt[4]{\gamma}$ $\sqrt[4]{\gamma}$ $\sqrt[4]{\gamma}$

ECx calculation for the bird reproduction studies

Executive summary

In the present study effect concentrations (EC_{10} and EC_{20}) were calculated from data of a reproduction study in northern bobwhite with exposure to Muopicolide (demplo et al., 2003) Calculations of EC10 and EC_{20} were conducted using ToxRat version 3.3.0.

Corresponding to the endpoints in the reproductive risk assessment for birds, the calculations have been performed based on achieved dietary doses, and the results are expressed accordingly in terms of mg/kg Ś bw/d. 0

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

To provide additional information on the reflability of EC, values the 'normalised width' or NW, which is the ratio of the confidence interval of the EC, and the median EC, was also calculated. The use of

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EC₁₀ and EC₂₀ of reproduction endpoints from Temple et al. (2003) in northern bobwhite quail exposed to fluopicolide.

	EC ₁₀ [mg/kg bw/d]	EC ₂₀ [mg/kg bw/d]
Endpoint	(95% confidence interval)	(95% confidence interval)
Eggs laid per hen	No statistically significant concentratio the relationship is not signif	on/response was found, i.e. the sope of
Eggs cracked per eggs laid	No adverse eff	ects detexted 1 2 2
Eggshell thickness	No statistically significant concentratio the relationship is not signif	n/respense was found i.e. the slope of a
Viable embryos per eggs set	No adverse eff	êcers detestied 1 2 4 5 4 5 4 5 4
Live 3-week embryos per viable embryos	120.2 (n.d. – n.d.)	127.0° NW· [™] (n.đ.) [™] (n.đ.) [™]
Hatchlings per hen	No statistically significant concentration	n/response was found, i.e. the slope of nearly different from zeres
Hatchlings per eggs set	No statistically significant concentration the concentration the signature of the second states of the second stat	Fresponse was found te. the slope of icanto different from zero.
Hatchlings per viable embryos	No statistically significant concentratio	n response was found, i.e. the slope of grantly different from zero.
Hatchlings per live 3-week embryos	No statistically significant concentratio	n/response was found, i.e. the slope of fication of first the slope of fication of the slope of fication of the slope of t
14-day survivors per hen	So statistically significant concentration the relationship is not significant	tresponse was found, i.e. the slope of icanto different from zero.
14-day survivors per eggo	No statistically significant concertizatio	neantly different from zero.
	No statistically significant concentration	» n/response was found, i.e. the slope of icapily different from zero.
Kapral hatchling bodyweight	69 469 → 129 → - 89.09 → NW: 1.3 →	n.d.
14-day survivor bodyw@jght	the gelation the pelation the pelation of the	on/response was found, i.e. the slope of ficantly different from zero.

n.d.: not determined due to mathematical reasons (inappropriate data) or value is beyond the tested concentrations ¹ No ECx values could be calculated since there was a positive response with increasing concentration, e.g. the number of eggs was slightly higher for bigher concentrations.

ECx calculations were only possible for \mathcal{D} endpoints (live 3-week embryos per viable eggs, initial hatchling bod weight). The lowest EC₁₀ was 46.7 mg/kg bw/d, albeit with a moderate fit (NW 1.3).

Assessment and conclusion by applicant:

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



Ø

Data Point:	KCA 8.1.1.3/04
Report Author:	
Report Year:	2019
Report Title:	Calculation of EC10 and EC20 for fluopicolide for reproduction endpoints in
	mallard
Report No:	19016-BAY-2
Document No:	<u>M-663971-01-1</u>
Guideline(s) followed in	None A A A
study:	
Deviations from current	Not applicable
test guideline:	
Previous evaluation:	No, not previously submitted \mathcal{K} $O^{\mathcal{V}}$ \mathcal{K} $\mathcal{A}^{\mathcal{V}}$
GLP/Officially	No, not conducted under GeP/Officially recognised testing facilities
recognised testing	
facilities:	
Acceptability/Reliability:	Yes O' L'

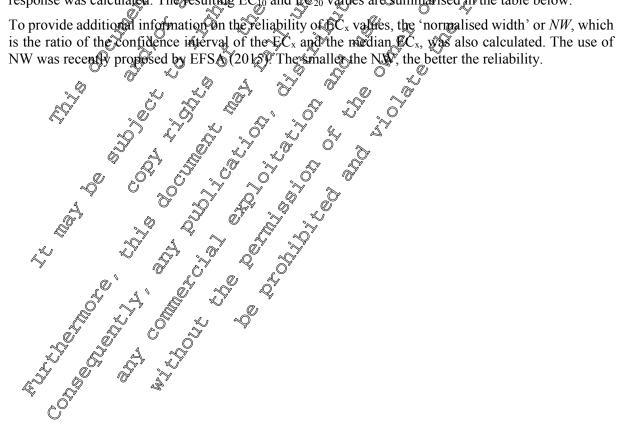
Executive summary

In the present study effect concentrations (EC_{10} and EC_{20}) were calculated from that of a reproduction studies in mallard with exposure to Puopicolide (Pemple et al. 2003) Calculations of EC Cand EC20 were conducted using ToxRat version 3.3.0.

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

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

To provide additional information on the reliability of \mathcal{C}_x values, the 'normalised width' or NW, which





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

F 1 • 4	EC10 [mg/kg bw/d]	EC ₂₀ [mg/kg bw/d]
Endpoint	(95% confidence interval)	(95% confidence interva)
Eggs laid per hen	No statistically significant concentration the relationship is not significant	on/response was found, i.e. the slope of ficantly different from zero.
Eggs cracked per eggs laid	No statistically significant concentration the relationship is not significant	on/response was found, ite the slope of a
Eggshell thickness	No statistically significant concentration the relationship is not significant	ficently different fom zero.
Viable embryos per eggs set	105.0 (n.d. – n.d.)	nd y y y
Live 3-week embryos per viable embryos	No statistically significant concentration the statistical significant concentration the statistical s	Wesponse was found, i.e. the stope of
Hatchlings per hen	32.2 (n.d.) (n.d	88.24 (n.d. – n.d. 4 – NW: 0.03
Hatchlings per viable embryos	No sofistically significant concentration	on/response wers found, i.e. the slope of
Hatchlings per live 3-week embryos	No statistically significant concentration the relationship is not significant	Wresponse was found, De. the slope of icantly different from zero.
14-day survivors per hen	32.3 (n.d., n.d.)	84.2 (n.d n.d.) NW: 0.76
14-day survivors por eggs		on/response was found, i.e. the slope of figure of figure of figure of the slope of figure of the slope of th
14-day survivors per hatchling	ONo statistically significant concentration	n/response was found, i.e. the slope of ficatily different from zero.
Initial hatchling	No statistically significant concentration the selationship is not signal	metersponse was found, i.e. the slope of ficantly different from zero.
14-day surviver	No statistically significant@oncentration the relationship is no significant	on/response was found, i.e. the slope of ficantly different from zero.

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

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

Assessment and conclusion by applicant?

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

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-02-1

CA 8.1.2 Effects on terrestrial vertebrates other than birds

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

CA 8.1.2.1 Acute oral toxicity to mammals

		. (%)_	Y
Table 8.1.2.1- 1: Acute ora	toxicity data for mamma	ls exnased to	fluonicoWde
Table 0.1.2.1 1. Heate of a	toxicity data for mamma	is exposed to	nuopiconuc

the toxicolog		a nave been cond	ducted with the active	substance muopic	
CA 8.1.2.1	Acute	oral toxicity to	o mammals	J.	
Table 8.1.2.1	- 1: Acute oral	toxicity data for	mammals exposed to fl	uopicolide	
Test species	Test design	Ecotoxicologic	al Endpoint 🖇	Reference	
Rat	Acute, oral	LD ₅₀	> 5000 mg a.s./kg bw	KCA 5.2 1/01	0005 <u>M-197224-015</u>

CA 8.1.2.2 Long-term and reproduction toxicity to mammals

The wild mammal long-term risk assessment endpoint has been previously set at 20 mg/kg bw/d, being the NOAEL from the rabbit developmental poxicity study (Table 8.1.2221). Thus, the position paper by . 2006; <u>M-268483-01-1</u>) is no longer relevant

Table 8.1.2.2-	1: Long-term t	oxicity data for m	ampals exposed to fluo	picolide
Test species	Test design 🃎	Ecotoxicological	Endpoint S	Reference
Rabbit	Long-term	NOAE	20 mg a.s./kg bw/d	<u>2004; M-202513-</u> KCA 5.6.2/04

Current EFSA guidance (EFSA 2015 Technical report on the outcourse of the pesticides peer review meeting on general recurring issues in ecotox cology recommends formalising the presentation of the toxicity profile of relevance for selecting the wild mammal reproductive risk assessment endpoint in Ô form of a tabled overview. 3 X 2

According to Appendix A of EFSA (2013), the results of 28 d oral toxicity studies, the sub-chronic oral toxicity studies, the molti-generation toxicity study and the developmental toxicity studies shall be compiled and evaluated.

Therefore, this overview presented in Table 8.12.202, followed by more detailed information on the potentiall@relevant effects observed in each of these studies.

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



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

1) Body weight change ¹ , behavioural effects and systemic toxicity ² 9 to (() N g st () C	28-day oral oxicity study OECD 407) 00-day oral oxicity study OECD 408)	Img/kg bw/d] Rat: M-199377-01-1 17.7: hepatocytic hypertrophy 179: BWG ↓, FC ↓, WC, clinical chemistry Mouse: M-197343-01-1 115 (hepatocytic hypertrophy, liver weight ↑, clinical chemistry) Rat: M-197622-01-1 114: urinalysis, clinical chemistry Inical chemistry Rat: M-197622-01-1 Inical chemistry		Large spacing between LOAL and LOAL and NOAEL NO relevant effects op to top dose Effects at LA mg kg ow/d not relevant for with mammal populations Food consumption
weight change ¹ , behavioural effects and systemic toxicity ² 9 to () N g sub- () N g sub- () N g sub- () N g sub- () N g sub- () N g sub- () N Sub- () N N Sub- () N N N N N N N N N N N N N N N N N N	OECD 407) OECD voral O-day oral oxicity study	Rat: M-199377-01-117.7: hepatocytic hypertrophy179: BWG \downarrow , FC \downarrow , WC, clinical chemistryMouse: M-197343-01-1115 (hepatocytic hypertrophy, liver weight \uparrow , clinical chemistry)Rat: M-197622-01-1114: urinalysis, clinical chemistryI14: urinalysis, clinical chemistrykidney weight \checkmark splet weight, hepatocellularhypertrophy, histopathology in bone joints, histopathology in kidneys1671: \downarrow BW, \downarrow BWG, \downarrow FC, \uparrow WC haematology, clinical chemistryMouse M-20579-02137.8 clinical chemistry161 \uparrow liver weight, hepatocellularyour weight, hepatocellularMouse M-20579-02137.8 clinical chemistry161 \uparrow liver weight, hepatocellularyour weight, hepatocellularMouse M-20579-02137.8 clinical chemistryyour weight, hepatocellularyour weight, hepatocellular <t< th=""><th>Mouœ. ≥ 1341</th><th>spacing betweed LOAL and NOAL and NOAL and NOAL and NOAL Services Se</th></t<>	Mouœ. ≥ 1341	spacing betweed LOAL and NOAL and NOAL and NOAL and NOAL Services Se
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y to (() N g s (() C	oxicity study	Rat: M-197622-01-10 114: urinalysis, clinical chemistry ↑ kidney weights ↓ splech weight ↑ kidney weights ↓ splech weight hypertrophy, histopathology in bone joints, histopathology in kidneys 1671: ↓ BW, ↓ BWG, ↓ FC, ↑ W@ haematology, clinical dremistry Mouse M-206 79-024 37.8 clinical chemistry 161* ↑ liver weight, hepatocellula, hypertrophy Ø70: ↓ BWG, ↓ BW		Effects at KJ4 mg/kg bw/d not relevant for wat mammad populations Food consumption
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	\$ \$	37.8 clinicationemistry 1612↑ liver weight, hepatocellulat hypertophy Ø70: ↓BWG, ↓BW	Afouse: @ 161 5	consumption
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g st (())))))))))))))))))	. a.	Mouse: M-127623-01-1 V O	Mouse:O	Second
g st (())))))))))))))))))		46; hepatocellular hypertrophy	P60 🔊	mouse study
g st (())))))))))))))))))	Q	46P. ↓ BWG (f), Chinical chemistry, ↑ lixer	s s s s s s s s s s s s s s s s s s s	conducted
g st (())))))))))))))))))	**	Aveight S Q A	29	
g st (())))))))))))))))))	Multi- 🖉 🧳	Rat: M-232532-01-1 3 3	Rat: 25.5	Effects at
	generation	255 (hepatocyte kypertrophy)	¥`	25.5 mg/kg
	study 🗸	109.4: ↓BW, ↓BWG, ↓PC, ↑ lizer weight, ↑		bw/d not
	@ECD_416)	Andney weights splgen weight		relevant for
				wild
				mammal
	.0 .			populations
	Developmental 2	Rat: M202155-02-1	Rat: 60	Very large
	studies	$700; \downarrow BW_{C}BWG \hookrightarrow FC$	Rat. 00	dose spacing
	OE OD 414)	Rabbit: M-202515-02-1	Rabbit:	uose spacing
(0EQD 414)	Rabult. M-202313502-1		
		Ø: mortality, ↓ BW, ↓ BWG, ℃FC	20	
2) Indices of ON	Multi 🖉 🚬 🖉	<u>Rat. M-232532-014</u>	Rat:	Dose from
	generation 🗞	2000 ppm 2↓ pup BW, ↓ pup BW G	35.8	F0 females
	study 🔊			during
pup and	OECD 416)			gestation
litter				and lactation
weight ³ [Developmental	<u>Řat: M-202155 2-1</u>	Rat: 60	Effects
- Sž	tudies /	€ 700: J@nean feetal body weights, ↓ crown rump		secondary to
-Că	оеср 414) 🖉	lengths & 1 placental weights)		maternal
*				toxicity
Ŕ		Kabbit: N-202513-02-1	Rabbit:	Effects
Ŭ I	ñ ^y Ö	$560: \downarrow$ mean foetal body weights, \downarrow crown rump	20	secondary to
~~ ~~ ~~	ž z ~C	lengths		maternal
A P		ion Stills		toxicity
			1	toxicity
	6			



Endpoint	Studies to	Dose: observations	NOAEL	Comment
Endpoint (phase)	check		proposal	Comment
· ·		[mg/kg bw/d]	1_	
3) Indices of	Multi-	<u>Rat: M-232532-01-1</u>	Rat:	Dose from
viability,	generation	no effects up to highest dose	127.3	F0 M prior
pre- and post-	study (OECD 416)		Å.	to paring
implantation	Developmental	Rat: M-202155-02-1	Rat: 700	
loss	studies	no effects up to highest dose	Rut. 700	5° 53° 6
	(OECD 414)	<u>Rabbit: M-202513-02-1</u>	Rabbit:	15/23/dams
	()	60: ↑ premature delivery	20 0	aborted 2
4) Embryo/	Multi-		20 0 Ratz 120.3	Dose from 0
foetal	generation	no effects up to highest dose	Ratz = 120.3	F0 Meprior
toxicity	study		Q A	to pairing
including	(OECD 416)			
teratological	Developmental	Rat: M-202155-02-1	Rat 050	₹ffects of
effects	studies	700: increase inominor skeletal stelects		questionable relevance
	(0100 414)		C O	for wild
		Rat: M-232532-01-1 no effects up to highest dose		mammads.
			Û S	large dose
			S. S	spacing
		Rabbit: M-202513-02-1	Rabbie 200	No effects
		60: no effects a gran of the construction of t	200	were seen
	Â	20: no effects		up to the
				highest
				dose; however,
			1 B	high
			N	mortality in
				the high
	JÝ VO			dose (60
				mg/kg bw/d)
·				precludes an
, Ø				adequate
	D 2			assessment of this group
5) Number	Multi-	$\mathbf{R}_{att} \mathbf{M}_{-232} \mathbf{A}_{2-01} \mathbf{M}_{-4} \mathbf{A}_{-4} \mathbf{A}_{-4}$	Rat [.]	Dose from
aborting	generation	Rat M-232 32-01-1	127.3	F0 M prior
and number	study			to pairing
delivering				
early 🚿	Developmental	Rat: M-202755-02-7	Rat:	
A	studies Or	no effects up to bighest Cose	700	
	(OECD 404)	Rabbit M-2021 3-02-1	Rabbit:	15/23 dams
		⁸ 60:≪ premature dett©ery Rato M-232532-0421	20	aborted
6) Systemic toxicity	Multa generation	23.5: hepatocyt Chypertrophy	Rat: 25.5	Effects at 25.5 mg/kg
and effects		103.4 BW 3° BWG, \downarrow FC, \uparrow liver weight, \uparrow	23.5	bw/d not
on adult	(OECD) 416)	kiduoj weights, \downarrow spleen weight		relevant for
hody weight				wild
	S C	S Y		mammal
		D ⁻		populations
	Developmental	<u>Rat: M-202155-02-1</u>	Rat: 60	Large dose
	studies *	700: \downarrow BW, \downarrow BWG, \downarrow FC	D 111	spacing
	(OECD 414)	$\frac{\text{Rabbit: M-202513-02-1}}{(0) \text{ martality } \text{ PWC} \text{ FC}}$	Rabbit:	
		60: mortality, \downarrow BW, \downarrow BWG, \downarrow FC	20	



Endpoint	Studies to	Dose: observations	NOAEL proposal	Comment
(phase)	check	[mg/kg bw/d]		
7) Indices of	Multi-	<u>Rat: M-232532-01-1</u>	Rat:	Dose from
post-natal	generation	no effects up to highest dose	127.3	F0 M prior
growth ⁴ ,	study		ð	to paying 🔬
indices of	(OECD 416)		S	4 0
lactation	Developmental	Not relevant (no post-natal data)	0	
and data on	studies		\$	
physical	(OECD 414)		4	
landmarks		The second se	ڭ ر	
8) Survival	Multi-	<u>Rat: M-232532-01-1</u>	Rat: \$2.9	N N O
and general	generation	127.3:↓BW,↓BWG	,O	
toxicity up	study		N N	S O
to sexual	(OECD 416)			
maturity	Developmental	Not relevant (nopost-nate) data)		
	studies	O ^N U ^N A ^N A ^N		
	(OECD 414)			The start of the s
) V d I	

comparison with controls More information on the most sensitive and relevant effects unless stated otherwise) in the studies tabled above.

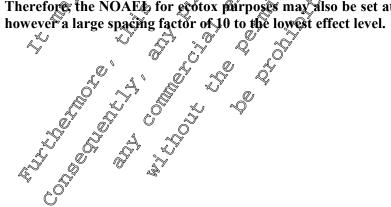
and systemic Phase 1: Body weight changes, behavioural effects

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

0, 20, 200, 2000 & 20000 ppm corresponding with @1.78, 07.8, 079 & 1770 mg/kg bw/d

Body weight gain (days 1-29) and absolut body weight (day 29) were aduced at 20000 ppm by 32/37% and 14/13% respectively in M/F. At 2000 ppm body weight gain and absolute body weight were reduced in females only, by 30% and 14% respectively (body weight charges were most marked at the start of the study for both doses). Food consomption at 20000 ppp was reduced by 41/28% in M/F during week 1. Water consumption was increased at 20000 ppm by 27/32% in M& F and at 2000 ppm by 18% in M. At 20000 ppm and 2000 ppm cholesterol was increased 20 & E whilst ALT was increased in M only. At 2000 ppm cholesters was acreased in M & F. Laver weights were increased at 20000 ppm by 25/23% (absolute) and 47/35% (relative) in M/F. Sentrilooular hypertrophy was noted in both sexes from 200 ppm and changes associated with the accumulation $oO\alpha^2\mu$ -globulin were noted in the kidneys of male rats (male rat specific finding) A NOAEL of 200 ppm (equivalent to 17.8 mg/kg bw/d) was proposed for toxicological purposes, however body weight offects observed in females at 2000 ppm may also be considered as ecotoxicologically relevant *b*a $\hat{\rho}$

Therefore, the NOAED for ecotox purposes may also be set at 200 ppm = 17.8 mg/kg bw/d, with





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

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

No effects on body weight, food consumption or haematology. ALT was increased at 640 ppm and \$400 ppm in M and F (ALP was slightly, non-statistically significantly increased at 6400 ppm). Liver weights? were increased at 6400 ppm by 33/50% (absolute) and 42/58% (relative) in MP respectivel At 640 ppm absolute and relative liver weights were increased in females only by 19%. Hepatocellular hypertrophy was noted from 640 ppm.

A NOAEL of 64 ppm (11.6 mg/kg bw/d) was proposed for toxicological purposes, however so findings of ecotoxicological relevance was observed up to the top dose level (6400 ppm, equivalent to 1111 mg/kg bw/d).

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

×1671 mg/kg@w/d 0, 100, 1400 & 20000 ppm corresponding with 0, 7, 9 114

Body weight gain (days 1-92) and absolute body weight day 92) were reduced at 20000 ppm 30/08% and 41/29% respectively in M/F (most marked at week). Foge consumption at 20000 ppm was reduced by 54/48% in M/F. Water consumption at this dose was increased by 44% in F (week 4) At 2000 ppm haematology parameters (Haematocht, MCH, MCHC) were reduced in M &F and activated partial prothrombin time was slightly increased in M. At 20000 ppm Tholesterol, total protein and GGT were increased in M & F and at 1400 ppm cholesterol was increased in M. Some Changes to urinalysis parameters were noted at 140% ppm & 20000 ppm (increased epighelial cells in M & increased volume and specific gravity in F). Liver weights were increased at 20000 ppm by 22% in F (absolute) and 51/49& (relative) in M/F) At 1400 ppm absolute liver weights were increased by 15% in M. Spleen weights were decreased at 2000, ppm by 45/40% in M/F and relative spleen weights were decreased by 24/29% in M/F. At 1400 ppm absolute spleen weights were reduced 10/16% in M/F and relative weights by 19% in F. Relative kidney weights were also increased by 1/2% in M at 1400 ppm. Histopathological findings comprised hypertrophy in the adrenal cortex at 20000 pppr, heperocellular hypertrophy from 1400 ppm, trabecular hyperostosis if the bone joint from 1400 ppm and a2µ-globulin accumulation in the kidney of Of (mate rat specific effect). × P

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

M-205579-02-4: More 90-day or al toxicity study

0, 50, 200, 800 & 3200 ppm corresponding with 0, 19.4, 37.8, 161 & 770 mg/kg bw/d in males & 0, 12.6, 52.8 207 & 965 mg/kg bw/d in females

Body weight gain (days 1-90) was reduced by 7/12% in M/F (most marked during weeks 1 and 2 being reduced in males by 88% during days 108 and i females by 87% and 85% during days 1-8 and 1-15 respectively). Absolute body weight (day 8) at 3200 ppm was reduced by 10/7% in M/F (only 3% lower in both sexes on day 90). No food consumption was measured. At 3200 ppm, ALP was increased in males by 29%, chotesterol was reduced by 50/16% in M/F and albumin was reduced by 13% (both sexes). At \$00 ppro cholesterol was reduced by 48.23% in M/F and albumin by 13/10% in M/F. At 200 ppm choksterol@was reduced by 25/21% in M/F. Absolute and relative liver weights were increased at 3200 ppm (20025% and 30/24% respectively in M/F) and 800 ppm (10/13% and 14/16% respectively in M/FD Hepatocellular hypertrophy was noted from 800 ppm.

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



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

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

Body weight gain was reduced at 6400 ppm by 20/32% in M/F (most severe at week 1; 74/64 in M/F or @ days 1-8) and at 3200 ppm by 22% females only. No effect on absolute body weight or food consumption. At 6400 ppm ALT and creatinine were increased in both sexes and AST and ALP were increased in males only. At 3200 ppm ALT was increased in both sexes, AST was increased mates and creatinine was increased in females. At 6400 ppm absolute liver weights were increased by 42,60% in M/F and relative liver weights were increased by 50/78% m M/F. At 3200 ppm absolute liver weights were increased 33/44% in M/F and relative weights were increased 12/36/59% in M/F. Hepatocellular hypertrophy was noted from 320 ppm. A NOAEL of 320 ppm (46/69/mg/kg bw/dm M/F) was proposed. for toxicological purposes, however body weight effects observed in females at \$200 ppm may also be considered as ecotoxicologically relevant.

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

<u>M-232532-01-1</u>: Multi-generation stody (0, 100, 500 & 2000 ppm)

Body weight gain was reduced at 2000 ppmpin males by a maximum of 0% (week 0.5), whilst absolute body weights were reduced by 6% (week). In gemal at 2,000 ppm body Weight gain was reduced by 14% during premating (weeks (10) and by max. 17% during gestation (GD 0-6) whilst absolute body weights were reduced by max 7% during premating (week 10) and max 8% during gestation (GD 13). Food consumption was reduced at 2000 ppm during premating (week (1) in males by 8% and in females by 9%, and in females during gestation (8% days 0-5) and lactation (13% days 7-15). At 2000 ppm liver weights were increased by 20/04% in M/F (absolute) and by 36/21% in M/F (relative), Kidney weights were increased by 10% in M (absolute) and by 167% if M/F (relative) and spleen weights reduced by 12/15% in M/F (absolute) and by 8% in F (relative). depatocyte hypertrophy was noted from 500 ppm in males and at 2000 ppm in males and females and histopathology findings in the kidneys were noted in both sexes 2000 ppm. A NOABL of 500 ppm was proposed for toxicological purposes; however, body weight changes at the LOAEL of 2000 ppm (equivalent to lowest achieved doses of 103.4/127.3 mg/kg bw/d in M/F) are also considered as ecoloxicologically relevant.

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

M-202155-02 : Rat developmental toxicity study

0, 5, 60 & 400 mg/kg bw/d

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

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

perspective with



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

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

At 60 mg/kg bw/d 3 animals were found dead and 15 were killed following abortion. Clinical signs comprised decreased defecation, hypoactivity, bristling coat, pultaceous faeces and discoloures urine? At 60 mg/kg bw/d body weight gain was reduced by 86% (days 6-29), whilst absolute bod weights were reduced (max. 7% on day 29). Food consumption was decreased at 600mg/kg bw/d (max: \$4% days 26-29).

A NOAEL of 20 mg/kg bw/d was proposed for maternal toxicity, which is also relevant for ecotoxicology. Phase 2: Indices of gestation, litter size, pup; and litter weight M-232532-01-1: Multi-generation study 0, 100, 500 & 2000 ppm

There was no effect on gestation length, gestation index on little size in either 1 or 2 pup. Body weights of F1 pups were reduced at 2000 ppm from day 14 by approximately 8% in males and females and body weight gain at 2000 ppm@days 1-28) was lower by 9.6/7.9% in M/F Body weights of F2 pups were reduced at 2000 ppm from day 14 max 3% in males and 26% in tomales whilst body weight gain (days 1-28) was reduced by 14/11% in M/F (began on weaning and possibly related to palatability of the test substance in the diet).

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

M-202155-02 O Rat developmental toxicity study

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

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

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

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

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

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

C

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



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

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

0, 100, 500 & 2000 ppm

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

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

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

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

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

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

At 60 mg/kg bw/d the incidence of premature deliveries was increased (13/23 dams) secondary to maternal toxicity at this dose. Therefore, a NGAEL of 20 mg/kg bw/d is selevant for ecotoxicology.

Phase 4: Embryo/foetal toxicity including Deratological Offect

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

0, 100, 500 & 2000 ppm ^O

There was no evidence of an adverse effect of thropic orde or necropsy of offspring. Some absolute organ weights of offspring at 2000 ppm were tower than controls: Spleen 11%/17% in M/F, thymus 11%/9% in M/F (related to lower body weights). The relevant NOAEL for ecotoxicology is therefore > 2000 pm (equivalent to 103,40,27.3 mg/kg bw/d in M/F for F0 females prior to pairing).

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

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

There was an increase in minor skelejal detects at /00 mg/kg bw/d, secondary to maternal toxicity (aplastic dysplastic or fused thoracic vertebral arches (0/148, 0/150, 1/153, 4/142), aplastic, dysplastic, fragmented, fused or dislocated thoracic vertebral centres (0/148, 0/150, 1/153, 10/142), fragmented or longitudinally displaced sternebra (0/148, 0/150, 1/153, 3/142), aplastic, dysplastic, shortened, fused or primordium of only 9 ribs (0/148, 0/250, 1/153, 6/142) as well as wavy and/or thickened ribs (1/148, 1/150, 0/153, 5/142). There was no evidence of an increase in any major skeletal malformations, or in any major or minor externel or visceral malformations.

Therefore, the SOEL is 60 mg/kg bw/d, but considering only major malformations, the relevant NOAFA for solvey may be 700 mg/kg bw/d.



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

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

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

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

Phase 5: Number aborting and number delivering arly

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

for this endpoi No effects on abortions or early deliveries. NQAEL

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

or this endpoint is No effects on abortions or early deliveres. NOAEI

M

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

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

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

Phase 6: Systemic toxicity and effects on ad

-232532-01-1: Multi-generation study

Ś 02-1: Rat developmental foxicity study

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

See Phase 1 (Body weight charges, behavioural effects, and systemic toxicity) for details on systemic and body weight effects for

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

M-232532-061: Matri-generation/study (0, 100, 500 & 2000 ppm)

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

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



Phase 8: Survival and general toxicity up to sexual maturation

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

There was no effect on survival up to sexual maturation. In F1 pups body weight was reduced from day 14 to day 28 (max -8.7%/7.7% in M/F on day 28). Body weight gain (days 1-28) was also lower than the first than the second secon controls in males (-9.6%) and females (-7.9%). In F2 pups body weight gain was reduced from day 0.4(max -13% in males on day 21 and day 25 and max. -12% in females on day 20); body weight gain was also reduced (days 1-28) by -14% in males and -11% in females).

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

Higher Tier endpoint for seed eating mammals

Based on the different feeding behaviour of rabbits and rodents (make) and the different observed effects in the toxicity studies, further described in M-68911449-1 (and summarized in MCP FLG+FX &FS 350), it is considered justified to employ distinct risk assessments for rabbit scenarios (herbivores) with the rabbit endpoint, and for granivoro scenarios of seed eating mice with the corresponding rodent endpoint.

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

For the use in the Toxicity Exposure Ratio (TER) calculation, 2-week body weight effects were derived with a benchmark fose (BMD) calculation. For this purpose, body weight data for the first 3 weeks were excerpted from all dietary studies with fluopicolide in rodents (28 d, 90-d, chronic, reproduction) which include a comparable exposure setting in the initial 21 days.

BMDs were calculated with the tools recommended by EFSA (2017), and the reliability of the fit was assessed based on the criterion of normalized widtle EFSA 2015) As a point of departure for the refined risk assessment, the BMD \mathbb{I}_{40} is propose \mathbb{Q}^{1} .e. the left confidence limit of the BMD for 10% effect on body weight. The lowest reliable BMDL10 was 119 mg/kg bw/d.

This endpoint of 119 m²/kg bŵ/d was used as a refinement step for the seed eating mammal scenario. The seedling eater scenario was conducted with the rabbit endpoint of 20 mg/kg bw/d.

Toxicity of plant metabolites of fluopicolide

The available information of the toxicity of fluopicolide metabolites in leafy substrates of potential relevance for the representative uses and wild mammal risk assessment is compiled in Table 8.1.2.3-3. The M-01 metabolite (ADC653941, BAM) is sufficiently characterized as to allow a wild mammal risk assessment with the specific pxicity endpoints compiled below (LD50 1470 mg/kg bw; NOAEL 7.5 mg/kg/w/d) The totheity data for metabolites is M-02, M-04 and M-05 are less extensive but confirm that they are not 100 more toxic than their parent, as would normally be assumed in the absence of data. Only metabolites M-06 and M-09 cannot be considered as characterised with experimental data, so that assuming 10x higher toxicity may be appropriate as a surrogate endpoint in case a quantitative risk assessment were required.



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

	nt (same study collection as for the parent).
Data available	Endpoint derived
M-01 (AE C653711, BAM)	
Acute oral toxicity rat	LD ₅₀ : 2000 mg/kg (males) & 500 mg/kg bw (females)
<u>M-225484-01-1</u>	
Acute oral toxicity/range	LD ₅₀ : 1470 mg/kg bw (males) & 2330 mg/kg bw (femates)
finding study rat	
<u>M-228905-01-1</u>	
13-week study rat	NOAEL: 14 mg/kg bw/d (1 BW BFC, 1 muscle fone at the LOAEL of 49
<u>M-234461-01-1</u>	mg/kg bw/d)
3-generation reproduction	NOAEL 7.5 mg/kg bw/d (slight ↓ BW in dates and offspring at LOAEL of 04
study rat, <u>M-301025-01-1</u>	mg/kg bw/d)
Teratology study in rabbits,	NOAEL: 30 mg/kg bw/d/priortality, abortion, clifeical signs, body weight loss and
<u>M-301030-01-1</u>	reduced food consumption at the LOAEL of 90 mg/kg bw/d). No effects on
	development & g a a c a c a c
M-02 (AE C657188, PCA)	
Acute oral toxicity rat	LD50: >2000 mg/kg bw/m
<u>M-197257-02-1</u>	
28-day study rat, M-204953-	NOAEL: 1580 mg/kg bw/de/no effects at highest dose tested)
<u>03-1</u>	
M-04 (AE C657378)	
Acute oral toxicity rat	LD50? \$ 2000 mg/kg/bw & & O & O & O
<u>M-221558-01-1</u>	
28-day study rat	NOAEL: 159.2/230.6 mg/kg bw/d in M/ (no effects at highes@dose tested)
<u>M-221960-01-2</u>	
M-05 (AE 1344122)	
Acute oral toxicity rat	LD50: > 2000 mg/kg bw
<u>M-235328-01-1</u>	
28-day study rat	NOAR: 152/167 mg/kg bw/d in M/F (\ BWG, kidney histopathology at
<u>M-222343-01-1</u>	LOAFL of 29,000 ppm)
M-06 (AE C643890)	
No experimentar data	
M-09 (AE B102859) Acute or at toxicity	
Acute grat toxicity	LO50: 1030 mg/kg.bw
<u>M-685650-01-1</u>	

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

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



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

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

Amphibians - Aquatic data

A 48-hour LC₅₀ test was performed with *Xenopus laevis* tadpoles with a LC₅₀-48 hours > 0 mg a.s./L. These data indicate a greater sensitivity to fluopicolide exposure of fish compared to tadpoles (LC_{50} for LC_{5

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

		o v	K A		- S L	4
Test species	Endpoin			Refere	\bigcirc^{γ}	
<i>Xenopus laevis</i> - tadpoles	LCQ -48	× . ~ -		2010: N	<u>1-@/3869-@</u> \$8/01	<u>1-1</u>
Oncorhynchus mykiss - rainbow trout		6 hogers: 0.200 n	ng ag A		20701	2003;
		y l				

Amphibians – Terrestrial exposure (adult ble stage)

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

- Anternal dose; Calculated LD 50 amphilinen
- exposure rate: assume 100 % dermal absorption of full over-spray (no spray interception by vegetation or highing by burrowing)
- available aquatic toxicity data for the specific chemical
- standardised species-specific allometric information on relevant skin surface area (3.041 cm²) and bodyweight (1.393 g), as suggested in Weltje et al. (2017)

Test species	Codpoint	Reference
Xenopus laevis	C Q LCQ-48 hours: > 1 mg a.s./L	<u>2010; M-393869-01-1</u> KCA 8.2.8/01
Legemis nucrochinas	BCFss: 65 L/kg (whole fish, lipid normalised)	2003; M-241273-01-1 KCA 8.2.2.3/01



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

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

, Ç

Then rate and species-specific exposure information are combined to indicate the potential derival dose. **Dermal dose** = Application rate (kg/hectare) \hat{x} (exposed skip (cm²)/body reight(g)) = 0.1 x (3.041/1.395) = 0.218 mg/ko

0 A simple toxicity: exposure ratio (TER) calculation using these derived values indicates low concern for terrestrial stage amphibians, as the TER acute trigger value of 10 and TER fonic trigger value of are 0 clearly exceeded: Ô Ø Ň

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

$$= 298$$

$$\int_{0}^{\infty} \int_{0}^{\infty} \int_{$$

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

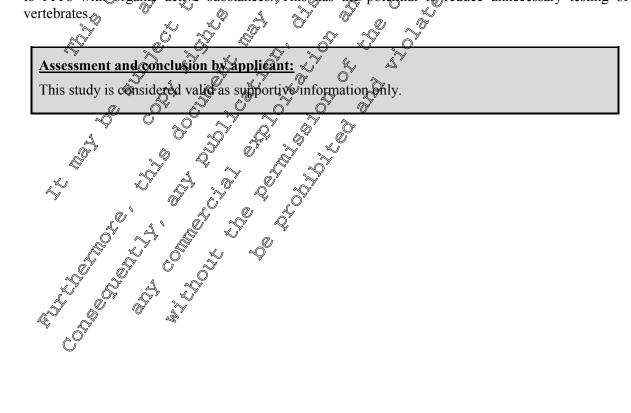
Consequently, food intake risk to reptiles is considered to be lower than for species with higher food intake rates, such as birds, and therefore reptiles can be considered as protected by the avian risk assessment.



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

Executive Summary

A study was performed to predict acute dermal toxicity of plant protection products (PPPs) to terrestrial amphibian life stages from (regulatory) fish data. By combining existing concepts, including interspecies correlation estimation (ICE), allometric relations, tethal body burden (LBB) and bioconcentration modelling, an equation was derived that predicts the amphibian median lethal dermal dose (LD₅₀) from standard acute toxicity values (96 h LC₅₀) for the and bioconcentration factors (BCF) in fish. Where possible, fish BCF values were corrected to 5% lipit, and to parent compound. Then, BCF values were adjusted to an exposure duration of 96 h, in case steady state took longer to be achieved. The derived correlation equation is based on 32 D_{50} values from acute dermal toxicity experiments with 15 different species of anuran amphibiants, comprising 15 different PPPs. The developed ICE model can be used in a screening approach to estimate the acute risk to amphibian terrestrial life stages from dermal exposures to PPPs with organic active substances. This has the potential to reduce unnecessary testing of vertebrates.





Data Point:	KCA 8.1.4/02
Report Author:	
Report Year:	1987
Report Title:	Field metabolic rate and food requirement scaling in mammals and birds
Report No:	A74185
Document No:	<u>M-152436-01-1</u>
Guideline(s) followed in	none
study:	
Deviations from current	Not applicable
test guideline:	
Previous evaluation:	No, not previously submitted 🕅 🖉 🧳 🦧
GLP/Officially	not applicable
recognised testing	
facilities:	
Acceptability/Reliability:	$\frac{Y}{Yes} \qquad \qquad$

Executive Summary

Field metabolic rates (FMRs or H_F), altomeasured using doubly labeled water, of 23 species of eutherian mammals, 13 species of marsupial mammals, and 25 species of birds were summarized and analyzed allometrically (log₁₀—log₁₀ regressions). FMR is strongly correlated with body mass in each of these groups. FMR scales differently than does based or standard metabolic rate in eutherians (FMR slope = 0.81) and marsupials (FMR slope = 0.58), but not in birds (FMR slope — 0.64 overall, but 0.75 in passerines and 0.75 in all other birds). Medium—sized (240—550 g) outherians, marsupials, and birds have similar FMRs, and these are ≈ 17 times as high as FMRs of tike—sized ectothermic vertebrates such as iguanid lizards. For endothermic vertebrates the energy cost of surviving in nature is enormous compared with that for ectotherms. Within the eutherians, marsupials, or birds, FMR scales differently for the following subgroups: rodents, passerine birds, herbivorous eutherians, herbivorous marsupials, desert eutherians, desert Ords, and seabilds. Equations are given for use in predicting daily and annual FMR and food requirement of a species of terrestriar vertebrate, given its body mass.

Assessment and conclusion by applicant:

This study is considered valid as supportive information only?

CA 8.1.5 Endocrine disrupting properties

Potential endocrine-disrupting properties of fluopicolide are being evaluated according to EU Regulation 2018/605 (*ED criteria?) following recommendations of the ECHA-EFSA Guidance for the identification of endocrine disruptors in the ontext of Regulations (EU) No 528/2012 and (EC) No 1107/2000



CA 8.2 Effects on aquatic organisms

	lpoints used in risk assessi abolites	nent and additional valid s	tudies for fluopicolide and its of
Test substance	Test species	Endpoint	Reference
	Fish, acute Oncorhynchus mykiss	96 h 0.36 mg a.s./L LC50 (mm) NOEC 0.16 mg a.s./L (mm) (mm)	2003; M-24080(Q)1-1 KCA & 2.1/01
	Fish, acute Lepomis macrochirus &		240805-01-1 *KCA&2.1/02 *
	Fish, acute	96 [°] h <i>Q</i> 3 mg as./L (mm LC ₅₀ NOE [©] 0.25 mg a.OL (com)	M-21-9743-01-4 KQ2 8.2.1003
	Fish, acute 5 Brachadanio rofio	96 h 1.8 mg a.s./L LC ₅₀ (mn0) NOBC 1.0 mg a.s.L (mm) 2	<u>2003;</u> <u>M-204508-61-2</u> KCA 8.2. P04
	Fish, acute Oryzłaś latines	96 h 0.7 mg a.s./£ LC& (nm) NOEC 0/44 mg a.s./L & S (mm)	<u>2003; M-</u> <u>234510-01-2</u> KCA 8.2.1/05
Fluopicade	Fish, acute Cyprinodon variegatus	96 h 0,49 mg a&/L L 30 (mm) SOEC 0.20 mg a.s./I (mm)	<u>2003; M-</u> <u>223359-01-2</u> KCA 8.2.1/06
	Fish acute Prinephates prometas	9607 194 mg a.s./L LC ₅₀ (nom) ² NOEC 0.313 mg a.s./L (nom)	<u>2015; M-533292-01-1</u> KCA 8.2.1/10
	Fish, acute Prinephates prometas Fish, anonic (ELS) Pinephales prometas Fish, Bor flow	33.0, 0.155 mg a.s./L NOEC (mm) PC10 0.278 mg a.s./L (mm)	<u>2003;</u> <u>M-241190-01-1</u> KCA 8.2.2.1/01 <u>2018; M-</u> 643769-01-1
	Fish, B ØF flow	∛ (mm)	Calculation of EC ₁₀ endpoint. KCA 8.2.2.1/02
	Fish, BEF flow through Lepomis macrochirus	BCFss, lipid 65 L/kg (whole normal fish) ised	<u>2003; M-241273-01-1</u> KCA 8.2.2.3/01
	Invertebrate, acute Daphnia magna	48 h > 1.8 mg a.s./L EC ₅₀ (mm)	<u>2003; M-240807-01-1</u> KCA 8.2.4.1/01



Test substance	Test species	Endpoint	Reference
	Invertebrate, acute Crassostrea virginica	96 h > 2.6 mg a.s./L EC ₅₀ (mm)	2003; M- 225445-01-1 KCA-8.2.4.2/01
	Invertebrate, acute Americamysis bahia	96 h 3.2 mg a.s./L LC ₅₀ (mm)	2003 XI- 220513-01-2 KCA 8.2.42702
	Invertebrate, chronic Daphnia magna	21 a 0.19 mg a.s./L SOEC (mm) EC ₁₀ Cannot be catevilated	$\begin{array}{c} 2003 \underbrace{\text{M}-241191-010}_{\text{K}} \\ \text{K} \\ \text{K} \\ \text{K} \\ \text{K} \\ \text{K} \\ \text{S} \\ \text{R} \\ \text{S} \\ \text{R} \\ \text{S} \\ \text{C} \\ \text{A} \\ \text{S} \\ \text{S} \\ \text{S} \\ \text{C} \\ \text{A} \\ \text{S} \\ \text{S} \\ \text{S} \\ \text{C} \\ \text{S} \\ \text{S} \\ \text{C} \\ \text{S} \\ \text{C} \\ \text{S} \\ \text{C} \\ \text{S} \\ \text{S} \\ \text{C} \\ \text{S} \\ \text{C} \\ \text{S} \\ \text{S} \\ \text{C} \\ \text{S} \\ \text{S} \\ \text{C} \\ \text{S} \\ \text{C} \\ \text{S} \\ \text{C} \\ \text{C} \\ \text{S} \\ \text{C} \\ \text{C}$
	Invertebrate, Bronie Americamytis bahia	28 d [©] 0.34 mg a. CL NOEC (com) EC 10 V.18 mg/a.s./P (mm)	2019; MO 544290-024 CCA 8,255.2/014
	Sediment dweller, chrome & S Lumbriculus variegatus	28 d 1.98 mg a.s./kg NOEC (nom)	<u>601529-03-1</u> KCA & 2.5.4/02
	Algae	2h 3.0 3.0 3.0 3.0 3.0 5.16	<u>2003;</u> <u>M-219737-01-2</u> KCA 8.2.6.1/01
	Pseudokirchnoriella subcapitata Green algae	NOE (mm)	2018; M- <u>643768-01-1</u> Endpoint recalculation. KCA 8.2.6.1/05
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	$\begin{array}{c} \begin{array}{c} & & & & \\ & & & & \\ & & & & \\ & & & & $	72 h 0.073 mg a.s./L E 50 (pom) 96 h 0.0612 mg a.s./L	
	Skeletonoma costatum & (Marine diatona)	$\begin{array}{c c} & E_yC_{50} & (nom) \\ \hline 72 & 0.0160 \text{ mg a.s./L} \\ & E_{05} & (nom) \\ \hline 72 & h & 0.0424 \text{ mg a.s./L} \\ \hline & F_rC_{10} & (nom) \end{array}$	KCA 8.2.6.2/07
	Algae, Skeletonema costatum ( (Marine diatom) Algae, Algae, Navialla pelliculosa (Freshwater diatom)	$\begin{array}{cccc} & 72 \ h & 0.121 \ mg \ a.s./L \\ E_r C_{50} & (mm) \\ 72 \ h & 0.068 \ mg \ a.s./L \\ E_y C_{50} & (mm) \\ 72 \ h & 0.043 \ mg \ a.s./L \\ \end{array}$	<u>2020; M-678011-01-1</u>
		$\begin{array}{ccc} NOE_{r}C & (mm) \\ 72 h & 0.064 mg a.s./L \\ E_{r}C_{10} & (mm) \end{array}$	KCA 8.2.6.2/08



Test substance	Test species	Endpoint		Reference	
	Aquatic macrophytes, Lemna gibba	7 d ErC50 NOE _r C	> 3.2 mg a.s./L (mm) frond number & dry weight 3.2 mg a.s./L (mm)	2003: 00 220201-01-2 K&A 8.2.7/01	
	Amphibian larvae, acute <i>Xenopus laevis</i>	48 h LC ₅₀ NOEC	(nom)	2010: M2 93860 01-1 KCA 8-2.8/01	
	Fish, acute Oncorhynchus mykiss	64	240 mg.p.m./10 (nom)	<u>2001, M-224311-07, 2</u> KCA 22.1/07, 2	
	Invertebrate, acute	LC 50	d. @% "	2001; M-23, 506-010 KCA \$2.4.1/92	
M-01	Algae Pseudokirchneriella subcapitata Geen algae	zŷh .	120 mg p.m./L (prom) 60 mg p.m./L (prom) 40 mg p.m./L (nom) 49 mg p.m./L 49 mg p.m./L	2001; M-294304-01-2 4 CA 8 2 6.1/03	
(2,6-dichloro- benzamide (BAM; BCS-AA65(84))	Algae, Navienta pellisilosa (Freshwater diatom) Aquatic macrophytes, Lemina sibba	72 h FrC50 72 h EyC50 72 h VOErC 72 h ErC10	92 mg. m./L (mm) 46 mg.p.n./L (mm) 90 mg.p.m./L (mm) 42 mg.p.m./L (mm)	2020; M-678377-01-1 KCA 8.2.6.2/10	
	Aquatic macrophytes, Lemina sigba	✓ d ErCa ErCa ErC10	<b>97.6 mg p.m./L</b> (nom), frond number 71.8 mg p.m./L (nom) 25.0 mg p.m./L (nom) 51.0 mg p.m./L (nom)	<u>2003:</u> <u>M-219725-01-2</u> KCA 8.2.7/02 <u>2018; M-</u> <u>664031-01-1</u> Endpoint recalculation. KCA 8.2.7/03	



Test substance	Test species	Endpoint	Reference
M-02	Fish, acute Oncorhynchus mykiss	96 h > 102 mg p.m./L LC ₅₀ (mm)	<u>M-218631-01-2</u> KC@8.2.1/08
(3-chloro-5- trifluoromethylpyridin e-2-carboxylic acid (PCA, BCS- AB43478))	Algae, Navicula pelliculosa (Freshwater diatom)	72 h       74 mg p.m./L $E_rC_{50}$ $faim$ 72 h       72 mg p.m./L $E_yC_{5e}$ (mm)         72 h       42 mg p.m./L         NOE _r C (mm)       72 h         72 h       48 mgp.m./L $F_rC_{10}$ (mm)	
L.S.: active substance; p.m.: p nom = nominal concentration	pure metabolite hs, mm = mean measured confi	ErCso (mm) 72 h 72 mg p.m./L E _y Cso (mm) 72 h 42 mg p.m/L 92 h 48 mg p.m./L F ₁ C (p) (mm) 40 f 40 f	



#### CA 8.2.1 Acute toxicity to fish

Data Point:	KCA 8.2.1/01
Report Author:	
Report Year:	2003
Report Title:	The 96 hour acute toxicity to the rainbow trout, Oncorhyrchus mykiss, is a static
-	system; AE C638206 Technical 97.1 percent w/w
Report No:	B003802
Document No:	M-240806-01-1
Guideline(s) followed in	OECD: 203 (1992); USEPA (=ERA): 72-1 (1982
study:	
Deviations from current	Method: Deviations from curent guideline SaNCO/3029/9@rev.4:
test guideline:	Recoveries were only determined at two different concentrations in duplicate.
	However, the obtained data demonstrate very good recoveries and the precision
	calculated form these data accounts for 9.9 (AE C638296) an 08.2 % (AE
	C653711), respectively. The method can therefore be regarded as fit for purpose
	Study: Current Guideline: DECD 293 (2019)
	The pH of all test solutions was greater than 8.5 on days and 1 (8.6 to 8.8).
	This deviation had no impact on the study since the validity exteria are met.
Previous evaluation:	yes, evaluated and accepted in the second seco
	in DAR (2005)
GLP/Officially	Yes, conducted under GLP/Officially recognised testing facilities
recognised testing	
facilities:	
Acceptability/Reliability:	Yes a by a b
\$	
Data Point:	KCA 8.2.141 6 6
Report Author:	
Report Year:	
Report Title:	Statement - Certificate of analysis for luopicalide acute toxicity study on
	rainbow trout (Young & Abedi, 2003; M-240806-01-1)
Report No: 🐎 🛷	MG63470@01-1%
Document No:	M-634800-01 1 2 2 2
Guideline(s) followed in	
study:	
Deviations from current	Not applicable of the A
test guideline:	
Previous evaluation:	No for previously submitted
GLP/Officiant	Out applicable y
recognised testing	
facilities?	
Acceptability/Reliability:	No not previously submitted ont applicable Yes Yes y
· ¥	

#### Executive summary

An acute toxicity test was performed with the rainbow trout (*Oncorhynchus mykiss*) in a static system. Junvenile rambow trouts were exposed of fluopicolide (tech.) at nominal concentrations of 0.65, 1.1, 1.8 3.0 and 50 mg a /L in well water for a 96- hour period. Additionally, a negative control was included. All treatments had 10 fish per test vessel (10 fish per treatment level). Test solutions were not renewed. Mortality, toxicity values and behaviour were recorded at time points 3, 6, 24, 48, 72 and 96 hours Recoveries at 0 and 96h were below 80% so the biological results are based on mean measured concentrations of fluopicolide. There was no fluopicolide residue found in the dilution water or control samples. Analytical data indicated that the fluopicolide metabolite M-01 (AE C653711) did not form in the study samples. All samples were analysed by Gas Chromatography with MS detection (GC/MS).



The arithmetic mean measured concentrations of fluopicolide were 0.16, 0.29, 0.44, 0.70, and 1.2 mg a.s./L.

The study fulfils all validity criteria of the current version of OECD 203 guideline. Mortality occurred at 0.44 mg a.s./L and above, with 100% mortality observed at 3h at 1.2 mg a.s./L. Sublethal effects were observed at 0.29 mg a.s./L and above all over the study in surviving fish. The 96-hour LC₅₀ of fluopicolide technical to rainbow trout is calculated as 0.36 mg a.s./L (95%CP = 0.29) to 0.44 mg a.s./L). The lowest observed concentration with mortality is 0.44 mg a.s./L. The highest concentration without mortality is 0.29 mg a.s./L. The NOEC (highest concentration without subtethal effects) is 0.16 mg a.s./L.

	I. MATERIAL AND METHODS:
Test material	I. MATERIAL AND METHODS:
Guideline(s) adaptation	None specified
Test species	Rainbow trout (Oneorhymenus mykiss) A 6 1
Acclimation	At least 14 days and a set of the
Organism age/size	In control figh at the end of the study:
at study initiation	Rainbow trout ( <i>Orioorhymchus mykiss</i> ) At least 14 days Health during acclimation: less than 1% mortality In control fish at the end of the study: Mean length: $4.6 \pm 0.41$ cm Mean body weight: $1.321 \pm 0.3545$ g Nominal concentrations: $0.65 - 1.1 - 1.8 + 3.0 - 5.00$ mg a.s./L. Corresponding mean measured concentrations: $0.16 - 0.29$ 0.44 - 0.70 and
Test solutions	Nominal concentrations: $0.65 - 1.1 - 1.8$ , $3.0 - 520$ mg a.s./L. Corresponding mean measured concentrations: $0.16 - 0.292$ , $0.44 - 0.70$ and $1.2$ mg a.s. / L. Samples were taken from all test chambers on day 0 and day 4. Controls: water The stock solution was filtered through a 0.45 µm filter as it was dispensed into the test chambers to remove undissolved test substance. This is the reason why the measured concentrations at 10 are in the range of 22 to 25% of nominal concentrations. After the filtration of the primary stock solution, there were no
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	additional problems with solubility throughout the study.
Replication	No. of vessels per concentration (replicates): 1
Organisms per replicate	No. of organisms per vessel: 10
	Static Table exposure duration 36 hours
Test Vessel Loading	0.377 g tish/L test medium
Feeding during test	
Test/conditions	Temperature: 12.7 – 139°C Destarshind: 76 hours doil
	Water kardness: 94 mg CaCO ₃ /L Dissoved oxygen: 67 -113% saturation
	Conductivity: 1000 to 1300 µmhos/cm



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

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II. RESU	тто		DICA	VICCI	$\overline{\mathbf{n}}$
II. KESU	LIN	AAD	DISK	JUSSI	L W D
		"(Of		/	

Validity criteria of OECD 203 (2019)	Required	Obtained S
Measured concentration of the test substance	Mandators, 5	Performed and results are based on mean measured concentrations
Mortality in control during test	<u><</u>	©*
Dissolved oxygen saturation	$\geq 60\%$	© 67-113%
		55

<u>Analytical results</u>: Recoveries at 0 and 6h were below 80% see table below) so the biological results are based on mean measured concentrations of flyopicolide. The results are reported on the basis of arithmetic mean measured concentrations. According to the EFSA technical report (2015), the results should be based on geometric mean measured concentrations. However, the concentrations are stable over the 96 h of the test and in these conditions, both arithmetic and geometric means are similar when rounded, so there

is no need to recalculate the endpoints? There was no fluopicelide, residue found in the dilution wate or control samples. Analytical data indicated that the fluopicelide metabolite M-01. (AE C653711) did not form in the study samples.

		CoorOtrio		
Nominal conc (mg a.s./L)	Arjthmetic mean (mg a.s./L)	Geometric mean maga s. (3)	[%] of nominal ♥ 0 hour	96 hour
0.65	0.18	0.16	23	26
1.14	0.29	0.22	24	29
1.8		Ø .44	23	25
3.0	Q.70 5 4	0.70	23	23
5.0	\$1.2 O \$	1.9	25	24
3.0 5.0 5.0 5.0 5.0 5.0 5.0 5.0 5				

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



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

Biological results:

Observations

At 3-hours, sub-lethal effects such as loss of equilibrium, erratic swimming, and swimming ceased were observed in the 0.29, 0.44, and 0.70 mg/L treatments. At 26 hours, sub-lethal effects were still observed in the 0.29 and 0.44 mg/L treatments. At 48 hours, sub-lethal effects were observed in the 0.29 mg/L treatments. At 72 or 96 hours, 10% sub-lethal effects continued in the 0.29 mg/L treatment.

A

Mortality

wortdifty			nor'	\sim $$	Q', O	a a
Exposure time (hours)	3	6 &	24 ° 2	48 4	72 0 ~	
Arithmetic mean measured conc. (mg a.s./L)	No of dead (%)	No of dead (%)	Nov of dead	No of dead	No of dead	No of dead (%)
Control	0 (0)	0 (00 6		0 (0) 0	0.00	0 (0)
0.16	0 (0)	Ø(x0) °	0(0)			B (0)
0.29	0 (0)			0 (0)	0,000	0 (0)
0.44	0(0)	0 (0)	9 (90)	10(100)	10(100)	10 (100)
0.70	3 (30)	60) J	ĴO (100)	10 (100)	10 (100)	10 (100)
1.2	10 (100)	10 (100)			10,000)	10 (100)
				0× %		

AII. CONCLUSIONS

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

LC ₅₀ % Hours (95% GL):
lowest concentration with mortality 7 7.44 mg a.s. / L
highest concentration workdut mortality 0.20 ng a SL
NOEC: highest concentration without sublethal of 16 moa.s. / L

Assessment and conclusion by applicant:

The study is reliable and the CC_{50} of 0.36 mg a.s./L and NOEC of 0.16 mg a.s./L can be used in risk assessment.

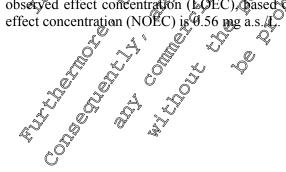
The second secon



Data Point:	KCA 8.2.1/02
Report Author:	Young, B. M.
Report Year:	2003
Report Title:	The 96 hour acute toxicity to the bluegill sunfish, Lepomis macrochirus, in a static of
	system; AE C638206 Technical 97.1 percent w/w
Report No:	B003801
Document No:	<u>M-240805-01-1</u>
Guideline(s) followed in	OECD: 203 (1992); USEPA (=EPA): 72-1 (1982)
study:	
Deviations from current	Method: Deviations from current guideline SANCO/3029/99 reg 4: S
test guideline:	Recoveries were only determined at two different concentrations in deplicate
	However, the obtained data demonstrate very good recoveries and the precision
	calculated form these data accounts for 2.4 (AE C 28206 and 7.2% (AE
	C653711), respectively. The method can therefore be regarded as fit for purpose.;
	Study: Current Guideline: OECD 203 (2019)
	The pH is greater than 8.5 (up to 8.7) on day of and Oin the highest
	concentrations. Since the mariation within the study was very limited (before a 34
	and 8.7) and validity criteria are met, it is unlikely that this deviation had a
	significant impact on the results of a start of the second start o
Previous evaluation:	yes, evaluated and accepted a solution of the
	in DAR (2005) 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2
GLP/Officially	Yes, conducted under GLP/Officially recognised testing facilities
recognised testing	
facilities:	
Acceptability/Reliability:	Yes & Cy & a a a

Executive summary

O SAN Executive summary An acute toxicity test was performed with the blue still surfish, (Lepomis macroschirus) in a static system. Juvenile bluegill sunfish were exposed to fluopicolide (tech Vin nominal concentrations of 0.65, 1.1, 1.8, 3.0, and 5.0 mg a.s. L, in well water for a 96 hour period. Additionally, a negative control was included. All treatments had 10 fish per test vessek (r.e., 10 fish per treatment level). Test solutions were not renewed. Mortably, toxicity values and behaviour were recorded at time points 3, 6, 24, 48, 72 and 96 hours. Each test chamber was sampled for analysis of flappicolide (tech.) and the metabolite M-01 (AE C650711) at 0 (prfor to the introduction of the test organisms to the test chambers) and 96 hours (study termination). Samples were taken at mid-depth and did not include any extraneous materials. All samples were analysed by Gas Chromatography with electron capture detection (GC/ECD). Recoveries were below 80% thus the results were based on mean measured concentrations of fluopicolide (tech.) which were 0.24, 0.3 50.56 P.0, and 1.7 mg a.s. 6. The order fulfils all validity criteria of the current version of OBCD 209 guideline. Sub-lethal effects were observed from 3 h to 24h at 1.0 mg a.s/L and above. Montality occured at the same soncermation Analysis of the mortality data by the trimmed Spearman Karber method gave the following result: The 96-hour LC₅₀ of fluopicolide (tech.) technical to bluegill sunfish was calculated as 0.75 mg a s/L (95%, CL = 0.56 to 1.0 mg a.s./L). The lowest observed effect concentration (LOEC), based on sublethal effects, is 1.0 mg a.s./L. The no observed





I. MATERIAL AND METHODS:

Test material	Fluopicolide (tech.)
	batch: 2050190//PP241024/2
<u>a :1 ::</u>	purity 9/.1 % w/w
Guideline(s)	None specified
adaptation	
Test species	Bluegill sunfish (Lepomis macrochirus)
Acclimation	At least 14 days
~ ·	Health during acclimation: less than 2% mortality
Organism	Mean length: 2.4 cm (2.1 – 2.6 cm) at the end of the study $\sqrt{2}$
age/size at	Mean body weight: $0.294 \text{ g}(0.17 \text{ g} + 0.397 \text{ g})$ at the end of the study 2
study initiation Test solutions	Nominal concentrations: 0.65 1 1 2 20 5 0 10 a c 12 O
Test solutions	Corresponding mean measured consentratives: 0.24 0.27 0.28 1.0 and 1.17 mg
	corresponding mean measured concentrations. 024 - 0.59 - 0.00 - 1.0 and 120 mg
	Samples were taken from all test champers or day 0 and day 4
	Controls: water
	Evidence of undissofted material The stock solution was filtered through an 45
	urn filter as it was dispensed into the test chambers to remove undissolved test
	Fluopicolide (tech.) batch: 2050190//PP241024/2 purity 97.1 % w/w None specified Bluegill sunfish (<i>Lepomis macrochirus</i>) At least 14 days Health during acclimation: less than 2% mortality Mean length: 2.4 cm (2.1 – 2.6 cm) at the end of the study Mean body weight: 0.294 g (0.177 + 0.397 g) at the end of the study Mean body weight: 0.294 g (0.177 + 0.397 g) at the end of the study Mean body weight: 0.294 g (0.177 + 0.397 g) at the end of the study Mean body weight: 0.294 g (0.177 + 0.397 g) at the end of the study Mean body weight: 0.294 g (0.177 + 0.397 g) at the end of the study Mean body weight: 0.294 g (0.177 + 0.397 g) at the end of the study Mean body weight: 0.294 g (0.177 + 0.397 g) at the end of the study Mean body weight: 0.294 g (0.177 + 0.397 g) at the end of the study Mean body weight: 0.294 g (0.177 + 0.397 g) at the end of the study Mean body weight: 0.294 g (0.177 + 0.397 g) at the end of the study Mean body weight: 0.294 g (0.177 + 0.397 g) at the end of the study Mean body weight: 0.294 g (0.177 + 0.397 g) at the end of the study Mean body weight: 0.294 g (0.177 + 0.397 g) at the end of the study Mean body weight: 0.294 g (0.177 + 0.397 g) at the end of the study Mean body weight: 0.294 g (0.177 + 0.397 g) at the end of the study Mean body weight: 0.294 g (0.177 + 0.397 g) at the end of the study Mean body weight: 0.294 g (0.177 + 0.397 g) at the end of the study Samples were taken from all test chambers on day 0 and day 4. Controls: water Evidence of undissolved material The stock solution was filtered through s0.45 urn filter as it was dispensed into the test chambers to remove undissolved test substance. After the filtration of the primary stock solution, there were no additional problems with solubility throughout the stody.
	problems with solubility throughout the stady.
Replication	problems with solubility throughou the stady.
1	No. of vessels per control (replicates): 1
Organisms per	No. of organisms per ressel: 00
replicate	
Exposure	No. of vessels per control (replicates): 1 No. of organisms per vessel: 20 static Total exposure duration 96 hours
	Total exposure duration 96 hours 2 0 4
Test Vessel	static Total exposure duration 96 hours \$196 stish/L test medium Note
Loading 💍	
Feeding during	Nope Temperature 21.6 22.2°C Photoperiod: 16 hours light / 8 hours dark Light intensity 4650 lox
test	
Test conditions	Temperature 21.6 22.2°C Photoperiod: 16 hours light / 8 hours dark Light intensity 4650 lox
RY'	Photoperiod. 16 hours light / 8 hours dark
	Light intensity 4650 km 2 4
Ő	pH: 8,4 – 8.7 Water hardness: 120 mg CaCO A.
0,	Disolvectoxygen. 77,-98% saturation
~Ç~	Conductivity: 900 to 000 µS cm
Parameters	Observations for death, and for abnormal appearance and behaviour, were
Measured /	performed at 3, 6, 24, 48, 72, and 96 hours (± 1 hour)
Observations	Discrete measurements of temperature, dissolved oxygen, pH, and conductivity
4	were obtained at test initiation, 24, 48, 72, and 96 hours, or within one hour of the
(D)	designated time.
Chemical	Samples were analysed by Gas Chromatography with electron capture detection
analysis	(GC/ ECD) for the actual concentration of fluopicolide and metabolite M-01 (AE
Data analysis	LQ50 values and the 95%-confidence intervals were calculated for 24-hour time
Data analysis	points with Spearman-Karber method and for the time points 48, 72 and 96 hours
L. Q.	with Isonominal test method using CT-Tox version 1.1. The LC ₅₀ was estimated,
	using one of four statistical techniques: moving average, binominal, Spearman
U	Karber analysis or Probit analysis. The appropriate method was determined
	according to the data characteristics.



II. RESULTS AND DISCUSSION:

Validity criteria according to OECD 203 (2019)	Required	Obtained of the second
Measured concentration of the test substance measured concentrations	Mandatory	Performed and results based on mean measured concentrations
Mortality in control during test	<u><</u> 10%	
Dissolved oxygen saturation	<u>> 60%</u> /0	<u>77 - 98% </u>
not tight regulation	al a	

Analytical results:

Recoveries were below 80% (see table below). The results should be expressed on the basis of geometric mean measured concentrations, but they were reported on the basis of arithmetic mean concentrations. The table below shows that there is no significant difference, between the 20 nears and a recalculation of the EC_{50} would not provide a different value so the biological results are based on arithmetic mean measured concentrations of fluopicolide. There was no fluopicolide residue found in the anilution water or control samples. Metabolite M-01 (BAM, AE C653711) was not quantified in the samples.

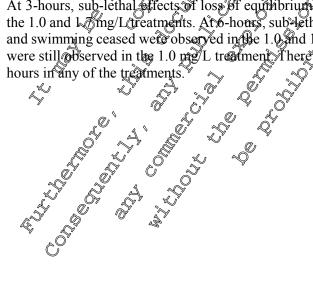
Nominal conc. (mg a.s./L)	Arithmetic mean (mg a.s./L)	Geometric mean (mga.s./LO	% of yominal conc 0 hour	entrations 96 hour
0.65	0.24	0.24 0 2	B & O	36
1.1	0.37	0.37 ~ ~	*734 × ~ ~ ~	32
1.8	0.56	0.56 ~~~~	32	30
3.0	×1.0 5 × 5	$ \overline{\Psi}_{1} \qquad \qquad$	34-35 ×	35
5.0	Q 1.7 X X	2, 1.7 5 5 Q	35 0 4	33

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

Biological results

Observations

At 3-hours, sub-lethal effects of loss of equilibrium, erratic swimming, and lethargy were observed in the 1.0 and 1.6 mg/L treatments. At 6-hours, sub-lethal effects of loss of equilibrium, erratic swimming, and swimming ceased were observed in the 1.0 and 1.6 mg/L treatments. At 24-hours, sub-lethal effects were still observed in the 1.0 mg/L treatment. There were no sub-lethal effects observed at 48, 72 or 96 hours in ny of the treatments.





Mortality

Exposure time (hours)	3	6	24	48	72	96 ° °			
mean measured conc. (mg a.s./L)	No of dead (%)	No of dead (%)	No of dead (%)	No of dead (%)	No of dead (%)	No 64 dead (%)			
Control	0 (0)	0 (0)	0(0)	0(0)	^v 0 (0)				
0.24	0 (0)	0 (0)	0(0)	0(0)	0(0)				
0.37	0 (0)	0 (0)	0(0)	0(0)	0 (0)				
0.56	0 (0)	0 (0)	0 (0)	0(0) 0	0,000 ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~				
1.0	0 (0)	0 (0)	2(20)	10 (100)	10 (100)	10 (100)			
1.7	0 (0)	0 (0)	¥0 (1069 🔬	Pio (1 40)	10 (1000)	10 (100)			
The study meet fluopicolide are	$\begin{array}{c cccc} 0.37 & 0(0)$								
LC50 96 hours	(95% C.I.):	a	9.75 mg a.s. /			·~~			
LOEC.	()- {\lambda}		° (0.56∂¥ 1.0 mg		jo ^o	1			
lowest concent	ration with an ef	fect S	1.67 mg a.87 / L						
NOEC:	tration without a	A Averse & fects	0.56 @g a.s.6	ř. V	^y S				
ingliest concern	S O	averse agricers of			L.Y				
Assessment a	andconclusion	by applicant;			D				
The study is reliable and the LC_{50} of 0.75 mg a.s. L and NOEC of 0.56 mg a.s. $/L$ can be used in the fluopicoliderisk assessment.									
						be used in the			



Data Point:	KCA 8.2.1/03
Report Author:	
Report Year:	2003
Report Title:	AE C638206: A 96-hour static acute toxicity test with the common carp (Cyprosus carpio)
Report No:	C036019
Document No:	M-219743-01-1
Guideline(s) followed in study:	OECD: 203 (1992); USEPA (=EPA): OPPTS 850.1075 (1996)
Deviations from current test guideline:	Method: Deviations from current grideline SANCO 3029/99 rev 4 Recoveries were determined at three different concentrations in triplicate However, the obtained data demonstrate very good recoveries and the precision. The method can therefore be regarded as fit for purpose. Study: Current Guideline: DCD 203 (2019) Dissolved oxygen concentrations in the test solutions were >96% of saturation (8.3 mg/L) at test initiation, but dropped to hightly less than 60% saturation in the second highest concentration by 24 hours. Since the mortality in this test concentration is within the accepted limit Q0%) for control and acration was provided afterwards, therefore the impact of this deviation is considered negligible. The size of the fish was bigget than recommended by OECP guidefine: mean of 4.5 instead of 3+// Put the weight of the fish is within the range specified in US EPA guidefine, below 3.0 g. The pH is greater than 8.5 (up to 8.7) mult test concentrations and controls at test start and, in some occasions later of. The start of the test were met so this slight deviation is not considered significant.
Previous evaluation:	ges, evaluated and accepted
GLP/Officially recognised testing facilities: Acceptability/Reliability/	Yes conducted under GLP officially recognised testing facilities

Executive summary

J. An acute wire test was performed with the common Garp (Cprinus carpio) in a static system. Juvenile common carpy were posed to fluopicolide in nominal Concentrations of 0.13, 0.25, 0.50, 1.0 and 2.0 mg a.s./L, in well water for a 96 hour period. Additionally, a negative control and a solvent control were included. All treatments had 100 fish per test vessel (i.e. 10 fish per treatment level). Mortality, toxicity values and behaviour were recorded at tome points 3, 24, 48, 72 and 96 hours.

Samples were taken from all test chambers on day 0, day 2 and day 4. Samples were analysed by High Performance Liquid chromatography wing an ultra colet detector for the actual concentration of fluopicolide in the test medium Recoveries in the media were between 85.3 and 103% and no residues above the limit of quantification were measured in the controls.

The arithmetic mean measured concentrations of Iluopicolide were 0.12, 0.25, 0.50, 0.98, and 2.0 mg a.s./D. There was a slight deviation from the validity criterion on oxygen saturation which is not considered to have impacted the reliability of the results. Common carp in the negative and solvent control groups appeared heathy and normal throughout the test. Sub-lethal effects were observed at 3h in the 2 highest concentrations, they had disappeared at 24h for the fish exposed to 0.98 mg a.s./L but were still observed in the surviving fish exposed to 2.0 mg a.s./L. All these fish were dead at 48h. At 0.98 mg@.s./L I fish died at #8 h. At 0.50 mg a.s./L, sublethal effects were observed in 1 fish at 96h. The 96-hour PC₅₀ value is 1.3 mg a.s./L, with a 95% confidence interval of 0.98 to 2.0 mg a.s./L. The no mortality concentration is considered to be 0.50 mg a.s./L, and the NOEC is 0.25 mg a.s./L.



I. MATERIAL AND METHODS:

Test material	Fluopicolide
	batch: OP 2350005; purity 99.4 %
Guideline(s)	None specified
adaptation	None specified
Test species	Carp (<i>Cyprinus carpio</i>)
Acclimation	At least 14 days Health during acclimation: a single mortality in the 7-day period prior to the test In control fish at the end of the study Mean length: $4.5 \text{ cm} (3.9 - 5.2 \text{ cm})$ Mean body weight: $1.1 \text{ g} (0.64 - 4.7 \text{ g})$ The fish used in the test slightly exceeded the OECD guideline recommended
	Health during acclimation: a single mortality in the 7-day period prior to the test
Organism	In control fish at the end of the study of t
age/size at	Mean length: 4.5 cm $(3.9 - 5.2 \text{ cm})^{3/2}$
study initiation	Mean body weight: 1.1 g $(0.64 - \frac{4}{3}\sqrt{7} g)$
-	The fish used in the test slightly exceeded the OECD guideline recommended
	length of 3.0 ± 1.0 cm but where well within weight recommendations for both
	OPPTS and ASTM guidelines (<3.0 g and 5.0 g, respectively)
Test solutions	Nominal concentrations: $(3 - 0)^{2} - 0^{2} - 10^{2} - 10^{2} - 20^{2}$
	Corresponding mean measured concentrations: 0.12 - 0.25 - 0.56 - 0.98 and 29
	mg a.s. / L. Samples were taken from all test chambers on day 0, day 2 and day 4. Controls: water Solvent controls 0.1 mm L dimethyl formamide
	Controls: water & & & & & & & & & & & & & & & & & & &
	Solvent controls 0.1 m/2 dimethyl formanide
	ALTEST INITIATION TAKE SOLIDOONS ADDEATERS CLEAFSAND CONOTIESS WITH SOME WHILE
	precipitate evident on the barryce of the 1.2 and 2.0 mg/ 2 boldstons. It test
	termination, the 0.13, 6.25 and 0.50 mg/L solutions @ere clear and eolorless, while
	the 1.0 and 2.0 mg/L solutions were slightly cloudy white, increasing in intensity
	with micreasing concentration of the two second sec
Replication	No of versels per concentration (replicates)
	1 Sto. Of Vessels ther control (repricates). 2
	No. of vessel@per solvent control (replicates): 2
Organisms per	No of organisms per vessel: 10 2 2 5
replicate 🔊	
Exposure	Bratic & S S S S
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	Total exposure duration: W hours
Test Vessel	$0.38$ g fish L test medium $3^{\circ}$ $3^{\circ}$ $3^{\circ}$
Loading	1 otal exposure duration: 96 hours 0 10 0.38 g fish L test medium 7 2 0
Feeding during	$\mathbb{P}^{\mathrm{NONE}}$ $\mathcal{P}^{\mathrm{V}}$ $\mathcal{P}^{\mathrm{V}}$ $\mathcal{P}^{\mathrm{V}}$
test	
Test conditions	Temperature: $24.7 - 292^{\circ}C_{2}$
4	Photoperiod to house light 8 hours dark Light intensity: 379 lux
- A	Light intensity: 379 lux
	pH 28.3 - 8.7.
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	Water thardness. 130 mg CaCO ₃ /L
* >	Dissoved oxygen: $45 \text{ mgV} = 8.5 \text{ mg/L}$
ر ©`	Gentle actation was added to each test chamber to achieve $> 60\%$ saturation
Y	throughout the test
Damage	Condictivity: 320 unhos/cm
Parameters C	Observations for death, and for abnormal appearance and behaviour, were
Measured 5/	performed at 3, 24, 48, 72, and 96 hours
Observation	Discrete measurements of temperature, dissolved oxygen and pH were obtained at
Chamica	Vest initiation, 24, 48, 72, and 96 hours.
Chemicat analysis	Samples were analysed by High Performance Liquid chromatography using an
allalys 4 5	ultraviolet detector for the actual concentration of fluopicolide and in the test
	medium on day 0, day 2 and on day 4.



O

Data analysis	LC ₅₀ values and the 95%-confidence intervals were calculated for 24-, 48-, 72-
	and 96-hour time points with binominal test using nonlinear interpolation between
	0.98 and 2.0 mg/L with the computer program of C. E. Stephan. The no mortality
	0.98 and 2.0 mg/L with the computer program of C. E. Stephan. The no mortality concentration and the no-observed-effect-concentration (NOEC) were determined
	by visual interpretation of the mortality and observation data δ^{γ}
1	

II. RESULTS AND DISCUSSION:

Validity criteria according to OECD 203 (2019)	Required	Č	Obtained 27 7 29
Measured concentration of the test substance	Mandatory		Performed and results are based on mean measured conce@fations
Mortality in control during test	<u>≤</u> 10%		
Dissolved oxygen saturation	$\geq 60\%$ 0 ²		45 mg/L 8.5 mg/L*
*A dissolved oxygen concentration of 5.2 n	ng/L represents	60% saturation at 22% in fr	eshwater.

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

Analytical results:

Recoveries in the media were between 855 and #3% (see table below). The biological results are based on arithmetic mean measured concentrations of fluopicolide.

	S. O		S :	s v	Ô 4
Nominal conc. (mg a.s./L) Č	Mean meas concentrati (mg.a.s./L)	ured % of	Coominat Contrations	nominal	f individual ments (% of)
0.13				× 850 -92	5 ⁰
0.25	0.25	L 5700	°°	@ 4.1 , 10	3
0.50	0.500	م 🖉 🖉		√° 97,1010	13
1.0	9 .98			93 .1 - 10	2
2.0	2.0			96.5 - 98	3.9
	DY D			<i>°</i>	

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

Biological results:

Observations

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



Exposure time (hours)	3	24	48	72	96
Mean measured conc. (mg a.s./L)	No of dead (%)	No of dead (%)	No of dead (%)	No of dead (%) 🏷	No of the ad
Control	0 (0)	0 (0)	0 (0)	0 (0)	
Solvent control	0 (0)	0 (0)	0 (0)	0,(0)	
0.12	0 (0)	0 (0)		\$(0)	
0.25	0 (0)	0 (0)	0 (0)		
0.50	0 (0)	0 (0)			
0.98	、 <i>,</i>				× //
	0 (0)		1(5)		$\begin{array}{c c} \begin{array}{c} \begin{array}{c} \begin{array}{c} \begin{array}{c} \end{array} \end{array} \end{array} \end{array} = \begin{array}{c} \begin{array}{c} \begin{array}{c} \end{array} \end{array} \end{array} \left(\begin{array}{c} \begin{array}{c} \end{array} \right) \end{array} \left(\begin{array}{c} \end{array} \right) $
2.0 ot considered as trea	1 (5)	16 (80)	20 (106) 20	20 (P00) C	20 (1060)
he endpoints base	ed on arithmetic	mean measured	LUSIONS: concentrations from mg a.s. / L 3 2.0 mg a.s. / L mg a.s. / L		
LC ₅₀ 96 hours (95	5% C.I.):	<u>لا يور م</u>	<u>3 2.0 mg</u> a.s. / 14		
NOEC:		م م م م الم الم الم الم الم الم الم الم	mg a.s. YL	, ⁶	
ingliest concentrati					
Assessment and	conclusion by	applicant			
The study is relia fluopicolide risk	able and the I C	Sof 1 2 mg 2 &	Land NOF Of) 25 mg & S./L ca	n be used in the
fluopicolide risk	assessment.			J@J IIIgaç S./L €a	in be used in the
			L and NOE of (J.	

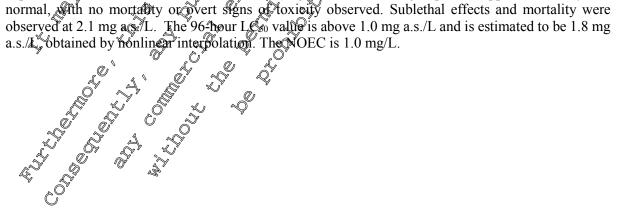


Data Point:	KCA 8.2.1/04
Report Author:	
Report Year:	2003
Report Title:	AE C638206: A 96-hour static acute toxicity test with the zebra fish (Brachydonio
	rerio)
Report No:	M-234508-01-2
Document No:	<u>M-234508-01-2</u>
Guideline(s) followed in	OECD: 203 (1992); USEPA (=EPA): OPPTS 850.1075 (1996)
study:	
Deviations from current	Method: Deviations from current guideline SANCQ/3029/99 rev
test guideline:	Limited sets of validation recoveries were analysed. However, the average
	recoveries were within the acceptable range of 00-110% and the RSD values were
	below 20%. The analytical method can be regarded as fit for purpose.
	Study: Current Guideline: Study: CD 203 (2019)
	Some fish were bigger than recommended by OECD guideline range 2 6 to 3.4
	instead of 2+/-1 cm. But the weight of the fisk is within the range specified in US
	EPA guideline, below 3.0 g. a b b b b b b b b b b b b b b b b b b
	The pH is greater than 8.5 Jup to 87) in all test concentrations and controls at test
	start and, in some occasions, later on. The validity criteria of the test were met so
D ' 1'	this slight deviation is not considered significant
Previous evaluation:	in DAR (2005)
OLD/0.00 · 11	111 DAR (4003)
GLP/Officially	Yes, conducted under GLP/Officially revognised testing facilities
recognised testing	
facilities:	
Acceptability/Reliability:	Yes & B & Y & Y

Executive summary

0 N , ^e Ô An acute toxicity test was performed with the zebra fish, Brachydanio Erio) in a static system. Juvenile zebra fish were exposed to fluopoolide in nominal concentrations of 0.13, 0.25, 0.50, 1.0 and 2.0 mg a.s./L in well water for a 96-hour period. Additionally, a negative control and a solvent control were included. All treatments had 10 fish per testovessel (i.e., 10 fish per treatment level). Mortality, toxicity values and behavious were accorded at time points 4, 24, 48, 70, and 96 hours. Samples were analysed by High Performance Liquid Chromatograph CHPLC with UV-detection for the actual concentration of fluopicolide in the test mediam on day 0, day 2 and on day A Recoveries in media were between 93.9 % and 109% of nominal concentrations and no residues above the limit of quantification were measured in the controls. The mean measured concentrations of fluopicolide were 0.12, 0.25, 0.51, 1.0, and 2.1 mg a.s./L

The study fulfils all variety griteria of the entrent fersion of the OECD 203 guideline. Zebra fish in the negative and Solvent Controb groups appeared healthy and normal throughout the test. After 96-hours of exposure, fish in the 0.12, 0.25 0.51 and 1.6 mg a K/L treatment groups also appeared healthy and normal, with no mortality or overt signs of toxicity observed. Sublethal effects and mortality were





I. MATERIAL AND METHODS:

Tost motorial	Eluaniaalida (taah)
Test material	Fluopicolide (tech.)
	batch OP 2050046;
	purity 96.1 % w/w
Guideline(s) adaptation	batch OP 2050046; purity 96.1 % w/w None specified Zebra fish (<i>Brachydanio rerio</i>) At least 14 days Health during acclimation: 0 % mortality In control fish at the end of the study: Mean length: 3.0 cm (2.6 cm - 3.4 cm) Mean body weight: 0.25 g (0.14 Å - 0.36 g) Nominal concentrations: 0.13 - 0.25 - 0.50 - 1.0 - 2.0 mg a.s./L Corresponding mean measured concentrations: 0.12 - 0.25 - 0.51 - 1.0 and 2.1 mg a.s. / L.
Test species	Zebra fish (Brachydanio rerio)
Acclimation	At least 14 days
rectimation	Health during acclimation: 0 % mortality
Organism	In control fish at the end of the study:
age/size at	Mean length: $3.0 \text{ cm} (2.6 \text{ cm} - 3.4 \text{ cm})$
study	Mean body weight: $0.25 \text{ g} (0.14 \text{ g} - 0.36 \text{ g})$
initiation	
Test solutions	Nominal concentrations: 0.13 - 0.25 - 0.50 - 1.0 - 2.0 mg a.s./L
	Corresponding mean measured concentrations: 6/12 - 6/25 - 0/51 - 1,0 and 2.1 mg a.s. / L. Samples were taken from all fest chambers on day 4 day 2 and day 4
	a.s. / L. Samples were taken from all test chambers on day Φ , day δ and day 4.
	Controls: water and share and an
	Solvent control: 0.2 mil/12 dimetary forma mide
	Solutions appeared clean and colourless at test initiation and termination
Replication	No. of vessels per concentration (repricates), 2 , 0 , 0
Organisms	No. of organisms per vessel: 10
per replicate	
Exposure	static Station: 96 hours
	Total exposure duration: 96 hours
Test Vessel	0 2 g fish/L test median 5 5 0 4
Loading	
Feeding	None & & y y y y y
during test	Temperature: $21.7 - 23.7^{\circ}$ Photoperiod 16 hoars light 8 hours dark Light intensity: 141 lux pH 8.4 \times 8.7 \times 9
conditions	$\frac{1}{2} \frac{1}{2} \frac{1}$
	Light interestry: 142 lux
K.	$n \neq 84 \rightarrow 87 \neq 10$
	Water hardness 132 mg CateO ₂ /LO
Ć	Water hardness: 132 pag CatoO ₃ /LO Dissofwed of ygen 3.7 mg/L (67% of saturation)– 8.9 mg/L
Ø	Conductivity: $326 \mu mh $ cm $^{\circ}$
Parameters	Observations for death, and for abnormal appearance and behaviour, were
Measured /	performed at 4, 24, 78, 72, and 96 hours
Observations	Discrete measurements of temperature, dissolved oxygen and pH, were obtained at
L.	test initiation, 24, 48, 72, and 96 hours.
N	Hardness, alkalinity and specific conductance were measured in the dilution water
Q	at the beginning of the test.
Chemical	Samples over analysed by High Performance Liquid Chromatography (HPLC) with
analysis &	UV-detection for the actual concentration of AE C638206 in the test medium on
Ű.	day @ day 20md on day 4.
Data Analysis	LQ ₅₀ values and the 95%-confidence intervals were calculated for 48, 72- and 96-
	Four time points with binominal test using nonlinear interpolation between 1.0 and
the state of the s	² .1 myL with the computer program of C. E. Stephan. There was <50% mortality
Č, ⁰ [*]	in any treatment group at 24 hours, which precluded the statistical calculation of the 24 hours LC ₂ , value. Therefore, the 24 hours LC ₂ , the no mortality concentration
V	24-hour LC_{50} value. Therefore, the 24-hour LC_{50} , the no mortality concentration and the no observed effect concentration (NOEC) were determined by visual
	and the no observed- effect-concentration (NOEC) were determined by visual interpretation of the mortality and observation data.
	interpretation of the mortanty and observation data.



II. RESULTS AND DISCUSSION:

Validity criteria according to OECD 203 (2019)	Required	Obtained	
Measured concentration of the test substance	Mandatory	Performed and results are based on mean measured concentration	
Mortality in control during test	<u>≤</u> 10%	0%	
Dissolved oxygen saturation	\geq 60% $\sqrt[6]{2}$	5.7 mg/L - 8.9 mg/L	

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

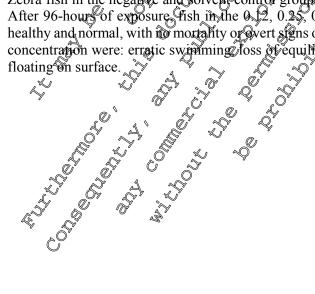
Analytical results:

Recoveries in media were between 93.9 % and 109% of nomital concentrations (see table below). The biological results are based on mean measured concentrations of fluopicolice (AEC638206). There was no fluopicolide residue found in the control samples.

Nominal conc. (mg a.s./L)	Mean measured concentration % of nominal individual (mg a.s./L)
0.13	0.12 0 4 0 492 0 0 93.9 97.9 4
0.25	0.25 0.25
0.50	0.51 0 2 102 2 24.1 - 109
1.0	
2.0	<u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u></u>

Full details and acceptable validation data to support this method accepted within document M-CA 4, which comply with the EU regulatory requirements outlined are presented within document M-CA and acceptable exceptions only. <u>Biological results:</u> Observations Zebra fish in the negative and folven control groups appeared healthy and normal throughout the test. After 96-hour of evolution for the first the first

After 96-hours of exposure fish in the 0.2, 0.25, 0.51 and 1.0 mg/L treatment groups also appeared healthy and normal, with the mortality or evert signs of oxicity observed. Observed effects at the highest concentration were: erratic swimming loss of equilibrium, lethargy, fish lying on bottom, surfacing or floating on surface





Mortality

Exposure time (hours)	4	24	48	72	96 ° ô
mean measured conc. (mg a.s./L)	No of dead (%)	No of dead (%)	No of dead (%)	No of dead (%)	No of dead
Control	0 (0)	0 (0)	0 (0)	0 (20)	80)
Solvent Control	0 (0)	0 (0)	0 (3)	Q(0)	
0.12	0 (0)	0 (0)	0(0)	0 (0)	
0.25	0 (0)	0 (0)	0 (0)	0 (0)	6Q0) 0 4
0.51	0 (0)	0(0)	0 (0)		
1.0	0 (0)	0 (0)	0(0)	× 0 (0) 0 ×	Q ((9)
2.1	0 (0)	9 (45)	13 (65)	140(70)	14 (70)
he study meets the voncentrations are: LC ₅₀ 96 hours (95% NOEC:					
LC 50 90 nours (95%			2.1 mg a.s. / 1)		Ő [¥]
NOEC: highest concentration	n without advorse	effects by mg	; a.9: / L 👋 👡		
Assessment and c The study is reliand fluopicolide risk as	puclusion by ap ble and the LOS ₀ ssessment	plicant:	and NOPEC of	0 mg, a.s./L ca	n be used in the
LC ₅₀ 96 hours (95% NOEC: highest concentration Assessment and c The study is relief fluopicolide risk as				¢ ,	
		N N N N N N N N N N N N N N N N N N N			

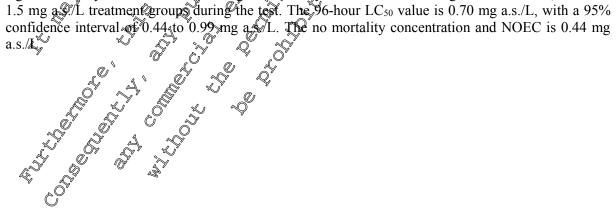


Data Point:	KCA 8.2.1/05
Report Author:	
Report Year:	2003
Report Title:	AE C638206: A 96-hour static toxicity test with the ricefish (Oryzias latipes)
Report No:	M-234510-01-2
Document No:	<u>M-234510-01-2</u>
Guideline(s) followed in	ASTM: E729-88a (1994); OECD: 203 (1992); USEPA (FEPA): OPPTS \$50.1005
study:	(1996)
Deviations from current	Method: Deviations from current guideline SANCQ/3029/99 rev.4:
test guideline:	I imited sets of validation recoveries were analysed However, the average
-	recoveries were within the acceptable range of 70–110% and the RSD Glues were below 20%. The analytical method can be regarded as fit for purpose Study: Current Guideline: OFCD 203 (2019)
	below 20%. The analytical method can be regarded as fit for purpose
	Study: Current Guideline: OF 203 (2019)
	The temperature range and size of medaka recommended of OECD guideline 203
	from 2019 have changed in comparison to previous version of 1992. The fish in
	the test measure 2-3 cm instead of 1-2 cm and the temperature in the range 21.8
	- 23.3 which is not in the cuffent range of 23-27°C. The pH is greater than 8.5 (up
	to 8.8) in all test concentrations and controls in almost all samples The varidity
	criteria of the test were met so these slight deviations are not considered
	significant.
Previous evaluation:	yes, evaluated and accepted in the second seco
GLP/Officially	in DAR (2005) y y y y y y y y y y y y y y y y y y y
recognised testing	
facilities:	
Acceptability/Reliability:	Yes & B & a & a &

Executive summary

0 An acute toxicity test was performed with the refishe (*Oryzias latipes*) in a static system. Juvenile ricefish were exposed to fluopicolide in nominal concentrations of 0.30, 0.44, 0.67, 1.0 and 1.5 mg a.s./L, in well water for a 96-hour period. Additionally, a negative control and a solvent control were included. All treatments had 10 fish per testovessel (i.e., 40 fish per treatment level). Mortality, toxicity values and behaviour were recorded at time points 3.5, 24, 48, 32, and 96 hours.

Samples were analysed by High Performance Diquid Chromatography with UV detection for the actual concentration of fluopicolide present in the test medium of day 0, day 2 and on day 4. Recoveries in media were between 90.8% and 103 %. The results were based on the arithmetic mean measured concentrations of theopicolide which were 0.2% 0.44 0.65, 0.99, and 1.5 mg/L. The study fulfils all validity criteria of the current version of QECD 203 guideline. Ricefish in the negative and solvent control groups appeared healthy and normal throughout the test. After 96-hours of exposure, fish in the 0.28 and 0.440 mg a.S/L treatment groups also appeared healthy and normal, with no mortality or overt signs of toxicity observed. Subte hal signs of exicit were observed among fish in the 0.65, 0.99 and 1.5 mg a S/L treatment groups during the test. The 96-hour LC50 value is 0.70 mg a.s./L, with a 95%





I. MATERIAL AND METHODS:

Test material	Fluopicolide (tech.)
	batch: OP 2050046
	purity 96.1 % w/w
Guideline(s)	None specified
adaptation	
Test species	Fluopicolide (tech.) batch: OP 2050046 purity 96.1 % w/w None specified Ricefish (<i>Oryzias latipes</i>) At least 14 days Health during acclimation: no sign of disease or stress In control fish at the end of the study: Mean length: 2.6 cm (2.0 – 3.0 cm) Mean body weight: 0.15 (0.055 – 0.22 g) Nominal concentrations: 0.20 – 0.44 = 0.675 1.0 -21.5 mg d.s./L Corresponding mean measured concentrations: 0.28 – 0.44 – 0.65 – 0.99 and 1.5 mg a.s./L.
Acclimation	At least 14 days
	Health during acclimation: no sign of disease or stress
Organism	In control fish at the end of the study:
age/size at	Mean length: 2.6 cm $(2.0 - 3.0 \text{ cm})$ Mean body weight: 0.15 $(0.050g - 0.22 \text{ g})$
study initiation	Mean body weight: 0.15 (0.05 g) \sim 0.22 g) \sim 0^{2} $\sqrt{2}$ $\sqrt{2}$
Test solutions	Nominal concentrations: $0.30 - 0.44 - 0.67 - 1.0 - 1.5$ mg a.s./L Corresponding mean measured concentrations: $0.28 - 0.44 - 0.65 - 0.99$ and 1.5 mg a.s./L. Samples were taken from all test chambers on day θ , day 2 and day 4.
	Corresponding mean measured concentrations: 0/28 - 0/44 - 0/65 - 0,99 and 1.5
	mg a.s. / L.
	mg a.s. / L. Samples were taken from all test chambers on day 0, day 2 and day 4.
	Controls: water of the water of the second s
	Solvent control: 0 kml/k dimethyl formamide 0
D 1: +:	Solutions appeared clean and covoriess at test initiation and termination $\sqrt[n]{2}$
Replication	Solvent control: 0 Rml/k dimethyl formamide Solutions appeared clear and colorless at test initiation and termination No. of vessels per concentration (replicates): 2
Organisms per	No. of organisms per vessel: 10
replicate	
Exposure	static Y A A A A A
Exposure	static Total exposure duration: 96 hours
Test Vessel	0,06 g fish/L test median 5 2 0 4
Loading	
Feeding during	None 4 4 7 7 7 7 7 7 7
test 🔊	
Test conditions	Temperature: 21.8 – 23.3° Photoperiod 16 hours light 8 hours dark Light intensity: 138 lux pH 8.5 – 8.8.
	Photoperiod 16 hours light 8 hours dark
and the second s	Light interprity: 138 lux
Č	Water hardness: 134 mg CaCO ₃ /L \bigcirc Dissolved sygen 7.8 mg/L – 8.4 mg/L \bigcirc
Ma	$Conduction y: 320 \mu mhos/cm_{\odot}^{\odot}$
Parameters	Observations for death, sign of tox city and for abnormal behavior were performed
Measured	at 3.5 24 48 72 and 96 hours $\sqrt{2}$
Observations	Discrete measurements of temperature, dissolved oxygen and pH were obtained at text initiation. 24, 48, 22, and 96 hours
	test initiation, 24, 48, 72, and 96 hours
L.	Hardness, alkalinity and specific conductance were measured in the dilution water
Q)	at the beginning of the test.
Chemical	Samples were analysed by High Performance Liquid Chromatography with UV
analysis \mathcal{L}	detection for the actual concentration of AE C638206 present in the test medium on
U Ő	day 0 , day 3 and on day 4.
Data analysis	Lesso values and the 95%-confidence intervals were calculated for 24, 72- and 96-
Data analysis	your time points with binomial test and for the time point 48 hours with probit
E Q	analysis using the computer program of C. E. Stephan (EPA). The no mortality
$e^{o^{v}}$	concentration and the no-observed-effect-concentration (NOEC) were determined
<u> </u>	by visual interpretation of the mortality and observation data.
	i



II. RESULTS AND DISCUSSION:

Validity criteria according to OECD 203 (2019)	Required	Obtained	e° &
Measured concentration of the test substance	Mandatory	Performed	J.
Mortality in control during test	$\leq 10\%$	Q 9 %	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
Dissolved oxygen saturation	$\geq 60\%$	7.8 mg/L –	%4 mg/€¥

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

Analytical results:

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

Nominal conc. (mg a.s./L)	Mean measured concentration (mg a.s./L) // // // // // // // // // // // // //
0.30	
0.44	
0.67	
1.0	0.99
1.5	1.5 4 0 4 100 4 10 999 - 101

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

Biological results: Observations Ricefish in the negative and solvent control groups appeated healthy and normal throughout the test. After 96 hours of exposure, fish in the 0.28 and 0.44 mg a.s./Lareatment groups also appeared healthy and normal, with nonortality or overt signs of toxicity observed.

Sublethal signs of toxicity were observed among fish in the 0.65, 0.99 and 1.5 mg/L treatment groups

Sublethal signs of toxicity were observed among fish in the 0.65, 0.99 and 1.5 mg/L treatment groups during the test, these signs were: erratic symming, loss of equilibrium, fish surfacing or lying on bottom.



Mortality

Exposure time (hours)	3.5	24	48	72	96	
Mean measured conc. (mg a.s./L)	No of dead (%)	No of dead (%)	No of dead (%)	No of dead (%)	No of dead	
Control	0 (0)	0 (0)	0 (0)	0(0)	0 (0)	
Solvent control	0 (0)	0 (0)	0 (0)	0 (0)	0(0)	
0.28	0 (0)	0 (0)	0 (0)	0 (0)		ŵ,
0.44	0 (0)	0 (0)	0(0)	(O)	0(0) 5 ⁹	
0.65	0 (0)	0 (0)	Q ¹ (5)	4 (20)	07 (35) 🔨 👔	°, °
0.99	0 (0)	5 (25) _{QD}	()		20 Q00) 🔊	<u> </u>
1.5	0 (0)	20 (100)		20 (100)	20 (100)	

III, CONCLUSIONS:

ĺ,

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

	L.	<i>®</i>	°∕ `	\sim \sim	\sim	S S	
LC ₅₀ 96 hours (95% C.I.):		0.70 mg (0.44 – Ø.	.s. / L 99 mg.a.	s. / b)	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~		°~~ 4.
NOEC:	ř ř	0.4 4 9mg a		Ý "Ô	N DA		Š C
Highest concentration without e	ffeot	() -	LSE/L	r 4	م م	Ô Ô	-
	0		Ű	<i>,</i>		¥ . ¥	



Data Point:	KCA 8.2.1/06
Report Author:	
Report Year:	
Report Title:	AE C638206 - Acute toxicity to sheepshead minnow (Cyprinodon variegatus)
	under static conditions
Report No:	M-223359-01-2
Document No:	<u>M-223359-01-2</u>
Guideline(s) followed in	USEPA (=EPA): FIFRA 72-3 (1982), OPPTS Draft 850.1075 (1996)
study:	
Deviations from current	Method: Deviations from current guideline SANCO/3029/99 rev 4
test guideline:	Limited sets of validation recoveries were analysed. However, the average recoveries were within the acceptable range of 00–110% and the RSD values were
	below 20%. The analytical method can be regarded as fit for purpose.
	Study: Current Guideline: SECD 203 (2019)
	The temperature range in the test 22-23 does not fulfil the recommendations of
	the OECD guideline version 2019 (23:37°C) but is compliant with the OCSPD
	850.1075 guideline. Smilarly, the size of the fish is bigger than recommended 2.0-
	3.5 cm instead of k -2 cm but the figh meet he requirements of the OCSPP
	guideline: weight 3.0 g and longest fish less than twice the size of the smallest.
	Since these despations are due to the lack of consistency between the guidelines
	and validity of the study are met, the study is considered as acceptable.
Previous evaluation:	yes, evaluated and accepted a 2 2 5 5
	in DAR (2005)
GLP/Officially	Yes, conducted under SLP/Officially recognised testing facilities
recognised testing	
facilities:	
Acceptability/Reliability:	objes O' 2' C' 2' 2' 2'
v~	
Data Point: 🔬	K(\$ 8.2.1) 2 5 5 5 6 5 5
Report Author:	
Report Year:	2018 $\sqrt{2}$ $\sqrt{2}$ $\sqrt{2}$ $\sqrt{2}$ $\sqrt{2}$ $\sqrt{2}$ $\sqrt{2}$ $\sqrt{2}$ $\sqrt{2}$
Report Title:	Statement Certificate of analysis for fluopfeolide acute toxicity study on
	Sheepshead minnow (Catarella, 2003; M223359-01-1)
Report No:	M-634697-01-1
Document No:	<u>M-634697-01-1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 </u>
Guideline(\$) followed in	
study:	Not applicable 4
Deviations from current	Anot applicable a solution of the solution of
test guideline:	
Previous evaluation:	No not proviously submitted
GLP/OILICIAIly	Onot appricable and a comparison of the comparis
facilities	
Acceptability/Reliability/	Mes X AV
Accoptability/Renautivy.	
	Ad-05-4097-01-1



Executive summary

An acute toxicity test was performed with the sheepshead minnow (*Cyprinodon variegatus*) in a static system. Sheepshead minnow were exposed to fluopicolide in nominal concentrations of 0.26, 0.43, 272, 1.2 and 2.0 mg a.s./L, in natural filtered seawater for a 96-hour period. Additionally, a negative control and a solvent control were included. All treatments had 10 fish per test vessel (i. 10 fish per treatment) level). Mortality and biological observations were recorded at time points 0, 6, 24, 48, 72 and 96 hours. Samples were analysed using gas chromatography equipped with electron capture detection (GC/ECD) for the actual concentration of fluopicolide present in the test medium on day 0 and on day 4. Recoveries in media were between 69% and 88%. The biological recolts are based on arithmetic mean measured concentrations of fluopicolide. These mean measured concentrations of fluopicolide were 220, 035, 0.58, 1.0, and 1.6 mg a.s./L. The study fulfils all validity criteria. Mortalities occured at 635 mg a.s./lk and above. Sub lethal effects were observed at 6 h at the 2 highest concentrations, until the fish died. At 0.58 mg/L effects started at 72 h and all fish were Wad at 96h. The lowest observed effect concentration (LOEC), is 0.41 mg a.s./L. The no observed effect concentration (NOPC) is 0.20 mg a.s./L. ×, 0×

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	I. MATERIAL AND METHODS:
Test material	I. MATERIAL AND METHODS: Fluopicolide (tech.) Lot No.: 2050190//PP241024/2 purity 97.7 % w/v None specified Sheepshead minnôw (<i>Cyprinodon variegatus</i>) At least 14 days Health during acclimation: No mortality was observed enving the 48-hour period prior to test Autitation Mean length: 2.8 om (2.0 cm - 9.5 cm) Mean body weight: 0.5/ g (0.21 g - 3.1 g)
Guideline(s) adaptation	None specified
Test species	Sheepshead minnow (Cyprinodol variegatus)
Acclimation	Sneepsnead minnow (Cyprinoadw variegatus) At least 14 days Health during acclimation: No mortality was observed during the 48-hour period prior to test hitiation Mean length: 2.8 cm (2.0 cm -3.5 cm) Mean body weight: 0.57 g (0.21 g -3.1 g)
Organism	Mean length: 2.8 cm (2.0 cm -3.5 cm) \bigcirc \bigcirc \checkmark
age/size at	Mean body weight: 0.5% g (0.21 g -3.1 g)
Test solutions	Normal concentrations: 7.26 - 6.43 - 0.72 - 1.02 - 2.0 mg a.s./L. Corresponding mean measured concentrations: 0.20 - 0.35 - 0.58 - 1.0 and 1.6 mg
	a.s. / L. Samples were taken from all test chambers on day 0 and day 4. Controls: patural filtered seawager
4 Y	Solvent control, 0.10 mL/L dimethy formamide in seawater
Č	All exposure solutions were observed to be clear and colorless with no visible undersolved test substance.
Replication	No. of vessels per concentration (replicates): 1 No. of vessels per copprol (replicates): 1
Organistos per	No of organizems por vest 10 V
replicate	
Exposure	Static Static Total Exposure duration: 90 hours
Test Vessel	
Loading O	
test	vone v v v
	None
Cĩ	



Test conditions	Temperature: 22 - 23°C
	Photoperiod: 16 hours light / 8 hours dark
	Light intensity: $76 - 90$ footcandles
	рН: 7.5 - 7.9
	pH: 7.5 - 7.9 Salinity: 32 ‰ - 33 ‰
	Dissolved oxygen: 4.3 mg/L (60% saturation) – 7.2 mg/L (gentle aeration was
	initiated at the 72-hour observation interval to maintain dissolved oxygen
Parameters	concentrations > 60% of saturation) Observations for death and sublethal effects were performed at 0, 6, 24, 48, 72, and 96 hours.
Measured /	96 hours.
Observations	Discrete measurements of temperature, dissolved oxygen, pH, and salinity overe
	measured daily in each test vessel \mathbb{Q}^{\vee}
Chemical	Samples were analysed using gas chromatography equipped with electron capture
analysis	detection (GC/ECD) for the actual concentration of fluopicolide present in the test
2	medium on day 0 and on day 4. 2° 2° 2° 2° 2°
Data analysis	The LC ₅₀ was estimated, using one of three statistical echniques: moving average,
-	binominal or probit analysis. The appropriate method was determined according to
	the data characteristics. The 96-hour LCS value was determined by non-knear
	interpolation with 8% confidence intervals determined by promotion probability.
	For the calculations a computer program (Stephan 1982 F.S. FPA) was used.
	The NOEC is empirically determined.

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	LTS AND DISCUSSION:
Validity criteria according to OECD 293 (2019)	Required Obtained
Measured concentration of the test substance	Mandatory Performed
Mortality and subternal effects in controls	
Dissolved oxogen saturation	$\geq 600^{\circ}$ \Im $3 \text{ mg/L} - 7.2$ \Im 2 mg/L

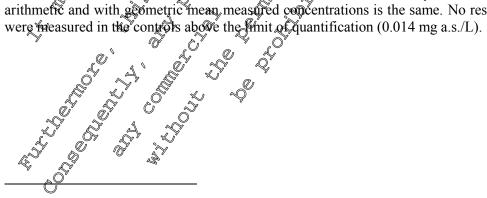
* A dissolved oxygen concentration of 4,3 mg/L represents 60% saturation at 23° G and 32 ‰ in freshwater.

Analytical results: Analytical results: Recoveries in media were between 69 % and 88% (see table below). The biological results are based on arithmetic mean measured concentrations of fluopicolides

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According to the EFS Rtechnical report (2015)², the concentrations should be calculated as geometric mean measured concentrations because the test is static, and the measured concentrations are below 80% of nominal. The geometric mean measured concentrations are provided below, they are very similar to the arithmetic mean measured conceptrations. The LC50 calculated by non-linear interpolation with arithmetic and with geometric mean measured concentrations is the same. No residues of fluopicolide



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



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

Full details and acceptable validation data to support this method are presented within document 4, which comply with the EU regulatory requirements outlined within SANG 0/3029/99 rev 4 with

minor acceptable exceptions only. <u>Biological results</u>: Observations At 72-hours, sub-lethal effects of loss of equilibrium where observe for two fish and one fish exhibited erratic swimming in the 0.58 mg/L treetment At 24 hours are fishered in the literation of the second sec erratic swimming in the 0.58 mg/L treatment. At 24 hours two fish exhibited partial loss of equilibrium and several fish showed a complete loss of equilibrium in the 1.0 mg/L treatment. In the 1.6 mg/L treatment several fish already showed partial or complete loss of equilibrium already after 6 hours.

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Mortality			Ø Å		9
Exposure time (hours)	6	24 5 6	48 5 6	72 59	96
Arithmetic mean measured & conc. (mg a.s./L)	No of dead	No of dead		No of dead	No of dead (%)
				0 (0) 0 (0)	0 (0) 0 (0)
0.20	,0Q(0) , ()			0 (0)	0 (0)
0.35			2 (26)	1 (10) 7 (70)	2(20) 10 (100)
1.0 Q 1.6 A			10 (100) 210 (100)	10 (100) 10 (100)	10 (100) 10 (100)
			(100)	10 (100)	10 (100)



III. CONCLUSIONS:

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

LC 0(horns (050/ C)	0.41 mg a.s. / L
LC ₅₀ 96 hours (95% C.1	.): (0.20 – 0.58 mg a.s. / L)
NOEC:	heurt educate 0.20 mg a.s. / L*
highest concentration with	hout adverse effects
0.19 mg a.s./L as geometric r	Image: concentrations Image: concentrations Image: concentrations Image: concentrations
Assessment and conc	usion by applicant:
The study is reliable an	nd the LC ₅₀ of 0.41 mg a.s, and the NOBC of 0.20 mg/a.s./Lycan be used in
the fluopicolide risk as	ad the LC ₅₀ of 0.41 mg a.s. and the NOBC of 0.20 mg a.s./L can be used it sessment.
Data Point:	KCA 8.2.1/10 A & & Q & Q & A & Q & Q & A & A & A & A
Report Author:	
Report Year:	
Report Title:	Acute toxicity of fluopicolide technical to the fatherd mintow (Pittephales
•	promelas funder static conditions γ γ γ γ
Report No:	007SRLS14C38 0 0 0 0 0 0 0
Document No:	<u>M-53@292-014</u> 0 2 0 0 0 4
Guideline(s) followed in	EU Directive 91/404/EEC; Regulation (EC) No. 1207/2009, US BPA OCSPP
study:	EU Directive 91/404/EEC; Regulation (EC) No. 1207/2009; US BPA OCSPP 850.1075 (1996) DECD Guideline 203 (1992) The afore mentioned guidelines
0	were harmonized for various test parameters (i.e. temperature light, etc.) to
A. I.	achieve optimal environmental conditions for the test organisms. Scientific
Q	dispretion was implemented where guideline parameters the not fully converge
Deviations from current	Method & S S
test guideline:	none Study Current Guide ine: QECD 203 (20)
. ~ ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	Study Current Guideline: (DECD 203 (20) 99
ð S	The size of the fish was by get than recommended $(2 \text{ cm } +/-1)$, however it is
ю" Ср	compliant with OCSPP \$50.1058 guideline which specifies a fish weight less than 3.0 g. Therefore, this deviation is not considered to be relevant.
Previous evaluation:	No bot presously submitted
Previous Valuation:	
GLP/Officially	Ves, conducted under GLP/Officially recognised testing facilities
recognised testing	
facilities:	
Acceptability Beliability:	
A A A A A A A A A A A A A A A A A A A	

Justification for new vertebrate test performed after 14 June 2011: the study was conducted to fulfil US EPA request as stated in September 2019 in Fluppicolide Final Work Plan, Registration review, Case number 7055.

Executive summary

An acute toxicity test was performed with the fathead minnow (*Pimephales promelas*) in a static system. Juvenile fathead minnows were exposed to fluopicolide in nominal concentrations of 0.156, 0.313, 0.625, 4.25 and 2.50 mg as /L in soft processed water for a 96-hour period. Additionally, a negative control and a solvent control were included. All treatments had 10 fish per test vessel. Mortality and subjectual offects were recorded at time points 4, 24, 48, 72 and 96 hours. Samples were analysed using Liquid Chromatography Mass Spectrometry/Mass Spectrometry (LC-MS/MS) to determine the actual concentration of fluopicolide present in the test medium on day 0 and on day 4. Recoveries in media were between 88% and 100% and no residues above the limit of quantification were measured in the



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controls. Biological results are based on nominal concentrations of fluopicolide. The study fulfils all validity criteria of the current version of the OECD 203 guideline. Sublethal effects were observed in the test concentrations of 0.625, 1.25 and 2.50 mg a.s./L from 4 h to the end of the test. Mortalities occured in the 2 highest concentrations. Analysis of the mortality data based on the nominal test concentrations gave the following result: The 96-hour LC50 value is 1.34 mg a.s./L. The fighest concentration without observed effect (NOEC), is 0.313 mg a.s./L. The highest concentration without lethal effect is 0.625 mg a.s./L. Ø Ő

	I. MATERIAL AND METHODS:
Test material	Fluopicolide (tech.)
	Batch number: ETFP00273
	Specification: 102000016444-01
	Batch number: ETFP00273 Specification: 102000016444-014 purity 100.5 % w/w None specified Fathead minnow (<i>Pimephales promelas</i>)
Guideline(s)	Purity 100.5 % w/w O O O O None specified Image: Specified Image: Specified Image: Specified Image: Specified Fathead minnow (Pimephales prometary) Image: Specified Image: Specified Image: Specified
adaptation	
Test species	Fathead minnow (Pimephales prometary
Acclimation	12 days $\sqrt{2}$ $\sqrt{2}$ $\sqrt{2}$ $\sqrt{2}$ $\sqrt{2}$ $\sqrt{2}$ $\sqrt{2}$
	Health during acclimation: No moralities during 48 hours prior to testing, no
	12 days Health during acclimation to mortalities during 48 hours prior to testing, not treatments for disease. Length and weight were only measured at test end. Mean length: $34.4 \text{ mm} \pm 1.24 \text{ mm}$ Mean body wet weight: 0.3337 ± 0.0498 Nominal concentrations 156- 0.313 - 0.625 + 1.252 + 2.50 mg a s P
Organism	Length and weight wergonly measured at test end a set of the set o
age/size at	Mean length: $34/4$ mm ± 1.24 mm \bigcirc \bigcirc \bigcirc \bigcirc \bigcirc \bigcirc \bigcirc
study initiation	Mean body wet weight: 0.3937 ± 0.0498 g $^{\circ}$
Test solutions	Mean length: $34.4 \text{ mm} \pm 1.24 \text{ mm}$ Mean body wet weight: 0.3937 ± 0.0498 Nominal concentrations @.156 - 0.313 - 0.625 1.259 2.50 mg a.s.P.
	Corresponding mean measured concentrations: 0.148 - 0.307 - 0.613 - 1.16 and 2.23 mg a.s. (L. Control: Soft processed water Softwent control; 0.10 mL/L digethylformamide
	2.23 mg a.s. L .
	Control: Soft processed water of the second se
	Solvent control: 0.10 nJ/L digethylformamide
	No prespitates were present in any of the test levels
Replication	No. of vessels per concentration (replicates): 1 2 5
~~~	No of vessels per control (replicates) 1
	No of vessels per control (replicates), 1 No. of vessels per solvent control (replicates): 1 No. of organisms per vessel 10
Organisms per	No. of organisms per vessed 10
replicate	
Exposure	No. of organisms per vessel 10 Static Potal exposure duration: 96 pours
	Solar exposure duration: 96 pours O
Test Vessel	0.12 prish test medium
Loading <i>©</i>	
Feeding during	None of the second seco
test A Test C	
Test &	0.12 g/fish/L test medium None Temperature: 21.8 – 22 & C Photoperiod: 16 hours fight / 8 hours dark (30 min transition period) Light intensity: 660 & 815 fax pH: 7.3 – 80
conditions	Photopenod: 16 hours fight /8 hours dark (30 min transition period)
	Light prensity: $600 \approx 815 \text{ ms}$
, O	PH: $7.3 - 80$ Water haveness 46 -54 mg/L
O ^Y	Vale industries 70 - 34 mg/L
L.	Conductivity: 177.7 496.3 μmhos/com Dissolved oxygen: 92 – 95% saturation
Daramatara 🔊	Survival (aportality) and sublethal behavioural effects
Parameters Measured	Observation interval: approx. after 4, 24, 48, 72 and 96 hours
Observations	$\nabla$ $\Delta$
Cost i valitaris	Daily for dissolved oxygen, pH and temperature. Day 0 and 4 for hardness,
cO'	alkalinity and conductivity.
<u> </u>	unconductivity.



Chemical analysis	Samples were analysed using Liquid Chromatography Mass Spectrometry/Mass Spectrometry (LC-MS/MS) to determine the actual concentration of fluopicolide
2	
Data analysis	<ul> <li>present in the test medium on day 0 and on day 4.</li> <li>The LC₅₀ values were calculated using CETIS (version 1.8.7.4) statistical software. The NOEC, NOLEC and LOEC were empirically determined based upon observations data including lethal and sublethal effects.</li> </ul>
	upon observations data including lethal and sublethal effects

	II. RESU	LTS AND DIS	CUSSION:	A A	
Validity criteria according to OECD 203 (2019)	Required			Obtained	
Measured concentration of the test substance	Mandatory	Ą		Performed	
Mortality in control during test	<u>≤</u> 10%				
Dissolved oxygen saturation	<u>≥</u> 60%			92 \$ 95% \$	

### Analytical results:

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

Naminalaana	Arithmetrc	[™] Afnominal concentrations
Nominal conc. (mg a.s./L)	(mg a.s./L)	
0.156	ð.¥48	<b>Q hour 7 0 48 hour 7 9</b> <b>94 5 7 95 5 5</b>
0.313	~0.307~°	98 × 99 × ×
0.625	0.613	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
1.25	P.16 2	897 396 OT 59
2.50	2.230	
, Ô		

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

## Biological resul

# Observations

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

effects, which remained until study end



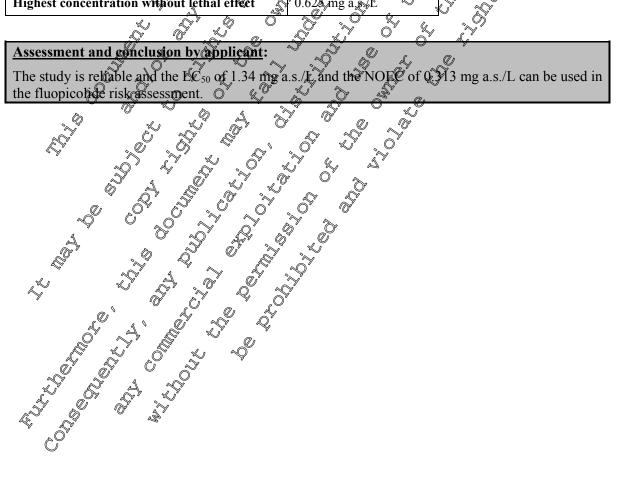
## Mortality

Exposure time (hours)	4	24	48	72	96 ° č
Nominal conc. (mg a.s./L)	No of dead (%)	No of dead (%)	No of dead (%)	No of dead (%)	No of dead
Control	0 (0)	0 (0)	0 (0)	0 (6)	0(0)
Solvent control	0 (0)	0 (0)	0 (0)	<b>A</b> (0)	
0.156	0 (0)	0 (0)	(0) کی (0)		x 0,07 x
0.313	0 (0)	0 (0)	<b>P</b> 0 (0)	Q 0 (0) Q	
0.625	0 (0)	0 (0)	0 (0) y	0(0)	
1.25	0 (0)	1 (10)	3 (30)	3 (3.0)	o [™] 4 (40)
2.50	3 (30)	8 (80)	° 10 (100) ×	> 10 <b>0</b> 100) 🏷	-10 (100) S
		III. Conci		- 20° - 67	of the the

# III. CONCLUSIONS:

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

LC50 96 hours (95% C.I.):		/) I.J I 44195 4.D. //	ng a.so ^{(L} )	
NOEC:		0.212 mg 0.5	U V D	, O [*]
highest concentration without ob	served effects	0.313 mg a.s.		× 0
Highest concentration without le	thal effect	[*] 0.62 <b>\$</b> mg a.s.	L V S	
L C	j ĝ O		× 4.	~) `)





Data Point:	KCA 8.2.1/07
Report Author:	
Report Year:	2001
Report Title:	2,6-dichlorobenzamide (BAM): Acute toxicity to rainbow trout (Oncorhynchus mykiss)
Report No:	M-234311-01-2
Document No:	<u>M-234311-01-2</u>
Guideline(s) followed in study:	OECD: 203 (1992); USEPA (=EPA): E 72-4 (1982), OPPTS 850.1075 (1996)
Deviations from current	Method: Deviations from current grideline SANC 3029/99 reverses and set of the set of th
test guideline:	Limited sets of validation recoveries were analysed. However, the average recoveries were within the acceptable range of 06–110% and the RSD values were below 20%. The analytical method can be regarded as fit for purpose. Study: Current Guideline: DECD 203 (2019)
Previous evaluation:	yes, evaluated and accepted $6^{\circ}$ $5^{\circ}$ $4^{\circ}$ $6^{\circ}$ $5^{\circ}$ $4^{\circ}$ in DAR (2005) $0^{\circ}$ $6^{\circ}$ $6^{\circ}$ $6^{\circ}$ $6^{\circ}$ $6^{\circ}$ $6^{\circ}$ $6^{\circ}$ $6^{\circ}$
GLP/Officially	Yes, conducted under GLP Officially recognised testing facilitie
recognised testing	
facilities:	
Acceptability/Reliability:	Yes Q V X X V V A
Executive summary	as participant with the rainbour trait (Orleginhout thus multics) Order sami static

## **Executive summary**

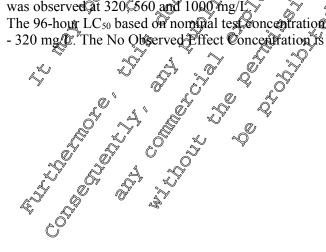
Executive summary An acute toxicity test was performed with the rainbow trout (*Opeorhymphus mykiss*) under semi-static conditions. Juvenile rainbow trouts were exposed to M-@ (2,6-dichlorobenzamide (BAM)) for a period of 96 hours, in groups of twenty (2 replicates often fish), to an aqueous solution of the test material over a range of concentrations of 100, 180, 320,560 and 1000mg/K, The control group was maintained under identical conditions but not exposed to the test material. The number of mortalities and any sublethal effects of exposure in each est and control Vesses were determined 3 and 6 hours after the start of exposure and there daily throughout the test until termination after 95 hours.

Water samples were taken from the control and all test groups with surviving fish at 0 (fresh media), 24, 48, 72 (old and frest media) and 96 (old media) hours for quantitative analysis by high performance liquid chromatography (HPLC with UV detector). Measured concentrations were in the 80-120% range of nominal concentrations and no residues above the light of quantification were measured in the controls. Recoveries were greater than 80% so the biological results are based on nominal concentrations of M-01 (2,6-dichlorobenzamide (BAM)).

The study fulfils all validity criteria of the OECD 203 guideline.

Sub-lethal effects of exposure were observed at test conceptrations of 180, 320 and 560 mg/L. Mortality was observed at 320 560 and 1000 mg/L

The 96-hour LC₅₀ based on nombal test concentrations is 240 mg/L with 95% confidence limits of 180 - 320 mg/D. The No Observed Effect Concentration is 100 mg/L.





### I. MATERIAL AND METHODS:

Test materialM-01 (2,6-dichlorobenzamide (BAM)) AE C653711 (M-01) Batch: FUX001000/FUN81G02C Purity 99.5 % w/wGuideline(s) adaptationNone specifiedTest speciesRainbow trout (Oncorhynchus mykiss)	0
Batch: FUX001000/FUN81G02C Purity 99.5 % w/w	
Purity 99.5 % w/w	Ę,
	Ø
Guideline(s) None specified	5
adaptation	9
Test species Rainbow trout ( <i>Oncorhynchus mykiss</i> )	<u>Q</u>
рологияние и при на	
AcclimationAt least 14 days to test conditions Health during acclimation: no mortalities in the 7 days prior to the start of the testOrganism age/size at the end of theMean body weight: $1.07 \pm 0.19$ g	
OrganismMean length: $4.5 \pm 0.2 \text{ cm}$ $\swarrow$ $\checkmark$ $\sim$ $\sim$ $\sim$ $\sim$ $\sim$ $\sim$ $\sim$ $\sim$ $\sim$ <	0″
age/size at the Mean body weight: $1.07 \pm 0.19$ g	, O
end of the $\sqrt{2}$ $\sqrt{2}$ $\sqrt{2}$ $\sqrt{2}$ $\sqrt{2}$	×,
definitive test	
Test solutions Nominal concentrations: 100 – 180 – 320 – 560 1000 mg/L	_
Controls: water	, ° 1
	e
clear colourless solutions throughout the duration of the test.	
Replication No. of vessels per concentration (replicates): 2	
No. of vessels per control (replicates). 2 2 5 5	
Evidence of undissolved material: The prepared test media were all observed to be clear colourless solutions throughout the duration of the test.         Replication       No. of vessels per concentration (replicates): 2         Organisms per replicate       No. of organisms per vessel 20	
Exposure Semi-static (daily renewar)	
$101a1 \in x = 0 = 0 = 0 = 0 = 0 = 0 = 0 = 0 = 0 =$	
Test Vessel 0.54 g Pish/Latest medium (at the cord of the test)	
Loading & As a construction of the second se	
Feeding during None	
Test Temperature 140°C (discrete measurements), 12.8 to 13.8 °C (continuou	IS
conditions of measurement in one control vessel) of a measurement in one control vessel) of a measurement in one control vessel) of a measurement of the second sec	
Protoperiod: 16 hours light / 8 bours dark, with 20 min transition period Light intensity: not peported	15
Photoperiod: 16 hours light / 8 bours dark, with 20 min transition period Light intensity: not reported pH J.4 – 59. Water hardness 144-160 mg CaCO ₂ /L	
Water hardness 144-160 mg CaCO ₂ /L	
Missolved oxagen: 69-95% asturation	
Dissolved oxygen: 69%-95% saturation Conductivity: 495/uS/cm	
Parameters <i>Oppervations</i> for death, and for abnormal appearance and behavior, were performe	d
Measured $\sqrt{2}$ at 3 6.54 48 $\sqrt{2}$ and 6 homes $\sqrt{2}$	
Observations Discrete measurements of temperature, dissolved oxygen and pH were obtained a	at
test initiation and after 24, 48, 72, and 96 hours, in fresh and old media	a.
Temperature was also measured continuously in one control replicate.	
Chemical Water samples were taken from the control and all surviving test groups at 0 (fres	h
analysis wedia); 24, 48, and 72 (old and fresh media); and 96 (old media) hours for	
guantitative analysis by high performance liquid chromatography (HPLC with UV	V
jetector ,	
Data analysis The C ₅₀ value and associated confidence limits at 24 hours were calculated by th	e
trimmed Spearman-Karber method using the ToxCalc computer software packag	e
$\sqrt{5}$ and at $\sqrt{3}$ , 6, 48, 72 and 96 hours the LC ₅₀ value was calculated using the geometri	
trimmed Spearman-Karber method using the ToxCalc computer software packag and at $3, 6, 48, 72$ and 96 hours the LC ₅₀ value was calculated using the geometric mean method (geometric mean of the concentration showing 0% mortality and the concentration showing 100% mortality). The confidence limits are these	
concentration showing 100% mortality). The confidence limits are these	2
C concentrations.	



## **II. RESULTS AND DISCUSSION:**

Validity criteria according to OECD 203 (2019)	Required	Obtained	
Measured concentration of the test substance	Mandatory	Performed	Å Å
Mortality in control during test	<u>&lt;</u> 10%		
Dissolved oxygen saturation	$\geq 60\%$	@ 69 - 95%	

Analytical results:

¢ V Recoveries were greater than 80% (see table below) (so the biolog@al results are concentrations of M-01 (2,6-dichlorobenzamide (BAM)). There was no M-01 (2,6-dichlorobenzamide (BAM) residue found in the control samples

Nominal			% %	of nominal c	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	ons ¹ 0		
conc. (mg a.s./L)	0 hour	24 hour- old	24 kour-	48 hour old	48 hour-	372 hours old	72 hour-	96 hour
100	103.5	101.5	Q1016	j. 2012 ~	× 103 5		\$ 105 <b>5</b>	105.5
180	105	104	10195	×102 ×	104.5	ð 107 d	168.5 ू	پ [*] 104
320	103.5	101	n.d.	n.d.	n.d.	ne.	o n.d	n.d.
560	102.5	190.5	n.d	n.d.	n.d	ي يn.d.	n.O	n.d.
1000	102.5	چ 100 ^م	the.	Ø n.d. Ø	n.d. «	n.đ.y	<b>Q</b> .d.	n.d.

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

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

# Biological results:

Observations

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

Exposure time			24	48	72	96
Nominal conc.	No of dead	No of K	No of dead	No of dead	No of dead	No of
(mg/L)	^	dead (%)	(%)	(%)	(%)	dead (%)
Control		0.0	0 (0)	0 (0)	0 (0)	0 (0)
600		0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
× 180 Å	A 0 Ø	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
5 326 O	Q(0)	0 (0)	9 (45)	20 (100)	20 (100)	20 (100)
\$60	0 (0)	0 (0)	20 (100)	20 (100)	20 (100)	20 (100)
1000	20 (100)	20 (100)	20 (100)	20 (100)	20 (100)	20 (100)

Mortality



## **III. CONCLUSIONS:**

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

LC50 96 hours (95% C.I.		) mg/L 0 – 320 mg/L)	) (Å	in the second se
NOEC:	100	mg/L	Ô	
highest concentration with	out adverse effects	ing/E		
		Č.	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	
Assessment and conclu		T.		
The study is reliable and	d the $LC_{50}$ of 240 mg/L ca	n be used in th	e MOOI risk assessn	hent of the other
		$\mathcal{A}$		Contraction of the contraction o
Data Point:	KCA 8.2.1/08		NY W N	
Report Author:		~ ****		N N
Report Year:	2003	K Û	a sa a	A A L'
Report Title:	AE C657188: A 96 hour \$ (Oncorhynchus(nykis\$)) M-218631-012	atic acute toxici	ty test with the rainbo	w trout
	(Oncorhynchus(mykiss))	N Ö		
Report No:	M-218631-01 2 &	\$ <u>\$</u>		A O
Document No:	$M-218631 OI^{-2}$			
Guideline(s) followed in	OECD: 203 (1992); ASTN	I Standard E729	-88a (0°994) 🖉 🔬	
study:				
Deviations from current	Method: Deviations from o	current guideline	SANCO/3029/99 rev	1.4.
test guideline:	Limited sets of validation	recoveries were	analyse@. However, th	ne average
	recoveries were within the	acceptable rang	e o£70–110% and the	RSD values were
	below 20%. The analytical	method can be	regarded as fit for pur	pose.
K)	Study: Current Guideline:	QECD 203 (201		
- S	The pH at 40 was 8.6 in the	stest concentrati	ons of V3 mg/L and a	bove, this is
	greater than 8.5		S Chan an	
	Since there were no mortal			
	test, this slight deviation has	no impact on	the study; which fulfi	is all validity
	critteria.			
Previous evaluation:	yes, evaluated and accepte	·• _0 _	Ŵ.	
GLP/Officially	Yes conducted under GL	Officially reco	nised testing facilitie	S
recognised testing	N & N			
facilities:				
Acceptability/Religibility:-	Yes V			
		<u>)</u> <u> </u>		

# Executive summary

An acute toxicity test was performed with the rainbow trout (*Oncorhynchus mykiss*) in a static system. Juvenile rainbow trouts were exposed to M-02 (AP C657188) at nominal concentrations of 6.3, 13, 25, 50 and 100 mg /L for a 96 hour period. Additionally, a negative control (dilution water) was included. One test chamber was maintained in each treatment and control group, with 10 trout in each test chamber. Observations of mortality and other signs of toxicity were made approximately 2, 24, 48, 72 and 96 hour after test initiation. Samples were taken at test initiation, after 48 hours and at test termination from all test chambers. Samples were analysed by high performance liquid chromatography (HPLC) using @V detection. Measured concentrations were in the 80-120% range of nominal concentrations for the study were 6.3, 13, 25, 51 and 102 mg/L, representing 100, 100, 100, and 192% of nominal concentrations, respectively.

The study fulfils all validity criteria of the current version of OECD 203 guideline.



Biological results are based on mean measured concentrations. After 96-hours of exposure, trout in all of the M-02 (AE C657188) treatment groups appeared healthy and normal, with no mortality or overt

signs of toxicity observed. There were no effects observed at any concentration tested. The 96-hour LC₅₀ value is > 102 mg/s, the  $\sqrt{2}$ 

	I. MATERIAL AND METHODS:
Test material	I. MATERIAL AND METHODS:
Guideline(s) adaptation	None specified     Image: State of the specified       Rainbow trout (Oncorhynchus mykiss)     Image: State of the specified       At least 14 days to test conditions     Image: State of the specified
Test species	Rainbow trout ( <i>Oncorhynchus mykiss</i> )
Acclimation	Rainbow trout ( <i>Oncorhynchus mykiss</i> ) At least 14 days to test conditions
Organism age/size at the end of the definitive test	At least 14 days to test conditions Health during acclimation: no nortalities in the acclimation period Juveniles Mean length: 5.2 cm/range 4.5 – 58 cm/ Mean body weight 1.2 g (range 0.72 – 4.6 g) Nominal concentrations: 6.3 – 13 – 25 – 56 – 100 mg p. m/L.
Test solutions	Mean measured concentrations: $5^3 - 13^{\circ} 25 - 51 - 10^{\circ} \text{ mg pm./L} $ Controls: water Evidence of undissolved material: All test concentrations appeared clear and
Replication	No of vessels per concentration (replicates):
Organisms per replicate	Sto. of organishos per vessel: 10
Exposure	Static Static Office Static Of
Test Vessel Loading	
Feeding during test Test conditions	
	Photoperiod: 16 hours light / 8 hours dark, with 30- min transition periods Light mensity 274 loc (at test initiation)
	pH: 8.3 – 8.6 Water hardness: 122 mgCaCO/L (at test initiation) Dissolved oxygen: 7.4 mg/L - 9.2 mg/L Conductivity, 280 µmhos/cm (at test initiation)
Parameters Measured	Observations for mortality, signs of toxicity or abnormal behavior were made approximately 2, 24, 48, 72 and 96 hours after test initiation.
Observations	Measurements of temperature, pH and dissolved oxygen in test chambers were conducted every 24 hours. Hardness, alkalinity and specific conductance were measured in the dilution water at the beginning of the test.



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

II. RE	ESULTS AND DISCUSSION:
Validity criteria according to OECD 203 (2019)	Required of Obtained of O
Measured concentration of the test substance	Mandadory Performed
Mortality in control during test	$= \bigcup_{i=1}^{n} \bigcup_{j=1}^{n} \bigcup_{i=1}^{n} \bigcup_{j=1}^{n} \bigcup_{j=1}^{n} \bigcup_{i=1}^{n} \bigcup_{j=1}^{n} \bigcup_{j=1}^{n} \bigcup_{i=1}^{n} \bigcup_{j=1}^{n} \bigcup_$
Dissolved oxygen saturation	\$ 60% @ Q Q 7.4 nQ/L − 20 mg/L*
*A dissolved oxygen concentration of 6.5 mg/L ref	presents 60% saturation a012°C in freshwater.

### Analytical results:

Recoveries were between 80 and 120% (see table velow). Nevertheless biological results are based on mg/L). Å Ô Ŵ Ĩ.

Nominal conc.	*	<u>©</u>	ured S	% of nomfal con	centrations 2	,
(mg p.m./L)		conc. (mg p.nc.L)		<b>W</b> hour O	48 bour 2	96 hour
6.3	Š, Č	6.3	$\mathcal{I}$	1019 2	§9.9 Ø	101
13		13 %		99.8 A	99.3 ⁶	101
25	S.	AS 65		999.1.Ç Ö	28.6	99.5
50	×.		à c	101 ~~~~	Ø100	102
100	Ň	102		$\Phi_{02}$	101	103
•				X & S		

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

### Biologica results:

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



### Mortality

Mean measured conc. (mg p.m./L) Control 6.3	./L) (%)	No of dead (%)	No of dead		96 °
Control 6.3			(%)	No of dead (%) 📎	No of dead
	0 (0)	0 (0)	0 (0)	0 (0)	$\frac{(\%)}{0} \frac{(\%)}{0} (\%$
	0 (0)	0 (0)	0 (0)	0 (00)	670) 25 , (2
13	0 (0)	0 (0)	0 (3)	A W	<u>70 (0)</u> <del>2</del> <del>2</del>
25	0 (0)	0 (0)	0 (0)	0.0(0)	
51	0 (0)	0 (0)	0 (0)	0(0)	
102	0 (0)	0(0)	0(0)		0 (0)
The study meets the valid LC50 96 hours (95% C.I.) NOEC: highest concentration witho	y meets the validity criteria a hours (95% C.I.):	III. CONCLET ind the endpoints 102 fects 102 for the fect of	sions: based on mean ng p.m/L (notar p.m/L	measured concer plicable)	ntrations are
Assessment and concl	ment and conclusion by an	plicant: P	<u>~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~</u>		**
The study is reliable an	udy is reliable and the LC	f > 402 mg Ø.m.	/Locan be used i	the 102 risk a	ssessment.
	v meets the validity criteria a hours (95% C.I.): oncentration without adverse ment and conclusion by an ady is reliable and the LCV of the the the the the of the the the the the the the of the the the the the the the of the the the the the the the the the of the				

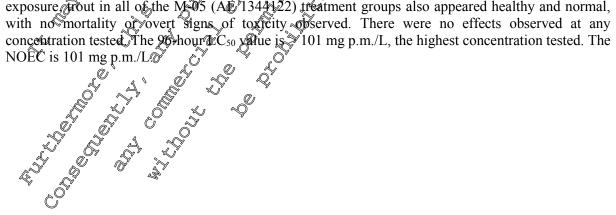
III.	Concretisions: A A A
The study meets the validity criteria and the	endpoints based on mean measured concentrations are
LC ₅₀ 96 hours (95% C.I.):	$\sim 102 \text{ mg p.m./L (not applicable) } \sim 102  mg p.m./L (not applic$
NOEC:	102/mg p*0/L ~ ~ ~ ~ ~
highest concentration without adverse effects	162/mg p.stor./L 2 2 2 2 2



Data Point:	KCA 8.2.1/09
Report Author:	
Report Year:	2003
Report Title:	AE 1344122: A 96-hour static acute toxicity test with the rainbow trout
	(Oncorhynchus mykiss)
Report No:	C035531
Document No:	<u>M-218630-01-1</u>
Guideline(s) followed in	OECD: 203 (1992); ASTM Standard E729-88a (1994)
study:	
Deviations from current	Method: Deviations from current $guideline SANC \sqrt[9]{3029/99}$ rev $4^{57}$ $\sqrt{2}$
test guideline:	Limited sets of validation recoveries were analysed. However, the average
-	recoveries were within the acceptable range of $\mathcal{O}^{-110\%}$ and the RSD values were $\sqrt{\mathcal{O}^{2}}$
	below 20%. The analytical method can be regarded as fit for purpose.
	Study: Current Guideline: DCD 203 (2019)
	The pH at t0 was 8.6 in all the test conceptrations, this is greater than 8.9.
	Since there were no mortalities or sign of stress in any of the fish included in the
	test, this slight deviation had to impact on the study which fulfils all validity
	criteria.
Previous evaluation:	yes, evaluated and accepted a A A
	in DAR (2005) 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2
GLP/Officially	Yes, conduced under GLP Officially recognised testing facilities
recognised testing	
facilities:	
Acceptability/Reliability:	Yes a gran for the second seco
Executive summary	

An acute toxicity test was performed with the randow Fout (Oncorhynchus mykiss) under static conditions. Juvenile ranbow pouts, were exposed to a geometric veries of five test concentrations of M-05 (AE 1344122) and a negative control (dilution water) for a 96-hour period. One test chamber was maintained in each treatment and control group, with 10 fout in each fest chamber. Nominal test concentrations were 63, 13, 25, 50 and 100 mg p.m./L. Observations of mortality and other signs of toxicity were made approximately 6,24, 48, 72 and 96 hours after test initiation. Samples were analysed by High Performance Liquid Chromatograph (PPLC with UV detector). Samples were collected from each test chamber at test mitiation, after 48 hours and at test termination. Recoveries in the aged media were between 99% and 107% and no residues above the limit of quantification were measured in the controls. The mean measured concentrations for the study were 6.4, 14, 26, 50 and 101 mg p.m./L, representing 102, 408, 104, 100 and 101% of rominal concentrations, respectively. The study fulfils all validity criteria of the orrent sersion of OFCD 20 guide me.

The biological results are based on writhmetic mean measured concentrations of the test item. Rainbow trout in the negative control group appeared healthy and normal throughout the test. After 96-hours of exposure frout in all of the MOS (Ap 1344 22) treatment groups also appeared healthy and normal,





### I. MATERIAL AND METHODS:

Test material	M-05 (AE 1344122)
i est materiai	
	Batch: YG3228
	Purity 98.8 % w/w
Guideline(s)	None specified
adaptation	3-methylsulfinyl-5-trifluoromethylpyridine-2-carboxylic acid Batch: YG3228 Purity 98.8 % w/w None specified Rainbow trout ( <i>Oncorhynchus mykiss</i> ) At least 14 days
Test species	Rainbow trout (Oncorhynchus mykiss)
Acclimation	At least 14 days
Acclimation	no mortalities occurred and fish showed no signs of disease or stress
Organism	Moon longth: 57 am (ronger 51 69 am); at start Cormination
age/size at	Mean body weight: 1.6 g (range $4.1 - 1.9$ g); at test termination $3.7$
study initiation	Mean body weight: 1.6 g (range $4, 1 - 1.9$ g); at lest termination
Test solutions	
1 est solutions	Nominal concentrations: $6.3 - 13 - 25 - 50^\circ$ 100 mg p. mg/L. $6.4 - 26 - 50^\circ$ and $101 \text{ mg}$
	p.m./L. Controls: water
	Evidence of undissolved material. Will test solutions appeared clear and colorbess at
	test initiation and termination.
Replication	Controls: water Evidence of undissolved material; All test solutions appeared clear and colortess at test initiation and termination. No. of vessels per control (replicates): 1 No. of vessels per control (replicates): 1 No. of organisms per vessel? 10
i topiouron	No. of vessels per control (replicates): 1 $\beta$
Organisms per	No. of organisms per vessel. 10 2 0 0
replicate	
Exposure	static o o o o o o o o o o
I	Total exposure duration: 9 hours y y y y
Test Vessel	static Total exposure duration: 96 hours +
Loading	0.39 g fish test medium
Feeding during	Stone of the state
test 🖉	
Test conditions	Temperature: 110 – 12, &C Photoperiod: 16 hours light / 8 bours dark with 30- min transition periods
	Protoperiod: 16 hours light / 8 bours dark with 30- min transition periods
<u>`</u>	Light intensity: 289 fux
<u>í</u> Gi	Light intensity: 289 $\mu x$ $pH_8.2 - 86$ .
* ¥	Water hardness $128$ mg CaCO ₃ /L $\ll$ $\sqrt{3}$
	Dissolved oxygen: 7.8 – 9.3 mg/L O 72% saturation) Conductivity: 320 pmhos cm
	Conductivity: 320 pmhos cm
Parameters	Observations for death, and signs of toxicity or abnormal behavior, were performed
Measured / /	at 6, 24, 48, 72 and 96 hours after test initiation.
Observations	Discrete measurements of temperature, dissolved oxygen, pH were obtained at test
L.	initiation, 24, 48, 72, and 96 hours. Hardness, alkalinity and conductance were
<u></u>	measured at the Beginning of the test.
Chemical	Samples were analysed by high Performance Liquid Chromatograph (HPLC with
analysis 🦉	UV detector). Samples were collected from each test chamber at test initiation, after
	45 hours and at test termination.
Data analysis	The absence of mortality in this study precluded the statistical calculation of $LC_{50}$
Ŭ,	values at 24,348, 72 and 96 hours. Therefore, the $LC_{50}$ values were estimated to be
N R	greater than the highest concentration tested. The no mortality concentration and
	the no-observed-effect-concentration (NOEC) were determined by visual
-& _~	48 hours and at test termination. The absence of mortality in this study precluded the statistical calculation of $LC_{50}$ values at 24,48, 72 and 96 hours. Therefore, the $LC_{50}$ values were estimated to be greater than the highest concentration tested. The no mortality concentration and the no-observed-effect-concentration (NOEC) were determined by visual interpretation of the mortality and observation data.
$e^{o^{v}}$	
$\lor$	



### **II. RESULTS AND DISCUSSION:**

Validity criteria according to OECD 203 (2019)	Required			Obtained 🖉 💍
Measured concentration of the test substance	Mandatory			Performed and results are based on mean uneasured Concentrations
Mortality in control during test	<u>&lt;</u> 10%		A	
Dissolved oxygen saturation	$\geq$ 60%	Ö	a di la come di la com	$\geq$ 72% saturation
	1	- V	Q,	

Analytical results:

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

Nominal conc. (mg p.m./L)	Mean (mg p.m./L)	% of nominal concentrations         48 hours         48
6.3	6.4	
13	14	
25	26	
50	50 <u></u>	98 F Q 99.3 S 99 g Q
100		29.7 × 101 × 102 ×

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

Biological results:

Observations

Rainbow trout in the negative control group appeared healthy and normal throughout the test. After 96-hours of exposure front in all of the M-05 (AF 1344022) treatment groups also appeared healthy and normal, with no mortality or overt signs of toxicity observed.

Monunty	A 6	<del>Y OF S</del>				
Exposure time (house)	3	6~	240	48	72	96
time (hours)		v v	Ň			
mean	a a a a a a a a a a a a a a a a a a a		Ň			
measured	No of dead	No of dead 🏑	No of dead	No of dead	No of dead	No of
conc. 🗸		(%)	(%)	(%)	(%)	dead (%)
(mg p.m./L)						
Control		SØ (0)	0 (0)	0 (0)	0 (0)	0 (0)
6.4		0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
		0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
264 4	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
50	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
101	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

Mortality .«



## **III. CONCLUSIONS:**

The study meets the validity criteria and the endpoints based on mean measured concentrations are:  $\mathcal{Q}_{p}^{\circ}$ 

The study meets the valid	dity criteria and the endp	points based on mean measured concentrations are:
LC ₅₀ 96 hours (95% C.I	.):	> 101 mg p.m./L (not applicable)
NOEC: highest concentration wit	hout adverse effects	> 101 mg p.m./L (not applicable)       101 mg p.m./L     Image: Concentration of the second se
Assessment and concl	usion by applicant:	
The study is renable an	in the $LC_{50} > 101 \text{ mg p.i}$	m./L carbe used in the M-05 risk acsessment.
~		
CA 8.2.2 Long	-term and chronic to	specify to fish $\frac{1}{2}$ $\frac{1}{2}$ $\frac{1}{2}$ $\frac{1}{2}$ $\frac{1}{2}$ $\frac{1}{2}$ $\frac{1}{2}$
CA 8.2.2.1 Fish	early life stage toxicit	ty test
Data Point:	KCA 8.2.2.1/0	
Report Author:		
Report Year:		
Report Title:	AE C638206: An Carly L	The-Stage Toxicity Test with the Fathead Minnow Inder Bow-Through Onditions
Report No:		
Document No:	M-2-1190-01-1	
Guideline(s) followed in	QECD: 210 (1999); USE	A (=E@A): 850.1400 (1996)
study:		
Deviations from current	Method:	
test guideline:	กณฑลั 🔬 เ	
Į,	Study: Current Guideline	2 OECD 210 (2013)
S, C		d from the range of $25^{\circ}C \pm 10^{\circ}C$ during a three-day
		ures ranged from approximately 23.0 to 27.0°C, due to a
ð S		Since there was no corresponding increase in effects ins during this period (Pays 4-6 post-hatch), the variation
, Ô		
Previous evaluation:	yes evaluated and accept	ter S
	in $DAR(2005)$	
GLP/Officially	Wes, conducted under GI	P/Officially recognised testing facilities
recognised testing		
facilities:		
Acceptability Reliability:	Xes v v	У́ _А
Q ^Y Q		
		\$ [°]
\$ A \		
	J.	
	× ×	
	9	
Previous evaluation:		



Ô

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

### **Executive summary**

A fish early life stage study was performed with fathead minnow (*Pinephatos promelas*) in flow through conditions for 33 days. Fluopicolide was applied at concentrations of 0.938 – 0.075 – 0.150 – 0.300 – 0.600 mg/L. A water control and a solven control were also included. The test comprised 4 replicates of 20 fish for each group. Mortality, clinical signs of toxicity and abnormal behaviour were recorded daily and were used to derive

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

Concentrations of fluopicolide were verified by HPLC/UV on days 0, 7.14, 2028 and 33 for each concentration and control. Measured concentrations were by the 80-120% range of nominal concentrations and por residues above the LOQ were measured in the controls. The mean measured concentrations were 0.037 - 0.076 - 0.185 - 0.304 - 0.585 mg/L.

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

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

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

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



## I. MATERIAL AND METHODS:

Test metarial	Fluopicolide (tech.) Lot/batch: 2050190/PP241024/2 Specification not reported Purity 97.7% w/w None specified Fathead minnow ( <i>Pimephales promelaco</i> Embryos less than 24 h old Nominal concentrations: of 0.038 - 0.075 - 0.150 - 0.390 - 0.600 mg/t/ (not corrected for purity) Arithmetic mean measured concentrations: 0.037 - 0.076 - 0.155 - 0.304 - 0.585
Test material:	Lot/batch: 2050190/PP241024/2
	Specification not reported
	Purity 97.7% w/w
Guideline(s)	None specified
adaptation	
Test species:	Fathead minnow (Pimephales promela@
Organism Age	Tadicad minilow (1 internates prometals)
at	Embryos less than 24 h old
Experimental	
Start:	
Test solutions	Nominal concentrations: of $0.038 - 0.075 - 0.150 - 0.300 - 0.600 \text{ mg/t/ (not corrected for purity)} Arithmetic mean measured concentrations: 0.037 - 0.076 - 0.155 - 0.304 = 0.585^{\circ}$
i est solutions	corrected for purity)
	Arithmetic mean measured concentrations: $0.097 - 0.076 - 0.155 - 0.304 = 0.585$
	$mg/L$ $d \sim $
	Controls: water control and solvent control (dimensional distance 0.05 mL/L)
	Evidence of undistrived material, all of the test solutions appeared clear and
	colorless in the test chambers at test initiation and termination. A slight brown
	precipitate was observed in the diluter mixing chamber for the 6,600 mg/L
	replicates at termination.
Replication:	No. of vessels per control (repricates): 4
	No. of vessels per solvent control (replicates): 4
o ·	
Organisms per replicate:	Ng of fertilized eggs/embryos per vessel: 20 0 0 4
	Flow-through Total exposure durations, 33 days (5-day-hater and 28 d post-hatch)
· . ( ))	Flow through & A A A
Test Vessel	At the end of the test 0.19 of ish/L of water in the tank or 0.015 g/L per 24 h
Loading	
Feeding	Newly barched prvae were fed live brine shrimp nauplii (Artemia sp.) three times
during test	per day during the test. Rations were adjusted each week to account for losses due
e (	to mortality of a a a
Test 🔊	Temperature: 24.9 to 24.6°C (test chambers), temperature measured continuously in
condition	one water control replicate remained within the desired range of $25 \pm 1$ °C, with the
Q	exception of a three-day period in which temperatures ranged from approximately
	23 0 to 27.0 °C, due to a mechanical malfunction.
4	Protopetiod: 16.8 light dark with gradual intensity changes at dawn and dusk
° Ø	Light $\widehat{o}$ tensity? 80 lux $\widehat{o}$ pH: 8.0 to $\widehat{s}$ $\widehat{s}$
Ś	
	Dissolved oxygen % saturation: $\geq 89\%$ (7.3 mg/L)
	$\mathcal{C}$ conductivity 350 $\mu$ S/cm
	Begin of post-hatch period: day 5
E Q	
$e^{O^{v}}$	
$\checkmark$	



	· · · · · · · · · · · · · · · · · · ·
Parameters	Temperature was measured in each test chamber at the beginning and end of the test
Measured /	and at weekly intervals during the test, Temperature also was measured continuously
Observations	in one negative control replicate.
	Dissolved oxygen was measured in alternating replicates of each treatment and
	control group at the beginning and end of the test, daily during the first seven days
	of the test and at weekly intervals during the test. Measurements of pH were made
	in alternating replicates of each treatment and control group at the beginning and
	end of the test and at weekly intervals during the test. Hardness, akalinity and
	specific conductance were measured invalternating replicates of the water control
	and the highest concentration treatment group at the beginning of the test at weekly
	intervals during the test and at test termination.
	During the first day of exposure embryos were observed to the mortality and
	eggs with fungus. Thereafter, with hatching was complete, observations of embryo
	mortality and the removal of dead embryon were performed some Maily
	mortality and the removal of dead conbryos were performed once daily. During the 28-day post-hatch exposure period, the latvae were observed daily to
	evaluate the number of mortalities and the number of individuals exhibiting clinical
	signs of toxicity or abnormal behavior. From these observations, hatching success,
	time to hatch, and post-hatch growth and survival were evabilited.
	Post-hatch growth of the tathead minnows (total length, we and de weights) was
	evaluated at the sonclusion of the 28-day post hatclexposure period.
Somuling for	
Sampling for	On Days 0, 7, 14, 24, 28 and 33 (test termination), water samples were collected from one alternating replicate test chamber of each treatment and control group to
chemical	monitone anemating represented that test chamber of each treatment and control group to
allalysis	HPI CVI M
	A A A A A A A A A A A A A A A A A A A
Data analysis:	Test endpoints analysed statistically for the juvenile, fish were hatching success,
	lage al survival and growth (total length, wet weight and dry weight). Data from the
,	are gative and solvent control groups for each parameter were compared using a t-
	test. Since no differences were detected between the two control groups ( $p > 0.05$ )
^O	testmentarous
Q	Discrete-variable data were analysed using Chilkquare and Fisher's Exact test to
² Q ³	identify treatment browns that showed statistically significant difference ( $n < 0.05$ )
	from the controls. All continuous-variable data were evaluated for normality using
	Shapiro-Wilk test, and for homogeneity of variance using Bartlett's test ( $p = 0.01$ ).
(	Since the data passed the assumptions of normality and homogeneity, ANOVA and
Ø	Boderron is t-test were ased $(p < 0.09)$ . All statistical tests were performed with
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	TOXSTAT v3 5 or SAS v 8 2 software.
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	On Days 0, $(z, 14, 24, 28 and 33)$ (fest termination), water samples were collected from one alternating replicate test chamber of each treatment and control group to measure concentrations of the test substance. Enopieofide was measured by HPLCdW. Test endpoints analysed statistically for the divenite fish were hatching success, laval survival and growth (tot) length, wet weight and dry weight). Data from the degative and solven control groups for each parameter were compared using a t- test. Since no differences were detected between the two control groups ($p > 0.05$) for any of the parameters, the control data were pooled for comparison among the treatment groups. Discrete-variable data were analysed using Chil-square and Fisher's Exact test to identify treatment groups that showed a statistically significant difference ($p < 0.05$) from the controls. All continuous-variable data were evaluated for normality using Shapico-Wilks test, and for homogeneity of variance using Bartlett's test ($p = 0.01$). Since the data passed the assumptions of normality and homogeneity, ANOVA and Booferrontis t-test were lased ($p < 0.05$). All statistical tests were performed with TOXSTAT v3 for SAS v 8 2 software.
S. S	
õ	



Validity criteria	Required by OECD 210, 1992	Required by OECD 210, 2013	Obtained 🦉 🐣
Dissolved oxygen concentration throughout the test	Between 60% - 100% saturation	\geq 60% saturation	≥ 89% saturation
Temperature range for the species	$25 \pm 2^{\circ}$	$25 \pm 1.5^{\circ}$	Fulfilled with the exception of a three day period in which temperatures
Water temperature difference between test chambers or between successive days at any time during the test	± 1.5° max	± 1.5° nax	day period in which temperatures ranged from approximately 23.0 to 27.0°C, the to a mechanical malfurction. Since there was no corresponding increase in effects noted among the organisms during this period (Days 45 post-fratch), the variation in (temperature is not believed to have adversely affected the study
Analytical measure of the test concentrations	Compulsory	Computsory	Kone of the first of
Hatching success of controls	> 66%	>70%	83 and 85% 7 4 4
Post-hatch survival of controls	>70%	× 75% 3	~89 and 90% 55 55 4
Solubilising agent when used	No significant & effect on survival norany other adverse effects	Not required 4	Faimled S S

II. RESULTS AND DISCUSSION:

<u>Analytical results</u>: The recoveries are of the range 80,120% (see table below). The results of the study are based on the arithmetic mean measured test concentrations. No residues of duopiconde were measured in the controls above the limit of quantification (20.5 μ g/L).

Nominal Arithmetic Soft nominal concentrations							
(mg/L)	mean (mg/L)	Day	Day 7	Day 14	Day 21	Day 28	Day 33
0.038	0.0370	ð 8.2 . Č	96î Ö	96.00	94.2	97.5	95.9
0.075	0.076	99.6	Q103 Q	104	102	101	102
0.150	0.155	193 6	101 .	<u>9</u> 104	102	103	103
0.300	0.304	103 ~	103 .	103	98.7	102	98.9
0.609	0.585	101	97.1	95.1	99.6	90.8	101

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



Biological results:

Observations

With the exception of one fish that appeared weak from Day 0 to 2 post-hatch, all surviving control larvae appeared normal. In general, the majority of the fish in the 0.037, 0.076, 0.155 and 0.30 mg/L² treatment groups appeared normal throughout the test. There were a few sporadic observations of organisms that appeared smaller, weak or lethargic, or were swimming errationally or exhibiting a loss of equilibrium. These observations were few in number and did not occur in a concentration responsive pattern.

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

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

			~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
Arithmetic mean measured	Mean total length	Mean wet weight	Mean dry weight
concentrations (mg/L)	(mm) SD	(mg) ± SD	$(mg) \pm SD$
Water control	21,3≠0.61 ×	√68.1 ± 4.6 0 √	$12.7 \pm 1.3$
Solvent control	$205 \pm 0.28$	72, <b>4</b> ± 3.8	$12.8 \pm 0.9$
Pooled control	$21.4 \pm 0.45$	709:3 ± 4 8 ×	$12.8 \pm 1.0$
0.037	21.3 0.79 0 2	73.9±3.5	$13.0 \pm 1.6$
0.076	$21.3 \pm 0.54$	73.2 3.4	$13.9 \pm 0.6$
0.155	29.1 ± 0.39	$70.9 \pm 3.9$	$13.9 \pm 0.8$
0.304	C19.9 ± C.29*	\$9.8±3.0*	$11.6 \pm 0.6$
0.585#	13.8±1.1 ~ ~	17.5 1.4	$3.3 \pm 0.2$

# excluded from statistical analysis since there was significant effect on servival

* Statistically significant ( $p \le 0.05$ , Bon troni's trest)  $\mathcal{D}$ 

# Time to hatch and hatching success

The fathead minnow endpryos began katching on Day 4 of the test and larvae were released into the test chambers on Day 5, when >90% of the surgiving control embryos had hatched. There were no apparent differences in time to hatch between the control group and the 0.037, 0.076, 0.155 and 0.304 mg/L treatment groups. With the exception of one embryo in the 0.304 mg/L treatment group that hatched on Day 6, all surviving embryos hatched by Day 5 of the test. However, there was a slight, yet notable delay in time hatch in the 0.585 mg/L treatment group in comparison to the control groups. Only 42% of the surviving embryco in the 0.585 mg/L treatment group had hatched by Day 5 of the test, in comparison to 100% in the negative and solvent control groups. The majority of the surviving embryos (58%) in the \$\$85 mg/L treatment group hatched on Day 6 of the test.



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

Arithmetic mean measured concentrations (mg/L)	Mean % hatching success	Mean % hatching success of non fungused embryos	
Water control	85	85	
Solvent control	83	83	
0.037	56*	787 8 5	
0.076	66*	95	
0.155	83	85	
0.304	83	87 5	
0.585	81	81 ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	

* Statistically significant difference ( $p \le 0.05$ ) from the proved combol using Pisher (Exact re

Larvae survival on day 28 post-hatch The decrease in survival in the 0.585 mg/L treatment group was statistically significant in comparison to the pooled controls

Arithmetic measured	
Arithmetic mean measured concentrations mg/L	Mean % survival y
Water control	
Solvent control	
0.037	
0.076	
0.155	
0.304	BY C C
0.585	Q 17* 0 X

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



#### **III.** CONCLUSIONS:

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

	% hatching success	% post hatch survival	Dry weight	Wetweight	Total length
LOEC (mg/L) lowest concentration with an effect	> 0.585	0.585	0.585	0.304	0.304 C
<b>NOEC</b> (mg/L) <b>highest concentration</b> without adverse effects	0.585	0.304	0.304	0.155	155 ×
Assessment and conclu The study is reliable, ar	usion by applica	<u>.15 mg/[@See [</u> s	Q ["] Q" Now for addit	° 📣 L.	
Dutu I Olitt.	KCA 8.2.2 1903			<u> </u>	
Report Author:	<u> </u>	<u>oʻ '&gt;' `\</u>	<u>~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~</u>	<u> </u>	<u> </u>
Report Year:	2018		S O		$\sim$
Report Title:	(Palmer et al. (20)	or floopicoffee stud 3, M-241190-01	y on early life s	tages of Pimeph	ales promelas
Report No:	M-643769-01-1				
Document No:	<u>M-643789-01-6</u>				
Guideline(s) followed in study:	none				
Deviations from current	Not appreable		0	4	
test guideline:		~~	<u>s</u> <u>s</u>	Ø	
Previous evaluation:	No, fløt previously			¥	
GLP/Officially	No, not conducted	under GLP/Officia	all Precognised	testing facilities	
recognised testing		~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	U N	C	
facilities		, <i>s s</i>			
Acceptability/Reliability:	Yass y A	× × /.	1 N		
ECx calculations for the	Yes + +		7		

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

Endpoints for the following parameters overe statistically determined in the existing report:
Hatching success
Post-hatch survival
Length
Fresh weight
Dry weight
Dry weight
The NOEC for hatching success is the highest tested concentration (0.585 mg/L) and only 4.7% of effects were observed at this level. Therefore, it is not necessary to calculate EC₁₀ and EC₂₀ values for effects were observed at this level. Therefore, it is not necessary to calculate  $EC_{10}$  and  $EC_{20}$  values for this parameter, they are both > 0.585 mg/L.

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



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

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

			A.	
Endpoints (mg/L)	% post hatch survival	Dry veight	Wet weight	Total Jength
$EC_{10}$	No significant	0.307	0.278	2 <u>50.338</u> 0 ⁴
(95% confidence interval)	dose-response	(n.d.)	(0.144-0.350)	(%194-00408)
EC ₂₀ (95% confidence interval)	relationship so no ECx can be calculated	0.356 (n.d.)	0.328 0.203-0.397)	0,440
NOEC from the report	0.304	0.30	<b>0</b> .155	0.155
n.d. not determined due to mathemat	ical reasons 🖂 🔍			

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

The overall NOEC for the fish ELS study on fathead minnow with flyppicolitie is 0.455 mg/L based on effects on length and we weight. The lowest  $LC_{10}$  is 0.278 mg/L based on effects on wet weight.

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## Assessment and conclusion by applicant:

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

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

## CA 8.2.2.2

## Fish full life cycle test

Based on the triggers stated in the EU regulation 283/2013 on data requirements for active substances and the EFSA Aquatic Guidance Document, a fish full life cycle (FFLC) study is required for bioaccumulative and persistent substances. Quopicolide has a BCF < 1000, and therefore does not meet the trigger of Dioaccumulation and persistent. A FFLC may also be required due to endocrine disruption criteria. These are discussed in CA.8.2.9.

criteria. These are discussed in CA.8.2.9.



Data Point:	KCA 8.2.2.3/01
Report Author:	
Report Year:	2003
Report Title:	Bioaccumulation and metabolism of [2,6-14C-pyridinyl]- C638206 in Cruegillo sunfish, Lepomis macrochirus, in a flow-through system
Report No:	B004340
Document No:	<u>M-241273-01-1</u>
Guideline(s) followed in study:	OECD: 305 (1981); USEPA (=EPG): Subdivision 165-4 (1982)
Deviations from current	Current Guideline: OECD 305 (2012)
test guideline:	The concentration was not maintained with $\phi^{+/-20\%}$ of the mean for the high $\psi^{-1}$
	The concentration was not maintained within 4/-20% of the mean for the high concentration (see details in the validity part below) because of the technical failure on day 16 only. This deviation is not considered to have affected the overall validity of the test since the west case BCF were obtained from the fow concentration where no deviations were observed.
	failure on day 16 only. This deviation is not considered to have affected the overall
	validity of the test since the worst case BCF were obtained from the low
	concentration where no deviations were observed.
Previous evaluation:	yes, evaluated and accepted in DAR (2005) Yes, conducted under GLP/QPAcially recognized testing factories
GLP/Officially	Yes, conducted under GLP/Officially recognised testing facilities
recognised testing	Yes, conducted under GLP/QPAcially recognized testing fact ties
facilities:	
Acceptability/Reliability:	Yes Q & & & Q & Q & X
receptuolinty, itenuolinty.	
Executive summarv	

#### CA 8.2.2.3 **Bioconcentration in fish**

#### Executive summary

A bioconcentration study was performed with fuopicolide on blue fill sunfish (Lepomis macrochirus) using a 45-day flow-through system, including a 24-day uptake and a 21-day depuration period. Three groups of 144 juvenile fish each were separately exposed to three treatments: (1) solvent control at 0.1 ml/L dimethylformanide: (2) treated at a nominal concentration of 0.8 µg/L [14C]- fluopicolide in 0.1 mL/L dimethylformamide and (3) treated at a nominal concentration of 80 µg/L [14C]- fluopicolide in 0.1 ml/L dimeto for total radioactive residues by liquid soprillation counting on day 0 and every Monday, Wednesday, and Friday during the uptake and depuration phases and by HPLC or days 0, 1, 7, 14 and 21. Fish were sampled at regular intervals throughout the uptake and depuration periods for determination of total radioactive residues and lipid content. Fish samples consisting of three tissue groups - whole body, edible tissue (muscle, skin and skeleton) and nonedible rissue (rins, head and viscera) - were taken throughout the uptake and depuration phases Total radioactive residues in each tissue group were determined by combustion. Additional fish were explected, after achieving steady state, on days 21 and 24 of the exposure period for metabolite identification The identity of single major residue component, unchanged fluopicolide, was confirmed by LC-MS. The study family allovalidity criteria of the current version of the OECD 305 guideline Mean measured concentration and standard deviation of test material in the water during the uptake period was  $0.644 \pm 0.028$  ftg/L [¹⁴C]- fluxpicolide equivalents at the low treatment (0.8 µg/L) and  $1265 \pm 1.79$  µg/L [¹⁴C]- fluxpicolide equivalents at the high treatment (8.0 µg/L). HPLC analysis demonstrated that the parent compound fluppicolide was stable in the low and high treatment water throughout the uptake phase it was the orly radioactive component detected in the water. There was one mortality on the solvent solution treatment and two mortalities in the high treatment during the course of the study. These mortalities were not considered to be related to the test substance. The steady-state-BCF parent (based on whole toh) in the 0.8  $\mu$ g/L test level is about 117 L/kg, the lipid normalized BCF is 65 Lakg. Fr



#### I. MATERIAL AND METHODS:

Test material	Fluopicolide	F ₃ C ₊ (+), +), +), +), +), +), +), +), +), +),
	AE C638206	
	Batch code:	
	R001737	• = position of radiolabel
	Purity: 99.3%	
	Radiochemical	
	purity: 96.5%	
Guideline(s)	None specified	
adaptation		
Test species	Bluegill sunfish (Lepo	omis macrochirus) Q' 6° A A O L
Acclimation	Fish were acclimated	to the test dilution water and the test temperature (i.e. $22 \pm 0^{\circ}$
	$2^{\circ}$ C) for $\geq 14$ days pri	or to initiation of testing. There were less than 3% of
	mortality in the popul	$\operatorname{ation}_{\mathcal{O}^{*}} \mathcal{O}^{*} _{\mathcal{O}^{*}} _{\mathcal{O}^{*}} \mathcal{O}^{*} _{\mathcal{O}^{*}} _{\mathcal{O}^{$
Details on test	- Weight at study initi	ation: 1.077 g (range 0.724-1.604 g)
organisms	- Length at study initia	ation: 3,33° cm (range $3,0-3$ , $2$ cm) $0^{3}$
	- Lipid content at test	initiation: 17.2% (www) of whole fish
Test solutions	Nominal concentration	ns: Q.8 µg and 8 µg [14C] [Huopicolide/[ ]
	Measured concentration	onsol.844 and 7.265 µg 14C] Duopicolide/LS
	Solvent control. 0.1 m	th/L dimethyl tormanic Long to the second
	Evidence of undissolv	ed material pot reported Q ^v S
Replication	No. of vessels per con	anon: 5,55 cm (range $\gtrsim 0 - 5.9$ cm) initiation: 17.2% (ww) of whole fish ns: 0.8 µg and 8 µg [ ⁴ C] Huopicolide/L on $\approx 0.844$ and 7.265 µg [ ⁴ C] Huopicolide/L d/L dimethylformanide ed material pot reported $\sim$
	No. of vessels per solv	vent control (repficates): 1 v v v
Organisms	No. of organisms per	vessel \$144
per replicate	L Ś ^y Ż	
Exposure	Test type: Plow throu	vessel \$144
	Koute of exposure: ag	
	Total exposure duration	on: 24 days a a a start
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	i i opa ucpanation ana	ului 21 yays () a are
Test Vessel	Bomass loading rate:	2.2 g/L test tank at the start of the study and 0.43 g/L/day
Loading	based on the flow of t	est solution through the tantos
Test	Temperature: 2K2 - 2	4 C (continuous measurements), 21.6 – 22.7°C (discrete
conditions	measurements	
	Photoperiod 16:8 kou	Irs. uxQinitiaQight intensity at the surface of the test system)
\$	pH. 8.2-8.5 Water hardness: 72	
<i>A</i>	Water hardness: 72	220 mg/L as CaCO ₃
	Oxygen saturation: 80	-102%. The test aquaria were aerated during the study to
¹ ¹		ygen levels at or above 60% of saturation.
×¥	TOC: \$2.0 mg/L in d	wution water
c @	Conductivity; 1000a	S/cm*y
	Alkalinity 258 to 328	
Feeding	During the uptake and	Repuration phase, the fish were fed twice daily with
during test	Commercial tosh tood	at a rate of approximately 2% of mean body weight per day.
J L	I ne amount of food w	vas re-calculated (to adjust for loss due to mortality or
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	sampling each day ba	ased on the mean weight of fish sampled previously.
during test		
r O ^x		
$\bigcirc$		



Parameters Measured / Observations	Discrete water quality measurements of temperature, dissolved oxygen, pH and conductivity were obtained in each test tank throughout the study. Continuous temperature monitoring in solvent control test tank. Fish were observed initially and daily during the exposure and depuration period.
	for any mortality and/or abnormal appearance or behaviour. Total lipid content was determined in fish at study initiation and in treated fish sampled on days 7, 4, 21 24 of uptake and days 7 and 18 of depuration.
Sampling for chemical analysis	Water analysis: On Day 0 and every Monday, Wednesday, and Friday thereafter during the uptake phase, the concentration of total radioactive residues in the test water were calculated by direct LSC as [ ¹⁴ C]-fluopicolide equivalents. On certain fish sampling days of the uptake phase (Study Days 0,1,7,14,21, 24) and on Day 1 of the depuration phase, duplicate water samples of 100 fail were removed from each aquarium using a glass pipette and stored in glass bottles. Samples were analysed by HPLC with UV detection Fish analysis: Six fish were taken from each tank at each sampling and pooled into 2 groups of fish. Three of these fish were taken for whole fish analysis and lipid analysis (hpid analysis on treated fish only). The remaining three fish from the control and the eated tanks were weighed and dissected into enalysis of total radioactive residue. Additional samples of fish were collected from each tank for metabolite characterization on Day 21 and Day 24. These fish were weighed in groups of six and dissected into edible portions. Metabolites in extracts containing significant residues (>5%) were identified and guantified by comparison with authentic standards on HPLC, The identity of single major

Si O' VII. RESUETS AND DISCUSSION C							
Validity critoria V (OECD305, 1996)	Required () ()ECD (305, 2012)	Obtained					
Water temperature variation over the whole test period $2^{\circ}$ $2^{\circ}$		21.2 - 24°C*					
Dissolved oxygen % saturation in all test vessels	Å Å	80 - 102%					
Concentration of test substance in test chambers maintained within required of range of the measured values during the uptake phase	7	low concentration: 93-105% of the mean high concentration: 23 - 116%**					
The concentration of the test substance is below its limit of solubility of test substance of the solution of the test substance is	Test concentration < water solubility of test item in test water	Yes***					
Mortality or other adverse effects/disease in control an Oreated fish		< 10%					

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

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



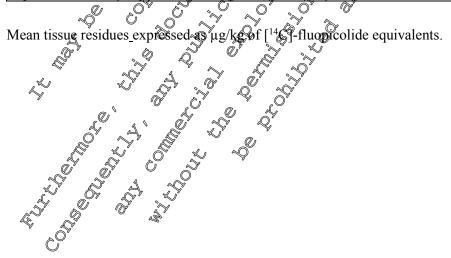
Analytical results:

Mean measured water concentrations as determined by LSC during the uptake period was  $0.844 \pm$ 0.0276  $\mu$ g/L [¹⁴C]-fluopicolide equivalents at the low treatment (0.8  $\mu$ g/L) and 7.265 ± 1.79  $\mu$ g/L [¹⁴C]fluopicolide equivalents at the high treatment (8.0  $\mu$ g/L). This represented 106% of the low normal concentrations and 91% of the high nominal concentration. Water concentrations ranged from 0.783  $\mu$ g/L to 0.888  $\mu$ g/L in the low treatment and 1.657  $\mu$ g/L to 8.452  $\mu$ g/L in the high treatment through the uptake phase. No radioactivity was detected in the solvent control tank.

Mean measured water concentration during day 1 and day 4 of depuration showed a decrease in S fluopicolide equivalents in both treated tanks. After day 4 of depuration, no water samples were taken since concentrations of radioactive residue were practically not detectable. At several times during the uptake phase (days 0, 1, 7, 14 and 21) water samples from both the low and high treatment aquaria were analysed by HPLC to monitor any degradation of the est compound in aquarium water. No degradation of the test compound was observed.

expressed Average daily concentrations of total radioactivity in water ing/L, ⁴C]_zffuopicatide , Ô equivalents) ×,

<u>G( 1 1</u>	Nominal concentration:         0.8 μg/b         Nominal concentration:         8.0 μg/b           0.898         8.405 or         3.0 μg/b         3.405 or         3.	A Color
Study day	Nominal concentration 20.8 μg/0 Nominal Concentration 8.0 μg/0	Ő
-3		~
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28	\$0.006 \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$	
Uptake Mean 🖗		



Study day	Nominal con	ncentration: 0.8	μg/L	Nominal concentration: 8.0 µg/L			
	Edible tissue	Whole fish	Non-edible tissue	Edible tissue	Whole fish	Non-edible ° tissue	
0	n.d.	$0.0 \pm 0.0$	n.d.	n.d.	$0.0 \pm 0.0$	n.d. 🔊 🦿	
1	$26.3 \pm 2.5$	$76.8\pm4.2$	$124.1 \pm 4.9$	$182.7 \pm 26.3$	594.1 46.7	256.2+89.6	
3	$23.9\pm3.7$	$128.3 \pm 15.2$	$127.3 \pm 27.1$	$165.5 \pm 22.4$	680 ± ± 1,14.2	107 8 ±	
7	29.1 ± 3.4	83.4 ± 10.5	156.6 ± 46.3	177.7 ± 13.6	<b>84</b> 7.0 ± 174.2 ≪	98.3 47.7	
10	$30.4 \pm 2.5$	$11.6 \pm 10.7$	151.9 ± 15.2 1	186.2 ± 10,7	720.3 ± 78	$6708 \pm 9745$	
14	$27.9 \pm 2.3$	91.6±1.4	$143.0 \pm 6.7$	193.3 ± 7.0 ×	708.9±4.4	A7.1 ± 59.6	
18	33.4 ± 2.9	$101.2 \pm 6.9$	151.5 ± 4,5	210.8 1 2.2	€30.0¥ 35.0√		
21	$43.3 \pm 5.1$	$108.0 \pm 7.7$	$186.6 \pm 23.7 \circ$	$33093 \pm 1660$	7666.6±88.9	1373.8 ± (* *17.7	
24	$44.8 \pm 3.6$	87.4 ± 9.5	159.3 ± 8.9	0301.8 <u>0</u> 26.7	825.9 143.2 0	136126 ± ° 74.20 °	
25	$15.0 \pm 0.4$	31.9 ± 4.5	51&₽2.9	848 ± 2.6	$179.3 \pm 23.0$	256.7 ± 29.6	
28	$9.2 \pm 0.3$	$11.4 \pm 0.4$	$17.2 \pm 1.3$	$64.9 \pm 10^{\circ}$	§3.0±90	¥07.8 4.7	
31	$7.5 \pm 0.2$	$8.4 \pm 0.3$	&13.1 ± 1.4	\$6.1 <b>± 0</b> .5	¥78.5 €6.3 🖉	$98.3 \pm 4.0$	
35	$5.9 \pm 0.2$	7.8±0,4	©9.1±0%6 ×	{ 47. <b>2</b> ¥ 0.8 ↔	47,59 ± 1.2,5	$67.8 \pm 6.4$	
42	$4.5 \pm 0.2$	4.6 ± 0.4	6.0@0.4	$383 \pm 0.90$	40.4 ± 9.6	°47.1 ± 6.6	

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

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

Depuration day	Domina	concentration.	N u a/I	Nominal	concentration: 8.	0 µ α/I
	Edible tissue	Whole fish	Non-edible	Edible tissue	Whole fish	Non-
Ő	Edible tissue		tissue			edible
( V						tissue
107	× 6	ř 64	67	72	79	81
	80 ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	87	89	78	89	92
	\$\$¥ 83 \$\$	90	92	81	91	93
Nº 11 0	10° 83	91	94	84	94	95
185	9Õ	95	96	87	94	97

Deputation (% of maximum uptake concentration at day 24)

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Depuration was fairly rapid in whole fish and non-edible tissue in both treatments, with greater than 90% of accumulated [¹⁴C]-residues being eliminated from tissues after 7 days of the depuration period. Edible tissue residues in both treatments was slower to depurate with approximately 90% of [¹⁴C]-residues eliminated from tissue after 18 days of depuration.

Fluopicolide was the only radioactive residue component in fillet tissues. Fluopicolide also represented from 67.7 to 75.9% of radioactive residue in viscera. Due to the low bioconcentration factors of AF C638206 determined in this study (<200 L/kg in non-edible tissue), identification of the minor metabolites was not pursued further.

#### Biological results (Bioconcentration part):

Mean lipid contents during the 45-day study period of whole fish were  $2.9 \pm 4.1\%$  for the low treatment and  $13.0 \pm 3.8\%$  in the high treatment. At the end of the uptake phase, the lipid contents were 9.0 and 9.8% in the low and high concentrations respectively. These values are used to normalise the BCF to 5% lipid.

Substance uptake, depuration constants and bioconcentration factors are given in the table below. The maximum observed bioconcentration factors for the low treatment were 53L/kg (Day 24). 156 L/kg (Day 3), and 221 L/kg (Day 21) for edible, whole fish, and non-edible tissues, respectively. The high treatment had maximum BCFs of 47 L/kg (Day 21), 14 L/kg (Day 24), and 187 L/kg (Day 24) for edible tissues, whole fish, and non-edible tissues, respectively. Due to the variability in tissue concentrations and the rapid bioconcentration, the steady-state values were based on the average concentration of the Day 18, 21, and 24 time points.

Contraction of the second seco		o an	4	<u> </u>	,	
	0.8 μgq		ridinyi]-		6- ¹⁴ C-pyric	
	∫× ∠fl	uopioclide/			opioclide/l	
	ediple	viscera 🔬	whote	~ dible	viscera	whole
	assue	tissue	د fish ک	<b>V</b> tissue	tissue	fish
Time to reach 90% of steady state (days)	n.d	ìn.d.	o [≫] 1.7 &	n.et.	n.d.	1.5
BCFss Steady-state BCF [ft] (L/kg)	n.d. 0 48	2197 @	117	¥0	175	104
Steady-state BCF [fr] (L/jeg)		\$ ³⁷¹⁹⁷ ,@			175	
Lipid content at day 24 (and of uptake)	∽n.d. "	n.d	9.0% ×	≥″ n.d.	n.d.	9.8%
Lipid normalized BCF (L/kg)	n.d	ðd.	§ 65	n.d.	n.d.	53
k ₁ Overall uptake rate constant [L kg] day 1	્યું.સ.	The second	164.4	n.d.	n.d.	152.1
k2 Overall depuration rate constant [day1]	∿ n.d.Ô	ñ.d.	©1.35	n.d.	n.d.	1.49
$BCF_{k}*(L/kg) (= k_{1}Q_{2})^{*} \qquad \qquad$	n.el.	≫n.d. ≈	122	n.d.	n.d.	102
Lipid normalised CFk* (L/kg)	≪n.d.	n.dO	68	n.d.	n.d.	57
Lipid normalised BCFk* AL/kg)	0 n.d.0	f£d.	0.51	n.d.	n.d.	0.47
n.d. = not determined $\bigcirc$						

* not calculated in the report

**WI. CONCLUSIONS:** 

The steady-state-BCF parent (based on whole  $\hat{P}$  is the 0.8  $\mu$ g/L test level is about 117 L/kg, the lipid normalized BGFss is 65 L/kg  $\hat{Q}$ 

## Assessment and concuision by applicant:

The study is reliable and the relevant endpoint for risk assessment is the lipid normalised BCF_{ss} of 654 kg.

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#### CA 8.2.3 **Endocrine disrupting properties**

Potential endocrine-disrupting properties of fluopicolide are being evaluated according to EU identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 510/2009. 

Four studies are currently ongoing;

			*		
Dossier node	Draft title	Study ID	R.	Planned sub	hission
KCA 8.2.3	<i>Xenopus</i> Eleutheroembryonic Thyroid Assay (XETA) analysis report	EBAC0095		Planned sub November 20 2 ^{re} Quarter 2	20/ 01 021 04 021 04
KCA 8.2.3	Rapid Estrogen ACTivity In Vivo assay (REACTIV) assay analysis report	VEBACO100		November 20	020 / 021 /
KCA 8.2.3	A la	EBA@101.		November 20 2 nd Quarter?	021
KCA 8.2.3	Fish Short-Term Reproduction Assay (FSTRA) study teport	EBAC 0097		Vovember 20 2 nd Quarter 2	020 /
	Adverse-outcome Reporter (RADAR) assay analysis report Fish Short-Term Reproduction Assay (FSTRA) study teport				



#### CA 8.2.4 Acute toxicity to aquatic invertebrates

#### CA 8.2.4.1 Acute toxicity to Daphnia magna

Data Point:	KCA 8.2.4.1/01
Report Author:	
Report Year:	2003
Report Title:	The 48 hour acute toxicity to the water flea, Daphnia magna, in a static system;
	AE C638206 technical
Report No:	
Document No:	<u>M-240807-01-1</u> & O ^V & O ^V & O ^V
Guideline(s) followed in study:	OECD: 202 (1992); USEPA (#EPA): 72-2 (\$982)
Deviations from current	Method: Deviations from current guideline SANCO/3029/99 rev.4:
test guideline:	Recoveries were only determined at two different concentrations in displicate
-	However, the obtained data demonstrate very good recoveries and the precision
	calculated form these data ccounts for 8 Q AE C638206) and 1 0 % (AP
	calculated form these data accounts for 8 Q(AE C638206) and 105 % (AF C653711), respectively. The method can therefore be regarded as fit for purpose.
	Study: Current Suideling: OF&D 202 (2004) ~ ~ ~ ~
	The test was performed in soft water, with a pardness of 40.98 mg/aCO3/P
	instead of 140-250 mg/L. These are not offimal conditions for Dephnia magna
	however thas been demonstrated that long term culturing (125 days) of this
	species in soft water with 50 mg CaC93 /L is possible with the effect on survival.
	Only reproduction is affected (Lewis and Maki, 1981). Therefore the results of the
	tests are considered to be reliable since the validity criteria are all met.
Previous evaluation:	ges, evaluated and accepted
	Mn DAR (2005) 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2
GLP/Officially	Yes conducted under GLPOfficially recognised testing facilities
recognised testing	
facilities:	
Acceptability/Reliability.	Yes y y y y y

### Executive summar

Ĩ An acute toxicity test was performed with water flea (Darhnia ragna) in a static system. Juvenile daphnies (less than 24 hours old), were exposed o nonitival concentrations of 0 (control) and 5.0 mg a.s./L of the test substance (hopicolide (tech) in well water (mean temperature of 19.5°C) for a 48-hour period. All treatments were in priplicate with 10 daphnics per test vessel. Test solutions were not renewed. Observations for impobility and for abnormal appearance and behaviour were performed at 3, 6, 24, and As hours. Samples of the freshly prepared test solutions from each treatment level were taken for analysis of fluoricolide (tech) and M-OI (BAOM (AE C653711)) at 0 hour. At 48 hours (study termination) old test solutions from each treatment level (pooled replicates) were analysed. Samples were taken at mid depth and did not include any extraneous materials. All samples were analysed by Gas chromatography with MS detection (GC/MS). The arithmetic mean measured concentration of fluopicolide (tech) was determined as 1.8 mg@s./L (36% of nominal concentration) over the course of the study. The study fulfils all validify criteria of the current version of OECD 202 guideline. The test was performed in softwater with advardness of 40-48 mg CaCO₃ /L instead of 140-250 mg/L. These are not optimal conditions for Daphnia magna however it has been demonstrated that long term culturing (125 days) of this species in soft water with 50 mg CaCO3 /L is possible without effect on survival. , 1981 [<u>M-669496-01-1</u>]- see section CA 8.2.4.1/03 for a Only reproduction is affected t summary of this publication). Therefore, the results of the tests are considered to be reliable since the validity criteria are all met. The substance is stable over the course of the study. Consequently, all toxicity values were calculated based on the arithmetic mean measured concentrations. No M-01 (BAM (AE C \$3711)) residues were detected in any test sample at 0 and 48 hours. Test solutions at 0 hour ranged from 35 to 37% of nominal concentration, while at 48 hours were at 36% of nominal concentration. No mortality or sub-lethal effects were observed in the control or 1.8 mg/L treatments



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

	I. MATERIAL AND METHODS:
Test material	I. MATERIAL AND METHODS:
Guideline(s) adaptation	None specified
Test species	Water flea (Daphnia magna)
Organism age/size at study initiation	Water flea ( <i>Daphnia magna</i> )
Test solutions	Limit test at 5 mg a.s. ((nominal concentration) corresponding to arithmetic mean measured concentrations of 1.8 mg a.s. L (maximum achievable concentration under test conditions). Controls: water control A stock solution was prepared in excess of solubility (5.0 mg/L) and stirred overnight to ensure maximum solubility. The stock solution was filtered and used directly as the greatment concentration. After the ultration of the primary stock solution there were po additional problems with solubility throughout the study.
Replication	No. of vessels per concentration (replicates): 3
Organisms per replicate	No. of árganisms per vessel: 10 Stanc
Exposure	Stanc Total exposure duration: 48 hours & & None & & & & & & & & & & & & & & & & & & &
Feeding during test	None None None None None None None None
Test conditions	Precording) 2
	and dusk Light intensity ca 719 lux pH: 7,3 - 7,9 Water hardness: 40 - 48 mg CaCO ₃ /L
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	Dissolved oxygen: 86@105% saturation Conductivity, 270 - 280 µScm
Parameters / Measured / Observations	abnormal appearance and behaviour were performed at 3, 6, 24, and 48 hours.



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

Data analysis Not applicable. no effects we	te observed during the study	
II RESULTS	AND PASCUSSION:	
Validity criteria (OECD 202, 2004)	Required Required	Obtained
Immobilisation and sub-lethal effects in control dur test		
Dissolved oxygen concentration at the end of the te	$ st \approx 2$ $ st \approx 3$	97.4 mg/L ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

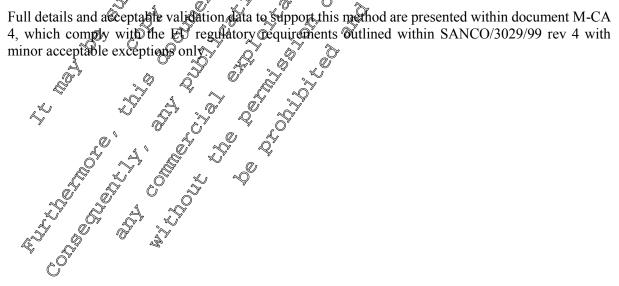
Analytical results:

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

Ø

Nominal Concentration (mg a.s./L)	Day 0 Measured Concentration (mg.a.s./L)	> ⁷⁰ ∞ Nomina	Day 2 Measured Concentration (mg as L)	Day 2 %	Arithmetic mean Measured Concentration (mg a.s./L)	% Mean Measured Concentration
5.0	1.77 5 × 1.840 5	35 A	1.80°	36 °	1.80	36
	\$' \$'	\$ ~		A°.		

Full details and acceptable validation data to support this method are presented within document M-CA





Biological results:

Observations

mg/L.	
Č Ű	
Immobility S	

Exposure time (hours)	0	3 6 8		48 ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~
Arithmetic mean measured conc. (mg a.s./L)	No of immobilized (%)	No of immobilized (%)	No of immobilized	No of Samolaized
Control	0 (0)			
1.8	0 (0)			°∂⁄(0)
		THI. CONCLUSIONS;		

	MI. CONCLUSIONS: A O K	
The study meets the validity		
concentrations are:		

EC50 48 hours (95% C.I.):	0> 1.8 mg a.s. L (not applicable)
LOEC:	
lowest concentration with affect	
NOEC:	
highest concentration without adverse effect	s 1.&mg as. / L 5

Assessment and conclusion by applicant: The study is reliable and the relevant endpoint for the Luopic of the risk assessment is the EC₅₀> 1.8 mg a.s./L.



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

Executive summary

An acute toxicity test was performed with water fiea (*Duphnia hagna*) in a static system. Juvenile daphnids (less than 24 hours old), were exposed to nominal concentrations of 0 (negative control) and nominal concentrations of 40, 18, 32, 56, 100, 180, 320, 560 and 1000 mg/L of the fest substance M-01 (2,6-dichlorobenzamide (BAMU) in decilorinated tas water (from tempetature (F21°C) for a 48 hour period. All treatments were fit fripleate with 10 daphnia/magna) in a static system. Juvenile diagonality of a design of



I. MATERIAL AND METHODS:

Test material	M-01 (2,6-dichlorobenzamide (BAM))
	Batch FUX001000/FUN81G02C
	Purity 99.5 % w/w
Guideline(s)	None specified
adaptation	
Test species	Water flea (Daphnia magna)
Organism	First instar neonates, less than 24 hours old
age/size at	
study initiation	
Test solutions	Batch FUX001000/FUN81G02C Purity 99.5 % w/w None specified Water flea (<i>Daphnia magna</i>) First instar neonates, less than 24 hours old Nominal concentrations: $10 - 18 - 22 - 56 - 100 - 180 - 320 - 560$ and 1000 mg/L Controls: Dechlorinated tap water Each jar contained approximately 200 mL of test solution
i est solutions	Nominal concentrations: $10 - 18 - 52 - 56 - 100$ 180 - 320 560 and 1000 mg/L Controls: Dechlorinated tap water, Each jar contained approximately 200 mL of test solution
	Controls: Dechlorinated tap water, Each jar contained approximately 200 mL of test solution
D 1: +:	
Replication	No. of vessels per concentration (replicates). $2 \neq 7 \neq 7 \neq 7$
- ·	No. of vessels per control (deplicates): 2
Organisms per	No. of organisms per vessel: 10° Q° Q° Q° Q° Q°
replicate	
Exposure	No. of vessels per control (replicates): 2 No. of organisms per vessel: 10 Static Total exposure duration: 48 hours None
	Total exposure duration: 48 hours $\sqrt{2}$ $\sqrt{2}$ $\sqrt{2}$
Feeding during	No. of vessels per control (ceplicates): 2 No. of organisms per vessel: 10 Static Total exposure duration: 48 hours None Temperature 21°C Photoperiod: 16 hours Light / 8 hours dark with gradial intensity changes at dawn
test	Temperature 21°C
Test conditions	Temperature 21°C 7 7 7 7
	and a a a a a a a a a a a a a a a a a a
	Photoperiod: 16 hours fight / 8 hours dark with gradical intensity changes at dawn and
	Water hardigess: 155 mg \Re CaCiO ₃ /L \sim \sim \sim \sim
	Water harddess. J 32 hig as CaCO ₃ /L 3 mg/L (93%) Dissolved oxygen: 8.0 mg/L (90%) $\stackrel{<}{\sim}$ 8.3 mg/L (93%) Alkalinity: 128 mg/L as CaCO ₃ /L 3
	Alkalintry: 128mg/12 as CaCO3/Lo
Parameters &	Immobilization or adverse reactions to exposure were recorded at 24 and 48 hours
Measured	after the start of exposure
Observations	Water temperature was recorded daily throughout the test. Dissolved oxygen
	conceptrations and and were recorded a the start and termination of the test. In
¢Q [™]	addition the temperature was recorded in one control vessel every hour
Chemical	Water satisfies were taken from the control and the test groups at 0 and 48 hours for
analysis	quantitative analysis of the test item by High Performance Liquid Chromatography
¢	(HPET) with UV detection
Data analysis	The C. walues and associated Confidence limits at 24 and 48 hours were calculated
	by the paying like the adverage mathed using the Tay Cale computer software
4	by the maximum-incontrol province to the toxe are computer software
Q*	
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s.	
Ŏ	
L.	
, Q ^v , Ô	
S R	
E S	
¢0 ^v	Dissolved oxygen: 8.0 mg/L (90%) 48.3 mg/L (93%) Alkalunty: 128 mg/L as CaCO ₃ /L Immobilization or adverse reactions to exposure were recorded at 24 and 48 hours after the start of exposure Water temperature was recorded at the start and termination of the test. In addition, the temperature was recorded in one control vessel every hour. Water samples were taken from the control and the test groups at 0 and 48 hours for guantitative analysis of the test item by High Performance Liquid Chromatography (HPEC) with UV detection The EC ₃ values and associated confidence limits at 24 and 48 hours were calculated by the maximum-likelihood probit method using the ToxCalc computer software package (Version 59.236, 1999).
\bigcirc	



II. RESULTS AND DISCUSSION:

btained 。	Required	Validity criteria (OECD 202, 2004)
obilisation no	$\leq 10\%$ 0 % data of the second secon	Immobilisation and sub-lethal effects in control during test
L - 8.10mg/L	\geq 3 mg/L	Dissolved oxygen concentration at the end of the test
L – 8.10m	\geq 3 mg/L \bigcirc .0	Dissolved oxygen concentration at the end of the test

Analytical results:

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

Nominal conc. (mg/L)	Arithmetic mean (mg/L)	% of nominal concentrations (mean of 2 measurisements) 0 hour 48 bours 7
10	(ing/L) 10.8	
18	19.7	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$
32	55.1	<u> </u>
56	58.9	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$
100		
180	188 💞 🌾	
320	336	
560	578	
1000	1019 \$	

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

Immobility

Exposure time (hours	$\begin{array}{c} & & & & \\ & & & & \\ & & & & \\ & & & & $	48
Nominal conc.	No of immobilized (%)	No of immobilized (%)
Control A		0 (0)
10 4		0 (0)
18		0 (0)
$\begin{array}{c} 18 \\ \hline 32 \\ \hline \end{array} $		0 (0)
56 7 7 100 7 7 180 7 7 320 7 7	ي چ 0 (0)	0 (0)
100 × × A	0 (0)	0 (0)
	5 (25)	13 (65)
320	15 (75)	18 (90)
560 [©]	19 (95)	20 (100)
1000	20 (100)	20 (100)



III. CONCLUSIONS:

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

EC50 48 hours (95% C.I.)	:	180 mg / L (150	mg/L - 210	mg /L)	N O
NOEC: highest concentration with	out adverse effects	100) mg / L	^A	
			4		
Assessment and conclu	<u>sion by applicant</u> :	Ĉ		Ĵ	
The study is reliable and	the relevant endpoir	nt for risk assessme	ent is the EC	50 of 180 mg	
	1	A CHARLES AND A CHARLE AND A CHARLE AND A CHARLE AND A CHARLE AND A CHAR			
Data Point:	KCA 8.2.4.1/03		<u>, 0</u>	~~ ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	\emptyset .
Report Author:		<u>k, 6°</u> .3°		<u>, 6, 4</u>	
Report Year:	1981		4.8	S.	4
Report Title:	Effects of water hardr laboratory culture	ness and diet on proc	betivity of da	phnia magna	strans vin 4°
Report No:	M-669496-01-4		~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~		
Document No:	<u>M-669496-06-1</u>			J L	× O
Guideline(s) followed in study:	not applica Qe			CUM	
Deviations from current test guideline:	not applicable				· ¥
Previous evaluation:	Noșnot previousla sul	bmitted			
GLP/Officially	No, not conducted un	er GLP Officially r	ecognised tes	ting facilities	
	No, not conducted un				
Acceptability/Reliability:	Ages 6 m	<u>~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ </u>		-	
Executive summary	Ales 6 2 2				

Executive summary

A Contraction of the second se This publication is included as it provides the additional information and justification that the study by 2003 (M-200807-01-1) see above CAC 8.2.4.001 is a valid study. The publication addresses the effects of different diet and water hardness, along and in combination in long-term (multigeneration) studies The summary below focuses only on the effects of water hardness.

The effects of diet approvate hardness, abone and in combination, on life history characteristics of Daphnia magna were determined in two laboratory tests. Number of young on the first day of reproduction, total young and the number of generations were greater with increasing hardness. At the maximum test hardness of 350 mg/L (as CaC $Ø_3$), approximately 65 percent more young were produced than at the lowest hardness of 50 mg/L (as CaC Q_3) Furthermore, time to sexual maturity was about one day shorter in the harder culture water. The only parameter not affected by water hardness is survival,

aay sporter in the Marder millure water. The only with around 100% in all Mardness conditions,



I. MATERIAL AND METHODS:

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

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

Water hardness

The chronic effects of four water hardnesses, 50, 125, 225 and 350 mg/L as Caco3 or D. megna productivity were determined for over 125 days (multi-generations). Filtered well water was blended with a deionized water to the desired test hardnesses. A supply of each hardness water was made prior to test initiation and used throughout the study to minumize variability in hardness during testing. Hardness of the well-water used in this study averaged 350 mg/l ($\pm \sqrt{5D} = 10^{\circ}$ mg/k). Before use total hardness of the test water was confirmed using a standard titration nothod. For each test hardness, three 1-L Pyrex beakers were utilized as first chambers At test initiation, 809 mL of the first water and 5 daphnids (<24 h old) were added to the chamber. The test chambers overe then covered with glass to prevent evaporation of the test. waters The test waters were renewed three times a week for the test duration. Daphnids were fed darly a combination of green algae Selenastrum capricornutum Printz) suspension and a trout chow dehydrated affalfa mixture. Time to sexual maturity, adult survival and number of newborns observed on the first day @reproduction were pronitized daily during the study. On the first day of reproduction after enumeration, all adults and all but five juveniles were discarded. The five remaining juveniles were then added to acleaned test chamber containing water of the same total hardness from which they were with grawn of initiate the next generation. The data from this test as well as that of the second test were staristically analysed using t-tests.

> Ø H. RESULTS AND DESCUSSION:

Dissolved oxygen exceeded 7. (King/L) 78% saturation) althe beginning of the tests. The pH ranged from 6.8 to 7.1 and the mean water temperature was 21° C/ \pm SD \oplus 2° C).

Total hardness 1	Total young	Mean number	Mean days to sexual maturity	Mean brood size ²	Adult survival %
چې 50	5259		8.7 (0.5) *	7.2 (0.3) *	98
125	5629	۵ [°] 48	8.3 (0.2) *	7.8 (0.1) *	100
225	7542	52	7.8 (0.04) *	9.7 (0.2) *	100
350	8600*3 5	~\$ ⁵⁵	7.6 (0.05) *	10.9 (0.4) *	100

As mg/b/CaCO

² Young observed on first day of reproduction per adult

³ Differs from 543

* = 3 gnificant difference at 0.05 level; () = \pm S.D.

Water hardness



0

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

The daphnids used in the hardness test were obtained from a culture unit where the hardness of the water averaged over 300 mg/L as CaCO₃. Therefore, the results observed in this study at the higher hardness may reflect adaptation by the daphnids to hard water. Other strains of D. magna grown and adapted to lower hardnesses may not react in the same way.

MI. Conclusions

High water hardness (225 or 350 mg (aCO₂/L) increases significantly the reproduction performance of Daphnia magna. The only parameter not affected by water hardness is survival, with around 100% ¢°°¢ Ô in all hardness conditions (50 to 3 mg CaCO₂/L). °

n

J. The publication provides supporting information to evaluate the valuate the study by Young and Abedi 2003 (M-240807-01-1) see above CAS 2.4. 101. It temonstrates that Daphnia survival is not affected by low water hardness in long-term studies, therefore no impact is expected in an acute study.

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CA 8.2.4.2	Acute toxicity to an additional aquatic invertebrate species	
CA 0.2.7.2	Acute toxicity to an additional aquatic invertebrate species	

Data Point:	KCA 8.2.4.2/01
Report Author:	
Report Year:	
Report Title:	AE C638206 - Acute toxicity to Eastern oysters (Crassostrea virginica) under
	AE C638206 - Acute toxicity to Eastern oysters (Crassostrea virginica) under flow-through conditions
Report No:	C038657
Document No:	<u>M-225445-01-1</u>
Guideline(s) followed in	USEPA (=EPA): FIFRA 72-3 (1985), OPPTS 859 1025 draft (1996)
study:	<u>v</u> <u>Q</u> <u>Q</u> <u>X</u> <u>X</u>
Deviations from current	Method: Deviations from current guideline SANCO/3029/99/rev.4:
test guideline:	Limited sets of validation recoveries were analysed. However, the average
	recoveries were within the acceptable range of 70-110% and the RSD values were
	Limited sets of validation recoveries were analysed. However, the average recoveries were within the acceptable range of 70–110% and the RSD values were below 20%. The analytical method can be regarded as fit for purpose.
	Study: Current Guideline: OCSPP 85691025 (1996) 2 C
	No deviations
Previous evaluation:	yes, evaluated and accepted a solution of the
	in DAR (2005) $\sqrt{7}$ $\sqrt{7}$ $\sqrt{7}$ $\sqrt{7}$ $\sqrt{7}$ $\sqrt{7}$ $\sqrt{7}$
GLP/Officially	Yes, conducted under GLP/Q Acially recognized testing facilities
recognised testing	
facilities:	
Acceptability/Reliability:	Yes \mathcal{A}
	KQX 8.2,4.2/03 20 6 6 6 7 6 7 7 7 7 7 7 7 7 7 7 7 7 7 7
Data Point:	KQA 8.2, 4.2/03
Report Author:	
Report Year:%Report Title:%	Statement - Gertificate of an Hysis to fluopicolide acute tookcity study on Eastern
Report The.	overers (Disonne, 2003; M 225445-01-1)
Report No:	M-634698-01
Document No:	M-634998-0147 2 2 2 2
Guideline(s) followed in	
study:	
	not applicable
test guideline:	
Previous evaluation:	No jot previously submitted of O
GLP/Officially	not appreable of the second seco
recognised testing	
facilities:	
Acceptability Reliability:	\mathcal{O} \mathcal{O} \mathcal{O} \mathcal{O} \mathcal{O} \mathcal{O}
× .1 `	
	Ď k v
y & A	
A N A	× ×
A A	not applicable
GLP/Officially recognised testing facilities: Acceptability Reliability	
\lor	



Executive summary

An acute toxicity test was performed with eastern oyster (Crassostrea virginica) under flow-through conditions. The oysters (similar age), were exposed to nominal concentrations of 0.39, 0.65, 1.1, 1.8 and 3.0 mg a.s./L. of the test substance fluopicolide in unfiltered natural seawater (salinity of 32% 33%) and temperature of 20°C - 21°C.) for a 96-hour period. Negative control (natural filtered sea water) and a solvent control (0.100 mL/L dimethylformamide) were included. All treatments include 20 organisms per test vessel. Biological observations were made at test initiation and at each subsequent 24-hour interval until termination of the test. All samples were analysed for fluopicality using gas? chromatography with electron capture detection (GC/ECD) at test initiation and after % hours. Analysis of the test preparations at 0 and 96 hours were between 2% and 100% of the nominal concentrations. Results reported are based on mean measured concentrations of the test substance. The mean measured concentrations were: 0.33, 0.64, 1.0, 1.6 and 2.6 mg as./L. The study fulfils all validity criteria of FIFR Guideline 72-3. No sublethal effects were observed among any of the prosed oysted throughout the test concentration range. A significant difference was detected between the dilution water control and the solvent control data. Therefore, treatment data was compared to solvent control data. At test termination, one dead oyster was observed in the 0.64 mg a.s. treatment level. No morganity was observed among oysters at any of the remaining weatment levels tested or the controls. The EC 50 at 96 hours for shell growth of eastern oysters (Crassostrea virginica) has been investigated and gives avalue of > 2.6 mg a.s./L. The No Observed Effect Concentration 13 2.6 mg a.s./L.

	QI. MATERIAL AND METHODS:
Test material	Fluopicolide Lot No: 2050190//PP241024/2+ Purity 97.7 % w/w &
Guideline(s) adaptation	None specified
Test species	Eastern oyster (Crassostreavirginica)
Acclimation	10 Grys prior testing. During this period the salingy was progressively increased from 15 to 32‰.
Q.	No mortality during the 7 days before test initiation
Organism age/size at study initiation	The ovsters were of similar agoind had a mean value height of 36 ± 4.6 mm (N = 30^{10} .
Test solutions	Nominal concentrations: 0.39 - 0.65 - 1. 0- 1.8 and 3.0 mg a.s./L Mean measured concentrations: 0.35 - 0.64 - 1.0 - 1.6 and 2.6 mg a.s./L Control natural unfiltered sea water. Solvent control: 0.100 mL/c dimetaylformamide
Replication	No. of vessels per concentration (replicates): 2 No. of vessels per control (replicates): 2
Organisms per replicate	No. of organisms percessed 20
Exposure	Flow through (flow rate of the recirculating test solution was 1.75 L/minute or about 5.25 L per oyster per hour) Total exposure duration: 96 hours
Feeding during	Concentrated volumes of algae (<i>Tetraselmus maculate</i>) suspension (approximately 0^7 cells/mL) were added to each test aquarium 3 times daily
tests	



T / 11/-	Temperature: 20 °C – 21 °C					
Test conditions	Photoperiod: 16 hours light / 8 hours dark with gradual intensity changes at dawn					
	and dusk					
	pH: 7.5 – 8.1.					
	Dissolved oxygen: 4.5 mg/L (60% saturation)– 7.5 mg/L (aeration was initiated at 10					
	the 72-hour observation interval to raise and maintain dissolved oxygen levels at					
	60% of air saturation)					
	Salinity: 32 ‰ – 33 ‰					
Parameters	Biological observations (e.g., visible abnormalities, such as excessive mucous production or a failure to siphon and feed, as evidenced by a lack of fecal and					
Measured /	pseudofecal production) and observations of the physical characteristic of the test					
Observations	solutions were made at test initiation and at each subsequent 22-hour intervenuntil					
	termination of the test. Sublethal effects were determined by a comparison of the					
	performance and appearance of the exposed oysters to that of the control oysters.					
	After 96 hours new shell growth was measured interoscopically to the nearest 0.1					
	mm. O L C A A C.					
	Immobilization or adverse reactions to exposure were recorded at 24 and 48 hours					
	after the start of exposure γ γ δ ζ γ δ					
	the pH, temperature salinity, and dissolved oxygen concentration were measured daily in each replicate aquatium. In addition, temperature was monitored					
	continuously in one replicate of the highest concentration					
Chemical	All samples @ere analysed for fluppicolide using gas chromatographs with electron					
analysis	capture detection (GC/ECD) at test initiation and after 96 hours					
Data analysis	During the exposure of eastern oysters to fluopeolide no concentration tested					
	caused > 50% reduction; the refore, the EC_{50} value was empirically estimated to be					
	greater that the bighest test concentration.					
	The No-Observed-Effect Concentration (NOEC) for the 96-hour exposure period					
L	Was statistically determined by using Williams' Test					
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	9 9. Results and Discussion:					

.0		A. RESULTS	AND DISC	USSION:	7,
$\ll$	~	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~		0,,«	)

Validity criteria (OCSPP 850.1025, 1996) O Required O	Obtained
Mortality and sublethal affects of control during test $0^{1} \leq 40\%$	0 %
New shell growth in Control by sters 2mm	2.2 mm (0.6 mm, SD)

Analytical results: Analysis of the test preparations are 0 an 096 hours (see table below) were between 82 % and 100 % of the nominal concentrations. Results reported are based mean measured concentrations of the test substance.

Nominal Concentration (mg a.s./L)	(mg a.s. (E)	Nominal	Day 0 Measured Concentration (mg a.s./L)	Day 4 Measured Concentration (mg a.s./L)
Control	V 0.028 x ~	) -	< 0.028	< 0.028
Solvent Control		-	< 0.028	< 0.028
0.39	\$.33 . L	85 %	0.34	0.32
0.65	0.64	98 %	0.62	0.66
1.1	1.0	91 %	1.1	0.94
1.8	1.6	88 %	1.7	1.5
3.0	2.6	86 %	2.7	2.5



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

**Biological results:** 

#### Observations

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

Nominal conc. (mg a.s./L)	Mean Shell deposition at 96h, mm (SD)
(ing a.s./1)	
Control	
Solvent Control	
0.33	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$
0.64	
1.0	$\begin{array}{cccccccccccccccccccccccccccccccccccc$
1.6	
2.6	$\begin{array}{c} & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & & \\ & & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & &$
NA – not applicable Immobility	

At test termination, one dead byster was observed in the 9.64 mg a.s. & treatment level. No mortality was observed among oysters at any of the gemaining treatment levels tested of the controls. 

III. CONCLUSIONS:

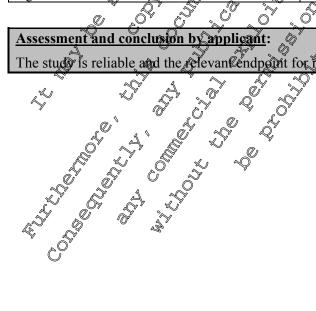
The study meets the validity criteria and the endpoints based of mean measured concentrations are:

	×, ×		0	
EC50 % hours (95%		2 40°.	2.0 >>>(not	6 mg/a.s. /L applicable)
NOEC:				
highest concentration	without adve	rse effects	2.0U	ng a.s. /L
	R N		Y Á	- Ar

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K.

The study is reliable and the relevance of the relevance of the state of the the the term  $E_{50} > 2.6$  mg a.s./L.





Data Point:	KCA 8.2.4.2/02
Report Author:	
Report Year:	2003
Report Title:	AE C638206 - acute toxicity to mysids (Americamysis bahia) under static conditions
Report No:	M-220513-01-2
Document No:	<u>M-220513-01-2</u>
Guideline(s) followed in study:	USEPA (=EPA): FIFRA 72-3 (1982)
Deviations from current test guideline:	Method: Deviations from current guideline SANCO 3029/99 rev4. Limited sets of validation recoveries were analysed. However, the average recoveries were within the acceptable range of 00–110% and the RSD values were below 20%. The analytical method can be regarded as fit for purpose. Study: Current Guideline: ACSPP 850.1035 (1996) The oxygen saturation has to be between 60 and 100% during the whole test. The saturation during the test dropped below 60% (4.1 mg/L for 2S) and 32‰ saturity) at 96h in all test vessels. The lowest saturation was around 44 %. The saturation in the highest test concentration was againficantly lower than in the other groups at 72h. The recommended saturity should be 20‰ but it was 32% in the test. The maximum solvent concentration should be 0.1mL/L but it was 0.25 mL/L during the test. The pH was slightly out of the recommended range 7.5 8.5, it was 7.4 in one replicate of 1 concentration at 96h. This slight deviation is not considered relevant. The validity criterion of the guideline is met but influence of the poor oxygenation on the test results cannot be ruled out as the saturation decreased with increasing concentrations. Consequently, the toxicity of floppicoline may be over-estimated.
Previous evaluation:	yes, evaluated and accepted of a start of the second secon
~	in Drak (2005) O S S S
GLP/Officially	Yes, conducted under GLSP/Officially recognise diesting facilities
recognised testing	
facilities:	
Acceptability/Reliability	

An acute foxicity test was performed with mysids *(imericanysi Bahia)* in a static system. The mysids (less than 24 hours ofd) were exposed to pominal concentrations of 0.65, 1.1, 1.8, 3.0 and 5.0 mg a.s./L of the test substance fluopicolide in filtered natural seawater (salinity of 32‰ and temperature of 25°C  $-26^{\circ}$ ) for a 96-hour period. Negative controls with natural filtered sea water and a solvent control with 0.250 ml/L dimethyl@rmanide were tes@d. AlD treatments include 10 organisms per test vessel. Biological observations were made at test initiation and at each subsequent 24-hour interval until termination of the test. All samples were analysed for fluopicolide using gas chromatography with electron capture detection (QC/ECD) at sest intriation and after 96 hours. Analysis of the test preparations at 0 and 96 hours were between 82% and 91% of the nominal concentrations. Results reported are based on mean measured concentrations of the test substance. The mean measured concentrations were: 0.57, 0.96, 1.6, 2.7 and 4.2 mg a.s./L. The study fulfils all validity criteria of the f 3.2 mg a.s./L / 2.7 - 4.2 mg a.s./L). The No Observed Effect Concentration is 1.6 mg a.s./L. U.S. EPA's Pesticide Assessment Guidelines (Subdivision E, Series 72-3; U.S. EPA, 1982). All surviving mostds exposed to the 2.7 and  $\pounds$ 2 mg a.s./L treatment levels were lethargic. The LC₅₀ 96 hours (95% C.L) of the test material to mysids (*Americamysis bahia*) has been investigated and gives a value of 3.2 mg a.s.



#### I. MATERIAL AND METHODS:

Test material	Fluopicolide Lot No 2050190//PP241024/2 Purity 97.7 % w/w
Guideline(s) adaptation	None specified
Test species	Mysid (Americamysis bahia)
Organism age/size at study initiation	Fluopicolide         Lot No 2050190//PP241024/2         Purity 97.7 % w/w         None specified         Mysid (Americamysis bahia)         The mysids were < 24 hours old.
Test solutions	Nominal concentrations: 0.65, 1 T, 1.8, 3.0 and 3.0 mg a.s./b Mean measured concentrations 0.57, 0.96,1 b, 2.7 and 4.2 mg a NL Controls: natural filtered sea water Solvent control: 0.250 mLP dimethylformamide No. of vessels per concentration (replicates): 2
Replication	No. of vessels per control (replicates): 2
Organisms per replicate	Controls: natural filtered sea water Solvent control: 0.250 mL/L diracthylformamide No. of vessels per control (replicates): 2 No. of vessels per control (replicates): 2 No. of organisms per vessel: 10 Static Total exposure duration: 96 hours Live brine shring naupin ( <i>Artemia salina</i> ) were added to each test vessel once daily
Exposure	Static Total exposure duration: 96 hours
Feeding during test	Live brine shrine naupin (Artemia salina) were added to each test vessel once daily
Test conditions	Temperature: 25 $\%$ – 26 $\%$ Photoperiod: 16 bours eight / Shours dark with gradual intensity changes at dawn and dust at 760 94 footcandles pH: 7.4 – 8.0 Dissolved oxygen 3.0 mg/L – 6.7 mg/L (40% saturation corresponds to 2.7 mg/L) Solvinity 32 ‰, Recommended salinity in the guideline is 20‰.
Parameters Meastred / Observations	Biological observations were made at test initiation and at each subsequent 24-hour interval until termination of the left. pH temperature salinity, and dissolved oxygen concentration were measured daily in each test yessel. In addition, temperature was monitored continuously in one replicate of the highest concentration.
Chemical of analysis	Ar sample's were analysed for fluopicolide using gas chromatography with electron capture detection (GQECD) at test initiation and after 96 hours
Data aparysis	The CC ₅₀ was calculated by a computer program (Stephan, 1982) by comparison of the three statistical methods: moving average angle analysis, probit analysis, and nonlinear interpolation with 95% confidence limits calculated by binomial probability. The 96 hour LC ₅₀ value was estimated by non-linear interpolation, with corresponding 95% confidence intervals calculated by binomial probability. The No-Observed-Effect Concentration (NOEC) during the 96-hour exposure period was also determined but the method is not reported.



#### **II. RESULTS AND DISCUSSION:**

Validity criteria (OCSPP 850.1035, 1996)	Required	<b>Obtained</b>	8
Mortality and sublethal effects in control(s) during test	<u>≤</u> 10%	0 % in control, 5 % in solvent control	Ĵ,

#### Analytical results:

Analysis of the test preparations at 0 and 96 hours (see table below) were between § the nominal concentrations. Results reported are based on mean measured concentrations Å 10 1 substance.

Nominal Concentration (mg a.s./L)	Day 0 Measured Concentration (mg a.s./L)	Day 4 Measured Concentration (mg a.s./D)	Mean measured Concentration	% of Nominal 2
Control	< 0.046	< 0.045	Y ~ A	
Solvent control	< 0.046	< 0045		
0.65	0.55	638 4 . 4	Q57 8 5	
1.1	0.93	0.99	0.96 2	87
1.8	1.6		1.6 0	91 6
3.0	2.7	2.7	2.7	80 0
5.0	4.2 S	4.1 2 4	4.2	×84 ×
	~ ~ ~	à õ õ		

Full details and acceptable validation datage support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4 with exposed to the 2.7 and 4 2 mg a 5/L treatment levels were lethargic. minor acceptable exceptions only.

Biological fesults:

Observations

All surviving mysids

Immobility

Exposure time (hours)	240 0 0 0		72	96
Arithmetic mean measured conc. (mg a.s./L)	Cumprative Mortality (%)	Cumulative Mortality (%)	Cumulative Mortality (%)	Cumulative Mortality (%)
Control		0	0	0
Solvent Control		<u></u>	0	5
0.57		0	0	0
0.96	Ĩ, Ĉ	0	0	5
46	0 🗳	0	0	0
2.7 °	0	0	0	15
4.2	0	35	45	95



### **III. CONCLUSIONS:**

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

The study meets the vand	ity criteria and the ef	
LC50 96 hours (95% C.I.)	:	3.2 mg a.s. /L (2.7 – 4.2 mg a.s./L)
NOEC: highest concentration with	out adverse effects	1.6 mg a.s. /L
Assessment and concl	usion by applicant:	
The study has deficie	ncies which may h	ave resulted in toxicts over-estimation. The relevant
endpoint for the fluopic	colide risk assessmer	nt is the $2C_{50}$ of 3.2 mg a.s./L.
CA 8.2.5 Long	-term and chronic	toxicity to aquatic invertebrates
СА 0.2.5.1 Керг		lopment toxicity to Daphnia magna
Data Point:	KCA 8.2.5 1901	
Report Author:	2002	
Report Year:	2003 Q	de of the water dea (Dephnia nagna) a static renewal
Report Title:	system AE C638206	technical 97.7 percent w/w
Report No:	B004236 0	
Document No:	<u>M-241191-01-6</u>	
Guideline(s) followed in	OECD; 211 (1998); (	SEPA@=EPA 72-4 (1986)
study:		
Deviations from current	Method: Deviations f	ronocurrent guideline SANCO/3029/99 rev.4:
test guideline:	Limited sets of Valida	tion receiveries were analysed. Rowever, the average
	recoveries were with	n the acceptable range of $70\sqrt{100\%}$ and the RSD values were
		ytical method can be regarded as fit for purpose with regard
	to this toxicity study.	× 05 m 211 (2012)
	Nastration	One: OECD 21 (2012)
Dravia Vavaluation &	No deviations	
Previous evaluation: GLP/Officially recognised testing facilities: Acceptability/Reliability:	yes, evaluated and ac	× & A
GLP/Officially	Yes, winducted under	CLP/Official@recognised testing facilities
recognised testing	1 N 8 N	
facilities:		
Acceptability/Reliability:	Stes Q	
Jon v		
Acceptability/Reliability:		A A A A A A A A A A A A A A A A A A A
		$\mathcal{O}^{*}$
	U N Q	
	)* <i>*</i> ~9	
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y & A	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	
ST & B	X	
¢Q°		
$\checkmark$		



Data Point:	KCA 8.2.5.1/02	
Report Author:		
Report Year:	2018	
Report Title:	Statistical re-evaluation of fluopicolide daphnia reproduction study (Young &	Ő
	Abedi, 2003; M-241191-01-1)	F
Report No:	M-617757-01-1	
Document No:	<u>M-617757-01-1</u>	
Guideline(s) followed in	none a a a a a a a a a a a a a a a a a a a	*~
study:		2
Deviations from current	Not applicable	a
test guideline:		Å
Previous evaluation:	No, not previously submitted $\sqrt{2}$	Oʻ
		1
GLP/Officially	not applicable	
recognised testing		
facilities:		
Acceptability/Reliability:	Yes O V V V V A	

#### **Executive summary**

A chronic toxicity of fluopicolide (tech) test was performed with water fles (Dapthia magna) in a semistatic system. First-instar neonates daphards (less that 24 hours old) were exposed to nominal concentrations of 0.028, 0.056, Q11, 0.23, 0.45 and 0.90 mg/L thiopicofide in well water (mean temperature of 20.4°C) for 21 days. A water control was included. All reatments consisted of ten individual replicates with a single daphild per test chamber and there group replicates with five daphnids per test chamber. Observations for death immobility, and abnormal appearance or behaviour were performed daily on each replicate chamber (both individual and group). Statting of day 6, all individual daphnids were checked daily for initial nonate production. Samples of the freshly prepared test solutions from each treatment were taken at test institution and each week on Friday. Samples from old test solutions from each treatment were taken at study termination and each week on Monday. Fluopicolide and the metabolite M-01 (BAM), were measured by Gas Chromatography with Electron Capture Detector (GC&CD). No residues of fluopicolide were preasured in the control above the limit of quantification (LOQ = 0.025 mg a.s./Ly. There were no NG01 (BOAM) residues found in any test solution greater than the limit of quantification (0.025 mg a.s./L). The arithmetic mean measured concentrations are 0.024, 0.047, 0.096, 0.19, 0.37 and 0, 74 mg a.s./L. The statistical analysis of the results presented in the report does not fulfil current standards and leads to inconclusive results for the most sensitive endpoint: mumber of off-spring per adult. Therefore, a new analysis of the results has been performed according to the OECD guideline 211 (2002). No sub-lethal effects were observed in any of the treatments during the study? Survival of adult daphnids was not significantly reduced at any of the treatment concentrations when compared to the control group. There were no immobile or dead neonates observed in any reatment lever. Total living neonates per adult were significantly reduced at the 0.048 0.37, and 0.74 mg/L mean breasured concentration when compared to the control group. In a static renewal exposure for 21 days, fluor colide has chronic effects on the reproduction of the water flea De magna at a mean measured concentration of 0.74 mg/L. No effects are observed at a mean measured concentration of 0.370 g a.s./L. However, the overall NOEC is 0.19 mg a.s./L on the basis of off-spring production



#### I. MATERIAL AND METHODS:

Test material:	Fluopicolide (tech.) Lot/batch: 2050190//PP241024/2 Code No: AE C638206 00 1C99 0005 Purity 97.7% w/w None specified Water flea ( <i>Daphnia magna</i> ) 1 st instar neonates less than 24 h old
	Lot/batch: 2050190//PP241024/2
	Code No: AE C638206 00 1C99 0005
	Purity 97.7% w/w
Guideline(s)	None specified
adaptation	
Test species:	Water flea (Daphnia magna)
Organism Age	1 st instar neonates less than 24 h old $\bigcirc$
at	
Experimental	
Start:	
Test solutions:	Nominal concentrations: $0.028^{\circ}$ 0.056 - 0.14 - 0.28 - 0.45 $\circ$ 0.90 mg a.6/L
	Arithmetic mean measured concentrations $9.024 - 0.048 - 0.06 - 0.19 - 0.37 - 0.048 - 0.096 - 0.19 - 0.37 - 0.000 - 0.000 - 0.000 - 0.000 - 0.000 - 0.000 - 0.0000 - 0.0000 - 0.0000 - 0.0000 - 0.0000 - 0.0000 - 0.0000 - 0.0000 - 0.0000 - 0.0000 - 0.0000 - 0.0000 - 0.0000 - 0.0000 - 0.0000 - 0.0000 - 0.0000 - 0.0000 - 0.0000 - 0.0000 - 0.0000 - 0.0000 - 0.0000 - 0.0000 - 0.0000 - 0.0000 - 0.0000 - 0.0000 - 0.0000 - 0.0000 - 0.0000 - 0.0000 - 0.0000 - 0.0000 - 0.0000 - 0.0000 - 0.0000 - 0.0000 - 0.0000 - 0.00000 - 0.00000 - 0.00000 - 0.00000 - 0.00000 - 0.00000 - 0.00000 - 0.0000000 - 0.00000 - 0.000000 - 0.00000 - 0.00000 - 0.00000 - 0.0000000 - 0.00000000$
	0.74 mg a.s./L $O^{\vee}$ $O^{\vee}$ $A^{\vee}$ $A^{\vee}$ $A^{\vee}$ $A^{\vee}$ $A^{\vee}$
	Controls: water control
	Arithmetic mean measured concentrations: $0.024 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.048 - 0.04$
Replication:	No. of vessels per concentration (replicates): 10 ordivioual replicates and 3 group
	No. of vessels per control (replicates). 10 individual replicates and 3 group
· · · ·	replicates $\sqrt{2}$ $\sqrt{2}$ $\sqrt{2}$ $\sqrt{2}$
Organisms per	No. of organisms per vessel. 1 for the individual replicates and 5 for the group
replicate:	replicates a first of the second seco
Exposure:	Semi-static test renewal every Monday, Wednesday, Friday
Test Vessel	Total exposure duration: 21 days
Loading:	80 mL of test solution / daphnid
Feeding during	Green algae (Pseudolgrchneriella abcapitata) daily: 2.0 × 10 ⁷ cells/daphnid/day
test:	For Q 23 mg C/daphnid/day and fish food suspension at each medium renewal at
	5.0 mg dro solidomL or 0.22 mg C/daphnid/day
Test	Temperature: 19.8 to 20.8°C in frest medium and 9.4 to 20.6°C in aged medium
conditions,	Photoperiod 16:8 light: dark with gradua@intensity changes at dawn and dusk
A CONTRACTOR OF THE CONTRACTOR	Light intersity: 639-700 lux
4 Y .	pt 7.4 to 7.8 in fresh medium and 8.1 to 8 of in aged medium
	Water, hardness: 170° to 1807 ang/L as CaCO3
(	Dissoftwed oxygen b saturation: 66% (54 mg/L) to 94% in fresh medium and 75
Ŵ	$t_0 D^2 0\%$ in aged medium $s O' O'$
, ~ Ç	Conductivity: 800 to 2000 µS/cm
Parameters	Discrete measurements of temperature, dissolved oxygen, pH, and specific
Measured /	conductivity were obtained at each renewal period. Water quality parameters were
Observations	measured on the newly prepared parent stock solutions and on one replicate of
- S	each old test solution alternating the replicate each time.
	Observations for death, in mobility, and abnormal appearance or behavior were
Å.	performed daily on each replicate chamber (both individual and group).
, Å	Starting on Day 6, all individual daphnids were checked daily for initial neonate
Ű ő	production. The time to first brood was recorded for each replicate. Following the
5 Z	onset of nonate production for the individual daphnids, all living, dead, or
Å .0	in mobile neonates were counted and removed from test chambers each day.
E R	At termination of the study, surviving adult daphnids were measured from the
	apex of the helmet to the base of the spine using a dissecting microscope and
$\lor$	ocular micrometer. Adult daphnids for each replicate were then dried and weighed.
	Aunt dapinnus for each replicate were men uneu and weighed.



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

## II. RESULTS ANICOISCUSSION

		X Û		10° L	°
Validity criteria (OECD 211, 2012)		Required		Obtained	
Mortality of the parent animals in contra	rol at the end of	f 20%		8%	
the test					
Mean number of living off-spring prod	Wed per parent			Sac a	Ô
animal surviving in control at the end	of the test		Ö Ö		
Y			$\sim$ $\sim$		· \
0	× '0'		Q. U	$\sim \sim$	

Analytical results:

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

Few recoveries were observed to be below 80% but the substance was proved to be stable over the renewal interval (variation between new and old medium less than 20%), therefore according to EFSA the technical report (2015), arithmetic means can be used to express the results. The geometric mean measured concentrations are 0.024, 0.047, 0.096 0.19, 0.37 and 0.742 mg/L, they are not significantly different from the arithmetic mean measured concentrations used to express the results (see table below).

Nominal conc.	Arithmetic	% of nor	ninal côn co	entrations	<u>s</u> y			
(mg a.s./L)	ng a AL)	Day 0 🗡 New	Day 3 Aged	Day 7 New ()	Day 10 Aged	Day 14 New	Day 17 Aged	Day 21 Aged
0.028	e.024 c	\$°~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	0 [°] 86~~0	92	90	85	85	88
0.056	0.048	° 76 ∠S	89	85	95	85	87	92
0.11	<u>0</u> ,0996	86	81	<b>9</b> 1	93	85	90	86
0.23	\$0.19	76	80~	86	89	77	86	86
0.45	0.30	79 🔗	<u>B</u>	89	92	78	79	80
0.90	0,74		Q ⁹ 79	85	92	77	81	84

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

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

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



#### **Biological results:**

#### Observations

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

#### Growth

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

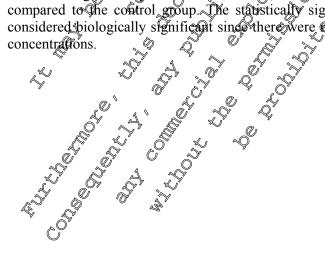
Arithmetic mean measured concentrations (mg a.s./L)	Mean dry weight (mg)± SD	Stean length (nom) ± 50
Control	$0.90 \pm 608$	$50 \pm 0.16$
0.024	0.87 + 0.14	\$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$
0.048	0.26 ± 0.15	<u>0</u> 4.9 ±0.21 0 0°
0.096	$0.78 \pm 0.21$	$40 \pm 0.40$
0.19	0.87 ±0.09 ×	\$ 0.23 A
0.37	0.87 0.11	5.0 ± 0.11
0.74	$(1.99) \pm 0.15$	$4.9 \pm 0.12$

### Adult survival

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

	√° (s.	à à		×, 0,
Arithmetic mean measured concentrations (mg a.s./Ľ)		soprvival ort day	21 × ×	
Control 🔬	\$* <u>\$</u>	0 <u>9</u> 2		
0.024		۵۷ می 292		$0^{*} ^{*}$
0.044		100		
0.096		~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~		
0.19		8° x96		~~~
0.37		, [©] 96	Å Õ	<i>©</i>
0.74		A & 96 '		T T
Reproduction data				_

There were no inamobile or dead neometes observed in an Otreatment level. Total living neonates per adult were significantly reduced at the 0.04\$, 0.37 and 074 mg/L mean measured concentration when compared to the control group. The statistically significant difference at 0.048 and 0.37 were not considered biologically significant since there were go effects at the 0.096, and 0.19 mean measured





Arithmetic mean measured concentrations (mg a.s./L)	Mean number of living neonates per adult ¹	Day of 1 st brood (mean) ¹	
Control	236	8.5	
0.024	214	8.7	
0.048	207#	8.5	A 10°
0.096	197	8.6	
0.19	212	8.8	
0.37	210#	8,7	
0.74	184*		

# Significant difference (P < 0.05) from the control group by Wilcoxon Rank Sum Test bowever, not considered biologically significant.

* Significant difference (P < 0.05) from the control group by Wilcoxon's Rank Sum dest.

¹ SD not reported

The statistical analysis of the results presented in the report and in the table above does not fulfil current standards and leads to inconclusive results for the most sensitive endpoint.

Therefore, a new analysis of the results has been performed according to the OECD guideline 211 (2012), i.e. determination of effects on the total number of living offspring per introduced parent, and on time to 1st brood, in addition to the parameters already analysed in the report total number of living offspring per surviving parent, immobility at 21 days (called parent survival), dry weight and length. Moreover, new endpoints such as  $FC_{10}$  and  $EC_{20}$  were determined when mathematically possible. All calculations were performed with ToxRat 3.2.

M. Coxclusions:

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

Endpoint (mg/L)	From thenew analysis	Y From the report
Endpoint (mg/L) NOEC	Immobility at 21 d	$\mathcal{Y}$ $a$ ,
NOEC	0.74 ~ ~	5° 074
EC ₁₀	Cannot be calculated dess than	Not available
EC ₂₀	\$ 10% of effects at the highest	No available
1020	concentration N 0	
	Sumber of off spring per introdu	ucedparent
NOEC	, 0.19 S S	Not available
EC ₁₀		
$\frac{EC_{10}}{EC_{20}}$	A response relationship	Not available
<i>Q</i> ₁	Number of off-spring per surviv	ring parent
NOEC 🔊		0.37
$EC_{10}$	Cannot be calculated: needose	Not available
EC ₂₀	🔬 response relationship	Not available
	Time to 1st prood	
NOEC		Not available
EC ₁₀	Cannot be calculated for	Not available
EC ₂₀	A Mathematical reasons	Not available
	Cannot be calculated: needose response relationship Time to 1st brood 0.37 Cannot be calculated for reathernatical reasons	



Endpoint (mg/L)	From the new analysis	From the report	
	Dry weight		
NOEC	0.74	0.74	l dî de
EC ₁₀	Cannot be calculated: less than	Not available	
EC ₂₀	10% of effects at the highest concentration	Not available 🔗	
	Length	1 Cr	
NOEC	See comment below	0.74	
EC10	Cannot be calculated: less than	🖒 Not available 🔬	
EC ₂₀	10% of effects at the highest concentration	NoQvailable	

The statistical analysis concludes that statistically significant effects on length are observed at the lowest (0.024 mg/L) and at the highest concentration (0.74 mg/L). The jevels of effects at these 2 concentrations were 3.1 and 2.9% inhibition, respectively. However, at 0.090 mg/L, the inhibition was higher (3.6%) despite no statistical significance. The inhibition on fength range of rom 0.1% at 0.37 fpg/L to 3.6% at 0.096 mg/L with no dose response relationship? Since no significant effects on dry weight was determined and since the inhibition on Tength is very low (less than 4%) and not concentration dependent, these statistical effects are not considered to be biologically relevant.

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

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



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

Data Point:	KCA 8.2.5.2/01
Report Author:	KCA 8.2.5.2/01
Report Year:	2019
Report Title:	Fluopicolide - Life-cycle toxicity test with mysids (Am@camysis bahia)
Report No:	
Document No:	M-544290-02-1
Guideline(s) followed in	OCSPP Draft Guideline 850.1350
study:	U.S. Environmental Protection Agency's OCSR 850.1350 (Deaft, State of a state
	U.S. EPA, 1996) and the Standard Guide for Conducting Life-Cycle Toxicity
	Tests with Saitwater Mysics (ASTM, 2003)
Deviations from current	Method: No deviation $\sqrt{2}$
test guideline:	Study: Current Guidelines: OCSPP 85001350 (1996) and ASTN 1191 03 (2008)
	The study was performed according to both OCSPP \$50.1350 and ASTM E
	1191-03 guidelines. The validity cruteria of ASTM guidelines were applied to this °
	study. The photoperiod was selected according to ASTM guideline: 160 light
	whereas OCSEP, recomprends 14h. The temperature for the test was selected
	according to OCSPP guidelines: 25,26°C whereas ASTM girdeline recommends
	27°C. These conditions were selected to be consistent with the culturing
	conditions of the second
Previous evaluation:	No, not previously subunitted O 6 0 0 7
GLP/Officially	Yes, conducted under GLP/Officially recognised desting facilities
recognised testing	
facilities:	
Acceptability/Reliability:	Yes X X X X

### Executive summary

The objective of this study was to determine the chronic (full life eycle) toxicity to the mysid Americamysis Dahia Exposed to fluopicolide technical under flow-through conditions. Two generations of saltwater mysids F0 juveniles (starting < 24 bours post release continued to sexual maturity) and F1 (mysids were collected during reproductive phase) were continuously exposed to the test substance in diluted for 96 hours for observations of mortality. After 28 days, the F0 generation was terminated and the results of the exposure were evaluated for potential chronic effects. All treatments of replicates/each concentration and control) include 20 organisms per aquare Foo the definitive 28 day pronic toxicity test nominal fluopicolide concentrations of 0.08 8, 0.48, 0.35, 0.70, 1.4 and 2.8 mg a.s./L were selected. A negative control was included. All exposure solutions were malysed for thopicolide by gas chromatography with micronelectron capture detection (GC aECD) Recoveries were between 76 and 111%. Therefore, results were based of arithmetic plean measured concentrations. 0.076, 0.16, 0.34, 0.66, 1.2 and 2.3 mg a.s./L. No residues of fluopicetude were found in the control samples above the maximum detectable limit (MDL). The study fulfils all validity corteria of the STM E 1191-03 guideline. Based on mean measured concentrations of fluopicolide and E0 mean number of offspring per female (the most sensitive indicator of toxicity), the No-Observed-Effect Concentration (NOEC) is determined to be 0.34 mg a.s./L. The Lowest-Observed Diffect Soncentration (LOEC) for mysids was determined to be 0.66 mg a.s./L. The  $EC_{10}$  is 0.08 mg s./L.



#### I. MATERIAL AND METHODS:

Test material	Fluopicolide technical Batch No.: ETFP000273 Purity 100.5% w/w None specified Saltwater mysid ( <i>Americamysis bahia</i> ) from in-house culture Juvenile mysids, approximately 24 hours old
Guideline(s) adaptation	None specified
Test species	Saltwater mysid (Americamysis bahia) from in-house oulture
Organism age/size at study initiation	Juvenile mysids, approximately 24 hours old
Test solutions	
Replication	No. of aquaria per concentration (replicates): 40 Y
Organisms per replicate	Evidence of undissolved material: Nøt reported No. of aquaria per concentration (replicates): 4 No. of aquaria per control/(replicates): 4 No. of organisms per aquaria. 20 Immature mysids were held in foretention chamber per aquarium ontil sexual maturity. Then they were held in pairing chambers (max 5 per aquarium). During the reproductive phase, groups of up to 10 offspring per replicate, 40 per treatment were colleged and evaluated. Offspring were tenoved from adult mysid chambers in each replicate wessel and placed in separate pairing chambers within that replicate. One F1 group was established and monitored for each replicate yessel.
Exposure	Flow through 7.7 aquarium volume addition per day Total exposure duration 28 days
Loading rate	Maximum biomass loading did not exceed 0.002 cg/L flowing solution per day or 0.02 g/L of solution at an orime, in any coplicate exposure aquarium
Feeding during test	Live bring shrimp nauplin ( <i>Artonia</i> sp.), twice daily. At least one of these feedings was with bring shrimp nauplin enriched with Selco®, a substance high in saturated fatty acids.
Test conditions	Temperature: 25 27°C Protoperiod: 16 hours light shours dark with a 15- to 30-min transition period Light intensity: 230 a 360 fax pH, 7.4 – 82 Dissolved oxygen: 4.50 to 6.89 mg/L (62 to 94% saturation range) Salinity: 19 – 21 %
	<ul> <li>Ital topheae. Our 11 gloup cas established and monitored for each replicate vessel.</li> <li>Flow-through: 7.7 aquarium volume addation per day Total exposure duration 28 days.</li> <li>Maximum biomass loading did not exceed 0.0026/2/L flowing solution per day or 0.02/g/L of solution at any time, in any replicate exposure aquarium.</li> <li>Live brine shrimp nauplin (<i>Artennia</i> sp.), twice daily. At least one of these feedings vas with brine shrimp nauplin (<i>Artennia</i> sp.), twice daily. At least one of these feedings vas with brine shrimp nauplin (<i>Artennia</i> sp.), twice daily. At least one of these feedings vas with brine shrimp nauplin (<i>Artennia</i> sp.), twice daily. At least one of these feedings vas with brine shrimp nauplin (<i>Artennia</i> sp.), twice daily. At least one of these feedings vas with brine shrimp nauplin (<i>Artennia</i> sp.), twice daily. At least one of these feedings vas with brine shrimp nauplin (<i>Artennia</i> sp.), twice daily. At least one of these feedings vas with brine shrimp nauplin (<i>Artennia</i> sp.), twice daily. At least one of these feedings vas with brine shrimp nauplin (<i>Artennia</i> sp.), twice daily. At least one of these feedings vas with brine shrimp nauplin (<i>Artennia</i> sp.), twice daily. At least one of these feedings vas with brine shrimp nauplin (<i>Artennia</i> sp.), twice daily. At least one of these feedings vas with brine shrimp nauplin (<i>Artennia</i> sp.), twice daily. At least one of these feedings vas with selected with selecters. The selected selecte</li></ul>



D +	In order to observe the mysids during the exposure period, the numbers of dead
Parameters	and living organisms were counted and any abnormal appearance or behavior
Measured/	was recorded. Survival of the test organisms was estimated for the first 12 days $\alpha^{\circ}$
Observations	of the test, i.e. prior to pairing of the mysids. After males and females had been
	paired (day 13) definitive counts of survival were made and the number of dead
	males and females, the number of off-spring produced by each individual females
	and any abnormal appearance or behavior was recorded. Observations were daily
	throughout the study. At the time an F1 generation pairing chamber was established and taily thereafter for 96 hours, observations of stress, abnormal behavior (including 2000)
	At the time an F1 generation pairing chamber was established and daily
	thereafter for 96 hours, observations of stress, abnornal behavior including $\mathcal{O}$
	discoloration, immobilization and inability to maintain position in the water and inability to maintain position.
	At the time an F1 generation pairing chamber was established and daily thereafter for 96 hours, observations of stress, abnornal behavior (including discoloration, immobilization and inability to maintain position in the vater of the body length and dry weight were made determined at the end of the test of the body length and dry weight were determined at the end of the test of the body length and dry weight were made determined at the end of the test of the body length and dry weight were determined at the end of the test of the body length and dry weight were determined at the end of the test of the body length and dry weight were determined at the end of the test of the body length and dry weight were determined at the end of the test of the body length at the
	Body length and dry weight were determined at the end of the test
	Temperature, dissolved oxygen concentration, pH and salinity were measured in
	each replicate on day 0 and alternated between replicates daily thereafter
	throughout the exposure period for each treatment by el anothe control.
Chemical	Prior to the start of the definitive exposure samples were removed from one
analysis	replicate of each treatment level and the control and malysed for fluopic fide concentration. In addition, a sample of effluent from each of the saturator coumns
	concentration. In addition, a sample of ethiuent from each of the saturator coumns
	was also analysed during the pretest period. The set of
	During the inflife phase, samples were removed from alternating replicate
	solutions of each treatment level and the control on days 0, 7, 14, 21 and 28 for
	analysis of fluopicolide concentration. In addition, one sample of each saturator
	column' effluent was analysed at each sampling interval during the exposure
	period
	All exposure solutions were analyzed for fluopicolide by gas promatography with
	mieron-electron canture detection (Ge/uECD).
	Data for the survival endpoints (e.g. 28-day survival male and female survival
Data analysis	Sand For survival) were analyzed using Figher's Exact Test with Bonferroni-Holm's
Ô	Adjustment For the other parameters the Dunnett's Multiple Comparison Test
<u> </u>	was conducted 0 ETIS M was used constrain the statistical computations
Or Or	Was conducted. CE Has was used to period in an engrander of Calculations were possible
, Q	$EC_x$ values were determined by inparimetal regression. Calculations were possible
	only for the number of on-spring per ternale because of the lack of dose response
K~y ^v	recations support the other parameters.
	a ne initial report has been amended to exclude 3 pairing chambers from the
Ô	statistical analysis of reproduction data. These chambers were excluded because
	they either contained 2 males or 2 temales and were therefore not capable of
	producing offspring.
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Å	All exposure solutions was analysed for fluopicolide by gas chromatography with meron-electron capture detection (GC/µECD). Data for the survival indpoints (e.g. 28-day survival, male and female survival and b survival) were analysed using Fisher's Exact List with Bonferroni-Holm's Adjustment. For the other parameters, the Dunnett's Multiple Comparison Test was conducted CETIS ¹¹ was used to perform the statistical computations. EC, values were determined by not-linear regression. Calculations were possible only for the number of off-spring per temale because of the lack of dose response relationship for the other parameters. The initial report has been amended to exclude 3 pairing chambers from the statistical analysis of reproduction data. These chambers were excluded because they either contained 2 males or 2 females and were therefore not capable of producing offspring
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II. RESULTS AND DISCUSSION:

Validity criteria (ASTM 1191-03 Guideline, 2008)	Required	Obtained	
F ₀ survival	$\geq 70\%$	84	
Reproductively active females	$\geq 75\%$	100	
Reproductive performance (offspring per female)	≥ 8	21.5	4 . 4

Analytical results:

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

Nominal Concentration (mg/L)	Arithmetic mean measured concentration mg/L/ 0.076
0.088	
0.18	0.16 0 4 5 5 91 0 5 5 5 - 11 5 0
0.35	0.34% 0 0 0 0 0 0 0 0 0 0
0.70	0.66
1.4	1.2 4 5 4 7 86 4 5 3 9 - 93
2.8	

Õ 0″

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

Biological resorts:

Ľ, At test termination, mean survival of \$4% was observed among male mysids in the control. Mean survival of 85, 69, 87, 92, 80 and 78% was observed among make mysids exposed to the 0.076, 0.16, 0.34, 0.66, 1.2 and 23 mg/L treatment levels, respectively. Fisher's Exact Test with Bonferroni-Holm's Adjustment determined no significant difference in male survival among organisms exposed to any of the treatment levels tested compared to the control (84%).

At test termination, mean survival of 94% was observed among female mysids in the control.

Mean surviva of 89, 100, 20, 86, 89 and 89% was observed among female mysids exposed to the 0.076, 0.16, 0.34 0.66, 1.2 and 2.3 mgP treatment levels, respectively. Fisher's Exact Test with Bonferroni-Holm's Adjustment determined no significant difference in female survival among organisms exposed to any of the treatment levels tested compared to the control (94%).

Following 28 days of exposure mean survivator 84% was observed among organisms in the control. Mean survival of \$5, 77, 90, \$0, 83 and 79% was observed among mysids exposed to the 0.076, 0.16, 0.34, 0.66, 1.24 and 2.3 mg/L freatment levels, respectively. Fisher's Exact Test with Bonferroni-Holm's Adjustment determined no significant deference in survival among organisms exposed to any of the treatment levels tested compared to the control (84%).

Following the 6-hour observation period, mean percent survival of 95% was observed among F1 myside in the control Mean percent survival of 100, 98, 95, 90, 85 and 73% was observed among F1 mysids exposed to the 0.076, 0.16, 0.34, 0.66, 1.2 and 2.3 mg/L treatment levels, respectively. Fisher's Exact Test with Bonferroni-Holm's Adjustment determined no significant difference in F1 mysid survival among organisms exposed to any of the treatment levels compared to the control (95%).



At test termination, the mean number of off-spring per female for organisms in the control was 21.5. The mean number of off-spring per female was 18.8, 16.4, 16.4, 12.9, 9.4 and 6.0 among mysids exposed to the 0.076, 0.16, 0.34, 0.66, 1.2 and 2.3 mg/L treatment levels, respectively.

Dunnett's Multiple Comparison Test determined a significant difference in the mean number of offspring per female among organisms exposed to the 0.66, 1.2 and 2.3 mg/L treatment levels compared to the control (21.5).

The average total body length of male mysids in the control was 7.14 mm. The average total body length of male mysids exposed to the 0.076, 0.16, 0.34, 0.66, 1.2 and 2.3 mg/L treatment levels was 7.35 7.64 7.41, 7.04, 7.00 and 6.89 mm, respectively. Dunnett's Multiple Comparison Test determined no significant difference in the total body length of male mysids exposed to any of the treatment levels , , compared to the control (7.14 mm).

The average total body length of female mysids in the control was 7/41 mm. The overage total body length of female mysids exposed to the 0.076 0.16, 0.34, 0.66, 1.2 and 2.3 mg/L treatment levels was 7.67, 7.81, 7.46, 7.19, 7.24 and 7.00 mm respectively. Dunnett's Multiple Comparison Fest determined a significant difference in the totakbody length of female mysics exposed to the 2.5 mg/L treatment level compared to the control (7.41 mm). treatment level compared to the control $(7.41 \text{ mm})_{\text{control}}$

of male mysids exposed to the 0.076, 0.16, 0.34, 0.66, 12 and 0.3 mg/D treatment levels was 0.81, 0.90, 0.83, 0.80, 0.77 and 0.76 mg, respectively. Dunnett's Multiple Comparison determined no significant difference in the dry body weight of male mysids exposed to any of the treatment levels compared to the control (0.78 mg).

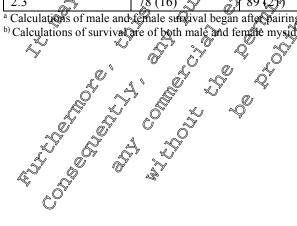
The average dry body weight of female mysids for the control was 1.10 mg. The average dry body weight of female mysids exposed to the 0.0%, 0.16, 0.34, 0.66, 12 and 2.3 mg/L treatment levels was 1.08, 1.39, 1.11, 1.01, 1.00 and 0.96 mg, respectively Dunnett's Multiple Comparison Test determined no significant difference in the dry body weight of female mysids exposed to any of the treatment levels compared to the control ($\mathbb{P}.10 \text{ mg}$).

Survival (F0)) <u> </u>		
Arithmetic mean measured conc (mg/L)	Mean male survi@l ^{a)} +(SD) [%]		Mean post pairing survival ^{b)} + (SD) [%]	Mean 28-day survival ^{b)} + (SD) [%]
Control N	84(16)	94 (7)	88 (4)	84(12)
0.076	Ø 5 (1,8) Ø 5 (1,8)	88,(10), O* &	87 (93)	85 (15)
0.16	°69 (4)	900 (0% %	83 (6)	77 (8)
0.34	87(11)		94 (5)	90 (4)
0.66	0 ² (20)	86(8) 5 8	81 (8)	80 (8)
1.2 2	80,00	89 (10) 3	87 (6)	83 (7)
2.3	78 (16)	89 (29)	83 (16)	79 (16)

Survival (Fa)

^a Calculations of male and ternale survival began after pairing

b) Calculations of survival are of both male and female mysid combined.





Survival (F₁)

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

Mean Number of Ysung Persurxiving Prmale 7(SD)

Reproduction

Arithmetic mean measured conc. (mg/L)	Percent of Females Producing Young + (SD)Mean/Number of Young Per Surxiving Female \rightarrow (SD)100 (0) 21.5 (29)100 (0) 88 (2.7)100 (0) 88 (2.7)
Control	
0.076	Young + (SD) Female (SD) 100 (0) 21.5 (29) 100 (0) 18 (8/2.7) 100 (0) 18 (8/2.7) 100 (0) 18 (8/2.7) 95 (10) 164 (94)
0.16	
0.34	95(10) 3 3 3 $16.4(9.4)$ 3 0 3 0
0.66	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$
1.2	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$
2.3	$90(12)$ 6.0^{a} $3.1)$ 3.1

^a Significant difference compared to the control, based on Bunnett's Multiple Comparison Test Growth parameters after 28 days

O*			
Arithmetic mean	Mean total length ± SD (mm)	Mean dry weight ±	SD (mg)
measured conc. (mg/L	Males H (SD)	Males + (SD)	Females + (SD)
Control	7.14 (0,13) 7.4 (0.13)	0.78 (0.02)	1.10 (0.08)
00/6 4	27.31.69.09) 2 2.67 (0,69)	0.81 (0.02)	1.08 (0.14)
0.16	7.64 (0.12) 7.81.00.16)	0.90 (0.02)	1.39 (0.06)
0.34	7.41 (9.11) 7 46 (0.15)	0.83 (0.05)	1.11 (0.13)
0.66	7.04 (0.2) 7.19 (0.20)	0.80 (0.04)	1.01 (0.07)
1.2	200 (029) 7.20 (0.19)	0.77 (0.01)	1.00 (0.10)
2.3	6.8900.13) 800° (0.20)	0.76 (0.07)	0.96 (0.09)

^{2.5} <u>Tooky(0.13) 7 700° (0.20)</u> 0.76 (0.07) ^a Significant difference compare to the control, fased on Dunnett's Multiple Comparison Test

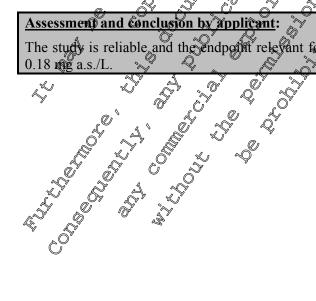


III. CONCLUSIONS:

The study meets the validity criteria. The most sensitive endpoint was mean number of off-spring per 41° female. EC_x values could be calculated for this parameter only. The results, based on arithmetic mean measured concentrations, are:

			~	
Parameter	NOEC (mg a.s./L)	LOEC (mg a.s./L)	EC ₁₀ (mg a.s./L)	EC20 (mg a.s./by
F ₀ male survival at test end	2.3	> 2.3	Cannot de calcul	ated Jack of Jose- 0
F_0 female survival at test end	2.3		Cannot be calcal	ated, tack of dose-
F ₀ survival at test end (male and female)	2.3		Caphot becalcul	ared, lack of dose-
F ₁ survival at 96h post-release	2.3		Canaot becalcul	fred, lack of dose-
Nb of offspring per female		0.66°	\$7.18 C	0.38
Body length of males		2.3 °		ated, lack of dose-
Body length of females		52.3 5 ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	Canno Die cateul	ated, lack of dose- oonse
Dry weight of males		2.3 °	Cannot be calcul	ated, lack of dose- bonse
Dry weight of females			Connot be calculated a contract of the calculated a contract o contra	ated, lack of dose-
ja ja				

The study is reliable and the endpoint relevant for the fluopicolide risk assessment is the EC₁₀ of 0.18 tog a.s./L.





CA 8.2.5.3 **Development and emergence in Chironomus riparius**

No study is avaiable and a study is not required.

CA 8.2.5.4 Sediment dwelling organisms

CA 8.2.5.4 Sedi	ment dwelling organisms
Data Point:	KCA 8.2.5.4/01
Report Author:	1 2 2 2 4 S
Report Year:	
Report Title:	AE C638206: A prolonged sedument toxicity test with Chironomus riparius range
Report No:	M-223565-01-2
Document No:	M-223565-01-2
Guideline(s) followed in study:	OECD: 218 (2001)
Deviations from current	Method: Deviations from carrent caideline SANCO/3029/99 rev.4:
test guideline:	However, the Obtained data demonstrate very good recoveries and the precision. The method can therefore be regarded as fit for purpose. Study: Cutrent Goodeline? DECD 218 (2004) The study does not meet the variability criteria
Previous evaluation:	yes, exaluated and accepted of a construction of the construction
GLP/Officially	Yes, conducted under GCP/Officially recognised testing Pacilities
recognised testing facilities:	
Acceptability/Reliability.	' Supportive only ' y y y y y y ' Supportive only ' y y

Executive summary 10

A prolonged sediment loxicity test was performed with widges (Chirgnomus riparius) using sediment spiked with the test substance fluopicolide. Groups of pidges were exposed to a geometric series of six test concentrations, a negative felilution water control and a solvent control (1.0 mL acetone/kg) for approximately 28 days Four replicate test chambers were maintained in each treatment and control group, with 20 midges in each chamber for a total of 80 individuals per test concentration. Nominal test concentrations were 0.50, 1.3, 21, 7.8, 20 and 49 mg fluppicolide/kg. The NOEC and LOEC were determined by visual interpretation of the dose-response pattern and statistical analyses of the mean development times, energence rates and development rates. Overlying water, pore water and sediment samples from the analytical sampling test champers were collected from the negative control, solvent control, the lowest test concentration (\$50 mg/a.s./kg) and the highest test concentration (49 mg a.s./kg) on day & day 7 and day 28. Samples were analysed by HPLC analysis. The endpoints were based on the nominal concentrations. The test doe on the validity criteria of the OECD guideline 218. All emerged midges appeared normal during the test, with no observations of abnormal behaviour before or after emergence free EC50 value base on percent emergence of midges (Chironomus riparius) exposed to sediment-incorporated fluopicolide is greater than 49 mg a.s./kg, the highest nominal concentration tested. There were no treatment related offects observed on mean development times, emergence rates and development states. The LQEC is greater than 49 mg a.s./kg and the NOEC is 49 mg a.s./kg.



I. MATERIAL AND METHODS:

	I. MATERIAL AND METHODS.
Test material:	Name of substance: Fluopicolide (AE C638206)
	Batch No.: OP 2050046
<u> </u>	Purity 96.1% w/w
Guideline	alpha cellulose was used as carbon source for the sediment instead of peat moss
adaptation	
Test species:	alpha cellulose was used as carbon source for the sediment instead of peat uposs Midge (Chironomus riparius) First instar larvae, one to four days old at exposure initiation Dry formulated sediment (1500 g) was weighed into seven 2000 mL plastic bottles
Organism age:	First instar larvae, one to four days old at exposure initiation
	Dry formulated sediment (1500 g) was weighed into seven 2000 mL plastic bottles
Preparation of spiked	to make batches of treated sediment for each test concentration. Each bottle received
sediment	1.5 mL of the appropriate acetome stock solution and then was placed in a rotary
seament	mixer. The sediment and test of bstance were mixed for approximately 39 hours.
	After mixing was complete, batches of sedurent were distributed among replicate
	test chambers. Sediment was added to the appropriate replicates to a depth of 2 cm
	from the bottom of the jar and then 600 mL of tiltraviolet sterilized well water was
	slowly added to the test chambers. Test chambers were then placed in an
	environmental chamber, covered, and allowed to settle for approximately 48 hours,
	prior to the addition of test organisms. Nominal sediment concentrations: 0.50, 1.3, 3, 1, 7.8, 20 and 49 mga.s./kg.sediment
Test solutions	dry weight.
	Arithmetic nean measured conceptration lowest and highest test tate
	- in sediment: 0.131 and 55.6 mg a.s./kg
	- in sediment pore water: 1418 and 3.517 mg as:/L
	Water control approx. 150 mL sedimentand 600 mL dilution water
	Sofvent control and test concentrations: approx. 50 mL sediment treated with
	acetone or the spock solution in acetone, 600 mL dilution water
(Evidence of undissolved material. At test initiation the overlying water in all test
ð°	chambers appeared clear and colourless. At test termination the overlying water in all test chambers appeared slightly cloudy tail.
	Vessels to measure biological response:
Replication:	No st vessels per concentration (replicates): 4
R.Y.	Nø of vessels per control (replicates): 4
	No of vessels per solvent control (replicates): 4
(S A Z Z Z O
	Adoptional Vessels with organisms for analytical sampling on day 7 and 28:
~\Q^	No. of Resels in the lowest and highest concentration (replicates): 2
Å	No. of vessels per control (replicates): 2
and the second s	No, or vessels per solvent control (replicates): 2
,≪	Additional vessels without organisms for analytical sampling on day 0:
~~	No. of Vessels in the lowest and highest concentration (replicates): 1
al c	No. of vessels percontrol (replicate): 1
Ú.	No of versels per solvent control (replicates): 1
Organisms per	No. of organisms per vessel: 20
replicate:	
	Static conditions
Exposure:	Total exposure duration: 28 days
Feeding	Larvae were fed $10 - 30$ mg of ground rabbit pellets throughout the test.
during test	Larvae were rea 10 – 50 mg of ground fabor penets unoughout the test.
auring 1051	



т	Water temperature: 20.4 to 20.6°C (daily measurements), 20 to 21°C (continuous
Test	Water temperature: 20.4 to 20.6°C (daily measurements), 20 to 21°C (continuous measurements) Photoperiod: 16:8 light: dark with a 30-min transition period Light intensity: 366 lux (at test initiation) pH: 7.9 to 8.5 Water hardness: 128 to 132 mg/L as CaCO ₃ Dissolved oxygen (mg/L): 6.1 to 8.6 Specific Conductance (µmhos/cm): 310-320 Alkalinity (mg/L as CaCO ₃): 182-186 Artificial sediment: 14% kaolin clay 80% industrial quartz sand 0.5% dolomite 5% alpha-cellulose 0.01% humic acid Temperature and dissolved oxygen were measured in the overlying water of one- alternating test chamber in each dreatment and control group at the beginning of the test, three times per week and at the end of the test. Temperature was also measured continuously in a before of water placed adjacen to the test chamber. The pHof the water was measured on samples of oxplying water collecter from one alternating replicate test chamber at the beginning of the test. Once per week and at the end of
conditions:	Photoperiod: 16:8 light: dark with a 30-min transition period Q_{μ}°
	Light intensity: 366 lux (at test initiation)
	pH: 7.9 to 8.5
	Water hardness: 128 to 132 mg/L as CaCO ₃
	Dissolved oxygen (mg/L): 6.1 to 8.6
	Specific Conductance (μ mhos/cm): 310-320
	Alkalinity (mg/L as CaCO ₃): 182-186
Codimont.	Artificial sediment:
Sediment	14% kaolin clay
	80% industrial quartz sand
	0.5% dolomite
	5% alpha-cellulose
	0.01% humic acid $\&$ \bigcirc \checkmark \checkmark \checkmark \checkmark
Parameters	14% kaolin clay 80% industrial quartz sand 0.5% dolomite 5% alpha-cellulose 0.01% humic acid Temperature and dissolved oxygen were measured in the overlying water of one
Measured /	alternating test chamber in each dreatment and control group at the beginning of the
Observations	test, three times per week and at the end of the test Temperature was also measured
005cl vations	continuously in a beaker of water placed adjacent to the test chamber The pHoof the
	water was measured on samples of overlying water collected from one alternating
	replicate test chamber at the beginning of the test, once per week and at the end of
	the test.
	Dissolved oxygen was measured on samples of overlying water collected from
	one alternating replicate test chamber at test initiation, three times per week during
	the test and at lest termination.
	The test chambers were observed three three per week during the first 13 days of
	the test to make visual assessments of any abnormal behavior (e.g., leaving
	sediment, unusual swimming) During the period of expected emergence following
ķ	Bay 13 test chambers were observed on a daily basis and the sex and number of
~ (⁽	fully emerged midges were recorded. The total number of midges emerged in each
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	repricate was determined at test termination
Sampling for	Overlying water, pore water and second nent samples from the analytical sampling test
chemical	chambers were collected from the negative control, solvent control, the lowest test
analysis	concentration (0.50 mg a s./kg) and the highes test concentration
	(49 mg a S./kg) on Da00, Day 7 and Day 28. Water samples were collected from
	mid-depth of the water column. Samples were analysed by HPLC analysis.
Data analysis.	The NOEC and LOEC were determined by visual interpretation of the dose- response sattern and statistical analyses of the mean development times, emergence
~Ģ~	rates and development rates
4	All comparisons were made between pooled control and treatment groups, as the
	Student's t-test showed no statistical difference between the negative and solvent
× 1	control group. Data were analysed using a two-tailed Dunnett's test, as appropriate,
4	to identify those treatment evels that were statistically different from the pooled
a	control group. In addition to the Dunnett's test, the development time was analysed
Ś	using the kruskal-Wallis test. A Chi-square test was performed to check normality,
	and the homogeneity of variance was checked using the Bartlett's test. All statistical
	procedures were performed using SAS version 8.2 and Toxstat version 3.5.
S. S.	
And And	
× Å	



### **II. RESULTS AND DISCUSSION:**

Validity criteria (OECD 218, 2004)	Required	Obtained
Emergence in the controls	≥ 70%	94% and 93%
Emergence period in the controls	between day 12 and 2	between da 14 and day 28
Dissolved oxygen % saturation at test end in all test vessels	> 60%	> 6.1 mg/L (66%) = 5.5 mg/L m test x conditions)
Water pH in all test vessels	between 6 and 9	Ø.9 to \$5
Water temperature variation over the whole exposure period	± 1°C	20 - 21°C
Analytical results: To fluopicolide residues were measured in second the the control and solvent control above the limits of grantit	overlaying water an	d sediment pore water of
Nominal test concentration (mg a.s./kg sediment dry weight) (mg a.s./kg sediment dry weight)	Dayo Day	
0.5 DM31	~~ <b>6</b> 8:4 -*	<u> </u>
49 455.6 Measured concentration was beneath the LOQ fimit of quantification	$\frac{110}{110} = 0.208 \times 10^{-1}$	2 118
	511 – 0,208 ga s./kg ~ 5	Ĵ.
S Measured concentrat	ion (mg a.s./b)	/ · · · · · · · · · · · · · · · · · · ·
Nominal concentration (mg a.s./kg sediment dry weight) Day 0 Day 7	Dray 28	Arithmetic mean
Overlying water of the second se	Ő "V	<u> </u>
0.5 $2$ $2$ $2$ $2$ $2$ $2$ $2$ $0$ $0$ $2$ $100$		< LOQ
49 6 ³⁷ 6 6712 6204	J.38	3.132
Sediment pore water 7 2 2 5 2 4	Y	
$0.5$ $\sqrt[3]{2}$	0.129	0.418
49 Q Q Q Q Q Q Q Q Q Q Q Q Q Q Q Q Q Q Q	2.820	3.517

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

All emerged midges appeared normal during the test, with no observations of abnormal behavior before or after emergence  $\frac{1}{\sqrt{2}}$ 



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

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

Nominal test concentration	Cumulative emergence after 28 days			
(mg a.s./kg sediment dry weight)	Mean Percent Emergence	Mean Male DEmergence Kate	Mean Female Emergence Rate	
Control	94	37 ₂ Q		
Solvent Control	93	34	0 40 0 [×]	
Pooled control	93	~~ @°	6 4 - 4	
0.5	91	39~	342 .0	
1.3	<b>\$</b> \$ <b>\$</b>	x 27 X	× ×	
3.1	95 ×	<u></u>	~ 36 ~ L°	
7.8	89~	38,4	33	
20	67 × 88 0	.~ 6 ⁷ ~ 7	\$ \$25 \$	
49	Q & 91 ~	1 × × × × ×	× × 42	

Mean development time of midges during the life-cycle exposure with fluopicolide (ÅE C683206) and midge (*Chironomus ripartits*) There were significant differences (p<0.05) in comparisons of development times between the pooled

There were significant differences (p<0.05) in comparisons of development times between the pooled control and the 3.1, 7.8, and 20 mg a.s./kg treatment groups using a two-tailed Dumett's test. However, there were no significant differences (p>0.055 between the pooled control and the 0.50, 1.3 or 49 mg a.s./kg treatment groups. The differences in development times were relatively small, were not concentration dependent and the range of values in the treatment groups overlapped with the pooled controls (with exception of the 20 mg a.s./kg group). Therefore, the differences observed in development time were not believed to be treatment related. There were no significant differences (p>0.05) in comparison of development rates between the pooled control and any of the treatment groups. Therefore, there were no treatment related effects on development rates.

Nominal test concentration (mg a.s./kg sediment dry weight)	Mean development rate [#]
Control 20.3	0.0521
Solvent Control 5 0 20.4 5	0.0524
Regiled control	0.0522
	0.0541
	0.0570
3.1 3 17.6*	0.0600
<i>√</i> 7.8 <i>√ √ √</i> 18.5*	0.0571
20 L 17.5*	0.0600
49 49 18.7	0.0563

*Dunnett's test shows statistical differences from pooled controls (p < 0.05)

#represents portion of Darval development per day.



# **III.** CONCLUSIONS:

The test does not meet the validity criteria of the OECD guideline 218. No adverse effects up to and including the highest concentration (49 mg a.s./kg) were observed. Endpoints based on nominal sediment concentrations are: Nominal test concentration (mg a.s./kg sediment dry weight) Endpoint NOEC LOEC Percent emergence  $\geq 49$ > 49Mean development  $\geq 49$ time Mean development  $\geq$  49 rate response relationship EC₁₀ and EC₂₀ values cannot be calculated because of Assessment and conclusion by applicant The study is not considered vali

Data Point:	ACCA 89.5.4/02 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2
Report Author:	<b>GRCA</b> 8.9.5.4/02 5 6 6 6 7 6 7 6 7 6 7 6 7 6 7 6 7 6 7 6
Report Year:	
Report Year:	Amendment 2 to final report - Europicolide, technical: A study on the chronic
	Yoxicity to the sediment dweller Lumbriculus variegatus
Report No:	19PA
Document No O	M-67152603-14 2 2 2
Guideline(s) follower in	"DECD Guideline 225: "Sediment-water Lumbriculus toxicity test using spiked
study: 🔬 🕺	, sediment", October 2007 "O O
Deviations from current	Method: Nodeviation St St St
test guideline:	Study: Current Gurdeline, OECD 225 (2007)
	No deviations O & A
Previous evaluation.	No, not previously submitted
	Xes, conducted under GLP/Officially recognised testing facilities
GLP/Official	Xes, conducted under CLP/Officially recognised testing facilities
recognised testing	
facilities:	
Acceptability/Reliability.	Yes y
	Yes a a a a a a a a a a a a a a a a a a a
×	
Exacutivo Summary	

# Executive Summary

A chronic study with sediment dwelling yorms (*Lumbriculus variegatus*) in a 28-day spiked-sediment test was investigated. Flopicotide was applied at nominal sediment concentrations of 0.126, 0.316, 0.79, 1.9 and 494 mg a.s./kg dry sediment. Additionally, a water control and a solvent control were included. The est comprised A replicates of 10 individual species for the control and the treatment and 6 replicates of 10 individual species for the solvent control. The total number of worms per replicate, survival and the total dry weight of the worms per replicate were assessed at test end (day 28).

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



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

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

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

	I. MATERIAL AND METHODS
Test material:	Fluopicolide, technical $\sqrt[6]{9}$ $\sqrt[6]{9}$ $\sqrt[6]{9}$ $\sqrt[6]{9}$
	Batch code: AE C638206-01-35
	Specification No.: Not reported
	Purity: 98.8 % w/w A to
Guideline(s) adaptation	I. MATERIAL AND METHODS
Test species:	Lumbriculus veriegation in the second s
Culturing	The culture conditions were $20 \pm 2^{\circ}$ C, 10 h light and 8 h dark
conditions	Cultured in crystallising dishes containing quartz sand and reconstituted water
	In the culture, the works are sed with fish food suspension, TetraMin®
Organism	Synchronised adult worms of similar size
age/size at study initiation:	
Preparation of	The test item (33 mg) was dissolved in 200 mL of accelene (stock solution of 165
spiked Sediments	mg(L) by stirring (10 mm) and manual shaking. A series of solutions to prepare
sediments 🔗	the different concentrations levels in the sediment was prepared by diluting the
	Stock solution with the same solvest used to presare the stock solution.
E.S.	Each of the application solutions of the different concentration levels was spiked on a defined quantity of the solument (10 g quartz sand per replicate). The spiked
**	Gand was left for evaporation of the solvent and was then mixed into the quantity
l l	^P of formulated sediment percessary for the replicates of one concentration level to
 	achieve the desired nominal concentration levels in mg/kg dry sediment. For each
~9	concentration level the same volume of the corresponding application solution per quantity of sediment was used. To ensure that the test item added to the sediment
A	was evenly distributed within the sediment, the bulk formulated sediments were
L. L.	thoroughly mixed using a power drill with stainless steel propeller.
Test	Nominal sectionent concentrations: 0.126, 0.316, 0.79, 1.98 and 4.94 mg a.s./kg dry
concentrations	sediment
Å	Control Cwater S
	Solvent control: aceterne (200 mL)
Replication:	No. Of vessels per concentration (replicates): 4
S G	No. of pessels per control (replicates): 4
1	No. of wessels per solvent control (replicates): 6
Organisors per	No. of organisms per vessel: 10
replicate:	



Exposure: Static conditions (with periodic compensation of evaporated water)	
Spiked sediment test	
Total exposure duration: 28 days	Ô
Feeding during test     Feed in sediment (Urtica powder and cellulose)	
Test conditions: Water temperature: 19.6-21.0°C (manual measurements iff test vessels) and 19.0- 20.7°C (automatic measurement; 1h intervals in a separate vessel) Photoperiod: 16 h light, 8 h dark Light intensity: 175 - 268 lux pH: 7.2 - 8.3 (overlying water) Water hardness: 286 - 339 mg/E CaCO ₃ (overlying water) Dissolved oxygen: 7.2 - 8.7 mg/L (> 81 % saturation) Ammonium content: max % 76 mg/L as NH ₄ ⁺ (overlying water)	Ş
Light intensity: 175 - 268 lux	
pH: 7.2 - 8.3 (overlying water) Water hardness: 286 - 339 mg/ CaCO ₃ (overlying water)	D'
Dissolved oxygen: 7.2 - 8.7 fsg/L (> 81 % saturation) Ammonium content: max 8/76 mg/L as NH4 ⁺ (everlying water)	
Sediment water ratio approx. 143.5 0 0 0 0 4	0
Sediment Artificial sediment according to OECD grideline No. 225 (2007); addition of reed to sediment before application. Composition in the dry weight sediment:	
Sediment       Artificial sediment according to OECD gudeline No. 225 (2007); addition of the disert to sediment before application. Composition in % dry weight sediment:         % peat: 4.8       % organic carbon: 2.5         % sand: 75       % sand: 75         % clay: 20       %         % Urtica powder: 0.3       %         % Cellulose powder: 0.3       %         % Cellulose powder: 0.3       %         % deionised water: 3@50       %         Parameters       Temperature, dissolved oxygen content and pb were measured in one test vessel of the controls at the start and the	
% sand: 75 3 3 3 5 5 5 5 5 5	
% clay: 20 %	
% Calulose powder: 0.13	
% Cellulose powder: 0.3 % Cellulose powder: 0.3 % Calcium carbonate: 023 % deionised water: 3@50	
& deignised water: 30250 5 2 0 0 4	
Parameters Temperature dissolved ovgen content and powere measured in one test vessel	
Observation of the exposure period and once per week. Total water hardness was measured in one replicate of the control and one test vessel at the highest concentration at	
the start and the end of the exposure period and once per week. Ammonium content	
was measured in one replicate of the control and one test vessel at the highest	
Concentration at the start and the end of the exposure period. Light intensity was measured once dyring the test over the testing area. Temperature was recorded	
additionally, at bourly ortervals, throughout the test.	
The total number of worms per replicate, survival and the total dry weight of the worms per beplicate were assessed at test end (day 28).	
Sampling for During the in-life phase of the definitive study, sediment, pore water and overlying	
chemical analysis even Dirbetly after spring the sediment samples were taken and kept as a	
analysis analysis water samples were taken from the control, solvent control and all concentration levels. Directly after spiking the sediment samples were taken and kept as a reserve. Dirther samples were taken at end of equilibration (day 0 of exposure), on day 14 and at the end of the exposure period; these samples were analysed. Chemical analysis of sediment, pore water and overlying water samples were performed by HPLC-MS/MS.	
Chemical analysis of sediment, pore water and overlying water samples were performed by HPLC-MS/MS.	



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Data analysis:	The total number of worms per replicate and the total dry weight of the worms per replicate were assessed. In order to estimate mortalities, the numbers of worms that did not react to a gentle stimulus were considered to be dead.
	To determine significant differences between the controls (control and solvent or control) the replicates of each control were tested for normal-distribution and for homogeneity of variances; thereafter a pair-wise comparison test was used. Since no statistically significant difference was observed between the control and solvent control for any of the parameters, the statistical evaluation of the effects was performed against the pooled controls.
	Normal distribution and variance homogeneity of the data were assessed by K-by- S test and Cochran's test, respectively. The Withlams test was used where variance homogeneity was confirmed The Multiple sequentially rejectore Welch-t-test after Bonferroni-Holm was used where variance homogeneity was not confirmed. Probit analysis was performed to calculate the EC _x -values.
	The statistical software package To Rat Professional 3.2.0 was used for these calculations. All statistical calculations were done based on the nominal test item concentrations.

		ê b
Validity criteria (OECD [*] 2 <b>2</b> 5, 2007)	Required S	<b>Obtained</b>
Average increase factor of the number of living worms in the control replicates at the end of exposure and the end of exposure		Obtained 2.3
Average increase tactor of the number of living worms in the solvent control replicates at the end of exposure		2.1
pH of the overlying water 4	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	7.2 - 8.3
Validity criteria (OECD 275, 2007) Average increase factor of the number of living worms in the control replicate at the end of exposure Average increase factor of the number of living worms in the solvent control replicates at the end of exposure pH of the overlying water Dissolved oxygen concentration measured in at least one replicate per concentration level and control at least once per week:	$\begin{array}{c} & & & & \\ & & & & \\ & & & & \\ & & & & $	≥ 81%
Dissolved øxygen concentration pleasured in at least one reallicate per concentration pleasured in at least least once per week		

	S D	<u>ĝ</u>	Ő	6	d.	<u> </u>	0
, Ø		107	a v		Ŵ.	0°	$\approx$
4	<b>UI. RES</b>				S C	ð,	
		eriste		CUSSR		i . C	



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# Analytical results:

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

inary ticar results.			I		1 alianti di seconda di	
Nominal	Day	0	Day 1	4		
sediment concentration [mg a.s./kg]	Measured concentration [mg a.s./kg]	% of nominal	Measure concentration [mg.@/kg]	% of nom@al	Measured concentration [mg@s./kg]	yo of S Snomital
Control	< LOD	-	COD	~- 8	€ LQIO	8 - Ú
Solvent control	< LOD	-	¢ < LQD ~		° < LOD	
0.126	0.139	111		<u>9</u> 6	9.117	∲93 <u></u> °
0.316	0.287	91	× 0.268	85-	0.255	8 8 8 8 1
0.790	0.798	461	× 0.649 ×		× \$	<b>3</b> 1
1.98	1.85	2 ⁹³ 2	°~1.58	×92 ×92 ×980 ×92 ×92 ×92 ×92 ×92 ×92 ×92 ×92	2 1.54	2 78
4.94	4.88	Q [*] _2\$		²⁷ x5 /		79

### Analytical results: Measured concentrations of fluopicolide in sediment

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

Analytical results: Measured concentrations of luopic fluopic fluopic fluopic syster and overlying water

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Nominal sediment concentrations	Day 0		Day 28
		ater - measured concer 9µg a,s//L]	tration
Control & &	^A < <u>b</u> OD ^A ≪LOD	_© _≲ ₽ÕD	< LOD
Solvent control	LODO ³ ×	C _ Q LOD	< LOD
	N 124 Y 4	<u>ه</u> 8.74	7.18
0.126 × × × × × × × × × × × × × × × × × × ×	29.5	<b>23.6</b>	21.2
Q.790 0 5 5		57.3	48.8
1.98	2 258 2	164	150
4.94	673 ×	520	448
	Q 2-00 A73 A Q A73 A Q A74	water - measured con	centration
Control C		[µg a.s./L]	
Control	YPOD	< LOD	< LOD
Control Contro	V Q LOD	< LOD	< LOD
	2.15	3.51	5.73
	5.70	13.6	14.0
29 20.790 A 59	15.7	30.6	34.0
0.129 0.129 0.129 0.16 0.190 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.790 0.7	38.4	91.7	106
2 0.790 3 0.790 3 0.790 4 0.96 4.94	122	260	307

LOD = Inflit of detection (0.15  $\mu$ g/L)



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

### **Biological results:**

Survival

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

### Reproduction

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

Ĉı

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

Nominal sediment concentration [mg a.s./kg]	$\mathbf{K}^{\text{rean}} (\mathbf{S}\mathbf{D}) = \begin{bmatrix} \mathbf{K}^{\text{rean}} \\ \mathbf{K}^{$
Control	
Solvent control	& <u>\$21.3 \$3.61 @ \$ \$ \$ 0</u>
Pooled control 😽 🧟	$2257 \pm 3.957$
0.126	22.3 ± 03 × 0 × -0.7
0.126 0.316 0.700 0.700	
0.700	$\begin{array}{c} 0 \\ 29.3 \pm 3.59 \\ \end{array}$
<u><u> </u></u>	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$
4.94	25.3*

SD: Standard deviation, * Stenificant difference to the booled Control (William Gest)

**Biomass** 

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

Concentration (mg a.s./kg)	A Mean (≠ SD)	Reduction in % of pooled controls
Contrôl	Q 41.8 ± 6.25	_
Solvent control	37.6 ± 2.75	-
Profed control		-
	\$ 35.5 ± 1.44	9.6
	$35.0 \pm 0.80$	10.8
0.790	$35.6 \pm 1.64$	9.4
1.98	$36.4 \pm 0.20$	7.5
4.94	$35.5 \pm 2.3$	9.6

SD: Standard deviation



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

### **III. CONCLUSION**

The study meets the validity criteria and the endpoints based on nominal sediment concentration

æ.	- 1	
Survival	Reproduction	Biomass (dev weight)
$> 4.9$ $\sqrt{2}$ $\sqrt{2}$ $\sqrt{2}$ $\sqrt{2}$	2 4 94 Mg a s /kg	4 94 mg a s 4 g
O' U' X		
4.94 mg a.s./kg	Q1.98 mg a.s./kg	04.94 mora.s./kg
2494 mg @.s./kg		
	Survival > 4.94 mg a.s./kg	Survival Reproduction > 4.94 mg a.s./kg A.94 mg a.s./kg

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

the second of th This study is considered reliable for risk assessment and the endpoint concentrations) is: NOEC (28 d) = 1.98 mg a 3 kg0 (based on nominal



# CA 8.2.6 Effects on algal growth

# CA 8.2.6.1 Effects on growth of green algae

The 72-hour toxicity test with the freshwater alga (*Scenedesmus subspicatus*) by 2004; M-227278-01-1 was originally submitted for the Annex Linelusion

- however since it was conducted with a straight SC 480 formulation is not considered relevant for this section.

Data Point:	KCA 8.2.6.1/02
Report Author:	
Report Year:	
Report Title:	AE C638206: A 96-hour toxicity test with the freshwater atea (Selenastrum)
	capricornotum)
Report No:	M-219737-01-2
Document No:	M 210727 01 2 %, Ø Ä Ä Å Å
Guideline(s) followed in	EU (=EEC): 92/69/EEC C 2 (1992) OECDO 201 (384); USEPA (=EPA) OPPTS ·
study:	850 5400 (1996) 🔍
Deviations from current	Method: Deviations from current guideline SANCO/3029/99 tev.4: Limited sets of validation recoveries were analysed However, the average of recoveries were within the acceptable range of 70 \$10% and the RSD values were below 20%. The Malutical method can be regarded as failed running of a
test guideline:	Limited sets Walidation recoveries were analysed However, the average
	recoveries were within the acceptable range of 70, 410% and the RSD values were
	below 20/y. The analytical method can be regarded as proto purpose.
	Study: (prrent&uideline: OF(2) 201 (2011) O O O
	The light intensity was slightly lowe than recommended in OECD guideline: it
	was \$900-4500 lux instead of 4440-8880 lax, but this is compliant with OCSPP
	guideline which recommends 4300 lux $+\frac{1}{2}$ 15% Since the growth of the controls
0	was satisfactors and not the validity criteria, this deviation is not considered
<i></i>	relevant.
Previous evaluation:	yes evaluated and accepted by of the former
Q ¹	$1 \text{ in } \text{DAR}(38,2005) = \emptyset$ $3^{12}$ $3^{12}$ $3^{12}$
GLP/Officially	Yes, conducted under GLP/Officially recognised testing facilities
GLP/Officially recognised testing facilities:	
facilities:	
Acceptability/Reliability:	Kes v v v v
× ×	
Executive summary 🖉	

The green alga, *Selenastrum capricornulum*, (syn *Pseudokirehneriella subcapitata*) was exposed to a geometric series of five test concentrations of fluopicolide a negative (culture medium) control and a solvent (dimethylformanide) control inder static conditions for 96 hours. Three replicate test chambers were maintained in each treatment group and six toplicates for each control group. The selected nominal test concentrations of fluopicolide were 0.31, 0.63, 1.2, 2.5 and 5 mg a.s./L. Fluopicolide was analysed by high performance liquid chromatography (HPLC) using an ultraviolet (UV) detector. Samples of the test solutions were collected at approximately 0 and 96 hours to measure concentrations of the test substance. Measured concentrations (LOQ) were measured in the controls. The mean measured concentrations, were: 0.30, 0.59, 1.2, 2.4 and 4.3 mg a.s./L. The study meets the validity criteria of the guideline OECD 201. Samples collected on day 0 had recoveries of 96, 94, 93, 94 and 87% of nominal concentrations, respectively. Mean measured test concentrations were 0.30, 0.59, 1.2, 2.4 and 4.3 mg a.s./L. The study meets the validity criteria of the guideline OECD 201. Samples collected on day 0 had recoveries of 96, 94, 93, 94 and 87% of nominal concentrations, respectively. Mean measured test concentrations were 0.30, 0.59, 1.2, 2.4 and 4.3 mg a.s./L. respectively. The 96-hour  $E_bC_{50}$  values. The 72-hour  $E_bC_{50}$  and  $E_1C_{50}$  values are 3.0 and > 4.3 mg a.s./L, respectively. The 96-hour  $E_bC_{50}$  and  $E_1C_{50}$  values are 3.0 and > 4.3 mg a.s./L, respectively. The 96-hour  $E_bC_{50}$  and  $E_1C_{50}$  values are 3.0 and > 4.3 mg a.s./L, respectively. The 96-hour  $E_bC_{50}$  and  $E_1C_{50}$  values are 3.0 and > 4.3 mg a.s./L, respectively. The 96-hour  $E_bC_{50}$  and  $E_1C_{50}$  values are 3.0 and > 4.3 mg a.s./L, respectively. The 96-hour  $E_bC_{50}$  and  $E_1C_{50}$  values are 2.7 and 4.3 mg a.s./L, respectively. The 72-hour NOAErC, is 2.4 mg a.s./L.



### I. MATERIAL AND METHODS:

Test material	Fluopicolide Batch No. OP2350005 Specification: AE C638206 00 1C99 0006 Purity 99.4 % None specified Algae <i>Pseudokirchneriella subcapitata</i> (UTCC 37) formerly <i>Selenastrum capricornutum</i>
i est material	Batch No. OP2350005
	Specification: AE C638206 00 1C99 0006
	Purity 99.4 %
Guideline(s)	None specified
adaptation	
Test species	Algae Pseudokirchneriella subcapitata (UTCC 37)
·····	formerly Selenastrum capricornutum (S
Culturing	Algal cells used in this test were obtained from cultures that had been actively
conditions	growing in culture medium for at least two weeks prior to test initiation? The corture
	was last transferred to fresh medium three days prior to test initiation The alga
	cells were cultured and tested in freshwater algal medium as defined in ASTM
	Standard guide 1218-90F $\sim$
Test solutions	Nominal concentrations: @1, 0, @, 1.3, 2.5, 5, 0 mg a@/L
	Corresponding mean measured concernation 0.30, 0.59, 1.2, 2.4, d.3 mga.s./L &
	Control: culture medium $2$ $2$ $3$ $3$ $3$ $3$ $3$ $3$ $3$ $3$
	Solvent control: 0.1 mil/L dipnethydrormarfuide
	All solutions were clear and colourless at test initiation. At test termination the 5.0
	mg/L solution had visible precipitation.
Replication	Corresponding mean measured concentration 0.30, 0.59, 1.2, 2.4, 4.3 mga.s./L Control: culture medium Solvent control: 0.1mil/L dimethytrormanide All solutions were clear and colourless at test initiation. At test termination the 5.0 mg/L solution had visible precipitation. No. of vessels per concentration (replicates): 3 No. of vessels per concentration (replicates): 6 No. of vessels per solvent control (replicates): 6 Static
	No. of vessels per control (replicates): 6
	No. of versels per solvent control (replicates) 6
Exposure	Static O' D' O' O' D' O' D' O' D' O' D' O' O' D' O' O' O' D' O' O' O' D' O'
	Total exposure duration: 96 hours 5 5 5
Initial cells	No. of vessels per control (replicates): 6 No. of vessels per solvent control (replicates) 6 Static Total exposure duration: 96 hours 1 × 40 ⁴ cells/mL fr each test group
density	
Test conditions	$\mathcal{A}$ empérature: $\mathcal{A}$ / $\mathcal{A}$ / $\mathcal{A}$ / $\mathcal{A}$ / $\mathcal{A}$
^C	Photoperiod continuous hight Light intensity: 5000 to 4500 fbx
Ŭ.	$\begin{array}{c} \textbf{BH}: 7.7 \stackrel{\frown}{=} 9.0 \\ \textbf{SH}: 7.7 \frown$
	Growth medium same as culture medium? Yes
žQ ⁴	Type of light: Cool white fluorescent tubes, O
Parameters	Test medium samples were collected from each replicate of the treatment and
Measured /	Zontrol group for the determination of algal cell densities. Samples were collected
Observations	at approximately 24-how intervals during the 96-hour exposure and were held for
	a maximum of one day under Pefrigerated conditions sufficient to inhibit growth
Ŷ	until cell counts could be pettormed.
À	Temperature was measured twice daily. Light intensity was measured at test
L.	initiation, pH was measured at lest initiation and termination.
Sampling for	Samples of the test solutions were collected at approximately 0 and 96 hours to
chemical	measure concentrations of the test substance. Samples at test initiation were
analysis 🖉	collected from the individual batches of test solution prepared for each treatment
_04	and control group prior to addition of the algae. At test termination, samples were
	collected from the pooled replicates from each treatment and control group.
Ű,	Fluopicolide was analysed by high performance liquid chromatography (HPLC)
j <u>p</u>	using an ouraviolet (UV) detector.
chemical analysis	
K S	
Ĉ	



Data analysis	The calculations, as well as all statistical analyses, were conducted using "The SAS
	System for Windows", Version 8.02 or TOXSTAT version 3.5. Nonlinear
	regression or linear interpolation was used to calculate EC50 values and their
	corresponding 95% confidence intervals. The data were evaluated for normality
	and homogeneity of variance (p=0.05) using the Shapiro-Wilk's and Levene's Jests, 10
	respectively. The treatment groups then were compared to the pooled control using
	Dunnett's test (p=0.05). If the assumptions of normality and homogeneity of
	variances were not met an attempt was made to contect the condition by log of
	transformation of the data. If the data failed the assumptions of normality and/or
	homogeneity of variances and transformations would not correct the problem
	Dunnett's test was still used to make the comparisons.

II. RESULTS AND DISCUSSIO	II. RESULTS	AND DISC	USSIQ
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D'unitette stebet was still abea to manp the company	
II. RESULTS AND DISCUSSION:	
Validity criteria (OECD 201, 2011)	red 🖉 🖉 Obfained 🖑
Increase of biomass in the control cultures	6 ° (7211), 365 96 h)
Mean coefficient of variation for section-by section	
specific growth rates (days 0-1, 1-2 and 2-3) in the $3$ in the	7.6% (72b), 12.0 (96h)
Coefficient of variation of average specific growth rates $\sqrt{79}$	% ≈ 3.2% €72 h) 2.0 (96h)
in replicate control cultures $\sqrt{2}$	
Analytical results:	

### Analytical results:

Nominal concentrations selected for use in this shirdy were 0.31, 0.63 Y.3, 2.5 and 50 mg a.s./L. Mean measured test concentrations were 0.30, 0.59, 42, 2.4 and 4.3 mg a.5 L representing 97, 94, 92, 96 and 86% of nominal, respectively. Mean measured test concentrations were used to calculate the EC₅₀ 0 values. Ň 0

No residues of fluor colide were deasured in the controls above the limit of quantification (201  $\mu$ g/L).

Nominal Concentration (mg a.s./L)?	(mg a _x s, /L)	Whour %	96-bour % Nominal
0.31	0.30	95.5 O ⁵⁷ 1	Ø5.5, O
0.63		94.2	93.3
1.3	51.2 A &	92.6	<b>.</b> 4
2.5	2.40 2.40	99.0 ° ′	94.1
5.0	4.3 8 8	87.2	84.4
<u>S</u>	6 8 0		

Full details and acceptable validation data to support this method are presented within document M-CA

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



# Biological results:

Mean measured	Biomass inhi	oition (%)	Growth rate in	hibition (%)	₽ ₽ Ø
concentrations		0.61			Dr.
(mg a.s./L)	72 h	96 h	72 h	96 h	
0.30	-3.2	0.17	-0.51	0.29	
0.59	5.2	6.9	0.97	1.6	Ĉn
1.2	9.4	17*	1.8	4.4# 57 ~ 57 5	Î
2.4	29*	39*	6.9# 🖉		Ŵ
4.3	81*	86*	39* 🐨		Š
# Although statistica this amount of inhib Exponential grow The study meet concentrations an	Ily significant a dition was consident with in the construction was solved by the construction of the solution of the solution	5.9 and 4.4% inhibit red to be within an trol: yes	tion from the contract of the	hibition (%) 96 h 0.29 1.6 4.4# 11* 36* cates using Dunnett's test rol way not considered to be treatment related since or <i>Selenastruk</i> he endpoints based on measured <b>s./Leptot calculable</b> L(2.8-32)	¥
ErC ₅₀ 72 hours,	96 hours (95%		>4.3 mg/a.	s./Lanot calculable	
$E_b C_{50}$ , 72 h	, Q		3.0 mg a.s./	L (2.8-3 )	
$E_b C_{50}$ , 96 h	×, 4		2 @ mg a 9	L (224-2.9)	
NOAEC 72 hour highest concentra	s (Growth rate)	verse effects	2.4 mg a.s.		
NOAEC 72 hour	Bioma Ce	ll density			
highest concentre	tion without ac	Verse effects	× 1.2 mg a:s.7		
Č,	oints were cal		the data require	ements, they are presented below.	
				9	

Report Author:	
Report Year: 🕡	
Report Title 🖓 🖉	Cx calculation for fluopicolide study on the green algae Pseudokirchneriella
2	Subcantata (Mesjardins et al @2003), M-219737-01-1)
Report No. 2	M-67-3768-64-1
Document No:	<u>M-643768-01-1</u>
Guideline(s) followed in	apone de la companya de la company
study:	
Deviations fron @urrent	Nor applicable
test guideline	
Previous evoluation:	No, not previously submitted
GLP/Officially	notapplicable
GLP/Officially recognised techng	
iugaotico.	$\checkmark$
Acceptab Wity/Reliability:	Yes
0	

Ĉ



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

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

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

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

, e	× 0/	KĨ Ŭ		"0" L	, A c	0
	A	<u>v</u>	Q 4 .	° 0'		1
ErC ₁₀ 72 hours (95% CI)		2.6 mg a.s. 1 (				
E _r C ₂₀ 72 hours (95% CI)	<u> </u>	3 mg a 3./L (	2,9-3.5)		, Q	
E _b C ₁₀ , 72 h (95% CI)		2.2 mg a.s./b)	1.5-20			
E _b C ₂₀ 72 hours (95% CI)		30 mg ats./L (	2 \$ 3.4)	ð %	1	
$E_rC_{10}$ 96 hours (95% CI)		1.7 m@a.s./L (	1.3-2.0			
E _r C ₂₀ 96 hours (95% CI)		20 mg a SiL (	×	- S		
E _b C ₁₀ , 96 h (95% CI)		§1.1 mg/a.s./L (	9.6-1.5	Ç,		
$E_bC_{20}$ 96 hours (95% CI)	$\mathcal{X}$	1.5 mg a.s.a. (	1.04.8)			
	Ő KOT	ý _b				

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

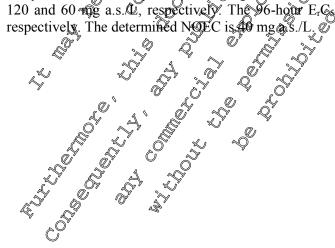
the endpoint relevant for the floor the endpoint relevant floor the endpoint relevant for the floor the endpoint relevant for the floor the endpoint relevant floor the endpoint relevant floor the endpoint relevant floor the endpoint floor the endpoint relevant floor the endpoint relevant floor the endpoint relevant floor the endpoint floor the endpoint floor the endpoint floor the endpoint floor the



Data Point:	KCA 8.2.6.1/03
Report Author:	
Report Year:	2001
Report Title:	2,6-dichlorobenzamide (BAM): Algal inhibition test
Report No:	1133/007
Document No:	<u>M-234304-01-2</u>
Guideline(s) followed in	EU (=EEC): 67/548/EEC; EU (=EEC): 92/69/EEC C.3 (2992); OECD: 201
study:	(1984); USEPA (=EPA): J 122-2, OPPTS 850.5400 (1996)
Deviations from current	Method: Deviations from current guideline SANCQ 3029/99 rev.4:
test guideline:	Limited sets of validation recoveries were analysed. However, the average
	recoveries were within the accept to be range of $M^{2}$ 110% and the KSD values were
	below 20%. The analytical method can be regarded as fit for purpose 2 2
	Study: Current Guideline: OFCD 201 (201)
	The pH in control increased by more than 1.3 unit at 96 how measurement was
	performed at 72h. Since tois change is the consequence of the important growth
	(factor 185 over 96h) and since the valuative criteria are met, this deviation is hot
	considered relevant. $O' = O' $
	The light conditions were not measured, but the test was performed with
	continuous illumination at approximatively 7000 lux, which is in the
	recommended range. However, the growth in control replicates was satisfactory so
	there is no impact of this deviation of the results of the test
Previous evaluation:	yes, evaluated and accepted 2 2 2 2 2
GT D (0.00 + 11	in DAR (2005)
GLP/Officially	Yes, conducted under JLP/Othicially recognised testing facilities
recognised testing	
facilities:	
Acceptability/Reliability:	loges O and a construction of the second sec

# Executive summary *K*

Executive summary The green alga, *Pseudokirchnerofilla subcapitata* was exposed to a geometric series of five test concentrations to the fluopicolide metabolite M-01-(2-6-dichlorobenzamide) and a negative (culture medium) control understatic conditions for % hours. After 96 hours a re-growth experiment of a maximum duration of 144h was performed. The selected nominal test concentrations of fluopicolide were 10, 20, 40, 80 and 160, mg a stL. From picolide was analysed by high performance liquid chromatography (HPLC) using an ultraviolet (UV) detector Samples of the test solutions were collected at 0, 24, 48, 72 and 96 hours 10 measure concentrations of the test substance. Measured concentrations were in the 80-120% range of nominal concentrations and no residues above the limit of quantification (LOQ) were measured in the control Therefore, results were based on nominal concentrations. The study meets the validity criteria of the guideline  $GECD_{201}$ . The 72-hour  $E_rC_{50}$  and  $E_bC_{50}$  values are 120 and 60 rog a.s. (1, respectively. The 96-hour  $E_r G_{50}$  and  $E_b C_{50}$  values are 140 and 62 mg a.s./L,





### I. MATERIAL AND METHODS:

Test material	M-01 (2,6-dichlorobenzamide, BAM, AE C653711) Batch number: FUX001000/FUN81G02C Purity: 99.5% None specified Freshwater green algae <i>Pseudokirchneriella subcapitato</i> Strain CCAP 278/4 The culture was maintained in the laboratory at ademperature of 21 ± °C under
Guideline(s) adaptation	None specified
Test species	Freshwater green algae <i>Pseudokirchneriella subcapitata</i>
Culturing conditions	continuous illumination (intensity approximately 9000 lux) and constant aeriation.
Test solutions	Nominal concentrations: $10 - 20 - 40 - 80 - 160$ mg/Iz $20 - 40 - 80$ mg/Iz $20 - 40 - 8$
Replication	No. of vessels per concentration (replicates): 3.
Exposure	Static Total exposure duration: 36 hours + re-growth experiment of 144 h without exposure
Initial cells density	$1 \times 10^{4}$ cells/mD in each test group $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{4}$ $3^{$
Test conditions	Protoperiod: continuous light Eight intensity approximately 7000lux pH at t0: 7.0-7.1&pH at 26h: 7.2, 9.8. In controls: 7.1, 9.7
	Water haulness: Dot specified Conductivity: not specified Growth medium same as culture medium. Yes Type of light: artificial (not specified)
Parameters Measured /@ Observations	The pH of each control and test flock was determined at initiation of the test and after 96-hour exposure. The temperature within the incubator was measured every hour Cell densities were determined at 0, 24, 48, 72 and 96 hours.
Sampling for chemical analysis	Water samples were taken from the control and each test group (individual replicate samples) at 0 and 96 hours for quantitative analysis by HPLC with UV detector.
Dată analysis	One-way analysis of variance incorporating Bartlett's test for homogeneity of variance and Dunnett's multiple comparison procedure for comparing several treatments with a control was carried out on the area under the growth curve data. The area under the growth curve data was transformed to its square root prior to analysis as statistical analysis of the untransformed data indicated that significant
Lang analysis	differences were apparent at 20 mg/1 but not 40 mg/L. All statistical analyses were performed using the SAS computer software package. $gc_x$ values were determined graphically. The 95% confidence limits were calculated using the method of Litchfield and Wilcoxon



### **II. RESULTS AND DISCUSSION:**

Validity criteria (OECD 201, 2011)	Required	Obtained 🖉 🖉
Increase of biomass in the control cultures	<u>&gt;16</u>	185
Mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3) in the control cultures	<u>≤</u> 35%	6.3% 4 5 5
Coefficient of variation of average specific growth rates in replicate control cultures	$\leq 7\%$	×.79%***

### Analytical results:

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

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		4	
Nominal Concentration (mg /L)	0-hour Measured Concentration (mg/L)	0-hour % Nominal	72-hour Measured 72-hour &
10	10.5	105	10,4 5 6 1046 5
20	20.8	x1,184 or	21,4 07 54 107 0
40	41.1	103	
80	81.5 0		84.2 10 105 2
160		100	163 5° 4 1402 5°

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

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

4				
Nominal	Biomass inhibition	Biomass	Growth rate	Growth rate
concentrations	Sat 724 (%)	inhibition at 96 h	inhibition at 72 h	inhibition at 96 h
(mg/L) **		(%)	(%)	(%)
10	5 0 0	6 O	1	0
20	417 Q ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	-Q6	3	0
40 O ×		11	0	-2*
80	v 67 6 🔊 🔊 🖓	65	23	15
160 0 0	96	98	79	74

prcrease compared to controls * growth

Exponential growth in the control: yes



Re-growth experiment:

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

### **III. CONCLUSIONS:**

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

ErC50 72 hours (95% C.I.):	
ErC ₅₀ 96 hours (95% C.I.):	120 mg/L     Image: Second secon
$E_rC_{10}$ 72 hours (95% C.I.):	
$E = \frac{72}{1000} hours (050/CL);$	62/mg/L (52-74 mg/L)
E _b C ₅₀ 96 hours (95% C.I.):	$\frac{62}{10} \frac{1}{10} $
E _b C ₁₀ 72 hours (95% C.I.):	$\frac{62 \text{ pag/L} (52-74 \text{ mg/L})}{\text{Ms mg/}}$
NOEC: highest concentration without adversed fects	
Assessment and conclusion by applicant. The study is reliable and the endpoint relev 72h-ErC ₅₀ of 120 mg/L	rant for the M-01 (AE C633711) risk assessment is the
	60 mg (p (51-7) mg/L 3 mg ( 40 mg /L 40 mg

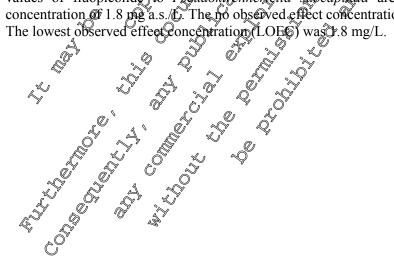


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Data Point:	KCA 8.2.6.1/04	
Report Author:		
Report Year:	2002	
Report Title:	Effect to Pseudokirchneriella subcapitata (green alga) in a growth inhibition test;	Ő
	AE C638206 technical 97.1 percent w/w	F
Report No:	B003804	
Document No:	<u>M-240808-01-1</u>	
Guideline(s) followed in	OECD: 201 (1992); USEPA (=EPA): 123-2 (1982)	2
study:		2
Deviations from current	Current Guideline: OECD 201 (2001)	
test guideline:	The study does not meet the validity criteria (see below) $\sqrt[n]{2}$	Ľ
Previous evaluation:	No, not previously submitted $\mathcal{L}$ $\mathcal{O}^{\vee}$ $\mathcal{L}^{\vee}$ $\mathcal{O}^{\vee}$	0
		<i>"</i>
GLP/Officially	Yes, conducted under GLB Officially recognised asting acilities	
recognised testing		
facilities:		
Acceptability/Reliability:	Supportive only O C C A C	
	$A  \mathcal{O}  \mathcal{Q}  \mathcal{Q}  \mathcal{O}  O$	

### **Executive summary**

Algal cultures of Pseudokirchneriella subcapituta with an initial rominal cell count of approximately  $1.0 \times 10^4$  cells/mL were exposed to mominal concentration of 50 mg/s./L or maximum achievable concentration of fluopicolide in AAP media for a 96-hour period. The sordy design included six replicate algal cultures each without test substance as the control treatment and six eplicate algal cultures each with test substance. At 24-hour intervals, the cell density (cells/mlQ of each culture was counted under a microscope using a haemocytometer. Samples were analysed by Gas Chromatography with MS detection (GC/MS) for determination of fluopfeolide and the metabolite M-01 (RE C653711). The recoveries were not in the range of 80-120% but the concentrations are stable over the study duration. Thus, biological results after 12 and 96 hours are based on arithmetic mean measured concentrations of fluopicolide. The arithmetic mean measured concentrations was 1.8 mg a.s./LyThe study does not meet the validity criteria of the current version of OECD 210 guideline, 1.8 mg a.s./L (arithmetic mean) represents 36% of the nominal concentrations. Test solutions all hour ranged from 29 to 33% of nominal, white at 96 hours they were 4 10 of pominal concentration indicating stability of the test substance. Since the highest concentration tested was prexcess of the maximum solubility of the test substance, the low percent of nominal was expected. In order to assure that fluopicolide (AE C638206) would for hydrolyse to the major metabolite Se 01 (AE C63711) under study conditions, the concentration of M-O1 (AE×C653711) in the samples are measured as well. The 72- and 96-hour endpoints are based on arithmetic mean. The  $\sqrt[3]{2}$  and 96-hour  $E_bC_{50}$  (biomass) and  $E_rC_{50}$  (growth rate) values of fluopfeolide to Pseudokinehner ella subcapitata are greater than the mean measured concentration of 1.8 pg a.s./C. The bo observed effect concentration (NOEC) is less than 1.8 mg a.s./L.





### I. MATERIAL AND METHODS:

Test material	Fluopicolide (AE C638206)         Batch number: 2050190//PP241024/2         Purity: 97.1%         None specified         Freshwater green algae Pseudokirchneriella subcapitata         Strain #1648         The culture was maintained in the laboratory at agemperature of 24.7 to 25.4 °C
Guideline(s) adaptation	None specified
Test species	Freshwater green algae <i>Pseudokirchneriella subcapitata</i>
Culturing conditions	under continuous illumination (intensity approximately 4800 to 5100 lux). The culture medium (AAP) used for the test was the same as that used to maintain the stock culture.
Test solutions	Nominal concentrations: 5.0 mg a \$\u00f5/L. Limit test at solubility limit Mean measured concentration: 1.8 mg a \$\u00e5/L unit Controls: AAP medium Evidence of undissolved material: Test solution was prepared in excess of solubility and filtered prior to study initiation. No evidence of undissolved material after filtering reported on the test solution.
Replication	No. of vessels per concentration (replicates), 6 0 0 0
Exposure	Static St
Initial cells density	Static Total exposure duration 96 hears $1 \times 104$ cells mL in each test group
Test conditions	Temperature of controls and test solution: 24.0 – 25.8°C 4 Photoperiod: continuous light Light mtensity 4200-4500 lux pHOn the control 7.6 788 Conductivity: 150 µmhos/cm Type of light, artificial (coor white fluorescent barlbs)
Paraméters Measured / Observations	Discrete, measurements of temperature, pH dissolved oxygen and salinity were obtained at test initiation and at test termination (i.e. 96 hours). Cell densities were determined at 24, 48, 72 and 96 hours.
Sampling for chemical analysis	Water samples were taken on day 0 and on day 4 (test termination). Samples taken at initiation were taken directly from parent test solutions after adequate mixing, and prior to the addition of algae. Samples taken at termination were composite from all deplicates within a treatment. Samples were analysed by Gas Opromatograph, with MS detection (GC/MS) for determination of fluopicolide and metabolite BAM.
Data analysis @	



### **II. RESULTS AND DISCUSSION:**

Validity criteria (OECD 201, 2011)	Required	Obtained 72 h	Obtained 96 h @
The increase of biomass in the control cultures.	<u>&gt;16</u>	50.8	1875
Mean coefficient of variation for section-by-section specific growth rates in the control cultures	<u>&lt;</u> 35%	47.0%	\$ <b>8</b> .9
Coefficient of variation of average specific growth rates during the whole test period in replicate control cultures	<u>&lt;</u> 7%	3.59%	
	Ĉ&	£ *	

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

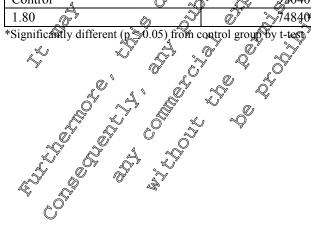
			E E		A A C
Nominal	Arithmetic mean	0-hour		96-hour	
concentration	measured	Measured	9-hour	Measured 🔬	96-hour &
(mg a.s./L)	concentration	Concentration	<b>% Nominal</b> C	Concentration	Nomina
(ing a.s./L)	(mg a.s./L)	(mg a.s./L)	S a	(mg a.s./L)	V à
5	1.8036	1.5625	31.25	Q.0446 0 , Š	¥ 40.9
	·~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~				

Full details and acceptable validation data to support this method are presented within occument M-CA 4, which comply with the EU regulatory requirements optimed within SANCØ/3029/99 rev 4. <u>Biological results:</u> <u>72 hours</u>

Arithmetic mean measured concentrat (mg a.s./L)	tion	Mean cell nu (cellomL &	mber P0 ⁴ )	Specific growth	h rste	Inhibition of average specific growth rate (%)
Control	× 1	50-8	ð, í	0.0544	J	-
1.80		S & .5		0.0510		6*
*Significantly different (*	6 0 05 Vafra	m control grow	hy t-fact			

Significantly different ( $p \leq 0.05$  strom control group by t-resp

Arithmetic mean n concentration (mg			nder the growt domass integra	162	Inhibition of Biomass integral (%)
Control 🐇		Y Q	9 <b>3</b> 04000		-
1.80	6 2	Ő	∕∼∕7484000		21*





### 96 hours

Arithmetic mean measured concentration (mg a.s./L)	Mean cell number (cells/mL × 10 ⁴ )		Specific gr (h ⁻		Inhibition of average specific growth rate (%)
Control	187	187		545	
1.80	124		0.05	501	8*5
*Significantly different ( $p \le 0.05$ )	from control group b	y t-test.			
			Ö		of Biemass 7
Arithmetic mean measured	Area unde		vth curve	Indibition of	of Biomass N
concentration	(biomass in	itegral) (		(integral (%	
(mg a.s./L)	27004000	-07	~		
Control	37804000	Q~	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~		
1.8036	26804000	K (			
*Significantly different ( $p \le 0.05$ ) The study does not meet the concentrations are:	<b>N</b> .	CONCLU	SIONS:	A points ba	seed on anothmetic mean
	S O	i Nj		ð ð	, 5 [°] , 4 [°]
ErC50 72 hours (95% C.I.):			80 mg@r.s./L.(	<b>p</b> .d.)	
LOE _r C 72 hours: lowest concentration with a compared to the control	gnificant effect		= 1.80 mg.a.s./		seed on arithmetic mean
NOE _r C 72 hours: highest concentration without effect compared to the como		~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	2.80 mg a.s./		1
ErC50 96 hours (95% C.I.):			80 mg a.s. (	n.d.)	
LOE _r C 96 bours: lowest concentration with a si			= 1.80 prig a.s.	Ø	
NOEr 96 hours: highest concentration without	a significant		¢1.80 mg a.s./		
$E_bC_{50}$ 72 hours (95% C). LOE _b C 96 hears:			80 mg/a.s./L (	n.d.)	
lowest concentration with a	gnificant effest		@.80 mg a.s./	L	
NOE _b C <b>%</b> hours:	a significant		< 1.80 mg a.s./	L	
EbC 56 96 hours (95% C.Ig		>1.	80 mg a.s./L (	n.d.)	
LOE _b C 96 hours. lowest concentration with a compared to the control	gnificant effect,	=	= 1.80 mg a.s./	L	
NOE _b CC6 hours. highest concentration without effect compared to the control		<	< 1.80 mg a.s./	L	

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

Assessment and conclusion by applicant:

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



Data Point:	KCA 8.2.6.2/01
Report Author:	
Report Year:	
Report Title:	Effect to Anabaena flos-aquae (blue-green alga) in a grow winhibition tes CAE
	C638206 technical 97.1 percent w/w
Report No:	B004237
Document No:	<u>M-241192-01-1</u>
Guideline(s) followed in	OECD: 201 (1992); USEPA (=EPG): 123-2 (1982)
study:	
Deviations from current	Method: Deviations from current guideline SANCO/3029/99 rev.4:
test guideline:	Limited sets of validation recoveries were analysed. However, the average
	recoveries were within the acceptable range of 70-7110% and the RSD values were
	below 20%. The analytical method can be regarded as fit for purpose with regard
	to this toxicity study.
	Study: Current Guidefine: QFCD 201 (2014)
	The study does not meet the validity criteria (see below)
Previous evaluation:	yes, evaluated and accepted a frequency of the second accepted and accepted a frequency of the second accepted accepted a frequency of the second accepted a frequency of the second accepted accepted accepted a frequency of the second accepted ac
	in DAR (2005) (10 10 10 10 10 10 10 10 10 10 10 10 10 1
GLP/Officially	in DAR (2003) Yes, conducted under GLP Officially recognised testing facilities
recognised testing	
facilities:	
Acceptability/Reliability:	Yes Q & Q Q Q Q Q

### CA 8.2.6.2 Effects on growth of an additional algal species

### **Executive summary**

**Executive summary** Algal cultures of Anabacya flos-aquae with an initial nominal cell count of approximately  $1.0 \times 10^4$ cells/mL were exposed to the nominal concentration. @ 5.0 kmg a.s./L or maximum achievable concentration of fluggicolideon Algel Assay Procedure (AAP) media for a 96-hour period. The study design included six replicate algar cultures each without test substance (control treatment) and six replicate algal cultures each with test substance. At 24-hour intervats, the coll density (cells/mL) of each culture was counted under a microscope using a baemocytometer. Water samples were taken on day 0 and on day 4. At test initiation they were taken directly from papent test solutions after adequate mixing, and prior to the addition of algae, Samples taken at termination were composite from all replicates within a treatment. The extracted flugscolide and fluopicolide metabolite M-01 (AE C653711) were measured by injection to gas chromatography with electron capture detector (GC/ECD). The recoveries were not in the range of 80-120% but the concentrations are stable over the study duration. Thus, biological results after 72 and 96 pours are based on arithmetic mean measured concentrations of fluopicolide. The arithmetic mean measured concentrations was: 2,2 mg as./L. The study does not meet the validity criteria of the Gurren Version of QECD 201 guideline All toxicity values were calculated based on the mean measured fluopicolide concentration of 2.2 mg a.s./L. Since the test solution was prepared in excess of solubility and altered prior to study initiation, the mean measured fluopicolide value represents the maximum achievable concentration under study conditions. The 72- and 96-hour endpoints are based on arithmetic mean. The 72- and 96-hour EbC30 (biomass) and ErC50 (growth rate) values of fluopicolide technical to Anabaena flos-aquae are greater than 2.2 mg a.s./L. The no observed effect concentration (NOEC) is 2.2 mg a.s./L. The lowest observed effect concentration (LOEC) could not be determined under the conditions of this study.



### I. MATERIAL AND METHODS:

Test material	Fluopicolide (AE C638206 (tech))
	Batch number: 2050190//PP241024/2
	Purity: 97.1% w/w
Guideline(s) adaptation	Batch number: 2050190//PP241024/2 Purity: 97.1% w/w None specified Freshwater blue-green algae (Anabaena flos-aquae strain 1444)
Test species	Freshwater blue-green algae (Anabaena flos-aquae strain 1444)
Culturing	Freshwater blue-green algae (Anabaena flos-aquae strain 1444) The algal cells were cultured and tested in freshwater AAP medium. Cultures were maintained at test temperature (2426 to 25 3°C) and under continuous light of
conditions	(approx. 1900 lux).
Test solutions	were maintained at test temperature (2426 to 25.3°C) and under continuous, light (approx. 1900 lux). Nominal concentrations: 5.0 mg a /L. Corresponding mean measured concentrations (0-96 h): 2.2 mg a.s. A. Controls: filtered medium control Evidence of undissolved material: Precipitate noted in stock solution of the preliminary test. Stock solution was filtered afterwards through a 0.45 µm filter to remove undissolved material. No information on precipitations given for main test, but stock solution for main test also filtered through a 0.45 µm filter.
Replication	No. of vessels per concentration (replicates): 6 5 5 5 5
Exposure	Static Total exposure duration: 96 doours of 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5
Initial cell	$1 \times 10^4$ cells at test initiation $2^{\circ}$ $2^{\circ}$ $2^{\circ}$
density	
Test	$1 \times 10^4$ cells in the st initiation $3^{\circ}$
conditions	Photoperiod continuous light Light intensity: 2300 to 2400 luc pla of controls (6-96 gours); 5.6 - 8.6
č	Conductivity: 900 μS cm Type of light: artificial (Cool white fluorescent Gubes)
Parameters 2	Test temperature was monitored continuous win a surrogate filled vessel within
Measured (	the environmental chamber. Discrete measurements of temperature, pH, dissolved
Observations	oxygen were obtained at test initiation, and at test termination (i.e. 96 hours). Cell
ES"	density was determined daily on a sub-sample from each flask by counting cells
<i>v</i>	with a hemacytometer and compound microseope.
Sampling for	Water samples were taken on day Oand on day 4. At test initiation they were
	taken directly from parent test solutions after adequate mixing, and prior to the
analysis 🦉	ad tion of algae. Sample's taken at termination were composite from all replicates
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	within a reatment. The extracted floopicolide and fluopicolide metabolite (AE
	C653711) were measured by injection to gas chromatography with electron capture detector (GC/ECD).
Data analysis	A STATISTICAL ABAILYSE SAMPLE DEPTORMED USING TITX NIA I \simeq 1 Version 3 41 With
Data analysis	All statistical analyses were performed using TOXSTAT [®] (Version 3.4) with conclusions of the statistical significance at the $\alpha = 0.05$ (95% confidence interval).

II. Results and Discussion:

Validity critecia (OECD 201, 2011)	Required	Obtained 72h	Obtained 96h
Inerease of Jomass in the sontrol cultures	<u>></u> 16	15.4*	53*
Mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3) in the control cultures	<u><</u> 35%	62.9%*	52.7%*
Coefficient of variation of average specific growth rates in replicate control cultures	<u><</u> 10%	4.28%*	4.46%*

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



Analytical results:

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

Nominal Concentration (mg a.s./L)	Arithmetic mean measured concentrations after 96 hours	% of nominal concentrations	
5.0	2.2	44* 2 44 & 2 44	ġ.

- 0.023 mg/L).		
Nominal Concentration	Arithmetic mean measured concentrations after 96	% of nominal concentrations
(mg a.s./L)	hours	0-hour 96-hour & a C
5.0	2.2	
Mean of two measu	rements (injections to GC/ECD)	
Full details and a	agentable validation data	A current this mathed are presented within doou from the wathed
Full details and a	contable validation datat	Support the method are presented within document M&A
Full details and a	contable validation datato	Support the method are presented within document M&A
Full details and a	contable validation datato	Support the method are presented within document M&A
Full details and a	contable validation datato	Support the method are presented within document M&A
Full details and a	contable validation datato	Support the method are presented within document M&A
Full details and a	contable validation datato	Support the method are presented within document M&A
Full details and a 4, which comply ninor acceptable <u>Biological results</u> 7 <u>2 hours</u>	cceptable validation data $\frac{1}{2}$ with the EU regulatory in exceptions only.	o support this method are presented within document MCA requirements outlined within SANCO 3029/99 rev 4 with
Full details and a 4, which comply ninor acceptable <u>Biological results</u> 72 hours Arithmetic mear	cceptable validation datation with the EU regulatory in exceptions only.	o support this method are presented within document MCA requirements outlined within SANCO 3029/99 rev 4 with
Full details and a 4, which comply ninor acceptable Biological results 72 hours Arithmetic mean concentrations	cceptable validation datation with the EU regulatory in exceptions only.	or support this method are presented within document MCA requirements outlined within SANCO 3029/99 rev 4 with
Full details and a 4, which comply ninor acceptable <u>Biological results</u> 72 hours Arithmetic mear concentrations (mg a.s./L)	cceptable validation datation with the EU regulatory in exceptions only.	o support this method are presented within document MCA requirements outlined within \$ANCO3029/99 rev 4 with
Full details and a 4, which comply minor acceptable Biological results 72 hours Arithmetic mean concentrations	cceptable validation datation with the EU regulatory in exceptions only.	o support this method are presented within document MCA requirements outlined within \$ANCO3029/99 rev 4 with imper after mo × 10% (tp ⁻¹) (%) 200 0 0.03788 -

Arithmetic mean	measured	OAreatund		inh	ibition of Biomass
concentrations (mg a.s./L)		curve (bio	mass integral)		integral (%)
Contro		£8 گ	3602 6 9° 🗸 .		-
2.2		<i>چ</i> ي ²⁴	60000 [#] 🏑 🔏	5	14
# Significantly differen	1 tost $n < 0.05$	Frame approx			

Significantly different?t-tes

96 hours

(1)

Arithmetic mea concentrations (mg a.s.*L)	ò	Nean cell number after 96 h (cells/tell × 109*	Specific growth rate (h ⁻¹)	Inhibition of average specific growth rate (%)
Control		529000 [°]	0.04120	-
2.2 🌹	, Or	596000	0.04253	-3

Arithmetic mean measured concentrations (mg a.s./b)	Area under the growth cucye (biomass integral)	Inhibition of Biomass integral (%)
Control 2 2	10812000	-
2.2 5 0 5 5	11128000	-3



III. CONCLUSIONS:

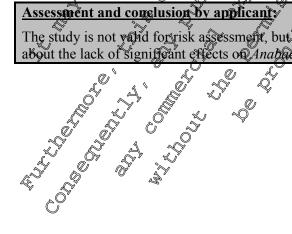
The study does not meet the validity criteria. The 72- and 96-hour endpoints based on arithmetic mean Ľ are: ð

uic.	
E _r C ₅₀ 72 hours:	> 2.2 mg a.s./L
LOE _r C 72 hours: lowest concentration with a significant effect compared to the control	> 2.2 mg a.s./L $> 2.2 mg a.s./L$ $> 3.2 mg a.s./L$
NOE _r C 72 hours: highest concentration without a significant effect compared to the control	2.2 mgra.s./L
E _b C ₅₀ 72 hours (95% C.I.):	$\frac{\Delta}{\sqrt{2}} = \frac{\sqrt{2}}{\sqrt{2}} \frac$
LOE _b C 72 hours: (lowest concentration with a significant effect compared to the control	$\sim 0^{\circ}$ $\sim 0^{\circ}$ 2.2 $\sim 0^{\circ}$ g a.s./L $\sim 0^{\circ}$ $\sim 0^{\circ}$ $\sim 0^{\circ}$
NOE _b C 72 hours: highest concentration without a significant effect compared to the control	
ErC ₅₀ 96 hours (95% C.I.):	6 2.2 mg a.s./L ~ ~ ~
LOE _r C 96 hours:	\sim 2.2 mg as. /L \sim 2.2 mg
NOErC 96 hours: highest concentration without a sign frcant effect compared to the control	57 57 22 mg a.S./L
EbC50 96 hours 95% C.I.):	> 2.1 mg a.s./L
LOELC 96 Baurs:	2.2 mg a.s./L
NOE _b C 96 hours: highest concentration without a significant effect compared to the sentrol	©2.2 mg a.s./L

Assessment and conclusion by applicanty

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

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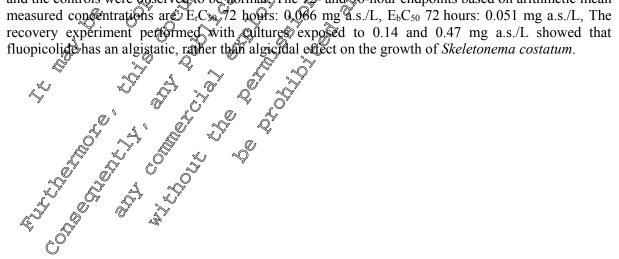




Data Point:	KCA 8.2.6.2/02
Report Author:	
Report Year:	2003
Report Title:	AE C638206 - Acute toxicity to the marine diatom, Skeletonema costatum, ander
	static conditions
Report No:	M-223563-01-2
Document No:	<u>M-223563-01-2</u>
Guideline(s) followed in	EU (=EEC): L383A - C.3 (1992); OECD: 201 (1984); USEPA (=EPA): QPOTS
study:	Draft 850.5400 (1996)
Deviations from current	Method: Deviations from current guideline SANCO/3029/99 rg/4: 2
test guideline:	Recoveries were determined at three different Oncentrations in triplicate.
	However, the obtained data demonstrate very good recoveries and the precision.
	The method can therefore be regarded as fit for purpose. $O' \qquad O' $
	The study does not meet the vehidity exteria 2 2 2 2 2
Previous evaluation:	yes, evaluated and accepted
	in DAR (2005)
GLP/Officially	in DAR (2005) Yes, conducted index GDP/Officially recognised testing facilities
recognised testing	
facilities:	
Acceptability/Reliability:	Supportive only of the transformed at the transform
Executive summary	

Executive summary

Executive summary The objective of this study was to determine the effect of fluopicolide on the growth of the marine diatom *Skeletonema costation* over 96h. For the culturing and the test the same medium was used: AES (artificially enriched seawater) prepared with sterile filtered natural seawater. Mominal concentrations were: 0.0012, 0.0041 0.014, 0.045, 0.15 and 0.50 mg a.s./L. Additionally, water and solvent controls (dimethylformamide at 0.1 μ L/mL) were included. The test comprised 3 replicates for each concentration/control. Samples analysed at 26 hours of exposure were removed from individually composited replicate solutions of the treatment levels and controls. Samples were analysed by gas chromatographic analysis with election capture detection (GC/ACD). Some recoveries were not in the range of 80 7 120% of nominal but the substance is stable over the test duration. Thus, biological results after 72 hours and 96 hours are based on arithmetic mean concentrations. The corresponding arithmetic mean measured concentrations (0 - % h): 0.0014 (0.0044, 0.01) 0.046, 0.14 and 0.47 mg a.s./L. The study is not considered valid according to the QECD 201 guideline. Cells exposed to the 0.14 and 0.47 mg a.s./L treatment levels were observed to be bloate Cells exposed to the remaining treatment levels and the controls were observed to be normal. The 72- and 96-hour endpoints based on arithmetic mean measured concentrations are E_rC_{36} , 72 hours: 0.066 mg a.s./L, E_bC_{50} 72 hours: 0.051 mg a.s./L, The





I. MATERIAL AND METHODS:

Test material Lot No: 2050190/PP241024/2 Purity: 97.7% w/w Guideline(s) adaptation Evaluation of recovery was included in the study		
Culturing conditionstemperature of $20 \pm 2^{\circ}$ C, a photoperiod of 14 hears light: 10 hours darkness and a light intensity of of 4300 to 5400 tux. For the culturing and the test the same medium was used: AES(dartificially enriched seaward) prepared with sterile, filtered natural seaware. Inoculum used to initiate the toxicity test with AE C638206 was taken from a stock culture that had been transferred to firsh medium six days before testing.Test solutionsNominal concentrations: 0/0012, 0/0041/0.014, 0.045(0.15 and 0.59 Mg a.s./L Corresponding withmetic mean measured concentrations: 0/0014, 0.0046, 0.016/0.046(0.14 did 0.47 Mg as:/L Controls: writer and solvent controls (dimethylformarbide at 011 µL/mL) Evidence of und/solveof material: AL fest solution, were observed to be clear, wolorless and did not containing visible undissolved test substance.ReplicationNo. of vessels per concentration (replicates): 3 No. of vessels per concentrations Recovery period up to 9 days for concentrations 0.45 and 0.50 mg a.s./LInitial cell density7.7 $\times 10^4$ cells/mL/det infinition Properative: 1821°C Protoperiod up to 9 days for concentrations 0.45 and 0.50 mg a.s./LParametersTemperature: 1821°C Protoperiod up to 9 days for concentrations 0.45 and 0.50 mg a.s./LParametersTemperature: 1821°C Protoperiod up to 9 days for concentrations 0.45 and 0.50 mg a.s./LParametersTemperature: 1821°C Protoperiod up to 9 days for concentrations 0.45 and 0.50 mg a.s./LParametersTemperature: 1821°C Protoperiod up to 9 days for concentrations 0.45 and 0.50 mg a.s./LParametersTemperature: 1821°C Protoperiod up to 9 days for concentrations 0.45 and 0.50 mg a.s./LParametersTemperature: 200 46000 lu	Test material	Fluopicolide (AE C638206)
Culturing conditionstemperature of $20 \pm 2^{\circ}$ C, a photoperiod of 14 hours light: 10 hours darkness and a light intensity of of 4300 to 5400 lux. For the culturing and the test the same medium was used: AES(dartificially efriched seawater) prepared with sterile, filtered natural seawater. Inoculum used to initiate the toxicity test with AE C638206 was taken from a stock culture that had been transferred to fresh medium six flays before testing.Test solutionsNominal concentrations: 0/0012, 0/0041, 0.014, 0.045; 0.15 and 0.50 kmg a.s./L Corresponding drithmetic mean measured concentrations: 0/0014, 0.0044, 0.016, 0.046; 0.14 did 0.47 kmg as:/L Controls: water and solved flat of 0.47 kmg as:/L Controls: water and solved flat of 0.41 light end of 0.0014, 0.0044, 0.016, 0.046; 0.14 did 0.47 kmg as:/L Controls: water and solved flat of 0.41 light end solved flat of 0.0014, 0.0044, 0.016, 0.046; 0.14 did 0.47 kmg as:/L Controls: water and solved flat of 0.41 light end solved flat of 0.0014, 0.0044, 0.016, 0.046; 0.14 did 0.47 kmg as:/L Controls: water and solved flat of 0.41 light end solved flat of 0.14 light and as:/L Controls: water and solved flat of 0.41 light end solved flat of 0.14 light and the test flat of 0.0014, 0.0044, 0.016, 0.046; 0.14 did 0.47 kmg as:/LReplicationNo. of Vessels per concentration (replicates): 3 No. of Vessels per concentration (replicates): 3 No. of Vessels per concentrations 0.45 and 0.50 kmg a.s./LInitial cell density7.7 $< 0^{\circ}$ cells/mL/delest infination Properiod up to 9 days for concentrations 0.45 and 0.50 kmg a.s./LInitial cell density7.7 $< 0^{\circ}$ cells/mL/delest infination Properiod up to 9 days for concentrations 0.45 and 0.50 kmg a.s./LParametersTemperature: 1821°C Protoperiod: go 1.20°C Protoperiod: go 1.20°C <br< td=""><td></td><td>Lot No: 2050190/PP241024/2</td></br<>		Lot No: 2050190/PP241024/2
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Exposure Static Total exposure duration: 96 hours Recovery period up to 9 days for concentrations 0.45 and 0.50 mg a.s./L Initial cell density 7.7 10 ⁴ celb/mL/aftest initiation Test conditions Temperature: 1821°C C Photoperiod: continuous light Light intensity: 4009-46000 lux PHor test and controls (0 – 96 b): 7.9 = 8.7 Salinity 30 +/-2 g/L Conductivity, 39 000 – 41 000 µmbos/cm Type of light: artificial Parameters Measured / Observations	Replication	No of vessels per control (replicates): 3
Exposure Total exposure duration: 96 hours Recovery period up to 9 days for concentrations 0.95 and 0.50 mg a.s./L Initial cell density 7.7 > 10 ⁴ cells/mL/attest initiation Test conditions Temperature: 18-21°C C Photoperiod: continuous light Light intensity: 4009-46000 lux Photoperiod: controls (0 – 96 b): 7.9 = 8.7 Salinity 30 +/*2 g/L Conductivity 39 000 – 41 000 µmbos/cm Type of light: artificial Parameters Measured / Observations		
Parameters Measured / Parameters Measured / Observations Temperature was measured continuously in a flask of water adjacent to the test flasks in the environmental chamber. Minimum and maximum	Evnogura	
Initial cell density 7.7 × 10 ⁴ celb/mL al test initiation Test conditions Temperature: 18×21°C Parameters Description Measured / Observations Very status Temperature: 18×21°C Parameters Temperature: 18×21°C Measured / Temperature: 18×21°C Observations Temperature: 18×21°C Parameters Temperature: 18×21°C Measured / Temperature: swere scorded daily. Light intensity was measured at hour 0 and ot to the 24 from interval during the expression period. Water anglity measured at hour 0 and ot to the 24 from interval during the expression period. Water anglity measured at hour 0 and ot to the 24 from interval during the expression period.		Total exposure duration: 96 hours
Test conditions Photoperiod: continuous light Light intensity: 4000-46000 lux pH of test and controls (0-96 b): 7.9=8.7 Salinity 30 +/×2 g/L Conductivity: 39 000-41 000 μmbos/cm Type of light: artificial Parameters Measured / Observations Temperatures were recorded daily. Light intensity was measured at hour 0 and at who 24 four intervaled ring the expression pariod. Water quality perspectators	Ĵ.	Recovery period up to 9 days for concentrations 0.45 and 0.50 mg a.s./L
Test conditions Photoperiod: continuous light Light intensity: 4000-46000 lux pH of test and controls (0-96 b): 7.9=8.7 Salinity 30 +/×2 g/L Conductivity: 39 000-41 000 μmbos/cm Type of light: artificial Parameters Measured / Observations Temperatures were recorded daily. Light intensity was measured at hour 0 and at who 24 four intervaled ring the expression pariod. Water quality perspectators	Initial cell deroity	7 7 x 10 ⁴ cells/mL/20 test initiation
Test conditions Photoperiod: continuous light Light intensity: 4000-46000 lux pH of test and controls (0-96 b): 7.9=8.7 Salinity 30 +/×2 g/L Conductivity: 39 000-41 000 μmbos/cm Type of light: artificial Parameters Measured / Observations Temperatures were recorded daily. Light intensity was measured at hour 0 and at who 24 four intervaled ring the expression pariod. Water quality perspectators		
Parameters Measured / Observations pH' of test and controls (9 – 96 b): 7.9 = 8.7 Salinity $30 + 42 g/L$ Conductivity: $39 000 - 41 000 \mu mbos/cm$ Type of light: artificial Temperature was measured continuously in a flask of water adjacent to the temperatures were recorded daily. Light intensity was measured at hour 0 and at a b 24 way interview of the average pariod. Water quality perspectators	Tast applifung	Temperature: 18-21°C°C 0 0
Parameters Measured / Observations pH' of test and controls (9 – 96 b): 7.9 = 8.7 Salinity $30 + 42 g/L$ Conductivity: $39 000 - 41 000 \mu mbos/cm$ Type of light: artificial Temperature was measured continuously in a flask of water adjacent to the temperatures were recorded daily. Light intensity was measured at hour 0 and at a b 24 way interview of the average pariod. Water quality perspectators		Photoperiod: continuous light
Parameters Measured / Observations	Ky y	Light intensity: 4009-46009 lux
Parameters Measured / Observations	<u> </u>	pH of test and controls $(0-96)$: 7.9 \approx 8.7
Parameters Measured / Observations	Q.	
Parameters Measured / Observations	Q .	Sconductivity 39 000 – 41 θ00 μmβros/cm
Measured / Observations dest flasks in the environmental chamber. Minimum and maximum temperatures were recorded daily. Light intensity was measured at hour 0 and at why 24 Your information the environmental chamber. Minimum and maximum	~Q~ C	Type of light: artificial /
Measured / Observations	Parameters	
Observations		
 at each 24 flour interval during the exposure period. Water quality parameters (pH and conductivity) were measured at test initiation and at the termination of the 96-how exposure period. At each subsequent 24-hour interval, cell counts were conducted on the three replicate vessels of the treatment and the control vessels using a hemacotometer and compound microscope. Recovery: the subcultures were microscopically examined every other day to determine whether or not cell growth had resumed. The subculture was discontinued after a substantial increase in cell density (i.e. > 10X) was observed. 		
 (pH and conductivity) were measured at test initiation and at the termination of the 96-hour exposure period. At each subsequent 24-hour interval, cell counts were conducted on the three replicates vessels of the treatment and the control vessels using a hemacotometer and compound microscope. Recovery: the subcultures were microscopically examined every other day to determine whether or not cell growth had resumed. The subculture was discontinued after a substantial increase in cell density (i.e. > 10X) was observed. 		at each 24 hour interval during the exposure period. Water quality parameters
of the 6-how exposire period. At each subsequent 24-hour interval, cell counts were conducted on the three replicate vessels of the treatment and the control vessels using a hemacytometer and compound microscope. Recovery: the subcultures were microscopically examined every other day to determine whether or not cell growth had resumed. The subculture was discontinued after a substantial increase in cell density (i.e. > 10X) was observed.	· @.\	(pH and conductivity) were measured at test initiation and at the termination
At each subsequent 24-hour interval, cell counts were conducted on the three replicate vessels of the treatment and the control vessels using a hemacorometer and compound microscope. Recovery: the subcultures were microscopically examined every other day to determine whether or not cell growth had resumed. The subculture was discontinued after a substantial increase in cell density (i.e. > 10X) was observed.	Å _	of the 96-hour exposite period.
replicate vessels of the treatment and the control vessels using a hemacorometer and compound microscope. Recovery: the subcultures were microscopically examined every other day to determine whether or not cell growth had resumed. The subculture was discontinued after a substantial increase in cell density (i.e. > 10X) was observed.		* At each subsequent 24-hour interval, cell counts were conducted on the three
Recovery: the subcultures were microscopically examined every other day to determine whether or not cell growth had resumed. The subculture was discontinued after a substantial increase in cell density (i.e. > 10X) was observed.		replicate vessels of the treatment and the control vessels using a
Recovery: the subcultures were microscopically examined every other day to determine whether or not cell growth had resumed. The subculture was discontinued after a substantial increase in cell density (i.e. > 10X) was observed.		nemacotometer and compound microscope.
determine whether or not cell growth had resumed. The subculture was determine whether or not cell growth had resumed. The subculture was determine whether a substantial increase in cell density (i.e. > 10X) was observed.	N S É	• Recovery: the subcultures were microscopically examined every other day to
Image: Second state in the se		determine whether or not cell growth had resumed. The subculture was
ODServea.		discontinued after a substantial increase in cell density (i.e. $> 10X$) was
	Ö	observea.



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

II. RESULOTS ANJODISCOSSION

			ð ⁱ A	
Validity criteria (OECD 201, 2011)		Required	Obtained 72h 🖉	Obtaine 96 k
Increase of biomass in the control cultures		$\sim 16^{\circ}$	× 80°	Ø,
Mean coefficient of variation for section-to growth rates (days 0-1, 1-2 and 2-3) of the	by-section specific -	J 5% D	80.8% 5	¥J10.5%
Coefficient of variation of average special replicate control cultures	jć grovah ratesom			^y 3.04%
	U' A A	× '' ''		

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

Analytical results:

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

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			0
Nominal Conceptration	Askithmethe mean measured encentrations after 96 hours	% of nominal	concentrations*
(mg [°] a.s./L)		🎸 0-hoŵr	96-hour
Control		< 0.000054	0.00037**
Solvent control		< 0.000054	0.00015**
0.0012		100.0	141.7
0.0041		92.7	122.0
0.0140	Q A 0.0¥60 S . Q	100.0	128.6
<i>√</i> y 0.0450	\$\$ \$ 0 460\$ \$	95.6	111.1
0.1500	~ 0.14 6 0 ~	86.7	100.0
0.50000	0.4700	92.0	98.0

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

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

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



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

Biological results:

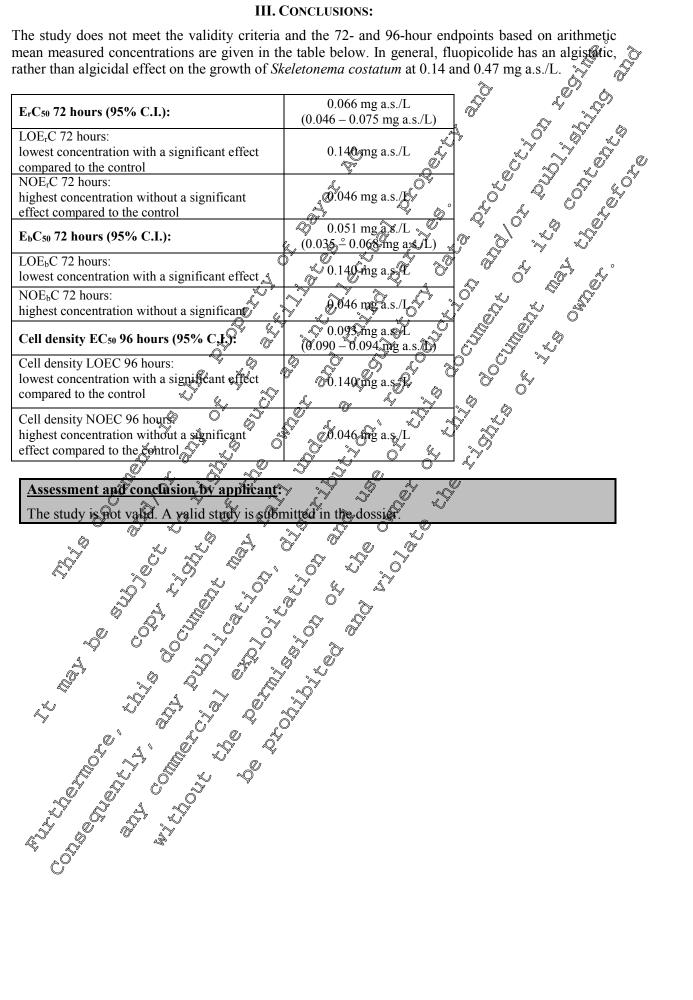
Biological results: Cells exposed to the 0.14 and 0.47 mg a.s./L treatment levels were observed to be bloated. Cells exposed to the remaining treatment levels tested and the controls were observed to be normal. Z2 hours Arithmetic mean (mg a.s./L) 0-72 h average specific (growth rates [days)) Inhibition of average (specific growth rate [%)) Water control 0.65 9 9 0.0014 0.62 5 9 0.0160 0.65 9 9 0.0160 0.65 9 9 0.0140 0.62 154 9 0.0160 0.65 154 9 # Significantly reduced compared to solvent control based on Within 's to' 154 9 Water control 0.65 186 9 Water control 0.65 9 9 Water compared to solvent control based on Within 's to' 154 9 9 Water control 0.955 186 9 9 Water control 0.956 186 9 9 Water control 0.956 186 9 9 Water control 9 9 9 95600^C Water control X 263700 Solvent control 0 289700 0.0014 <u>3</u>06000 0.004 331900 0.0960 Ò **@**0460 164000 38 \$33200 0.1400 151 0.4700 ≪*ا*-152**800** ¥58# # Significantly reduced compared to solvent control based on William's test Ô 96 hours 0 Arithmetic mean measured concentration Mean celonumber Inhibition of cell after 96 h per ml densities [%] (@ng a.s./L) 667500 Water control 768\$00 ≪Solvent control Pooled control 717900 0 C 0.00 \$18300 -14 709200 0.0044 1 **Ø**ۯ160 ≪ 684200 5 0.0460 839200 -17 0.1460 12500 98# 0.4700 1700 100#

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



III. CONCLUSIONS:

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



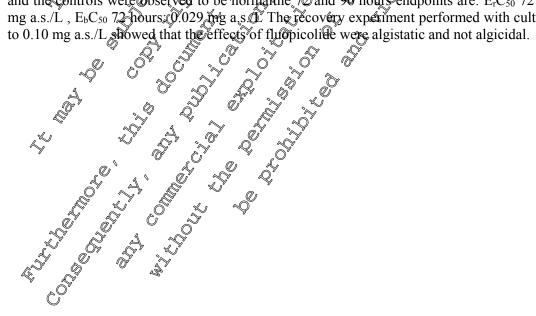


Data Point:	KCA 8.2.6.2/03
Report Author:	
Report Year:	2003
Report Title:	AE C638206 - Acute toxicity to the freshwater diatom, Navicula pelliculosa
Report No:	C037812
Document No:	<u>M-223560-01-1</u>
Guideline(s) followed in	EU (=EEC): L383A - C.3 (1992); OECD: 201 (1984); USEPA (=EPA): OPPTS
study:	Draft 850.5400 (1996)
Deviations from current	Method: Deviations from current guideline SANCQ 3029/99 rev.4;
test guideline:	Limited sets of validation recoveries were analysed. However, the average recoveries were within the acceptible range of 70–110% and the RSD dues were below 20%. The analytical method can be regarded as fit for purpose study: Current Guideline: OFCD 201 (2011)
Previous evaluation:	yes, evaluated and accepted in DAR (2005)
GLP/Officially	Yes, conducted under GLP/Officially recognised testing facilities
recognised testing	
facilities:	
Acceptability/Reliability:	Yes a way of the second

Executive summary

The objective of this study was to determine the effect of fluppicolide on the growth of the freshwater diatom *Navicula pelliculosa* over 96 h. Nominal concentrations were 0.0010, 0.0026 0.0064, 0.016, 0.040 and 0.10 mg a.s./L. Additionally, where and solvent controls (dimethylformamide at 0.1 μ L/mL) were included. The test comprised replicates for each concentration control.

Samples analysed at 0-hour were removed from the test and control solutions prove to division into the replicate test vessels. Samples analysed at 96 hours of exposure were removed from individually composited replicate solutions of the treatment levels and controls. Samples were analysed by gas chromatographic analysis with electron capture detection (G6/ECD). Some recoveries were not in the range of 80 - 120% of nominal but the substance is stable over the test duration. Thus, biological results after 72 hours and 96 hours are based on attimute mean concentrations. The corresponding arithmetic mean measured concentrations (0°_{\circ} 96 h): 0.0013, 0.0030, 0.0071, 0.018, 0.044 and 0.11 mg a.s./L. The study is not considered valid according to the OECD 201 guideline. Cells exposed to all treatment levels and the controls were observed to be normathe 70 and 96 hours are: E_rC_{50} 72 hours: 0.029 for a.s.0. The recovery experiment performed with cultures exposed to 0.10 mg a.s./L. showed that the effects of fluppicoline were algistatic and not algicidal.





Test material	AE C638206 (Fluopicolide)
	Lot No: 2050190/PP241024/2
	Purity: 97.7% w/w
Guideline(s)	Evaluation of recovery was included in the study
adaptation	
Test species	Lot No: 2050190/PP241024/2 Purity: 97.7% w/w Evaluation of recovery was included in the study
C1+	The AAP medium used to prepare the exposure solutions was formulated in
Culturing	the same manner as the culture modium. Culture conditions similar to testing
conditions	conditions (e.g. $24 \pm 2^{\circ}$ C, continuous lighting at $3900 - 4700^{\circ}$ lux)
	Nominal concentrations: 0.0040, 0.0026, 0.0064, 0.016, 0.040 and 0.10 mg
Test solutions	as/L The second se
	Corresponding arithmetic mean measured concentrations (0 - 96 h): 0.0013;
	0.0030, 0.0071, 0.018, 9.044 and 0.1 1 mg as /L
	Evidence of undissolved material. No visible undissolved test substance
	Controls: water and solvent controls (dimethylformamide at 0 HµL/mH) Evidence of undissolved material. No visible undissolved test substance observed in stock solution
Penlicotion	observed in stock solution No. of vessels per concentration (replicates): 3 No. of vessels per control (replicates): 3 No. of vessels per solvent control (replicates): 3 Static Total exposure duration: 96 hours
Replication	No. of vessel per control (replicates): 3 0 5 5 5
	No. of vessels per solvent control (replicates) S
Exposure	Static C & F O O S
Exposure	Total exposure duration: 96 hours 4
	Recovery period: 19 to 9 days for concentration 0.10 mg a.s./L
Initial cell density	No. of vescels per solvent control (replaces) 5° Static Total exposure duration: 96 hours Recovery period: up to 9 days for concentration 0.10 for a.s./L approx. 1×10^4 cells/net at test initiation
mittai cen density	
Test conditions	$\sqrt{1} \text{ emperature} 23-24, C \sqrt{2} \sqrt{2}$
	Photoperiod: continuous light 2 2
Test conditions	Light intensity: 4000 to 4600 bux
so a	\bigcirc H of controls and test solutions (0 – 96 \bigcirc 7.1 – 9.4
, Ô	Conductivity: 90 to 100 unhos/cm
Q ⁽	Oype of Tight artificial of the second secon
Parameters	Temperature was measured continuously in a flask of water adjacent to the
	test flasks in the environmental chamber. Minimum and maximum
Observations	temperatures were recorded daily. Light intensity was measured at hour 0 and at each 24-hour interval during the exposure period. Water quality parameters
	at each 24-nour interval during interval at test initiation and at the termination
4	(pHand conductivity) were measured at test initiation and at the termination of the 96 hour exposure period.
	At each subsequent 24-hour interval, cell counts were conducted on the three
	replicate vessels of the treatment and the control vessels using a
	hepacytometer and compound microscope.
× ×	
¢ v	determine whether of not cell growth had resumed. The subculture was
	discontinued after a substantial increase in cell density (i.e. $> 10X$) was
	observed.
	XY .
	45
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Sampling for chemical analysis	At test initiation and test termination one sample was removed from each test solution and the controls for analysis of AE C638206 concentration. Samples analysed at 0-hour were removed from the test and control solutions° prior to division into the replicate test vessels. Samples analysed at 96 hours of exposure were removed from individually composited replicate solutions of the treatment levels and controls. Samples were analysed by gas chromatographic analysis with electron capture detection. (GC/ECD).
Data analysis	EC _x values (e.g. $x = 50$) and confidence intervals were calculated for the standard exposure period, using a commercial program (TOXSTAT).

			Ove	8. uni (1 0 00 11	
		Ą	Q a		
	II. RESULTS&		USSTON: V		
Validity criteria (OECD 201, 2011		C C	Required	≪ Obtained 70°h ∡	VObtained
Increase of biomass in the control cu	ultures 🐥 🗞		¥6 1	\$24.6	£ 104 Ø
Mean coefficient of variation for s growth rates (days 0-1, 1-2 and 2-3)	in the control cu	ltures		64.5%	51/10%
The coefficient of variation of avera replicate control cultures	ge Decific prowt	h fates in	5,10% 5	£\$.08%\$	3.91%
				\approx δ .	V

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

Analytical results:

Some recoveries were not in the range of $80 \neq 120\%$ of normal (see table below) but the substance is stable over the test duration. Thus, biorogical results after 72 hours and 26 hours are based on arithmetic mean concentrations O (1) n

<u></u>		<u>~</u> 9 <u>6</u> <u>6</u>	
Nominal 🖉	Arithmetic mean measured	% of nominal	concentrations*
Concentration	soncentrations after 96 hours	0-hour	n 96-hour
(mg a.s./L)	🔊 🔍 (mg a.s./L)		<u> </u>
Control		0.000070**	< 0.000054
Solvent control		< 0.00005	< 0.000054
0.0010		130	130
0.0026			119
0.0064	0.140/1	¹⁰⁸	114
0.0160	0 0180 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	113	113
0,0400	Q 0.0440 X X	115	108
0.1000		120	99

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

A compound present in the matox is believed to give co-eluted at the same retention time as the analyte and separation was not ach Oved. 🗚 ~Q[®]

The 96-hour sample from the 2016 mg a.s./L (nominal) treatment level, without algae present, yielded a measured concentration of 0.017 mg a.s./L. The equivalent replicate 96-hour test solution with algae present was © 018 ppg a.s. L, indicating that the presence of algae in the test solution had no impact on the conceptration of fluopicolide.

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



Biological results:

Cells exposed to all treatment	nt levels tested and the cont	rols were observed to be nor	
72 hours			N O
			л <i>с</i> б
Arithmetic mean measured concentrations	0-72 h average specific	Inhibition of average	×
(mg a.s./L)	growth rates [days]	specific growth rate [%]	
Control	1.09		
Solvent control	1.09		
Pooled control	1.05		
0.0013	0.99 & Q		
0.0030	1.04	C & 1 & C	- A
0.0071	1.04		
0.0180	0r94# ~ ~		
0.0440	Q. 70# ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~		
0.1100	\$\colored{4}		
# Significantly reduced compared to		m's test y &	
Arithmetic mean	Area under the growth	Inhibition of Biomass	Ky (
measured concentrations	curve (biomass	integral (%)	0'
(mg a.s./L)	integral)	O 'Y N Y	Ŷ
Control 🍾	190000		p [*]
Solvent control 🔬	\$ \$38300 °	<u>, 0, % - 1, , 0,</u>	
Pooled control	164200	× 0 . v	
0.0013	· 128600	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	
0.0030	4 (143100 x x)	\sim	
0.0071	0 0°126200 ×	Ö 4 230	
0 ,0180	\$ 10 6 600#		
× 0.0440 Č	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	
0.1100 %	15200#	× × 91	
# Significantly reduced compared	to pooled control based an Willia	h's test	
A Standard		ð	
<u>96 hours</u>			
Arithmetic mean		» Inhibition of cell density	
measured concentration	Niewi cen miniber anter		
(jug a.s./L)	96 H per m t	[78]	
Control 😽	1038300	-	
Solvent control	914200	_	
Pooled control	976300	_	
0.0013	<u>م</u> ر 2006706	-3	
0.0630	1095000	-12	
0,0071	87\$300	10	
9.0180	806700#	17	
0.0440	Q 402500#	59	
	50000#	95	
# Significantly feduced compared	to pooled control based on Willia	m's test	
× ~~~			
\bigcirc			



Recovery for algistatic/algicidal properties:

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

III. CONCLUSIONS:

The study does not meet the validity criteria and the 72 and 96-hour endpoints based on arithmetic mean measured concentrations are given in the table below. The 4-day recovery phase conducted at termination of the definitive test indicated that the effects of fluopicolide (AE C63 206) were algostat and not algicidal. Ä

ErC ₅₀ 72 hours (95% C.I.): (0.069) mg a.s. f_{2} (0.069) mg a
LOE _r C 72 hours: lowest concentration with a significant effect compared to the control NOE _r C 72 hours: highest concentration without a significant effect compared to the control
LOE _r C 72 hours: lowest concentration with a significant effect compared to the control NOE _r C 72 hours: highest concentration without a significant effect compared to the control ExCap 72 hours (95% C L):
compared to the control
NOE _r C 72 hours: $\sqrt{2}$ $\sqrt{2}$ $\sqrt{2}$ $\sqrt{2}$ $\sqrt{2}$ $\sqrt{2}$ $\sqrt{2}$ $\sqrt{2}$
NOE _r C /2 hours: highest concentration without a significant effect compared to the control
effect compared to the control and the control of t
EbC50 72 hours (95% C.I.):
$\frac{E_bC_{50} 72 \text{ hours (95\% C.I.):}}{LOE (6.72)} \sqrt[6]{9} \sqrt[$
LOE _b C 72 hours: $(0,180,180,180,180,180,180,180,180,180,18$
lowest concentration with a significant effect
NOE _b C 72 hours: 3 3 3 3 3 3 3 3 3 3
Cell density EC 96 hours (95% C.I.):
Cell density LOEC 96 flours; O C C C C C C C C C C C C C C C C C C
lowest concentration with a significant effect 000180 mg a.s./1
Cell density NOEC % hours highest concentration without a significant effect compared to the control
Cell density NOEC % hours in the significant of the
effect compared to the control
Assessment and conclusion by applicant:
The staff is not will be all the staff of the design

and study is subplitted in the dossier.

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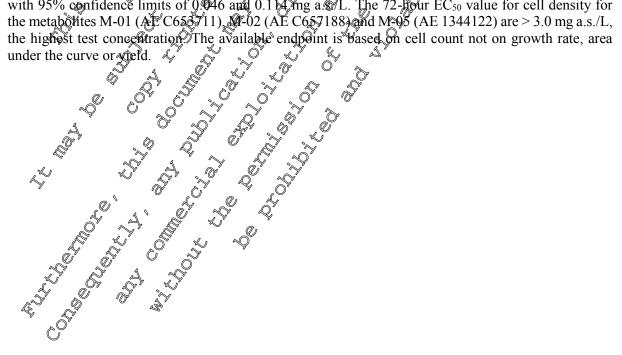


Data Point:	KCA 8.2.6.2/04
Report Author:	
Report Year:	2003
Report Title:	72-hour toxicity screening tests with the freshwater diatom (Navicula pelliculation) Codes: AE C638206, AE C653711, AE C657188, AE 1344122
Report No:	149A-181
Document No:	<u>M-225549-01-2</u>
Guideline(s) followed in study:	OECD: 201 (1984); USEPA (=EPA): OPPTS 850.5400 (1996)
Deviations from current test guideline:	Current Guideline: OECD 201 (2004) The study validity cannot be assessed due to the ack of information in the report (screening study).
Previous evaluation:	yes, evaluated and accepted accepted and acc
GLP/Officially	No, not conducted under GLP/Officially recognised testing facilities
recognised testing facilities:	
Acceptability/Reliability:	Yes Δ ∂ ∂ Q ∂ ∂ ∂' ∂'
Executive summary	

Executive summary

The purpose of this study was to evaluate the toxic effect of the patent compound fluopicolide in comparison to the toxic effect of the metabolites M-01 (AE Co53711) M-02 (AE Co57188) and M-05 (AE 1344122) on the freshwater diaton, Navigula pethculos, in a static system for 72 h, Algal medium supplemented with silica and selenium was used for culturing and the test. Algae were kept under continuous light. Nominal concentrations for the parent compound were. 0.03 and 0.3 mg a.s./L and for the metabolites 0.03, 0.3 and 3.0 mg/L. Additionally, water and solvent controls (dwnethylformamide at 0.1 µL/mL) were included. The test comprises 3 reputates for each concentration and control. Number of cells/mL were counted at \$2 hours using a haemocytometer and a meroscope. pH was measured on day 0 and day 4. No chemical analysis of test concentrations was done. The validity criteria of biomass increase in cultures was met according to OECD 200 guideline. Earther validity criteria could not be calculated on the basis of the data given in the report.

The 72-hour ICE 50 for Scell density for the parent compound fluepicolide is reported as 0.072 mg a.s./L, with 95% confidence limits of 0.046 and 0.1 Amg a. L. The 72-hour EC50 value for cell density for the metabolites M-01 (AE C655711), Q-02 (AE C657188) and M-95 (AE 1344122) are > 3.0 mg a.s./L,





Fluopicolide (AE C638206) M-01 (AE C653711 (BAM, 2,6-dichlorobenzamide)) M-02 (AE C657188 (PCA, 3-chloro-5-trifluoromethyl-pyridine-2-carboxylic
acid)) M-05 (AE 1344122 (3-methylsulfinyl-5-trifluoro-methylsyridine-2-carboxylic)
No further data on test materials
acid)) M-05 (AE 1344122 (3-methylsulfinyl-5-trifluoro-methylsyridine-2-carboxylic acid)) No further data on test materials None specified Freshwater diatom (<i>Navicula policulosa</i>) Medium used for culture and for: algal medium stanlemented with silica and
Freshwater diatom (Navicula policulosa)
selenium. Algae were lighted with artificial (cool white fluorescent light),
Nominal concentrations (parent compound) 00.03 and 0.3 mg/L Nominal concentrations (metabolites): 0.03, 0.3 and 3.0 mg/L Controls: water and solvent control (dimethylformamide at 0, 4 µL/mL)
Evidence of undersolved material: All test solutions were dear and colorless No. of vessels per concentration (replicates): 3 No. of vessels per control (replicates): 3 No. of vessels per solvent control (replicates): 3 Static Total exposure duration: 72 hours approx. 1x 10 ⁴ cells/misat test initiation
No. of vessels per solvent control (repretes) 3 0 0 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5
approx. 14, 10 ⁴ cells/ml at test mitiation
Pemperature 23.6 to 24.6 $\%$ Continuous Dight, 3890 – 4700 lpc pN of controls and solutions ($0 \neq 96$ k)? 7.5 $\oplus 3.0$ $\%$ Other of light artificial (cool white fluores pent light)
Number of cells/mL at 72 bours using a haemocytometer and a microscope.
pHymeasured on day 0 and day 4. Timing of forther physical measurements not specified in study report.
Screening Study; No chemical adalysis of test concentrations.
Calculation of EC ₅₀ values and confidence limits not specified in study report.
approx. 12, 10 ⁴ cells/mEat tear initiation Femperature 23.6 to 24.6°C Confinuous light, 3890 – 4700 lint pN of controls and solutions (0 + 96 k)? 7.5 - 99.0 Type of light: artificial/(cool white fluorescent light) Number of cells/mL at 72 fours using a haemocytometer and a microscope. pH measured on day 0 and day 4. Timing of further physical measurements not specified in study report. Screeching study: No chean cal analysis of test concentrations. Palculation of EC 56 values and confidence limits not specified in study report.



II. RESULTS AND DISCUSSION:

			551011	
Validity criteria (DECD 201, 2011)		Required	Obtained °
Increase of biomass test period.	s in the control cultures	16	139	
	of variation for section 0-1, 1-2 and 2-3) in the	<u><</u> 35%	Carbot be calculated from She data in the port	
	tion of average specif iod in replicate contro		<u>≤</u> 10%	Cannot be calculated from the data in the report
Biological results:				the data in the report
Nominal test		icolide 🖉		
concentration (mg a.s./L)	Mean cell density after 72 hours			
Control	1388333	A 0° 0	Ŷ,	
Solvent control	1126667		SA.	
Pooled control	1257500			
0.030	1175000	6.6 × ~		
0.30	9000	~ 99× ~		
* % inhibition relative	to the pooled control			

	× ľ		e			
Nominal 4ast	M-Q1 (ĂE	Č653711	M-02 (AE	C657188	M-05 (AE	1344122)
Nominal test concentration (mg a.s./L)	Mean cell density after 72 hours	Percent &	Mean cell density aftor 2 hours		Mean cell density after 372 hours	Percent inhibition *
Control	£1388333		[©] 13883333	<i>a</i> , <u>-</u>	1388333	-
Solvent control			1126667		1126667	-
Pooled control	\$ 2 \$7500 ^{~y}	~ ~ ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	_ł\$257500 ^{∞5}	~~ ~ [*]	1257500	-
0.030	Q1088339	0'13%	£1050000	17 ₀	1073333	15
0.30	⁰ 1110000 ¢		y 106 69 00	¥6	1065000	15
3.0	1128333	Orð C	1098333	~¶3	1045000	17

* % inhibition relative to the pooled option of a

MII. Conclusions:

The validity criteria of biomass increase in cultures was mer according to OECD 201 guideline. Further validity criteria could not be reviewed on the basis of the data given in the report. The 72 h endpoints based on nominal concentrations are given in the table below. The available endpoint is based on cell count not on growth rate, area under the curve or yield.

		, v					
Endpoint		A.	Fluopicolic	le 🌂	M-01	M-02	M-05
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	-	O C	, ⁷	ÕÝ.	(AE C653711)	(AE C657188)	(AE 1344122)
72-hour EC ₅₀ (n	æg∕L)	. 4	@ 0.072		> 3.0	> 3.0	> 3.0
95% Confidence		(mg/L)	× Ø.046 – Ø.	114	_*	_*	_*
* Confidence light		a a a a a a a a a a a a a a a a a a a	1 mills alot al ha	in a d			

* Confidence logits cannot be calculated with date obtained. Ô

# Assessment and conclusion by applicant:

the study is not acceptable and should not be used in risk assessment.

N

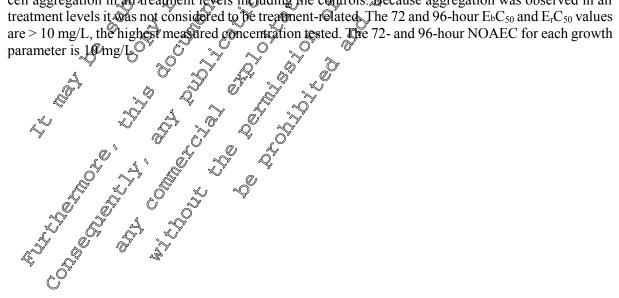
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Ø,



Data Point:	KCA 8.2.6.2/05
Report Author:	
Report Year:	2003
Report Title:	A 96-hour toxicity test with the freshwater diatom Navicula pelliculosa Final
	Report AE C653711
Report No:	M-225556-01-2
Document No:	<u>M-225556-01-2</u>
Guideline(s) followed in	EU (=EEC): Method C3 (1992); OECD: 201 (1984); USEPA (=EPA) OPPT
study:	850.5400 (1996)
Deviations from current	Method: Deviations from current guideline SANC 3029/99 rev 4
test guideline:	Recoveries were determined at two different concentrations in typlicate However,
	the obtained data demonstrate very good recoveries and the precision. The method $\sqrt{O}^2$
	can therefore be regarded as $4$ for purpose $3$
	Study: Current Guideline: DCD 201 (2011)
	The study does not meet the validity criteria
Previous evaluation:	yes, evaluated and accepted of the work of
	in DAR (2005) O' , O'
GLP/Officially	Yes, conducted under GLB Officially recognised testing facilitie
recognised testing	
facilities:	
Acceptability/Reliability:	Supportive any the second seco

Ś **Executive summary** The objective of this study was to determine the doxicity of M-D1 (2,6-dichlorobenzamide) to the freshwater diatom, *Navicula pelliculosa*, over a 96-hour exposure period under static test conditions for 96 hours. Eight replicates were maintained in the control group of the static test conditions for 96 hours. Eight replicates were maintained in the control group and four replicate rest chambers were maintained in each treatment group. M-dr (26-dichkrobenzamide) was applied at nominal concentrations of 0.43, 0.94, 2.07, 4.35 and 10 mol. Measured test concentrations were determined from samples of test medium colleged from each reatment and control group at the beginning and end of the test. Samples were analysed by high performance liquid abromatography (HPLC) using UV detection. Measured concentrations were in the so-120% range of nominal concentrations and no residues above the limit of mantification (LOQ) were measured in the controls. The arithmetic mean measured concentrations were: 0.43, 0.96, 2.08, 4.59 and 10 mg/L. The study does not fulfil the validity criteria of the current version of OECD 20P guideline. There were no noticeable changes in cell morphology in any of the tested concentrations when compared the control. There was evidence of cell aggregation in all treatment levels including the controls. Because aggregation was observed in all treatment levels it was not considered to be treatment-related. The 72 and 96-hour  $E_bC_{50}$  and  $E_rC_{50}$  values





Test material M-01 (2,6-dichlorobenzamide, AE C653711) Lot No: I8499A	A A A A A A A A A A A A A A A A A A A
Purity 98 %	
Guideline(s) None specified	
adaptation 0 ³	
Test species Navicula pelliculosa	
UTEX 667	A A
Culturing Algal cells used in this test were obtained cultures that had been active	to growing
conditions in culture medium for at least two weeks prior to test initiation. The	culture was
last transferred to fresh medium four days prior to test initiation. The	algal cells
were cultured and tested in free $f$ water algalymedium $f$ $\mathcal{Q}$ $\mathcal{Q}$	i a a a a a a a a a a a a a a a a a a a
Test solutions Nominal concentrations: 0,43 - 0.94 • 2.07 04.55 - 10 mg/L	
Test solutions Nominal concentrations: 0,43 - 0.94 - 2.07 4.55 - 10 mg/L Corresponding mean measured concentration: 0,43 - 0,96 - 2,98 - 4,59 Controls: culture medium (freshwater algal medium)	- 10 mg /L
Controls: culture medium (freshvater agal medium)	D' L'
All test solutions appeared clear and colourless A	- 10 mg /L
Replication No. of vessels per concentration (replicates): 8 0 2 2	L'AN A
No. of vessels percontrol (replicates):	
Exposure Static	S I
Total exposure duration: 96 pours & S o o o o	۶
Corresponding mean measured concentration: 04.3 - 0.06 - 2.08 - 4.59         Controls: culture medium (freshwater atgal medium)         All test solutions appeared clear and coloucless         No. of vessels per concentration (replicates): 8         No. of vessels per concentration (replicates): 8         No. of vessels per concentration (replicates): 4         Exposure         Static         Total exposure duration: 96 bours         Initial cells         1 × 10 ⁴ cells/mL inteach test group         Photoperiod continuous light         Light intensity at surface of test vessels. 3890 to 4760 lux, 7	
density	
Temperature: $24.1 - 24.4^{\circ}C^{\circ}$	
Test conditions Temperature: 24.1 - 24.4°C Photoperiod continuous light	
Test conditions Temperature: 24.1 – 24.4°C Photoperiod continuous light Light intensity at surface of test vessels. 3890 to 4700 lux	
1041: 7.5 – 7.8 $37$ $37$ $37$ $37$	
Growd medium same as culture medium? Yes	
	1
amples were concered as approximately 24-its in mervals during t	ne 96-nour
	performed.
Observations sufficient to infibit growth until cell counts could be physics measured at study stars and end. Temperature was measured co	
In the environmental chamber and force daily in a vessel adjacent to the t	est vessels
Sampling for Samples offest solutions were taken at fest initiation (0 hour) and at test t	termination
chemical (% hours) for analysis of AE C653711. Samples were analyse	d by high
analysis performance fiquid chromatography (HPLC) using UV detection.	
Data analysis The calculation of cell consities areas under the growth curve (bioma	ss), growth
rates and percent inhibition values, as well as all statistical anal	
Conducted using "The SAS System for Windows Version 8 02	<i>J</i> ,
	1.
$\mathbb{C}$ Non-linear regression was used to calculate EC ₅₀ values and their cor	responding
25% confidence intervals for cell density (EC ₅₀ ), biomass (E _b C ₅₀ ) and g	
Chr C - Stor and 34 know announce paried when accepted The 1-4-	e evaluated
(ErC ₅ ) for each 24-bour exposure period, when possible. The data wer	Wille's and
(ÉrC ) for each 24-bour exposure period, when possible. The data were for normality and homogeneity of variance (p=0.05) using the Shapiro-	-Wilk's and
$\langle ErC_{5} \rangle$ for each 24-bour exposure period, when possible. The data were for normality and homogeneity of variance (p=0.05) using the Shapiro- Levene's cests, respectively. The treatment groups then were compared using Dunnett's test (p = 0.05). The results of the	-Wilk's and ared to the
$\langle ErC_{5}\rangle$ for each 24-hour exposure period, when possible. The data were for normality and homogeneity of variance (p=0.05) using the Shapiro- Levene's pests, respectively. The treatment groups then were compa- begative control using Dunnett's test (p = 0.05). The results of the analysis as well as an evaluation of the concentration response pattern	-Wilk's and ared to the e statistical
for normality and homogeneity of variance (p=0.05) using the Shapiro Levene's Gests, respectively. The treatment groups then were compared	-Wilk's and ared to the e statistical , were used



#### **II. RESULTS AND DISCUSSION:**

Validity criteria (OECD 201, 2011)	Required	Obtained
Increase of biomass in the control cultures	16	119
Mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3) in the control cultures	<u>≤</u> 35%	
Coefficient of variation of average specific growth rates in replicate control cultures	<u>≤10%</u>	0.34%
	· · ·	

#### Analytical results:

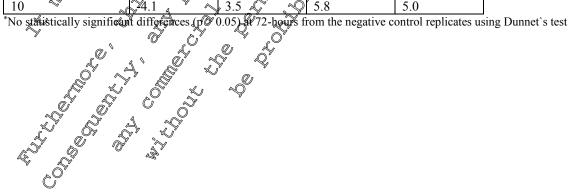
**Biological** results

Recoveries were 98 - 103% of nominal concentration (see table below). The biological results and on mean measured concentrations of M-01 (AE C653711). No residues of flugpicolide were measured in the controls above the limit of quantification (60 mg a.s./L)

Nominal Concentration	Mean Measured Concentration
(mg /L)	(mg/L)
0.43	
0.94	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$
2.07	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$
4.55	$4.59$ $\swarrow$ $102$ $\swarrow$ $100$ $\checkmark$ $\sim$ $0$
10	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$

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

Mean area under the	growth curve (t	promass) and pe	scent and ibition	¥
Mean measured	Inhibition %	InhOpition	Inhibition	Inhibition %
concentrations (mg) /L)	Inhibition & at 24 h	% at 48 h	at 72 h*	at 96 h
0.43	OM AN O	Ĵ-17 o ⁷ ô	*-10 🔗	-6.4
0.96 ~ (	-7.30	1.7 *	0:66	-0.052
2.08	-7.1 ⁰ _0″	6.4. 6	, Wb	0.92
4.59			-0.066	1.2
10	24.1 × ~	, 3.5 🖉 🔊	5.8	5.0





#### Algae growth rate

Mean measured Concentrations (mg /L)	Inhibition % at 24 h	Inhibition % at 48 h	Inhibition % at 72 h*	Inhibition % at 96 h	
0.43	-5.3	-4.2	-0.69	-0.33	
0.96	-3.2	0.82	-0.27	-0.041	
2.08	-3.3	2.5	-0.59	0.20	
4.59	-4.6	-0.39	0.42		
10	-1.9	1.3	1.6*	A0.27 Ö	

*Although statistically significant a 1.6% inhibition from the control was not considered to be treatment related since amount of inhibition was considered to be within an acceptable limit for Navicula.

III. CONCLUSIONS:
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	A	N N		O*
ErC50 72 hours, 96 hours (95 % CI)	× 18	mg /L v	not applica	ablé)
E _b C ₅₀ 72 hours, 96 hours (95 % CI)		mg/L (I	not applie	ble)
NOAEC:			y z	
highest concentration without adverse effe	cts 10 n	ng /L	S	
(based on biomass and growth rate)		Â,		5
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	-	O,		

III. CONCRESSIONS The study is not considered to be valid. The endpoints based on arithmetic means measured occentrations are:

 • Concretions are:

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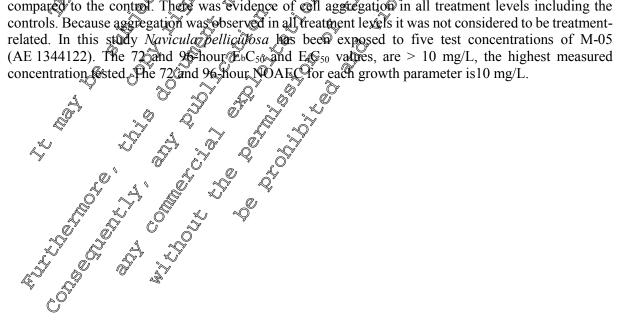
 • Concretions



Data Point:	KCA 8.2.6.2/06
Report Author:	
Report Year:	2003
Report Title:	A 96-hour toxicity test with the freshwater diatom Navicula pelliculosa Final
	Report AE 1344122
Report No:	M-225547-01-2
Document No:	<u>M-225547-01-2</u>
Guideline(s) followed in	EU (=EEC): Method C3 (1992); OECD: 201 (1984); USEPA (=EPA); OPPTS
study:	850.5400 (1996)
Deviations from current	Method: Deviations from current guideline SANCQ/3029/99 rev
test guideline:	Recoveries were determined at two different concentrations in typlicate However,
	the obtained data demonstrate gery good recoveries and the precision. The method $\sqrt{2}$
	can therefore be regarded as the for purpose of the second s
	Study: Current Guideline: DCD 201 (2011)
	The study does not meet the validity criteria (see below)
Previous evaluation:	yes, evaluated and accepted of the work of the second accepted of the second accepted of the second se
	in DAR (2005) O' , , , , , , , , , , , , , , , , , ,
GLP/Officially	Yes, conducted under GLP Officially recognised testing facilitie
recognised testing	
facilities:	
Acceptability/Reliability:	Supportive By K Z Z Z Z Z

Executive summary

The objective of this study was to determine the toxicity of M-09 (AE 1344122, 3-methylsulfinyl-5trifluoro-methylpyridine-2-carboxylic acid to the freshwater diatom *Navicuta pelliculosa*, over a 96hour exposure period understatic test conditions. Eight replicates were maintained in the control group and four replicate test chambers were maintained in each treatment group. The nominal test concentrations were 0.43, 0.94, 2.07, 4.55 and 10 me/L. Additionally a negative control was included. Samples of test solutions were taken at test initiation (0 hour) and at test termination (96 hours) for analysis of 3-methylsulfinyl-5-tuffluoramethylpyridine-2-carboxylic acid Samples were analysed by high performance liquid chromatography (HPLC) using UV detection. Recoveries were in the range of 80 - 120% of nominal. Thus, biological results after (2 hours and 96 hours are based on nominal concentrations. The study does not fulfillone of the validity criteria of the current version of OECD 201 guideline. There were no noticeable changes in cell morphology in any of the tested concentrations when compared to the control. They was evidence of cell aggregation in all treatment levels including the controls. Because aggregation was observed in all treatment levels it was not considered to be treatmentrelated. In this study *Navicula pellicitosa* has been exposed to five test concentrations of M-05 (AE 1344122). The 72 and 96 hour NOAEC for each growth parameter is10 mg/L.





	I. MATERIAL AND WETHODS:
Test material	M-05 (3-methylsulfinyl-5-trifluoromethylpyridine-2-carboxylic acid)
	AE 1344122
	Batch No: YG3228
	Specification: not reported
	Purity 98.8 %
Guideline(s)	None specified
adaptation	
Test species	Navicula pelliculosa 🖉 🖉 🖉
-	AE 1344122 Batch No: YG3228 Specification: not reported Purity 98.8 % None specified Navicula pelliculosa UTEX 667- Algal cells used in this test were obtained from cultures that had been actively for the test initiation. The
Culturing	Algal cells used in this test were obtained from cultures that had been actively
conditions	growing in culture medium for at least two weeks prior to test initiation. The
	culture was last transferred to fresh medium three days prior to test anitiation.
	The algal cells were cultured and tested fri freshwater algal modium
Test solutions	Nominal concentrations 0.43 0.94 -2.07 -4.55 -16 mg /
	Corresponding mean measured concentration: $0.46 - 0.97 - 2.12 \pm 4.71$ for mg/L
	Controls: culture medium (freshwater abgal medium)
Replication	No. of vessels per concentration (replicates): 8
	No. of vessels per control (replicates): 8° No. of vessels per control (replicates): 4 Static Total exposure duration: 96 hours
Exposure	Static Total exposure duration: 96 hours S
T '4' 1 11	Controls: culture medium (breshwater abgal medium) No. of vessels per control (replicates): 8 No. of vessels per control (replicates): 4 Static Total exposure duration: 96 hours
Initial cells	1×10^4 cells/mL in each test group $\sqrt{2}$ $\sqrt{2}$
density Test can ditions	Temperature 24.0, 24.4°
Test conditions	Photoperiod: continuous hight Light intensity at Surface of test vessels: 3890 to 4460 lux
	Photoperiod: continuous light kight intensify at Surface of test vessels: 3890 to 4460 lux
	Light intensity at \bigcirc surface of test vessels: 3890 to 4460 lux
Â	Growth meetium same as culture medium: Yes
, D	Type of light: gool white fluorescent tribes
Parameters 2	Samples were collected at approximately 24-hour intervals during the 96-hour
Measured /	exposure and were held for a maximum of three days under refrigerated
Observations	conditions sufficient to mhibit growth untilicell counts could be performed.
^A Q ^A	conditions sufficient to mhibit growth until cell counts could be performed.
4 Y .	sontiproously in the invironmental chamber and twice daily in a vessel adjacent
	to the test possels of a
Sampling for 🖉	Samples of test solutions were taken at test initiation (0 hour) and at test
chemical analysis	@rmination (96 hours) for analysis of test substance. Samples were analysed by
~~	high performance liquid coromatography (HPLC) using UV detection.
Data analysis	The calculation of cell densities, areas under the growth curve (biomass), growth
	rates and percent intobition values, as well as all statistical analyses, were
, KU K	Sconducted using The SAS System for Windows", Version 8.02.
× .	Non-Tinear regression was used to calculate EC ₅₀ values and their corresponding
[©]	95% confidence intervals. The data were evaluated for normality and
Ô ×	shomogeneity of variance ($p = 0.05$) using the Shapiro-Wilk's and Levene's tests,
	respectively. The seatment groups then were compared to the negative control $P_{\rm respectively}$ ($n = 0.05$). If the compared to the negative control
Û ÔS	using Duppett's test ($p = 0.05$). If the assumptions of normality and homogeneity
N R	of variances were not met an attempt was made to correct the condition by log
Á "O" á	Mansformation of the data. If the data failed the assumptions of normality and/or
A A	homogeneity of variances and transformations would not correct the problem
	Dunnett's test was still used to make the comparisons. The results of the statistical analyses, as well as an evaluation of the concentration-response pattern, were
\checkmark	used to determine the NOAEC relative to each parameter at 72 and 96 hours.
	used to determine the mOALE relative to each parameter at 72 and 90 hours.



II. RESULTS AND DISCUSSION:

Validity criteria (OECD 201, 2011)	Required	Obtained
Increase of biomass in the control cultures	16	115
Mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3) in the control cultures	<u>≤35%</u>	69 % 5 ⁷
Coefficient of variation of average specific growth rates in replicate control cultures	≤ 10% Ø	1.2 %
	· · · ·	

Analytical results:

Recoveries were 100 - 112% of nominal concentration see table below). The biological results are based on nominal concentrations of M-05 (AE 1344122) No residues of M-05 (AE 1344122) were measured in the control above the limit of quantification (0.2 mg/L).

$\begin{array}{c c c c c c c c c c c c c c c c c c c $	Nominal Concentration (mg /L)	Arithmetic mean Measured Concentration (mg /L)
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	0.43	$0.46 \qquad \bigcirc $
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	0.94	
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	2.07	
$10 \qquad 10 \qquad 10 \qquad 10 \qquad 10 \qquad 107 \qquad 107$	4.55	4.71 4.72 4.06 4.101 6
	10	

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

Biomass				
Nominal concentrations (mg/L)	Inhibition % at 24h	Thhibition % at 48 h	Anhibition Ø% at 92 h* ~	Anhibition % at 96 h*
0.43	A.5 0	*3.0	~1,9 & ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	-0.20
0.94	-1.9 0		j1.5 0	0.50
2.07	140 ⁴ ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	-5.7	-6.07	-4.3
4.55 *	↓ -6.7 · ·		ç î o	-7.3
10	-4.1	-7.00	-5.1	-4.3

10 -4.1 -4.3 *No statistically significant differences (p>0.05) at 72-hours and 96-hours from the negative control replicates using Dunnet Plest



Algae growth rate

Nominal concentrations (mg /L)	Inhibition % at 24h	Inhibition % at 48 h	Inhibition % at 72 h*	Inhibition % at 96 h*		
0.43	1.5	-1.1	0.40	0.29	~	
0.94	-0.23	0.85	0.22	-0.57		4
2.07	3.9	-2.5	-0.64	-0.30	O ^y	
4.55	-2.5	-3.6	-0.48	-0.93		
10	-1.3	-1.8	-0.31	-1.1		

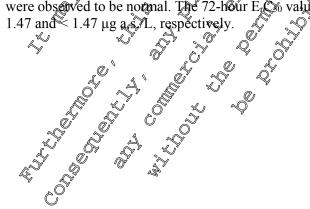
4.55	-2.5	-3.6	-0.48	-0.93	A	O' A' O
10	-1.3	-1.8	-0.31	<i>⊳</i> _A -1.1	<u> </u>	
No statistica	lly significant diffe	rences $(n > 0)$	05) at 72-hoi	irs and 96-hour	s from the neg	zative control replicates
ing Dunnet	s test	ences (p = 0.	<i>ce j ut 12</i> 1100	e		ative control replicates
8 - 4			n M	y 1	<u> </u>	× Q . O .
		I	IL CONCE	SIONS: Q	' a° a'	
		-	DO.			
he study i	s not considered to	be valid. The	ne endpoints	based or non	nimal concent	rations are.
						S A
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ErC50 72 ł	iours, 96 hours (95	% CI) 🕺		gal (not applie	cable)	
EbC50 72 1	10urs, 96 hours (95	% CD		/L (not applia	cable)	
NOEC		Q				
highest co	ncentration without	effects based		Hima N	N N	
on hiomas	s and growth rate)				o A	
on oronius	s und growin rute)					Õ (k.
	nours, 96 hours (95 ncentration without s and growth rate)	<u> </u>		<u> </u>	O	ř (Š
Assessme	ent and conclusio	n by applica	înt: 🔗	based or non c.L. (not applie to mg the formation of the mg the mg the mg the mg the formation of the mg th	Son Maria	
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The study	nours, 96 hours (95 ncentration without s and growth rate)		iot be used			» »
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Data Point:	KCA 8.2.6.2/07
Report Author:	
Report Year:	2015
Report Title:	Toxicity of fluopicolide technical to the saltwater diatom Skeletonema costatute
	during a 96 hour exposure
Report No:	007SRLS14C39
Document No:	M-533278-01-1
Guideline(s) followed in	EU Directive 91/414/EEC; Regulation (EC) No. 1107/2009; US EPA CSPP
study:	850.4500; OCSPP Guideline 850.4500 (2012), OECD Guideline 20 2006 The
	afore-mentioned guidelines were harmonized for various test parameters (1), temperature, light, etc.) to achieve optimal environmental conditions for the test organism. Scientific discretion was implemented where guideline parameters to
	temperature, light, etc.) to achieve optimal environmental conditions for the test
	not fully converge
Deviations from current	Method:
test guideline:	none Q , y , y , y
	Study: Current Guidelines: OPCD 2015 2011 COCSPP 850.4 00 (2012)
	The validity is assessed according to eriteria of both DECD 201 and OCSPP
	850.4500 The test conditions are compared to OCSPP 850.4500 only since no
	information is a variable for OECD 201 segarding maring species.
	There is a slight deviation of hight range: 4496-5100 hix instead of 3655-494 Iux
	recommended, However, all validity criteris are met so this deviation has no
<b>D</b> 1 4	influence of the study results. I a so so so
Previous evaluation:	No, not periously submitted a provide the submitted of th
CLD/000 - 1-11-	
GLP/Officially	Yes conducted under GLP/Officially recognised testing facilities
recognised testing facilities:	
Acceptability/Reliability:	Yes A S A S A

# Executive summary

A toxicity study was performed with sattwater diaton (Skelptonen) under static conditions for 96 hours. The following nominal (mean measured) concentrations of Iluopicolide were included in the study: 0.00147 (0.00139), 0.00470 (0.00463), 0.0150 (0.0135), 0.0481 (0.0446), and 0.154 (0.147) mg a.s./L. Additionally a control and solvent control was included. Number of replicates: 4 per toxicant level and 6 in the controls. Cell density counts were conducted daily in the control and all treatments till the end of the test. Aleal cellowere inspected with the help of a light microscope. Sampling was done on day 0 from bate Opreparation for each evel and on day 3 and 4 from composite samples from each level. The analysis was performed by Liquid Chromatography-Mass Spectrometry/Mass Spectrometry (LC-MS/MS). Recoveries were in the range of 80 120% of nominal. Thus, biological results after 72 hours and 96 hours are based on nominal conceptrations. The study meets the validity criteria of the current version of OECD 201 goideline. Cells exposed to all treatment levels tested and the controls were observed to be normal. The 72-hour  $E_1 \gtrsim 50$  value is 73.0 µg a.s./L with LOEC and NOEC values of 1.47 or  $2 \sim 1.47$  with LOEC and NOEC values of





Test material	Fluopicolide (technical) Batch No: ETFP000273 Spec No: 10200001644401 Purity: 100.5% w/w None specified Saltwater diatom ( <i>Skeletonema costatum</i> ) In-house 3-day old batch culture held under test conditions Nominal concentrations: 0.00147, 0.00470, 0.0150, 0.0081 and 0.154 mg a.s./L Corresponding arithmetic mean measured concentrations (0 – 96 h): 0.00139, 0.00463, 0.0135, 0.0446, 0.447 mg/a.s./L Controls: water and solvent controls (dimethylformanide at 0.1 µL/mL) Evidence of undissolved material: No precipitates No. of vessels per control (replicates): 6 No. of vessels per control (replicates): 6
	Batch No: E1FP0002/3
	Spec No: 10200001644401
	Purity: 100.5% w/w
Guideline(s)	
adaptation	None specified
T 4 ·	
Test species	Saltwater diatom (Skeletonema costatum)
Culturing	
conditions	In-house 3-day old batch culture held under test conditions $\sqrt{2}$ $\sqrt{2}$ $\sqrt{2}$
conditions	
	Nominal concentrations: 0.00147, 0.00470, 0.0150, 0.0081 and 0.154 mg
Test solutions	a.s./L
	Corresponding arithmetic mean mean fed concentrations (0 – 96 h) 0 00139
	0.00463 0.0135 0.0446 0.3447  msG s/L @ @ & A & o
	Controls: water and colvent controls (dimethylformaniale at 0 ul /al)
	Evidence of unditional protection of the protect
	Controls: water and solvent controls (dimethylformanude at 0.1 µL/mL) Evidence of undissolved material: No precipitates No. of vessels per concentration (replicates), 4 No. of vessels per control (replicates), 6 No. of vessels per solvent control (replicates) 6 Static Total exposure duration: 96 hours
Replication	No. of vessels per concentration (repricates): 4
1	No. of vessels per control (centrol) of a start of the second sec
	No. of vessels per solvent control (replacates) of
Exposure	Static Station of Gura &
Enposaro	Total exposure duration: 96 hours $\mathcal{V}$ $\mathcal{Q}$ $\mathcal{Q}$
Tu: 14: -111 -1 14	
Initial cell density	approx. 1 $^{\circ}10^4$ cells/mL at test initiation $^{\circ}$
	Temperature: 20.4-201°C
Test conditions	Photoperioe 14 hours light, 10 hours dark
le la	$1$ introductor $100 \text{ Juv} \approx 0$
, S	$\mathbb{R}^{H}$ of controls $(0 - 72 h)$ : 8.2 to 8.3 range for test concentrations: 8.2-8.6
. 0	Salinity: 3%
ð á	Growth medium same as culture medium: Yes enriched saltwater medium
. Ò	Type of Light: attrificial $Q$
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	type of augmining the second s
Parameters .	Pemperature was measured continuously during the test. Salinity and pH were
Measured / 🔊	measured on day 053 and 4.
Observations	Cell density counts were conducted daily in the control and all treatments till
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	the end of the jest. Algal cells were inspected via light microscope.
Sampling for C	Sampling on day 0 from bach preparation for each level and on day 3 and 4
Sumpring rop @	from composite samples from each level. The analysis was performed by
chemical analysis	Liquid Chromatography-Mass Spectrometry/Mass Spectrometry (LC-
	MŚ/MŚ).
	Shapiro-Wilks Test and Bartlett Equality of Variance Test were used to check
Dată analysis 👋	data for normality and homogeneity, respectively. ANOVA followed by
L .4	$L_{\rm EC_{\rm x}}$ estimates were done by linear interpolation. Statistical software used was
Q' .>	CEPIS v1.8.7.4
	Controls were pooled for the statistical analysis since they were not
	significantly different
jý _k	significantly different
A R	AP
pov .	
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#### **II. RESULTS AND DISCUSSION:**

Required	<b>Obtained</b> .
16	19
<u>≤</u> 35%	16%
$\leq 10\%$	1.9%
Required	Solution of the second
<b>3</b> 0	× ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~
0 < 15% v	Q 0.7% S
2 15%Q	

### Analytical results:

72 hour

Recoveries were in the range of 80 - 120% of nominal (see table below). Thus, biological results after 72 hours and 96 hours are based on nominal concentrations. No residues of foopicolide were measured in the controls above the limit of quantification  $(0.1 \mu g/L)$ . Ô

0

Nominal	Arithmetic @ean measure@ concentrations after 96 hours	ð	N. C		
Concentration	concentrations after 96 hours	% of norm	inal conce	entrations*	
(mg a.s./L)	(mg a.\${/L)	0-hour	72-houn	96-frour	Ŝ,
0.00147	0400139	2105 Ô	× 82	*86	2
0.00470	25 00.00469 0 Å		P03	85	
0.0150	δ <u>6</u> 0.0925 <u>5</u> <u>6</u>		@ 91	Ø3	
0.0481	0,0446 ~ ~	L ⁹ 95 ~		85	
0.154	5 0.14P 5 0	<u> </u>	699	93	

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

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

<u>72 IIOUIS</u>		
Nominal concentration	Moan growth rate (h ⁻¹ )	Inhibition of average
(mg a.s./L)		specific growth rate (%)
Control	L @0.04251	-
Solvent control	0.041840	-
0.00147	0 040592	3.3*
0.00470	0.040472	3.6*
0.00470	0.039976	4.8*
0.0481 67 6	0.037346	11.1*
0.15	-0.032106	176*

* Treatment group was significantly reduced when compared to pooled controls (Wilcoxon/Bonferroni Adj test;  $p \le 0.05$ ) ĉ



<u>96 hours</u>			
Nominal concentration	Mean growth rate $(h^{-1})$	Inhibition of average specific growth rate (%)  1.8* 3.9* 3.6* 3.6* 3.6* 12.3* 4 13.6* 4 12.3* 4 13.6* 4 12.3* 4 13.6* 4 12.3* 4 13.6* 4 12.3* 4 13.6* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4 12.3* 4	
(mg a.s./L)		specific growth rate (%)	
Control	0.046575	-	0
Solvent control	0.046364	-	Ó
0.00147	0.045636	1.8*	
0.00470	0.044677	3.9*	~~
0.0150	0.044795	3.6*	\$ \$
0.0481	0.040747	12.3	
0.154	-0.016859		$\sim$
* Treatment group was significat	ntly reduced when compared to pople	d controls (Witexon/Bonferron Adj te	$sop p \le 0$
	a C		∛ ¢°
			Ŷ
Nominal concentration	Yield	Infripition @ yield (%)	49 A
(mg a.s./L)	$(cells/mL \times 10^4)$ °		y W
Control	86.50 ° °		1
Solvent control	84.75		Q, y
0.00147	79.00 ~ ~ ~	~ 17.9× 0 ×	le la
0.00470	<b>@</b> 1.94, ~ <b></b>	016.0*	j A
0.0150	0 ⁹ 72 7∕5 √ √		No.
0.0491			L.
0.154	Q 49.00	deontrols Wilcoxon/Bonferroni Act te Anhibition of biomass integral (%) 9.24 0.187* 0.187* 0.187* 0.187* 0.187* 0.180* 103.0* d controls (Wilcoxon/Bonferroni Adj te	Ň
0.154	<u>0</u> <u>4</u> 0.98 <u>6</u> <u>6</u>	101.1°C	
* Treatment group was significant	ntly toduced when compared to poole	deontrol Milcoxon/Bonfertoni A@ te	st; $p \le 0.0$
Nominal concentration	A rog under the row the	Inhibition of Moments	
(mg a s / L)	an ca under the growin	antegral (%)	
Control			
Solvent control			
0.00147		9.2	
0.00470		1 <u>0</u> <u>1</u> 8.7*	
0.0150		Ø 0 ¹ 6.0*	
0.0481	\$\$ \$\$1047.6 \$\$ .	37.2*	
0.154	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	103.0*	
* Treatment group was significat	htly redified when compared to pose	d controls (Wilcoxon/Bonferroni Adj te	est; $p \le 0.0$ :
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#### 96 hours



# **III. CONCLUSIONS:**

The study meets the validity criteria and the 72- and 96-hour endpoints based on nominal concentrations are given in the table below.

ErC ₅₀ 72 hours (95% C.I.):	0.073 mg a.s./L (0.0667-0.0798 mg a.s./L)
E _r C ₂₀ 72 hours (95% C.I.):	0.0538 mg a.s./L (0.0522–0.0557 mg a.s./L)
E _r C ₁₀ 72 hours (95% C.I.):	0.0420 mg a.s./L (0.0330 - 0.0517 mg a.s./L)
E _r C ₀₅ 72 hours (95% C.I.):	6,016 mg a.s./L (0.00023-0.027 mg a.s./L) •
LOE _r C 72 hours: lowest concentration with significant effect compared to the control	$\begin{array}{c} 0.073 \text{ mg a.s./L} \\ (0.0667-0.0798 \text{ mg a.s./L}) \\ \hline 0.0538 \text{ mg a.s./L} \\ (0.0522-0.0557 \text{ mg a.s./L}) \\ \hline 0.042 \text{ mg a.s./L} \\ \hline 0.0330-0.0517 \text{ mg a.s./L} \\ \hline 0.016 \text{ mg a.s./L} \\ \hline 0.00147 \text{ mg a.s./L} \\ \hline $
NOE _r C 72 hours: highest concentration without significant effcet, compared to the control	$\sim 0.00447 \text{ mg a.s./L}$ $\sim 0.00447 \text{ mg a.s./L}$ $\sim 0.00447 \text{ mg a.s./L}$ $\sim 0.00547 \text{ mg a.s./L}$ $\sim 0.00529 - 0.009 \text{ mg a.s./L}$ $\sim 0.0392 \text{ mg a.s./L}$ $\sim 0.0392 \text{ mg a.s./L}$
ErC50 96 hours (95% C.I.):	0 < 0.00447 mg a.s./L 0 < 0.00447 mg a.s./L 0 < 0.0803 mg a.s./L 0 < 0.0547 mg a.s./L
E _r C ₂₀ 96 hours (95% C.I.):	0.0547 mg a.s./L 0 5 5 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4
$E_r C_{10}$ 96 hours (95% C.I.):	(0.0392  mg a.s.) $(0.0353 - 0.0428  mg a.s.)$
$E_r C_{05}$ 96 hours (95% C.I.) $\sim$	0.020 mg a.s./L (0.0)6-0.029 mg a.s./L)
LOE _r C 96 hours:	$\begin{array}{c} & (0.0803, \mbox{mg a.s}/\mbox{$\square$}) \\ \hline & (0.08.6 - 0.102 \mbox{ mg a.s}.\mbox{$\square$}) \\ \hline & 0.0547 \mbox{ mg a.s}.\mbox{$\square$} \\ \hline & 0.052 - 0.0393 \mbox{ mg a.s}.\mbox{$\square$} \\ \hline & 0.0392 \mbox{ mg a.s}.\mbox{$\square$} \\ \hline & 0.0392 \mbox{ mg a.s}.\mbox{$\square$} \\ \hline & 0.020 \mbox{ mg a.s}.\mbox{$\square$} \\ \hline & 0.020 \mbox{ mg a.s}.\mbox{$\square$} \\ \hline & 0.00047 \mbox{ mg a.s}.\mbox{$\square$} \\ \hline & 0.000447 \mbox{ mg a.s}.\mbox{$\square$}$
NOE _r C 96 hours highest concentration on thout significant effect compared to the control	- 0.00147 mea.s./L
EyC ₅₀ <b>26</b> hours (95% 64.):	0.0612 mg a.55L (0.0568 – 0.0651 mg a.s./L)
E _y C ₅₀ <b>96 hours (95% G.I.):</b>	© 0.0162 – 0.0236 mg a.s./L (0.0162 – 0.0236 mg a.s./L)
E _y C ₁₀ 96 hours(95% (0.))	© 0.0024 mg a.s./L > 0.000571 – 0.00410 mg
LOE _y C 96 hours: lowest concentration with significant effect	∞ 0.00147 mg a.s./L
NOE C 96 hours: highest concentration without significant effect	<0.00147 mg a.s./L
compared to the control	



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E _b C ₅₀ 96 hours (95% C.I.):	0.0687 mg a.s./L (0.0654 - 0.0717 mg a.s./L)	
E _b C ₂₀ 96 hours (95% C.I.)	0.0212 mg a.s./L (0.0152 - 0.0256 mg a.s./L)	
E _b C ₁₀ 96 hours (95% C.I.)	0.00185 mg a.s./L (0.000807 - 0.00362 mg	
LOE _b C 96 hours: lowest concentration with significant effect compared to the control	0.00147 mg a.s./L	
$NOE_bC$ 96 hours: highest concentration without significant effect compared to the control	< 0.00147 mg a.s./LQ	

#### Assessment and conclusion by applicant:

The study is reliable and the 72h-ErC50 of 0.073 mg ars./L can be used in risk assessment The NOErC is < 0.00147 mg a.s./L based on 3.3% or 1.8% of effects a 0.00147 mg a.s./L at 12 and 96h, respectively. The biological relevance of such a low level of effects is questionable. Moreover,

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very similar inhibition, 3.8 and 3.9% are observed at the next concentration. Therefore, this is representative of biological variability.

Bayer performed 18 other studies showing effects in the same laboratory between December 2003 and July 2018. 17 of these studies showed at least 5.0% of effects at the LOEC. Only one performed in April 2015 (the study performed just after the study on fluopic dide) showed similar profile with significant effects at 2.1 and 3.3% of inhibition. The median kalues for the inhibition at the LOEC were 14% and 11% at 72 and 96 respectively (ranges: 3.3 – 67.9% at 72 and 2.1 – 53.8 at 96h). This demonstrates that the NOEC determined in the study on fluopicolide, was artificially low.

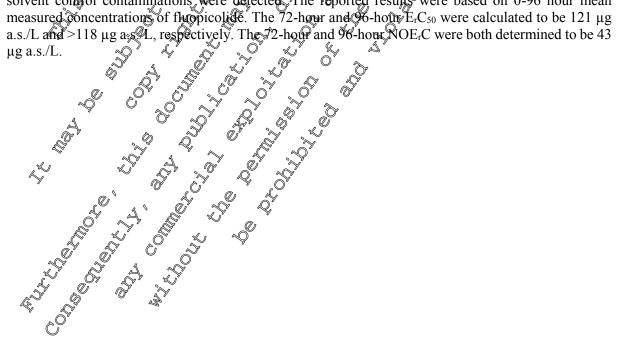
This demonstrates that the NOEC determined in the study on fluopicolide, was artificially low. Consequently, EC₀₅ values were calculated in the pertand are considered more biologically relevant than the NOEC. The EC₀₅ at 72105 0.040 mg as 7.L., this endpoint is relevant for the PBT assessment.



Data Point:	KCA 8.2.6.2/08
Report Author:	
Report Year:	2020
Report Title:	Fluopicolide (AEC638206): A 96-hour toxicity test with the freshwater diaton (Navicula pelliculosa)
Report No:	149P-120
Document No:	<u>M-678011-01-1</u>
Guideline(s) followed in study:	OECD 201 (2011); OCSPP 850.4500 (2012)
Deviations from current	Method: none ; Study: Current Guideline: OECD 201 (2011)
test guideline:	The pH in the control solution increased by greater than 1.5 units over the course of the study. The pH of the notation control solution increased by greater than 2.5 million over the course of the study.
	1 of the study. The prior the negative control solution was 9.8 at 72 hours and 9.0
	at 96 hours. Although the ploin the control groups increase by greater than 1.5 units after 72 hours of exposure, the increases in 100 documented for this study are commonly observed or this diatom species and all control validity criteria
	units after 72 hours of exposure, the increases in pol documented for this study
	are commonly observed for this diatom species and all control validity criteria
	were achieved despite the increase in pH. & & &
Previous evaluation:	No, not previously submitted to a final state of the second state
GLP/Officially	Yes, conducted under ODP/Officially recognised testing facilities
recognised testing	
facilities:	
Acceptability/Reliability:	$  Yes \downarrow 0, \forall \gamma $
Executive Summary	

The aim of the study was to determine the toxicity of fluopicolide (AE 638266) to the freshwater diatom (*Navicula pelliculosa*) expressed as NOEC. COEC and EC_x for growth rate and yield. *Navicula pelliculosa* with an initial cells density of 10,000 cells/pfl were exposed to fluopicolide for 96 hours. Nominal test concentrations were (control and solvent control), 2.0, 5.5, 15, 43, and 120 µg a.s./L. The cell density ineach applicate was counted at 24-pour intervals. The water samples were analysed with LC-MS/MS. Measured concentrations ranged from 91.0 to 103% of nominal concentrations. After 96 hours the mean measured concentrations were 1.8, 5.3, 15 43 and 118  $\mu$ g a.s./L. No control and solvent compol contaminations, were detected. The reported results were based on 0-96 hour mean measured concentrations of fluopicolide. The 72-hour and 6-hour  $E_rC_{50}$  were calculated to be 121 µg

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Test material	Fluopicolide (AEC638206)
	Batch code: AE C638206-01-27
	Purity: 98.7% Specification: 102000016444
Guideline(s) adaptation	Fluopicolide (AEC638206)         Batch code: AE C638206-01-27         Purity: 98.7%         Specification: 102000016444         None         Freshwater diatom (Navicula pelliculosa)
Test species	Freshwater diatom ( <i>Navicula pelliculosa</i> )
Culturing conditions	Algal cells used in this test have been actively growing in white medium under the same environmental conditions as used in this test for at least for weeks prior to test initiation.
Test solutions	Test medium: freshwater AAP medium with silica constituents Nominal concentrations: 0 (control) and solvent control, 2, 0, 5.5, 12, 43, and 120 µg a.s./ Corresponding mean measured concentrations 0–46 hours < LOO (control and solvent control), 1.8, 5.3, 15, 43 and 118 µg a.s. 2 Control: untreated test medium Solvent control: <i>N</i> , <i>N</i> -dimethal formanide (02 mL/E) Evidence of undissolved material. No particulates or surface-slices were observed in any experimental group.
Replication	Solvent control: <i>N</i> , <i>N</i> -dimethylformamide (04 mL/E) Evidence of undissolved material. No particulates or surface-slices were observed in and experimental group.
Exposure	Test type: state with the second seco
Initial cells density	Approx. 10,000 c@ls/mL cells/mL Algal cells for this study were taken from a culture that had been transferred to tresh medium three days prior to test initiation
Test conditions	Temperature 22.0–22.5 °C Photoperiod: contributous light Light intensity: 3880–4280 lux Type of light: dool white fluorescent lighting 5 pH: 73–9.8 pHon controls: 7.3–9.8 (over 72.18)
Parameters Measurer Observations	Cell density was determined at Q4-hour intervals using an electronic particle counter. Cells were also assessed for atypical morphology (e.g., changes in cell shape, size or color), aggregation or flocculation and adherence to the test chamber. The pH of the medium in each treatment and control group was measured at test initiation, at 2 hours, and a exposure termination. The temperature of a container of water adjacent to the test chambers in the environmental chamber was measured continuously. Light intensity was measured at test solution level at nine locations surrounding the test flasks at test initiation.
Sampling for chemical analysis	Samples were collected at approximately 0, 72, and 96 hours. At test initiation (0 hour),
	chambers At 72 Hours, samples were collected from surrogate replicates included in each treatment and control group. At test termination (96 hours), the remaining replicates from each respective treatment and control group were pooled and then sampled. The samples were analysed with by high performance liquid chromatography with tandem mass spectrometric detection (LC/MS/MS)



Data analysis	Average specific growth rate (r), area under the growth curve (biomass) and yield (y), were calculated for each test flask.
	$EC_x$ -values: $E_rC_x$ and $E_yC_x$ values and their corresponding 95% confidence intervals were calculated at 72 and 96 hours of exposure, when possible, using non-linear regression with
	treatment response (growth rate, area under the growth curve and yield) and exposure
	concentration data (0–96 hour mean measured concentrations).
	normality and homogeneity of variance ( $\alpha = 0.01$ ) using Shapiro Wilk's and Levene's tests,
	respectively. The treatment group means for the datasets which the assumptions of normality $\alpha$ and homogeneity of variance were compared to the pooled control using Duprett's test ( $\alpha \neq \beta$ )
	0.05). A non-parametric test, Wilcoxon test with Bonferron Holm correction ( $\alpha = 0.05$ ), was
	used to evaluate the treatment group means for the datasets which did not meet the assumptions of normality or homogeneits of variance
	assumptions of normality or homogeneity of variance

II. RESULTS AND DISCUSSION:

Validity criteria:

		~~ ~ ~C	
Validity criteria (OECD 201, 2011)		Required	Obtained S
Biomass in the control within the 72-hou	ngest period	~~≥ 16 ℃	269
Mean coefficient of variation for section growth rates in the control within the 72-	-hogo test period		25,5%
Coefficient of variation of average specifi	by growth rates within $\mathcal{L}$	10%gg	° 1.9%
72-hour test period in control replicates	Nº 4 m		
QO′ `~∆			N. C.

¢ Ø ۶ Analytical results: Recoveries ranged from 92.0 to 101% of nominal concentrations after 72 to and from 92.4 to 100% of nominal concentrations after 96 h (see table below). Results of the study are based on mean measured fluopicolide concentrations.

	~		, Q					
Nominal	Day 0	(New)	Day 3	(Old)	🔊 Day 4	(Old)	Mean	Mean
conc. [µg a.s./L]	Measured ⊘ conc.○ ]µg a.s./L]	% of nominal	Measured conC [µg.a.s./L]	, nominal© ©∑	Measured conc. [µg a.s./L]	% of nominal	measured conc. [µg a.s./L]	% of nominal
2.0	1.87	93.5	A.82	<u>_</u> 94_0	1.85	92.4	1.8	92.3
5.5	5.20	94.6	5.26	م م ب ب ب ب ب ب ب ب 5.1	5.47	99.5	5.3	96.4
AJ 5	4	\$103~~	Q\$.2 ~	y 101	15.1	100	15	101
43	@42.9	9 <b>9</b> ,8	€ 43.3 ×	101	42.8	99.6	43	100
120	148	§98.1 ×	[*] 120 [*]	100	117	97.1	118	98.3
Full details an 4, which com	d seceptab ply with the	le valudation E EV regula	n data to su tory requir	pport this n ements outl	nethod are p ined within	oresented w SANCO/3	rithin docun 8029/99 rev	ient M-CA 4.



#### **Biological results:**

There were no noticeable changes in cell morphology in any treatment group when compared to the control replicates during the microscopic examinations of the cells. Cells present in all fluogication treatment groups and in the solvent control on days 1, 2, 3, and 4, appeared normal when compared to cells present in the negative control. Adherence of cells to the test chambers was not observed any of the experimental groups. Flocculation or aggregation of cells was not observed in the control groups or , ° any of the Fluopicolide treatment groups. , Ş Ŝ

% Inhibition of growth ra	te and yield	, T		
Mean measured concentration		th rate		Field & O
[µg a.s./L]	72 h	96 h	72 h	∞96 h
1.8	0			
5.3	-4		\$ -29	
15	0			~~~-2 <u>~</u> ~
43	1 8			
118	48	32 ^{b)}	93 a)	(495 b)

a) Treatment group mean was significantly reduced (Duppett's test p < 0.93) when compared to the pooled control response. a) Treatment group mean was significantly reduced (Durgett's tesp p < 0.95) when compared to the pooled control response.</li>
 b) Treatment group mean was significantly reduced (Wilcoxon Bonferran-Holp test; p < 0.05) when compared to the pooled control response.</li>
 The endpoints based on mean measured concentrations are:

The endpoints based on mean measured concentrations are.

$\sqrt{2}$ $\sqrt{2}$ $\sqrt{2}$ Results – 0 to 72 hours	rs of a
<b>C</b> (95% CI):	
E4G20 - 72 hours	79 μg a.s./L (71 to 89)
$E_{r}C_{W} \neq 72$ hears $f = 1$	64 μg a.s./L (54 to 76)
IDEC - E hours lowest concentration with m effect based on growth rate	118 µg a.s./L
NOBC - 72 bours: 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	43 μg a.s./L
$E_yC_{50}$ - 72 hours	68 μg a.s./L (58 to 79)
$\mathbb{E}(\widehat{\mathbf{a}}_{20}^2 - 7\widehat{\mathbf{a}})$ hours $\mathbb{O}$	50 μg a.s./L (40 to 62)
F _y C _b 72 hoters	42 μg a.s./L (32 to 55)
LOEC - 32 hours: Jowest concentration with an effect (based on yield)	118 μg a.s./L
NOBC - 72 hours: kighest concentration without an effect (based on yield)	43 μg a.s./L
ČČ ^Ý	·

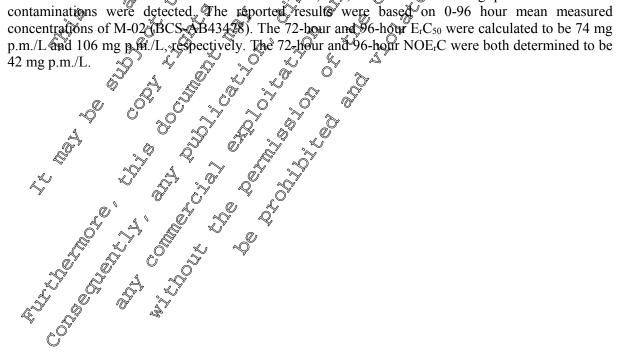


Results – 0 to 96 hou	rs
ErC ₅₀ - 96 hours	>118 µg a.s./L 。
(95% CI):	(NA)
$E_r C_{20}$ - 96 hours	113 µg a.s./L
(95% C.I.):	(112 to 114)
$E_rC_{10}$ - 96 hours	1μ0 μg a.s./L
(95% C.I.):	(Ĭ10 to 111)
LOEC - 96 hours: lowest concentration with an effect (based on growth rate)	118 μg a.s. μ
NOEC - 96 hours:	
highest concentration without an effect (based on growth rate)	43 μg/u/s./L 2 43 μg/u/s./L
E _v C ₅₀ - 96 hours	2 105 ag a.s. Az
(95% CI):	(103  to  106)
$E_v C_{20}$ - 96 hours	² 105 μg a.s. 4 (103 to 106) ² 495 μg a.s./L ² ² 495 μg a.s./L ²
(95% C.I.):	(9400 96) ( A
$E_yC_{10}$ - 96 hours	90γμg a.s./L & @
(95% C.I.):	(89 to 91)
LOEC - 96 hours lowest concentration with an effect (based on yield)	2 1180 g a.s. 6
lowest concentration with an effect (based on yield)	
NOEC - 96 ROURS:	²
$CI = confidence interval: NA = not applicable \gamma$	
* Extrapolated value. The 72-hour $\text{ErC}_{50}$ estimate is preater than the higher	st concentration tested, however, the estimated was
considered to be sensible based on evaluation of the dose response and the	precision of the 95% confidence interval.
Assessment and copelusion by a folicant	
Assessment and conclusion by applicant.	
The study is reliable and the $723$ ErC of 0.121 mg as./L	an be used in risk assessment.
	ŷ.
LOEC - 96 hours lowest concentration with an effect (based on yield) NOEC - 96 hours: highest concentration without an effect (based on yield) CI = confidence interval; NA = not applicable * Extrapolated value. The 72-hour E/Cso estimate is greater at an the aighe considered to be sensible based on evaluation of the dose response and the Assessment and conclusion by applicant: The study is reliable and the 72 her Cso of 0.121 mg ats./L	
$\lor$	



Data Point:	KCA 8.2.6.2/09
	KCA 8.2.0.2/09
Report Author:	
Report Year:	
Report Title:	BCS-AB43478: A 96-hour toxicity test with the freshwater diatom (Navicula)
Report No:	149P-121
Document No:	<u>M-678012-01-1</u>
Guideline(s) followed in study:	OECD 201 (2011); OCSPP 850.4500 (2012)
Deviations from current	Method: none; $(3)$
test guideline:	Study: Current Guideline: OECD 201 (2011) $\mathcal{O}$
-	The pH of the negative control solution was 9 $\bigcirc$ at 72 hours and 9.6 at 96 hours.
	Although the pH in the control groups increased by greater than 1.5 units after 72
	hours of exposure, the increases in pH documented for this study are commonly
	observed for this diatom species and all control validity criteria were achieved
	despite the increase in pH. $\bigcirc$ $\checkmark$ $\checkmark$ $\checkmark$
Previous evaluation:	No, not previously something and the second se
GLP/Officially	Yes, conducted under CIP/Officially recognised, testing facilities
recognised testing	
facilities:	
Acceptability/Reliability:	Yes $\mathcal{L}$ $\mathcal{P}$ $\mathcal{P}$ $\mathcal{P}$ $\mathcal{P}$ $\mathcal{P}$ $\mathcal{P}$ $\mathcal{P}$
Executive Summary	

Ô The aim of the study was to determine the toxicity of M-02 (BCS-AB43478) to the freshwater diatom (*Navicula pelliculosa*) expressed as NOEC LOEC and  $C_x$  for growth rate and yield. *Navicula pelliculosa* with an initial cells density of 10,000 cells/ml were exposed to M 02 (BCS-AB43478) for 96 hours. Nominal test concentrations were 0 (control) 1.0, 2.6, 6.4, 16, 40 and 100 mg p.m./L. The cell density in each replicate was counted at 24-hour intervals. The water samples were analysed with LC-MS/MS. Measured concentrations ranged from 97,4 to 113% of rominal concentrations. After 96 hours the mean measured concentrations were 1.1, 2.7, 69, 16,42 and 107 mg p.m./L. No control contaminations were detected. The reported results were based on 0-96 hour mean measured





Test material	M-02 (BCS-AB43478)
	Batch code: AE C657188-PU-02 Purity: 99.9%
Guideline(s) adaptation	Batch code: AE C657188-PU-02 Purity: 99.9% None
Test species	Freshwater diatom (Navicula pelliculosa)
Culturing conditions	Algal cells used in this test have been actively growing in cuture medium under the same environmental conditions as used in this test for at least two weeks prior to test initiation.
Test solutions	Test medium: freshwater AAP medium with silica constituents Nominal concentrations: 0 (control), 10, 2.6, 6.4, 16,40 and 100 mgp.m./L Corresponding mean measured concentrations 0–96 hours: LOQ Controb 1.1, 2, 7, 6.6, 66, 42 and 107 mg p.m./L Controls: untreated test medium. Evidence of undissolved material: All fest solutions appeared clear and coloutless, with no particulates or surface-slick visible? No. of vessels per concentration (replicates): 4 No. of vessels per control (replicates): 4 No. of vessels per control (replicates): 4 No. of vessels per control (replicates): 4 Approx. 10,000 cells/ml cells/ml Algal cells for this study were taken from a culture that had been transferred to fresh medium three days prior to test initiation
Replication	No. of vessels per concentration (replicates): 4
Exposure	Test type: static Of A State of State o
Initial cells	Approx. 10,000 cells/ml cells/ml
density	Approx. 10,000 cells/mL cells/mL Algal cells for this study were taken from a culture that had been transferred to fresh medium three days prior to test initiation
	Temperature: 21.0–23.0 C Photoperiod: continuous light Light intensity: 3910–4640 lux Type of light: cool white fluorescent lighting pdf: 3.7–98, pH in controls: 7.2-8.9 (over 72 h) Introduction of BCS-AB43478 to the test medium at chominal concentration of 100 mg p.m./O significantly reduced the pH of the test medium. The pH was not adjusted in order to provide the most conservative estimate of toxicity and the most realistic exposure scenario. Physical toxicity is highly likely at these pH levels, however, this was considered to be a treatment related effect since the test substance caused the reduction in pH.
Parameters Measured /	Cell density was determined at 24 hour intervals using an electronic particle counter. Cell's were also assessed for atypical morphology (e.g., changes in cell shape, size or color),
Observations	Aggregation or thocculation, and adherence to the test chamber. The phoof the medium in each treatment and control group was measured at test initiation, at 72 hours, and at exposure termination. The temperature of a container of water adjacent to the test chambers in the environmental chamber was measured continuously. Light intensity was measured at test solution level at nine locations surrounding the test
Sampling for	flasks at test initiation of some state of the second seco
chemical analysis	samples were collected for each treatment and control group prior to distribution into test
	charbers. Af 72 hours, samples were collected from surrogate replicates included in each treatment and control group. At test termination (96 hours), the remaining replicates from each respective treatment and control group were pooled and then sampled. The samples were analysed with by high performance liquid chromatography with tandem mass spectrometric detection (LC/MS/MS)



Data analysis	Average specific growth rate (r), area under the growth curve (biomass) and yield (y), were calculated for each test flask.
	$EC_x$ -values: $E_rC_x$ and $E_yC_x$ values and their corresponding 95% confidence intervals were of calculated at 72 and 96 hours of exposure, when possible, using non-linear regression with tractionary constraints are under the growth energy and visible) and even with the growth energy of the set of the
	treatment response (growth rate, area under the growth curve and yield) and exposure
	concentration data (0–96 hour mean measured concentrations).
	NOEC and LOEC values: The 72- and 96-hour growth rate and yield data were evaluated for $x = 0.01$ wing Sharing Values and Lower 24 to $x = 0.01$
	normality and homogeneity of variance ( $\alpha = 0.01$ ) using Shapiro Wilk's and Levene's tests, respectively. The treatment group means for the datasets which met assumptions of normality
	and homogeneity of variance were compared to the control using Dunnett's test ( $\alpha = 0.05$ ). A non-parametric test, Jonckheere-Terpstra Step-Down Trendlest ( $\alpha = 0.05$ ) was used to
	non-parametric test, Jonckheere-Terpstra Step-Down Trendflest ( $\alpha = 0.05$ ) was used to
	evaluate the treatment group means for the datasets which did not meet the assumption of
	normality or homogeneity of variance of the second se

Validity criteria:

Validity criteria (OECD 201, 2011)		Required >>>	& Obtained
Biomass in the control within the 72-ho	west period	2 16 ℃	\$ <u>323</u>
Mean coefficient of variation for section growth rates in the control within the 1/2	2-hour test period		25,5%
Coefficient of variation of average speci 72-hour test period in control replicates			00.60%
			L. G

Analytical results: Recoveries ranged from 594 to 193% of nominal concentrations after 72 brand from 97.4 to 113% of nominal concentrations after 96 h (see table below). Results of the study are based on mean measured concentrations of M-92 (BCS AB43478).

nominal concentrations after 90 if (Sec table period, results of the sector of the sec

				/ %	<u>A</u> '			
Nominal conc.	ېDay 🕰	New)		Old)	Ö Day 4 (	Old)	Mean measured	Mean % of
[mg p.m./L	Measured		Measured	× .		% of	conc.	nominal
A	conc. [mg p.m./L]	frominal"	eonc. [mg p.m./D]	nominal	conc. [mg p.m./L]	nominal	[mg p.m./L]	
1.00	1.04	A94	1.15	م 113	1.13	113	1.1	110
2.6	2%60	\$99.8°	Q.74	105	2.78	107	2.7	104
6.4	6.35	20.3	6.74C	105	6.75	105	6.6	103
16	15:0	£97.5 √	16.7	104	15.6	97.4	16	99.8
40	<b>40</b> .9 Č	102	~944.6	111	41.6	104	42	106
	2 ¹⁰⁴	~ <b>0</b> 4	112	112	104	104	107	107
$\sim$	Dr _C r.	<i>K</i> ″						

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



### Biological results:

Cells present in the 1.1, 2.7, 6.6, 16, and 42 mg p.m./L treatment groups on days 1, 2, 3, and 4, appeared normal when compared to cells present in the negative control. Lysed cells were observed in the 107 mg p.m./L treatment group on days 1-4. Adherence of cells to the test chambers was not observed in any of the experimental groups. Aggregation of cells was observed in the control and the 1.1, 2.7, 66, 16, and 42 mg p.m./L treatment groups. This is a normal observation for this diatom species, and this was not considered to be a treatment related effect since it was also noted in the control. A tack of aggregation in the 107 mg p.m./L treatment group was considered to be a treatment related effect bit was also related to reduced cell densities relative to the other experimental groups.

		Â		
<u>% Inhibition of grow</u>	vth rate and yield	Ą	<u>Q' 6°</u>	
Mean measured conc.		th rate 🗳 ion [%]		Yield X
[mg p.m./L]	72 h	● 96,b	^م 72%	َنَّ الْمَ 96 الْمَ الْمَ ⁰
1.1	0		à Al ć	
2.7	0			11 b)
6.6	0 0			
16	0 %		² 2 ⁰ -2 0	
42		~~~ 0° ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	10 ⁹ 5	0
107		5 <b>50</b> a) 7		99 ^{b)}

a) Treatment group mean was significantly reduced (Jonckheere-Terpstra Step-Down Trend Fest; p 50.05) when compared to the control response.

b) Treatment group mean was significantly reduced (Dunnett Stest;  $p^{\circ}$  0.05) when compared to the control response.

IIL CONCER

The endpoints based on mean measured concentrations are:

	<u> </u>
Results – 0 to 72 hor	's A
	5
(95) Č.I.): Č	54 mg p.m./L (54 to 55)
$\begin{array}{ccc} & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & & \\ & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & &$	48 mg p.m./L (47 to 49)
LOEC - 2 hours: lowest concentration with an effect (based on growth rate)	107 mg p.m./L
NOSC - 72 brours: highest concentration without an effect/based on growth rate)	42 mg p.m./L
$\begin{array}{c} \begin{array}{c} \begin{array}{c} \begin{array}{c} \begin{array}{c} \end{array} \\ \end{array} \\ \end{array} \\ \end{array} \\ \end{array} \\ \begin{array}{c} \end{array} \\ \end{array} \\ \begin{array}{c} \end{array} \\ \end{array} \\ \end{array} \\ \begin{array}{c} \end{array} \\ \end{array} \\ \end{array} \\ \end{array} \\ \begin{array}{c} \end{array} \\ \end{array} $	72 mg p.m./L (70 to 75)
$\begin{array}{c} & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & & \\ & & & & & & \\ & & & & & & \\ & & & & & & \\ & & & & & & \\ & & & & & & \\ & & & & & & \\ & & & & & & \\ & & & & & & \\ & & & & & & \\ & & & & & & \\ & & & & & & \\ & & & & & & \\ & & & & & & \\ & & & & & & \\ & & & & & & \\ & & & & & & \\ & & & & & & \\ & & & & & \\$	51 mg p.m./L (46 to 55)
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	44 mg p.m./L (35 to 49)
LOEC - 72 hours: west concentration with an effect (based on yield)	107 mg p.m./L
NOEC - 72 hours: highest concentration without an effect (based on yield)	42 mg p.m./L

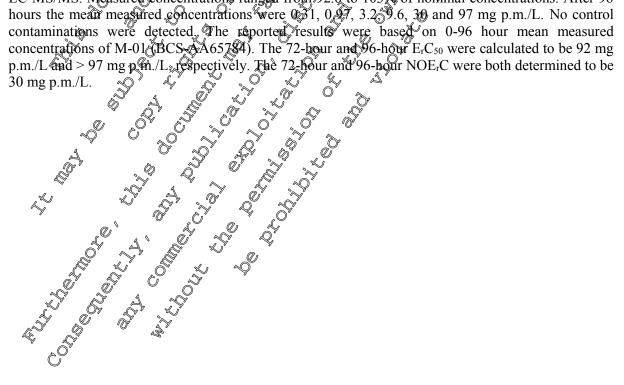


Results – 0 to 96 hour	rs
ErC ₅₀ - 96 hours	106 mg p.m./L
(95% CI):	(106 to 106)
$E_rC_{20}$ - 96 hours	89 mg p.m./L
(95% C.I.):	
$E_r C_{10}$ - 96 hours	86 mg p.m./L
(95% C.I.):	(85 to 87) 2 3
LOEC - 96 hours:	(8860 90) 86 mg p.m./L (85 to 87) 107 mg p.m./P
lowest concentration with an effect (based on growth rate)	
NOEC - 96 hours: highest concentration without an effect (based on growth) ate)	0 42 mg m./L 3 5 40
	2 106 girg p.m St
(05% CI):	
E.C.20 - 96 hours	2 106 mg p.m./L (106 to 106) 3 2 3 mg p.m./L 3 3 mg p.m./L 4 3 mg p.m./L 4 3 mg p.m./L 4 3 mg p.m./L
(95%  C.I.):	
E _v C ₁₀ - 96 hours	40mg p.m./L
(95% C.I.):	(42 to 48)
$E_yC_{20} - 96 \text{ hours}$ $(95\% \text{ C.I.}):$ $E_yC_{10} - 96 \text{ hours}$ $(95\% \text{ C.I.}):$ $LOEC - 96 \text{ hours}$ $(95\% \text{ C.I.}):$ $UOEC - 96 \text{ hours}$ $(95\% \text{ C.I.}):$	107. or nm d
LOEC - 96 hours lowest concentration with an effect (based on yield)	→ → → → → → → → → → → → → → → → → → →
NOEC - 96 kours:	∞ 2 mg € m./L ×
CI = confidence interval	
$\frac{(93\% \text{ CI})}{\text{E}_{y}\text{C}_{20} - 96 \text{ hours}}$ $\frac{(95\% \text{ C.I.}):}{(95\% \text{ C.I.}):}$ $\frac{\text{E}_{y}\text{C}_{10} - 96 \text{ hours}}{(95\% \text{ C.I.}):}$ $\frac{\text{LOEC} - 96 \text{ hours}}{(95\% \text{ C.I.}):}$ $\frac{\text{CI} = \text{concentration without an effect (based on yield)}}{(95\% \text{ CI})}$ $\frac{\text{CI} = \text{confidence interval}}{(95\% \text{ CI})}$	
Assessment and conclusion by applicant:	
Assessment and conclusion by applicant: The study is reliable and the 72h-E&C ₅₀ of 94 mgp.m./4. 96	r M202 can be user On risk assessment
	¢.
J Z A J	
Exclose 90 hours (95% C.I.): LOEC - 96 hours lowest concentration with an effect (based on yield) NOEC - 96 hours: highest concentration without an effect (based on yield) CI = confidence interval Assessment and conclusion by applicant: The study is reliable and the 72h-EaC 50 of 74 mg m./4. for the study is reliable and the 72h-EaC 50 of 74 mg m./4. for the study is reliable and the 72h-EaC 50 of 74 mg m./4. for the study is reliable and the 72h-EaC 50 of 74 mg m./4. for the study is reliable and the 72h-EaC 50 of 74 mg m./4. for the study is reliable and the 72h-EaC 50 of 74 mg m./4. for the study is reliable and the 72h-EaC 50 of 74 mg m./4. for the study is reliable and the 72h-EaC 50 of 74 mg m./4. for the study is reliable and the 72h-EaC 50 of 74 mg m./4. for the study is reliable and the 72h-EaC 50 of 74 mg m./4. for the study is reliable and the 72h-EaC 50 of 74 mg m./4. for the study is reliable and the 72h-EaC 50 of 74 mg m./4. for the study is reliable and the 72h-EaC 50 of 74 mg m./4. for the study is reliable and the 72h-EaC 50 of 74 mg m./4. for the study is reliable and the 72h-EaC 50 of 74 mg m./4. for the study is reliable and the 72h-EaC 50 of 74 mg m./4. for the study is reliable and the 72h-EaC 50 of 74 mg m./4. for the study is reliable and the 72h for the study is reliable and the study is r	
č ^{O.}	
-	



Data Point:	KCA 8.2.6.2/10
Report Author:	
Report Year:	2020
Report Title:	BCS-AA65784: A 96-hour toxicity test with the freshwater Diatom (Navicula
	pelliculosa)
Report No:	EBAC0075
Document No:	<u>M-678377-01-1</u>
Guideline(s) followed in	OECD 201 (2011); OCSPP 850.4500 (2012)
study:	
Deviations from current	Method: none; $(3)$ $(3)$ $(3)$ $(3)$ $(3)$ $(3)$
test guideline:	Study: Current Guideline: OECD 201 (2011)
	Study: Current Guideline: OECD/201 (2011) The pH of the negative control solution was 90 at 72 hours and 9.6 at 96 hours.
	There are presented by the present of the present o
	hours of exposure, the increases in pH documented for this study are commonly by
	observed for this diatom species and all control validity criteria were achieved
	despite the increase in pH. O S & S & S
Previous evaluation:	No, not previously submitted a start of a st
	$\underline{\Lambda}, \overline{\eta}, \overline{\theta}, \overline{Q}, \underline{\gamma}, \underline{\theta}', \underline{\eta}'$
GLP/Officially	Yes, conducted under ODP/Officially recognised testing facilities
recognised testing	
facilities:	
Acceptability/Reliability:	$Yes \qquad \qquad$
Executive Summary	

Õ The aim of the study was of determine the toxicity of M-01 (BCS-AA6578) to the freshwater diatom (*Navicula pelliculosa*) expressed as NOEC LOEC and  $C_X$  for growth rate and yield. *Navicula pelliculosa* with an initial cells density of 10,000 cells/mL were exposed to M-01 (BCS-AA65784) for 06 hours. Naviral were exposed to M-01 (BCS-AA65784) for 96 hours. Nominal test concentrations were 0 (control) 0.32 0 .0, 3.2, 10, 32 and 100 mg p.m./L. The cell density in each replicate was counted at 24-hour intervals. The water samples were analysed with LC-MS/MS. Measurer concentrations ranged from 92.6 to 103% of nominal concentrations. After 96 hours the mean measured concentrations were 031, 0, 97, 3.2, 9.6, 30 and 97 mg p.m./L. No control





#### I. MATERIAL AND METHODS:

Test material	M-01 (BCS-AA65784)
	Batch code: AE C653711 00 1B96 001
	CAS No.: 2008-58-4
~	Purity: 96.2%
Guideline(s) adaptation	Batch code: AE C653711 00 1B96 001       CAS No.: 2008-58-4       Purity: 96.2%       None       Freshwater diatom (Navicula pelliculosa)
Test species	
Culturing conditions	Algal cells used in this test have been actively growing in sufficient medium under the same environmental conditions as used in this test for at least to weeks prior to test initiation.
Test solutions	Test medium: freshwater AAP medium with silica constituents Nominal concentrations: 0 (control) (0.32, 1.0, 3.2, 10, 32 and 100 kg p.m./ Corresponding mean measured concentrations 0–96 hours < LOO (control), 0.34, 0.97, 32, 9.6, 30 and 97 mg p.m./L Controls: untreated test medium Evidence of undissolved material: Air test solutions appeared clear and colourless. No particulates or surface-slicks were visible No. of vessels per concentration (replicates): 4 No. of vessels per concentration (replicates): 8 Test type: static Total exposure duration: 96 hours Approx. 10,000 cells/mL cells/mL
Replication	No. of vessels per concentration (replicates): 4
Exposure	Test type: static Total exposure duration: 96 hours
Initial cells density	Total exposure duration: 96 hours Approx. 10,000 cells/mL cells/mL Algal cells for this study were taken from a culture that had been transferred to fresh medium three days prior to cest initiation Temperature: 21,3–22,1 °C Photoperiod, continuous light
	Temperature: 21, 3–22, 1 °C Photoperiod: Sontinuous light Light intensity: 3800–4690 ux Tope of light: coor white fluorescent lighting H: 7, 2–9.8
Parameters Measured /	Cell censity was determined at 24-hour intervals using an electronic particle counter. Cells were also assessed for atypical morphology (e.g., changes in cell shape, size or color),
Observations	aggregation or Rocculation, and adherence to the test chamber. The pivor the medium in each treatment and control group was measured at test initiation, at 72 hours, and at exposure termination. The temperature of a container of water adjacent to the test chambers in the environmental
~0	chambet was measured continuously. Light extensity was measured at test solution level at nine locations surrounding the test flasts at test initiation
Sampling for chemical malysis	flasts at test initiation Samples were collected an approximately 0, 72, and 96 hours. At test initiation (0 hour), samples were collected for each treatment and control group prior to distribution into test chambers. At 72 hours, samples were collected from surrogate replicates included in each
	treatment and control group. At test termination (96 hours), the remaining replicates from each respective deatment and control group were pooled and then sampled. The samples were analysed with by high performance liquid chromatography with tandem mass spectrometric detection (LC/MS/MS)
	samples were collected for each treatment and control group prior to distribution into test chambers. At 72 hours, samples were collected from surrogate replicates included in each treatment and control group. At test termination (96 hours), the remaining replicates from each respective reatment and control group were pooled and then sampled. The samples were analysed with by high performance liquid chromatography with tandem mass spectrometric detection (LC/MS/MS)



Data analysis	Average specific growth rate (r), area under the growth curve (biomass) and yield (y), were calculated for each test flask.
	$EC_x$ -values: $E_rC_x$ , and $E_yC_x$ values and their corresponding 95% confidence intervals were $\circ$
	calculated at 72 and 96 hours of exposure, when possible, using non-linear regression with
	treatment response (growth rate, area under the growth curve and yield) and exposure
	concentration data (0–96 hour mean measured concentrations). $EC_x$ -values: $E_rC_x$ and $E_xC_x$
	values and their corresponding 95% confidence intervals were calculated at 72 and 96 hours
	of exposure, when possible, using non-linear regression with treatment response (growth)
	rate, area under the growth curve and yield) and exposure concentration data (@96 hour
	mean measured concentrations).
	NOEC and LOEC values: The 72- and 96-hour growth rate and yield data were evaluated for
	normality and homogeneity of variance ( $\alpha = 0.01$ ) using Shapiro Wilk's and Levene's tests,
	respectively. The treatment group means for the datasets which met assumptions of normality
	and homogeneity of variance were compared to the powled control using Dubnett's test ( $\alpha \neq \beta$
	0.05). A non-parametric test, Wilcoron test with Bonferron-Holm correction ( $\alpha = 0.05$ ) was
	used to evaluate the treatment group means for the datasets which did not meet the assumptions of normality or homogeneity of variance as a sumption of normality or homogeneity of variance
	assumptions of normality of noniogenerity of variance. y
	II. RESULTS AND RISCUSSION: A O'
<b></b>	
Validity criteri	a:
Validity criteri	a (OECD 201, 2011) a g S S Required O Obtained
	control within the 72-hour test period $\sqrt[6]{259}$
Mean coefficien	nt of variation for section-by-section specific $\mathcal{O}$ (3.1%)
growth rates in t	the control within the /2-hour test period $\mathcal{L}$
Coefficient of v	ariation of average specific growth rates within $0 \le 10\%$ 1.5%
72-hour test per	10d in control regulates $\sim$
Analytical resu	
C	
Recoveries Can	ged from 92.6 to 99.5% of noninal concentrations after 72 h and from 93.7 to 97.9% of
nominal conce	ntrations after 26 h (see table below) Results of the study are based on mean measured
concentrations	of M 20 (BCS-AA65784) 2 2 2 2
	of MO01 (BCS-A665784) were measured in the control samples above the limit of
No residues o	t MIXII (RUN-A & 65 / X4) were measived in the control samples above the limit of

No residues of MOI (BCS-A665784) were measured in the control samples above the limit of quantification (0.100 mg p.m.45).

Nominal «	Day 0 (1	WW)	Day 34	Öld)	Day 4	(Old)	Mean	Mean	
conc.	Measured conc [mg psu./L]	%0f	Measured	م کې of کې of	Measured conc. [mg p.m./L]	conc. nominal		% of nominal	
0.32	0,314	98.9	0.312	97.5	0.301	94.2	0.31	97	
1.0	\$ 0.992 \	§9.3 🐇	§ 0.957	95.7	0.953	95.3	0.97	97	
3.2	3,29	S 103	~9.13	97.8	3.06	95.6	3.2	100	
10	Q.74	2 <b>0</b> .4	9.62	96.2	9.54	95.4	9.6	96	
30	9 4//14 .	^{≪9} 94.1	29.6	92.6	30.0	93.7	30	94	
\$100 S	94.5	94.5	99.5	99.5	97.9	97.9	97	97	
Â									

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



#### Biological results:

There were no noticeable changes in cell morphology in any treatment group when compared to the control replicates during the microscopic examinations of the cells. Cells present in all M-01 (BCS-AA65784) treatment groups on days 1, 2, 3, and 4, appeared normal when compared to cells present in the negative control. Adherence of cells to the test chambers was not observed in any of the experimental groups. Flocculation or aggregation of cells was not observed in the control groups or any of the AF01 (BCS-AA65784) treatment groups.

% Inhibition of grow	th rate and yield	Č V	
Mean measured conc.	Growt Growt inhibiti	on [%]	Yield S inhibition [%]
[mg p.m./L]	72 h	96 h	A h B h S h
0.31	-2		
0.97	-2	A OF L	
3.2	2		
9.6	0		
30	3		
97	53 ° , «	© 31 ª) Ç	2 95 b 8 4 b)
b) Treatment group mean	n was significantly reduce	Dunnett's test; p < 0.0	Holl Hereits; p2 0.05) when compared to control (5) when compared to the control response.
The endpoints base		Soncentration are:	
	ErC50 - 72 Hours (95% C1):		92 mg p.m./L (89 to 95)
L.	° €rC20 - 92 hours (95% C.L.		55 mg p.m./L (49 to 61)
Č,	E-C ₁₀ - 72 hours (95) C.I.):		42 mg p.m./L (35 to 49)
مې lowest <u>م</u> oncentrat	LOEO - 72 hours: ion with an effect (basi	ed on gowth gate)	97 mg p.m./L
	$\sim 100 \text{EC} - Q2$ hours.		30 mg p.m./L
	E ₄ S ₅₀ - 72 hours (95% CI):	Š , 0 ⁵	46 mg p.m./L
	(95%  e1): $\underline{A} E_y C_{20} = 72 \text{ hours}$	- Q	(36 to 58) 31 mg p.m./L
	$\mathcal{L}_{y}C_{J} \mathcal{L}_{y}C_{J} $	Q	(25 to 36)
	EC ₁₀ - 72 hours (95% C.I.):	<i>y</i>	26 mg p.m./L (17 to 30)
Le lowest concer	CLOEC - 72 hours:	based on yield)	30 mg p.m./L
highest concentr	NOEC - 72 hours: ation without an effect	(based on yield)	9.6 mg p.m./L



Results – 0 to 96 hour	S
ErC50 - 96 hours (95% CI):	> 97 mg p.m./L (NA)
$E_rC_{20}$ - 96 hours (95% C.I.):	81 mg p.m./L (775to 85)
$E_r C_{10}$ - 96 hours (95% C.I.):	64 mg p.m./L (58 to 71)
LOEC - 96 hours: lowest concentration with an effect (based on growth rate	97 mg p.m/L
NOEC - 96 hours: highest concentration without an effect (based on growth fate)	30 mg pm./L
E _y C ₅₀ - 96 hours (95% CI):	62 to XI) (62 to XI) (62 to XI) (62 to XI) (420to 54)( (420to 54)( (40to 54)(
$E_{y}C_{20} - 96 \text{ hours}$ (95% C.I.):	(420to 54) € (420to 54) € (420t
$E_yC_{10}$ - 96 hours	400mg p.m.7L & O
(95% C.I.): LOEC - 96 hours lowest concentration with an effect (based on yield)	97 pg p.m.()
NOEC - 96 kours: highest concentration without an effect (based on yield)	97 ng p.m.() 97 ng p.m.() 90 ng f.m./L
Assessment and conclusion by applicant: The study is reliable and the 72h-EAC ₅₀ of 92 me p.m./P.c BCS-AA65784) risk assessment	an be used in the M-01 (AE C653711 /



Data Point:	KCA 8.2.6.2/11	
Report Author:		
Report Year:	2017	
Report Title:	Solubility/stability study of BCS-AX86048 in algae test media	Ş
Report No:	EBAC0022	Y
Document No:	<u>M-611528-01-1</u>	
Guideline(s) followed in	none	
study:		2
Deviations from current	No guideline available	"
test guideline:		a
Previous evaluation:	No, not previously submitted	Ś
		)°
GLP/Officially	No, not conducted under GLEO Officially recognised testing acilities	
recognised testing	No, not conducted under GL ^W Officially recognised testing facilities $\sqrt[4]{2}$	
facilities:		
Acceptability/Reliability:	Yes & & X & X & X	

#### **Executive summary**

The objective of this study is to determine the technical feasibility of a strict with the algae *Navicula* considering the known hydrolytic properties of the substance M-03 (BCS-AX86048 / AF 0608000) The solubility and stability of the test item were assessed in the usual conditions finedium type duration, temperature, pH) of the OECD 201 est with diatoms. The 2 most common algae growth media i.e. AAP and OECD, both supplemented with situates were used in the test. The initial pH of the media was 7.5 and 8.1 for AAP and OECD medium, respectively. Concentrations of 10 and 100 µg/L were tested, these concentrations were chosen based on the concentration range of the test on fluopicodide. Samples were taken after 10 seconds of sturring (0), after 10 50, 240 minutes and on day 52, 3 and 4. The metabolite M03 was quantified by HPLC-MS/MS, the limit of cuantification was set at 0.0625 µg/L. It was possible to solubilize the test item in *Navicula* test media with the help of DMF stock solutions, however, the test item is very unstable and after 30 min, only 20 to 27% of the nominal concentrations remain in the systems. As the algae test is porformed in static conditions, it is not possible to carry out the test with the substance M03 (BCS-AX86048 / AE 0608000) over 72h as requested by the OECD 201 guideline.

	M-03 (BCS-AX86048, AE 0608000) Batch code: AE 06080000
Test material	M-03 (BCS-AX86048, AE (608000)
	P Batch/code AE 060800 00/1B970001
l õ	Putity: 969% www By and 200 µgg in AAP and OECD media supplemented with silicates.
Test solutions	for and \$20 µgg in AAP and OECIS media supplemented with silicates.
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	Solvent: 0.4 (in / b) dimethyllormamide
<u>A</u>	Test solutions: normal appearance
Exposure	4 days of the second se
Test conditions	As set in OECD 201 guideline for Navicula
Sampling for	After 10 sec of stirring (10), 10, 30, 240 minutes and on day 1, 2, 3 and 4.
chemical analysis	The test item was quantified by HPLC-MS/MS.
	After 10 sec of stirring(t0), 10, 30, 240 minutes and on day 1, 2, 3 and 4. The text item was quantified by HPLC-MS/MS.



II. RESULTS AND DISCUSSION:

Analytical results:

г			<u> </u>	below.
	OECD	medium	A P n	nedium 🔧 🔊
Sampling time	Nominal	Nominal	Nominal	Sominal
	concentration: 10	concentration: 100	concentration: 10	concentration: 100
	μg/L	µg/L ₹		
0	10.7	94.8	O10.8	V Q107 🖓 🖇
10 min	6.09	64	6. 86 °	Å 64.3 Å
30 min	2.07	20.3	T 2.59 T	\$26.9
240 min	< LOQ			\sim $< LQQ$
Day 1	< LOQ	~ 2 ≤ 200 ~ 0		
Day 2	< LOQ		C LOQX	LOQS LOQS
Day 3	< LOQ	LOQ S	~ <u>~ LOO 5</u>	₹ LQQ
Day 4	< LOQ			C ×LOO

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

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It was possible to solubly the test item in *Navicula* test media with the help of DMF stock solutions, however, the test item is vero unstable and after 30 min only 20 to 27% of the nominal concentrations remain in the systems. As the algae test is performed in static conditions, it is not possible to carry out the test with the substance M-03 (BCS AX86048 / AE 0608000) over 72h as requested by the OECD 201 guideline. The DT and DT 90 are approximated 15 and 45 minutes, respectively (see table below).

	@ @	Ş′ 4	x Q v		. 💥	A)			
Test media	Kinetic model		Pacameter Ak, β1, k2, g, tb, α,	error	O Prob &	, Lower CI	Upper CI	DT ₅₀ [minutes]	DT90 [minutes]
ОЕСД 10 µg/k	SFO	0.68	0.0554		∞ 7.60E-05	0.05411	0.05700	12.5	41.5
OFCD		0 10.7 0	o 18.78	0.8	n.r.	1.696	35.860	10.0	12 0
10 µg/L	FOMC	-	328.22	0.63°	n.r.	20.316	636.120	12.3	42.8
ОЕСD 100 µg/L	SFO X	96.51	🖇 k 0.04670 🖉		0.005412	0.03710	0.05600	14.8	49.3
OECEC	ar i	0	Q1.679E+04		n.r.	1.192E+04	21665		
	FOMC	4 6.51	β 3.596E+05	6.41	n.r.	3.594E+05	359806	14.8	49.3
балар 10 µgдО	×~ "(10.83	k 0.04684	0.91	0.000175	0.04513	0.04900	14.8	49.2
AAP			α 9.876E+03		n.r.	9.364E+03	10387.9		
10 μg/L	FOMC	10.83	β 2.108E+05	1.14	n.r.	2.108E+05	210835.7	14.8	49.2



Model Modelled vs observed Reference Referenc	Test media	Kinetic model	M ₀	Parameter (k, k1, k2, g, tb, α, β)	χ ² , %- error	Prob >t	Lower CI	Upper CI	DT50 [minutes]	DT90 [minutes]	8
100 μg/L FOMC 107.0 β 84.713 0.22 n.r. 67.644 441.782 13.5 4557 Reference And		SFO	106.3	k 0.04767	1.93	0.000780	0.04398	Ŭ.	14.5	₹ 48.3	ð
Model Modelled vs observed Reference DECD DFCD		FOMC	107.0		0.22	n.r.			13.9	-55.7 -55.7	Ê,
Model Modelled vs observed Reference DECD DFCD						inutes.	red acceptable	Û	\$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$		
Model Modelled vs observed Reference DECD DFCD	Plots and re	siduals for s	selected b	est fits.				<u>, Q</u>			
DECD DECD	Media Model	Modelled v	s observe	ed	ð.	Residuals		J. J		à 4°	
SFO AAP 10 μg/μ SFO AAP 10 μg/μ 10	Reference			¥			ç, ç				
DECD 100 μg/L SFO AAP 10 μg/L SFO AAP 10 μg/L SFO AAP 10 μg/L SFO AAP	OECD 10 μg/L SFO	Massured & Predicted Residues				y box				\$	
AAP 10 µg/to y to y	OECD 100 μg/L SFO	Makeupada a Predicted Residues						200 25	0 300		
	AAP 10 μg/k SFO	12 sanpisado a de la construcción de la construcció				-0.05 -0.10	50 100 1	0 50 200 22	0 300		
			ÿ §		//						1
Assessment and conclusion by applicant: The study is reliable and shows that a study on the most relevant test organisms (<i>Navicula</i>) is not	~										

The study of reliable and shows that a study on the most relevant test organis technically feasible with metabolite M-03.



CA 8.2.7	Effects on aquatic macrophytes

Data Point:	KCA 8.2.7/01
Report Author:	
Report Year:	
Report Title:	AE C638206 - 7-day toxicity test with duckweed (Lemna, Sbba)
Report No:	M-220201-01-2
Document No:	<u>M-220201-01-2</u>
Guideline(s) followed in	OECD: 221 (2000); USEPA (=EPA): OPPTS 850.4400 (1996)
study:	
Deviations from current	Method: Deviations from current guideline SANC O/3029/99 rev.4: S
test guideline:	Limited sets of validation recoveries were analysed. However, the average
_	recoveries were within the acceptable range of $70-110\%$ and the RSD values were
	below 20%. The analytical method can be regarded as fit for purpose.
	Study: Current Guideline. OECD 221 (2006) 5 m Study St
	The test was conducted with 19 fronds per repHcates ustead of 9-12 as in OECD
	221 guideline, to be compliant with CCSPP \$50.4400 guid@ine which requires 12 •
	to 16 fronds. Since the valuatity criteria of the OECD guideline aromet, this
	deviation had no impact on the study regults. $(\mathcal{D}^{\vee} \otimes \mathcal{D}^$
Previous evaluation:	yes, evaluated and accepted a start of the second
	in DAR (2005) (1 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2
GLP/Officially	Yes, conducted under GLD/Officially recognised testing facilities
recognised testing	
facilities:	
Acceptability/Reliability:	Yest A C O O
Data Point:	KCA.8.2.7/046 6 6 6 6
Report Author:	
Report Year:	
Report Title:	Statement - Certificate of analysis for fluopicolide toxicity study on Lemna gibba
, , , , , , , , , , , , , , , , , , ,	(Hoberg, 2003, M-220201-09)
Report No:	
Document No 2	<u>MQ63469Q01-1</u>
Guideline(s) followed in	
study: 👾	
Deviations from current	Not applicable
test guideline:	
Previous evaluation	No, no previõusly submitted
GLP/Officially	nov applicable of or or
recognised tesping	
facilities:	
Acceptability/Reliability	Yes V V V
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S & P	Y
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Executive summary

The purpose of this study was to determine the effects of fluopicolide on the growth of the duckweed Lemna gibba under static conditions for 7 days. On days 3, 5 and at test termination (day 7), fronds were counted, and observations were made. At test termination, frond density for each replicate treatment and control vessel were determined in addition to dry weight. The nominal test concentrations were 0.16, 0.31, 0.63, 1.3, 2.5 and 5.0 mg a.s./L. Exposure solutions and QC samples were abalysed for fluopicolide concentration using gas chromatography with electron capture detection (GC/ECD) Measured concentrations on day 0 and day 7 ranged between 64 and 98%. The highest concentration averaged 64% of nominal concentration which was believed to be the solubility limit of fluopicolitie in 20X AAP medium. The results are based on arithmetic mean measured concentrations: 0.16, 0.28, 0.57, 1.0, 2.2 and 3.2 mg a.s./L. The study fulfils all validity criteria of the current version of QPCD 22 guideline, On day 7, fronds exposed to all treatment levels tested and the controls were observed to be pormation Endpoints (Effect on mean frond number, effect of mean growth rate of frong number, and effect on mean dry weight) are based on arithmetic mean measured concentrations: $EC_{50} > 3.2 \text{ mg a s}/L$, LOEC > 3.2 mg a.s./L and NOEC = 3.2 mg a.s./L. × 0

	I. MATERIAL AND METHODS:
Test material	Fluopicolide (AEC638206)
Guideline(s) adaptation	not specified
Test species	Duckweed Lemna gibbay
Acclimation	Inoculum pre-culture, preparation 7 days before the start of the main test cultivation and the same conditions as in main test.
Culturing conditions	Duckweed <i>Lemna gibba</i> strain G3 Inoculum pre-culture, preparation 7 days before the state of the main test cultivation under the same conditions as in main test. 20X AAP medium 5500 ± 10000 Jux temperature of 24 \pm 2 °C pH 7.5 \pm 0.1 Nominal concentrations: 0.16, 0.31, 0.63, 4.3, 2.5 and 5.0 mg a.s./L Mean measured concentrations: 0.16, 0.28, 0.57, 1.0, 2.2 and 3.2 mg a.s./L Control: unreated medium
Test solutions	Nominal concentrations: 0.16 , 0.31 , 0.63 , 4.3 , 2.5 and 5.0 mg a.s./L
	Solvent control 0.1 mOL Dimethylformamid (DMF)
Replication	Evidence of andissolved material: not observed No of vessels per concentration (replicates): 3 No of vessels per control (replicates): 3
Orrenteredan	No. of dessels per solvent control (peplicates): 3
Organisms per replicate	No of fronds per vessel 35
Exposure	Static St
Test conditions	vessels: 290 mL Gystalfizing dishes with 100 mL test solution Temperature: 23 °C t@24 °C Photoperiod permanent light
	Light quality: fluorescent bulbs Light intensity: 7000 – 7800 lux pH: 7 328.9
	Water hardness: not specified
Ŭ	
	Conductivity: not specified Growth medium: 20X AAP



Parameters	On days 3, 5 and at test termination (day 7), fronds were counted, and observations
Measured/	were made. At test termination, frond density for each replicate treatment and
Observations	control vessel were determined. Fronds were counted and then removed from each
	vessel, blotted dry and transferred to pre-weighed aluminium pans. Fronds were
	dried in an oven at 67 to 69 °C for two days prior to dry weight determination
	Temperature was measured continuously with Fisher Scientific
	minimum/maximum thermometer located in a flask of water adjacent to the test
	vessels within the environmental chamber. Light intensity was measured with a lo
	General Electric type 214 light meter at 0 hour and at each subsequent 24-hour
	interval during the exposure period. The pH of all exposure solutions was preasured
	at test initiation and at test termination.
Sampling for	On test day 0 (test initiation) and day 7, one sample was removed from each
chemical	treatment and control solution to be analysed for fluoppeolide concentration
analysis	Furthermore, three quality control (QC) samples were prepared attest initiation and
	termination and remained with the appropriate set of exposure solution samples
	throughout the analytical process \mathcal{O} \mathcal{O} \mathcal{O} \mathcal{O} \mathcal{A} \mathcal{A}
	Exposure solutions and OC samples were analysed for fluopicolide concentration
	using gas chromatography with electron capture detection (CC/ECD) based on
	methodology validated at springborn Spathers.
Data analysis	A t-test was conducted to statistically compare the growth rate of the compol to the
5	solvent control
	For determination of the NOEC and LOEC the data were first checked for normality
	using Shapiro-Wilks' Test and for homogeneity of variance using Bartlett's Test. If
	the data sets passed the tests for homogeneity and pormality, then Williams' Test
	was used to determine the NGEC. If the data did not pass the tests for homogeneity
	and normality, then Kruskal-Wattes' Test was used to determine the NOEC. All
	statistical determinations were pade at the 95% levek of certainty, except in the case
	of Shapiro-Wilks and Bartlett's Tests, where the 99% level of certainty was applied.
	The EG85, EC59 and EC90 values were calculated for front densities, average growth
ć	rate and biomass at test termination. A computer program was used to perform both
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	the statistical (ODEC and DOEC determinations) and EC05, EC50 and EC90
<i>i</i> n	conculations and the second se



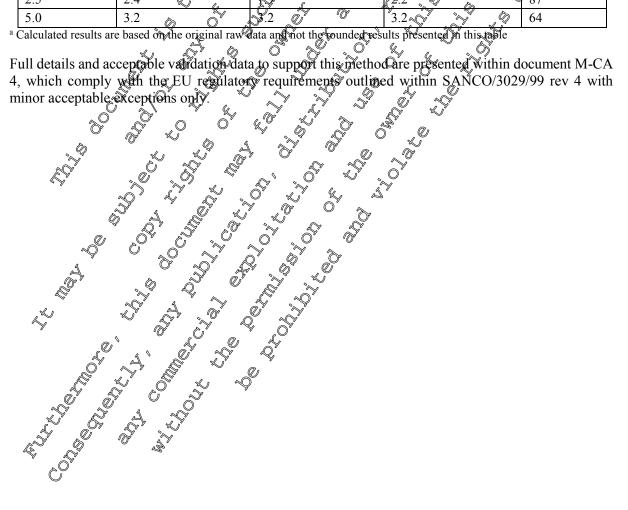
#### **II. RESULTS AND DISCUSSION:**

Validity criteria (OECD 221, 2006)	Required	Obtained
Doubling time	< 2.5 days	1.71 days

Analytical results:

Measured concentrations on day 0 and day 7 ranged between 64 and 98% and are presented below. highest concentration averaged 64% of nominal concentration which was believed to be the solubility limit of AE C638206 in 20X AAP medium. The results are based on arithmetic mean measured concentrations. Å

Nominal Concentration (mg a.s./L)	Day 0 Measured Concentration (mg a.s./ L)	Day 7 Measured Concentration (mg a.s./ L)	Mean Measured Concentration ^a (mg a.s./2)	Percent of C nominal
Control	< 0.0055	≤Q.00550° Q	Q , _A C	- 2 7
Solvent control	< 0.0055	× 0.0 <del>053</del> ~ 0	- , , , , , , , , , , , , , , , , , , ,	-
0.16	0.16			Q98 O
0.31	0.29	928 × × × ×	0.28 J J	91 🖏
0.63	0.53	0.60	0.570 6	90
1.3	0.97 🖉 🔍	1.1 0 5 0	1,6 0 20	81
2.5	2.4	h@r (		87
5.0	3.2	S.2 0 0	3.2	64





#### **Biological results:**

#### Observations

Mean measured concentration [mg a.s./L]	Frond number on day 7 ^b (SD)	7-day inhibition of frond number ª	Mean growth rate for frond number (SD)	7-day inhibition of growth rate	Mean dry weight (SD)	7-day inhibition of dry
Control	257 (21)	NA	0.41 (0.01)	NA A	0.044 (0.0069)_C	NAC
Solvent control	272 (42)	NA	0.42 (0.62)	NA	0.043 (0.00®7)	NA S
Pooled control	265 (31)	NA	0.45(0.02)	NA, Q	0.034	NØ OF
0.16	260 (24)	1.8	0.41 (001)		0.045 (0.0042)	
0.28	278 (21)	-5.1	0.42 (0.0)	2 ^{.4} ,	0-0048 0.00659	-90
0.57	294 (47)	-11 8	0.43 (0.02)	-4.9 [%]	0.040 (0.0089)	-6
1.0	272 (24)	-2.7 [°] 0	@42 (0.@)	\$ ^{2.4}	0.042 (0.00052)	X X
2.2	283 (23)	G6.9 5 5	0.42 (0.01)	-2,404	0.047	-8
3.2	304 (37)		0.43 (0.02)	x-4.9 2 2	0.050 (0.0082)	-14

On day 7, fronds exposed to all treatment levels tested and the controls were observed to be normal ð

^a Percent inhibition relative to pooled control, ^a Mean of 3 repletates, NX not applicable

III CONCEVSIONS.

Endpoints were calculated based or Arithmetic mean measured concentrations.

 $EC_{10}$  and  $EC_{20}$  cannot be calculated for this test because there is no dose response relationship. The  $E_rQ = 3.2 \text{ mg a}$  s/L and the NOE_rC of 3.2 mg i.s./L can be used in risk assessment. The calculated endpoints are not fully in hire with the corrent OECD guideline, more specifically the yield of frond number and dry weight or the growth rate of doy weight are not available. They were not re-calculated since no effects were observed in this set, so they are all assumed to be > 3.2 mg a.s./L.

		No.	
Endpoint to - / days)	ond number	Éffection mean growth rate of frond number	Effect on mean dry weight
EC 20 95% C.I.):	3.2 mg/a.s./L	≫3.2 mg a.s./L (NA)	> 3.2 mg a.s./L (NA)
LOEC: lowest concentration with	\$.2 mg & s./L	> 3.2 mg a.s./L	> 3.2 mg a.s./L
NOEC: highest concentration	2 mag a.s./bQ	3.2 mg a.s./L	3.2 mg a.s./L
NA = not applicable	0		

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#### Assessment antoconclusion by applicant:

The study is reliable and the  $E_rC_{50} > 3.2 \text{ mg}$  a.s./L and the NOE_rC of 3.2 mg a.s./L can be used in risk assessment.



Data Point:	KCA 8.2.7/02
	KCA 0.2.//02
Report Author:	
Report Year:	
Report Title:	AE C653711: A 7-day toxicity test with duckweed Lemna gibba G3
Report No:	149A-167A
Document No:	<u>M-219725-01-2</u>
Guideline(s) followed in	ASTM: 1415-91E (1991); OECD: 221 (2000); USEPA (SEPA): OPPTS \$50.4400
study:	(1996)
Deviations from current	Method: Deviations from current guideline SANCQ 3029/99 rev.4; 0
test guideline:	Limited sets of validation recoveries were analysed. However, the average and the sets of validation recoveries were analysed.
	recoveries were within the acceptible range of $76^{-1}10\%$ and the RSD acues were below $20\%$ . The analytical method can be range d as fit for purpose $3\%$
	below 20%. The analytical method can be regarded as fit for surpose $\mathcal{O}$
	Study: Current Guideline: OF 221 (2006)
	The light intensity of 4500,5450 lux was lower than recommended in the OECD
	guideline 221, 2006 (6569-10000 lux) but was compliant with the OCSPP
	850.4400 guideline (4200-6700 fux). Since the validity criterin are mey, this
	deviation had no impact on the results of the test.
Previous evaluation:	yes, evaluated and accepted Q Q
Flevious evaluation.	
	in DAR (2005)
GLP/Officially	Yes, conducted under GLP/Officially recognised testing facilities
recognised testing	
facilities:	
Acceptability/Reliability:	Yes $Q'$ $a$ $b$ $Q'$ $b$

	$\begin{array}{cccccccccccccccccccccccccccccccccccc$
Data Point:	KCA 8.257/03 0 4 0 4 5 6
Report Author:	KCA 8.257/03 0 4 7 7 7 7 7
Report Year:	
Report Title:	KCA 8.257/03 2019 Statistical re-evaluation of a Lemma study performed with AE C653711 (BAM) (Desjardurs et al 2003; M-219725-01-1)
	(Desjardurs et al 2003; M-219725-01-1)
Report No:	111-004931-014-1 ~ · · · · · · · · · · · · · · · · · ·
Document No: 🖉 🔊	
Guideline(s) followed in	
study:	
Deviations from current x	Not stopilicable $\mathcal{O}$ $\mathcal{O}$ $\mathcal{O}$
test guidenne:	
revious evaluation:	No, not previously submitted
GLP/Officially	
GLP/Officially	not applicable to the second sec
GLP/Officially recognised testing facilities:	not applicable
facilities:	
Acceptability/Reliability:	$O^{Y} es _{O}' _{A} G^{Y} _{C} G^{Y} _{C}$
	not applicable
×	



#### **Executive summary**

Fronds of duckweed, *Lemna gibba* G3, were exposed to a geometric series of five test concentrations of M-01 (2,6-Dichlorobenzamide / AE C653711) and a negative control (culture medium) under static conditions for seven days. Three replicate test chambers were maintained in each treatment and control group. The nominal test concentrations were 13, 25, 50, 100 and 200 mg a.s./L. Growth, defined as an increase in the total number of fronds in each replicate test chamber, was determined through direct counts on Days 0, 3 and 5 during the test and at the end of the test (Day 7) forcall treatment and control replicates. In addition, the total number of duckweed plants in each replicate test chamber was determined at test termination. Concentrations of M-01 (2,6-Dichlorobenzamide) in the samples were determined by high performance liquid chromatograph, with UV detection using an Agilent Medel 1100 High Performance Liquid Chromatograph (HPLC) equipped with an Agilent Series 1000 Variable Wavelength Detector. The results of the study were based on mean measured test oncentration of 12 25, 50, 101 and 203 mg a.s./L. The study fulfils all validity criteria of the current version of the OECD 221 guideline. In the highest treatment level, 100% of the test organisms were dead at the end of the study. Slight chlorotic effects (0.41-1.7%) were obserged in the control and in the treatments with 12, 25 and 50 mg a.s./L. In the treatment with 10 mg a.s./L 25% of plants sowed chlorosis. Slight signs of necrosis were observed in the control and the 50 mg als./L test concentration. In the treatment with 101 mg/L, 58% necrosis was observed. The E.C.50 are 97.6 mg a.s. D and 910.2 mg a.s./L for frond number and dry weight (biomass), respectively). The NOE C is 250 mg as./L for both frond number and dry weight.

Test material	M-01 (2,6-Dichlorobenzamide, AE C653711)
	Lot No. 48499AO
	Lot No. $48499AO'$ $a$ $b$
Guideline(s)	
adaptation	Duckweed (Leana gibba)
Test species	Duckweed (Lenna gibba) ~
	Buckword (Leyna gibba)
Acclimation	ELEMENTA THOUGHTS HANVIDEEDSACHVEAV STOWLUS HEXSUA HAAF CHITTEE HEUHHHT TOL ALTEAST :
6	two weeks prior to test initiation. 20X  AP medium $pH @5 \pm 0.0$
Culturing	$\frac{1}{20X} \text{ AP medium } $
conditions	$pH_{\mathcal{D}5} \pm 0.0^{\circ}  \bigcirc  \bigcirc  \bigcirc  \bigcirc  \bigcirc  \bigcirc  \bigcirc  \bigcirc  \bigcirc  $
Test solutions	Nominal concentration 13, 25, 50, 400 and 200 mg/L
	Mean measured concentrations: 12, 25, 59, 101 and 203 mg/L
<i>.</i>	Contol: untreated medium & S
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	Evidence of undissolved material: not observed
Replication	No. of cossels per concentration (replicates): 3
	No. of vessels per control (replicates): 3
	No of vessels per solvent control (replicates): 3
Organisms	No. of fronds per vessel. 12
per replicate	No. of plants of \mathcal{A}
Exposure	Static Q Q
Č ^y	Total exposure duration; 7 days
J.	No. of vessels per contentration (replicates): 3 No. of vessels per control (replicates): 3 No. of vessels per solvent control (replicates): 3 No. of fronds per vessel. 12 No. of of ants 4 Static Total exposure duration; 7 days
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I. MATERIAL AND METHODS



Test	vessels: 250 mL glass beakers with 100 mL test solution
conditions	Temperature: 24 °C to 25 °C
	Light quality: warm white fluorescent lighting
	Light intensity: 4500 – 5450 lux
	pH: 7.5-8.9
	Water hardness: not specified
	Dissolved oxygen: not specified
	Conductivity: not specified
	Growth medium: 20X AAP
Parameters	Growth, defined as an increase in the total number of fronds in each replicate test.
Measured /	chamber, was determined through Greet counts on Days 0, 3 and 5 during the test
Observations	and at the end of the test (Day 7) for all treatment and control peplicates. In addition,
	the total number of duckweed plants in each replicate test chamber was determined
	at test termination Observations of offects such as chlorosis, necrosis dead frends
	root destruction and break-up of duckweed colonies were performed on Days 0, 3.
	and 5 during the test and at the end of the test (Day 7). Biomas (dry reight in
	milligrams) was determined at test termination.
	The temperature of a container of water adjacent to the test chambers on the
	environmental chamber was recorded twice daily during the test. Light intensity was
	measured at five locations surrounding the test chambers on Day 9. The pH of the
	medium in each treatment and control group was measured at test initiation and at
	test termination. No and the second sec
Sampling for	Samples of the test solutions were collected at test initiation and test termination.
chemical	Samples at test mitiation were collected from the individual batches of test solution
analysis	prepared for the treatment and control group. At test termination, samples were
	collected from the pooled replicates from treatment groups and the control group.
	Concentrations of M-0 (AE \$653751) in the samples were determined by high
	Berformance liquid chromatography with UV detection using an Agilent Model 1100
. 0	High Rerformance Liquid Chromatograph (HPSC) equipped with an Agilent Series
Č.	1100 Variable Wavelength Detector.
Data analysis	Day 7 EC_{50} values were determined using linear interpolation with treatment
	response (frond number, growth rate and biomass) and exposure concentration data.
K, [™]	The Way (Opond numbers) growth rates and biomass were evaluated for normality
	and homogeneits of variances $(p = 0.05)$ using the Shapiro-Wilk's and Bartlett's tests,
	respectively. Deatment groups were compared to the control groups ($p = 0.05$) using
0,	analysis of statiance (ANUVAL and Bunnett's t-test (5). The least significant
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	diperence (LSD) detected by ANOVA was 22 for frond number; 0.023 for growth
A	rate; and 5.8 for biologies. However, the endpoints were re-calculated to meet the
LQ^ .'	
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Å Å	
4 ²⁵ 29	
$\bigcirc$	Concentrations of M-04 (AE-6653711) in the samples were determined by high performance Liquid Chromatography with UV detection using an Agilent Model 1100 High Performance Liquid Chromatograph (HPLC) equipped with an Agilent Series 1100 Variable Wavelength Detector. Day 7 EC ₅₀ values were determined using linear interpolation with treatment response (frond number, growth rate and bromass) and exposure concentration data. The Day 7 brond numbers, growth rate and bromass) and exposure concentration data. The Day 7 drond numbers, growth rates and bromass were evaluated for normality and homogeneity of variances (p = 0.05) using the Shapiro-Wilk's and Bartlett's tests, respectively. Treatment groups were compared to the control groups (p = 0.05) using analysis of variance (ANOVA) and Dunnett's t-test (5). The least significant difference (LSD) detected by ANOVA was 22 for frond number; 0.023 for growth rate; and 3.8 for biomass. However, the endpoints were re-calculated to meet the requirements of the correct euclidence.



#### **II. RESULTS AND DISCUSSION:**

Validity criter	ia (OECD 221,	2006)		Requi	red	Obtained 。
Doubling time				< 2.5 c	lays	1.9 days
Analytical result Nominal stock so	<u>s</u> : olution concentr	ations were	13, 25, 50, 100 a	nd 200 mg a	S./L. The re	estitutes of the study
were based on m	ean measured te	est concentra	ations of 12, <b>2</b> 5, 5	50, 101 and 20	03 mg a.s./	
Nominal Concentration (mg/L)	Measured Concentration	Day 0 Percent of Nominal (%)	Day 7 Measured Concentration	Day Rercent of Nominat	Mean Measure Conceptra (mg/E)	D´ Of Oĭ
Control	(mg/ L) < LOQ ¹	- «	< LOQU C	- 0 0	- ( <b>mg/</b> 2)	
13	12.1	93.3	12.37	94.8		\$92.3
25	24.8	99.2	(23.2 L) ~	101 0 ×	25	S 100
50	49.4	98.8	750.5 J	109 2	5.05	
100	98.5	98.5	103 0	A103 0		مَحْ 101
200	200	100~	206 8	103	2030	× 102

¹ The limit of quantitation (LOQ) was 6.12 mg/L calculated as the product of the concentration of the lowest calibration standard (6.00 mg/L) and the dilution factor of the matrix blank samples (1.00) corrected for purity (98%).

² Results were generated using Ficel 2000 in full precision mode Manual calculations may differ stightly.

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

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# Observations

In the highest treatment level, 100% of the test organisms were dead at the end of the study. Slight chlorotic effects (041-1,7%) were observed in the control and in the treatments with 12, 25 and 50 mg/L. In the treatment with 10 mg/D, 23% of plants showed chlorosis. Slight signs of necrosis were observed in the control and the 50 mg/L test concentration. In the treatment with 101 mg/L, 58% necrosis was observed?

	Day Frond number 155	7-day infribition of frond number &	DayO-7 Mean growth Oate for frond number	7-day inhibition of growth rate	Day 0 – 7 Mean Biomass (mg)	7-day inhibition of dry weight
Control	155	- 🔬 🔊	0.365	-	23.4	-
12	<b>1</b> \$7 Û	-0.86	0.366	-0.25	24.1	-3.1
25	F164 🔬 🖂	¥5.8	0.374	-2.2	24.0	-2.7
50 57 0 10\$ 57	135 20	13	0.346	5.3	17.4	25
	40*	74	0.171*	53	7.93*	66
203	13*	91	0.015*	96	2.63*	89

^a Mean of 3 replicates, NA not applicable

* Statistically significant difference on day 7 frond growth (p<0.05) from the control replicates using Dunnett's test.



To derive endpoints that meet current regulatory demands, the study with M-01 (AE C653711 also called BAM) and *Lemna gibba* by (2003; <u>M-219725-01-2</u>) has been re-evaluated.

The original study report includes endpoint calculations and estimates for the following response and measurement variables:

- 7-day EC₅₀ for frond number (80 mg/L), calculated based on frond count at the end of the pest: endpoint not in accordance with OECD 221 (2006)
- 7-day ErC₅₀ frond number growth rate (97 mg/L): endpoint in accordance with OEOD 224, 2006,
  7-day EC₅₀ for dry weight (80 mg/L), calculated based on dry weight at the end of the test endpoint named 'biomass' in study report, not in accordance with OECD 221 (2006).
- 7-day NOEC values for frond number, frond number growth rate and 'biomass' (50, 50 and 25 mg/L, respectively). Only the frond number growth rate NOEC is in accordance with OECD 221 (2006)

This report provides a full statistical re-evaluation of the study. The following recalculations were performed to meet current data and guideline requirements:

- $EC_{10}$ ,  $EC_{20}$ ,  $EC_{50}$ , NOEC values for the measurement variable frond number and the two response variables growth rate and yield  $\sqrt{2}$
- EC10, EC20, EC50, NOEC values for the measurement variable ory weight and the two response Variables growth rate and yield

Dry weight was not determined at test start therefore to be in accordance with OECD 221 (2006), it has been estimated from control data at the ond of the test. It was then used to calculate yield and growth rate  $EC_{50}$  for dry weight. This response parameter is called promass in the statistical report in appendix.

All recalculations were performed with the software ToxRat Professional Vers. 3.2.1, on the basis of nominal concentrations as for the originally reported endpoints. This approach is acceptable since all measured concentrations of M-01 (BAM) are in the range 80-120% of nominal concentrations.

The results of the recalculations based on noninnal concentrations are presented in the table below. Details on the statistical re-evaluation are provided in the Appendix.

In the original report  $EC_{50}$  were calculated according to binomial interpolation. Probit analysis is preferred in this re-assessment since the Ose-response surve covers a large effect range. Therefore, the recalculated  $E_rC_{50}$  for frond number is slightly different form the original value: 97.6 vs 97 mg/L.

Similarly, for he N@FC determination, the statistical method used in this re-assessment (Williams Multiple Sequential t-test Procedure) is considered more appropriate than the approach (Dunnett's t-test) in the original report.

And t-test Procedure) is considered in the statistica is considered in the original report.



Variable	Endpoint type	Result (mg/L)	95% confidence interval (mg/L)
Frond number	E _y C ₁₀	46.5	41.7 - 50.6
	E _y C ₂₀	54.5	501-58.4
	E _y C ₅₀	73.9	69.9 - 78.0
	NOE _y C	25.0	Not applicable
	$E_rC_{10}$	57.2	53.8-00.2 5
	$E_rC_{20}$	<u>68.7</u>	Q ^y • 659 - 71,4 Q
	ErC ₅₀	^{97.6}	95.5 99.8 v v
	NOErC		Notapplicable
Dry weight	E _y C ₁₀	33.87 O	
(biomass)	E _y C ₂₀	46.8 × Y	2 5 380 - 48 6 0 2 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5
	EyC50	₽´ [~] 71.8 [~] ~	× 5 56.5 - \$7.6 ×
	NOE _y C	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	Not pplicable
	ErC ₁₀	\$ 51.0°°	45.1-556.3
	ErČžo	5° 5.5 5°	60 <b>9</b> – 71.6
	$\widetilde{\mathcal{P}}_{r}C_{50}$ $\widetilde{\mathcal{O}}^{y}$ $\widetilde{\mathcal{O}}^{y}$	ې _م ې110.2 س	Au4.6 – 116.1
Ő	ST NOErC ST K		Not applicable
² 0 ⁰			
in the second se		III. Concousions:	

Endpoints were calculated based on nominal concentrations. The lowest  $E_rC_{50}$  is 97.6 mg/L, obtained for the measurement variable from number. For the second variable dry weight, an  $E_rC_{50}$  of 110.2 mg/L was calculated.

Endpoint (0-7 days)	Effect on mean frond number weight)
ErC ₅₀ (95% C.I.):	97.6 mg/L 3410.2 mg/L (9555 – 99.8 mg/L) (104.6 – 116.1 mg/L)
NOFAC	25:0 mg/L
<u> </u>	

### Assessment and conclusion by applicant:

The study is rollable. The E  $\mathcal{S}_0$  of 97.6 mg/L and the NOE_rC based on frond number and dry weight of 22 mg/L can be used in this assessment.

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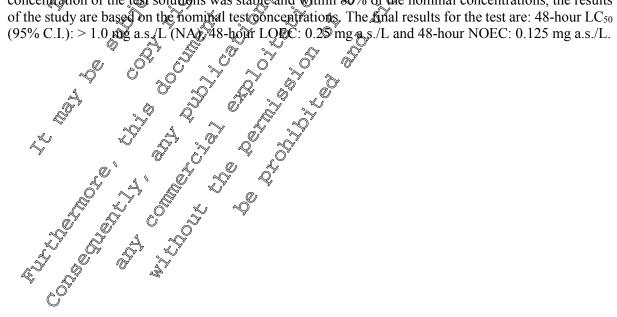


CA 8.2.8	Further testing on	aquatic organisms
011 0.2.0	i ui thei testing on	aquatic of Samonis

Data Point:	KCA 8.2.8/01
Report Author:	
Report Year:	
Report Title:	Acute toxicity of fluopicolide to Xenopus laevis under staric conditions 4
Report No:	EBACY001
Document No:	<u>M-393869-01-1</u>
Guideline(s) followed in	No formal English guideline exists for this test protocol. Methodologies from
study:	No formal English guideline exists for this test protocol. Methodologies from USEPA, OPPTS Guideline 850.4975 (1996), USEPA-FIFRA, 40 CFR, Part 158 Guideline No. 72-1 (1982) and OFCD Guideline 203 (1992).
	Guideline 10. 72 1 (1902), und OLCD Guideline 205 (1992), were considered and
	the development of this protocol. Scientific discretion was implemented where
	guideline parameters do not fully converge.
Deviations from current	Method: Deviations from current guideline SANCO/3029/99 rev.4:
test guideline:	Limited sets of validation recoveries were analysed. However, the average
	recoveries were within the acceptable range of 70-b)0% and the RSD values
	were below 20%. The analytical method can be regarded as fit for purpose?
	Study: Not applicable ' A A A
Previous evaluation:	were below 20%. The analytical method can be regarded as fit for purpose?
GLP/Officially	
recognised testing	Yes, conducted under GLP/Officially recognised Desting Socilities
facilities:	
	Yest ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~
Acceptability/Reliability:	Yes y y y y y y

#### **Executive summary**

An acute toxicity study was performed with fluopicolide (Xenopus Jaevis Funder flow-through conditions for 48 hours. The following nominal (nean measured) concentrations were included in the study: 0.063 (0.052) 0.125 (0.115) 0.25 (0.23) 0.50 (0.51) and 1.0 (0.88) mg a.s./L. Additionally a control and solven control was included. There were three applicates of 10 tadpoles in the control and each toxicant level. Survival (mortality) and sublethal behavioural effects were assessed after 6, 24 and 48 hours. No statistical calculations were necessary to determine the EC 50 for this study. The NOEC and LOEC were empirically determined based upon observation data including lethal and sublethal effects. The mean pleasured recoveries range from 84 to 103% of the norminal test concentrations. Since the concentration of the test solutions was stable and within 80% of the nominal concentrations, the results of the study are based on the nominal test oncentrations. The final results for the test are: 48-hour LC50





#### I. MATERIAL AND METHODS:

Test material	Fluopicolide
	Batch: PF90195670
	99.1%
Guideline(s)	No specific guideline is available. Methodologies from USERA, OPPTS Guideline
adaptation	850.1075, USEPA-FIFRA, 40 CFR, Part 158, Guideline No. 72-1, and OECD
1	Guideline 203 were considered in the development of this protocol. Scientific
	discretion was implemented where guideline parameters do not fully converge.
Test species	African clawed frog - tadpoles (Xenoply laevis)
Acclimation	4 days acclimation
	Mortalities less than 5% during holding period, no treatments for disease
	Not fed for 24 hours prior to testing
Organism	Tadpoles
age/size at	Size: 15.1 +/- 0.9 mm
study initiation	
Test solutions	Nominal concentrations: 0.063 0.125 0.25, 050 and 0.0 mg a.s./10
	Mean measured concentration $0.05\%$ 0.145, 0.23 $\neq$ 0.51 $\oplus$ 88 mg as /L $\approx$
	Nominal concentrations: 0.063, 0.125, 0.25, 0.50 and 9.0 mg a.s./Io Mean measured concentrations: 0.052, 0.155, 0.23, 0.51, 0.88 mg a.s./L Control: Untreated medium: hard processed water (reverse osmosis water blended
	Solvent control: Dimetholforniamide (0.1 mDL)
	Precipitates: None & & & & & & & & & & & & & & & & & & &
Replication	No. of vessels per concentration (replicates): 3 Q
*	No. of vessels per control (replicates): 3
	No. of vessels per solvent control (replicates): 3
Organisms per	No. of ørganisms pervesses 10 4 2 2
replicate	
Exposure	Static S & S & O 4
	$\mathcal{A}^{8} \mathbf{h} \overset{6}{\longrightarrow} \mathcal{A} \overset{6}{\overset} \mathcal{A} \overset{6}{\overset} $
Test Vessel	10 tadpoles in a testing volume of 7 L. S
Loading	None
Feeding during	None & A A A A
test	
Test	Temperature: $21.6^{\circ}$ 22.2 °C $^{\circ}$ $^{\circ}$ $^{\circ}$
conditións	Photoperiod: 16 hours fight, 8 hours dark, with a 30-min transition period
6	Dight intensity 570 to 832 box
, ,	VDH & 4 to 85 m s c s
~	Water hardness: 470 to 086 mgl
.1	Alkalinity range: 13400 1450ng/L O
Ĩ,	Conductivity range 486 to 313 µmhos/cm
	Dissolved by ygen saturation (%)? 91 to 102% saturation (8.1 to 8.9 mg/L)
Parameters	Survival (mortality) and subject al behavioural effects were assessed after 6, 24
Measured /	and 48 hours. Temperature was measured hourly, dissolved oxygen and pH daily.
Observations	Alkalinity, hardness and conductivity were measured on day 0 and day 2.
Sampling for	Out test day 0 and day 2 analytical samples from each test level were taken and
	analyses with Liquid Chromatograph / Tandem Mass Spectrometry system
chemical analysis	CLC/NS/MS: Day 0 samples were taken from the batch prepared solutions and
J R	the day 2 sample were collected were composites of the three replicates at each test concentration.
Deblanclinic	
Data analysis	No statistical calculations were necessary to determine the $EC_{50}$ for this study. The
Ô	NOEC and LOEC were empirically determined based upon observation data including lethal and sublethal effects.
	menumg tental and subtental effects.



#### **II. RESULTS AND DISCUSSION:**

Validity criteria as defined in the study plan	Required	Obtained		
Mortality during domestication period	$\leq$ 5%	< 5%	Č,	
Mortality in the control	≤ 10%	6.7%		
Dissolved oxygen content	≥ 5.8	≥ 8.1		
pH during the test	Constant	8.4 8.5		
		- A		

Analytical results:

Dial

Measured concentrations on day 0 and day 2 ranged between 83 and 104% and are presented below Therefore, results are based on nominal concentrations. No residues of fluopicolide were measured in the controls above the LOQ (Limit of Quantification = 0.006) fig a = 1/2.

Nominal Concentration (mg a.s./L)	Day 0 % of nominal	Dav 2 % of Mean pominal Concentration 183 0,052 84
0.063	84	
0.125	89	94 0° \$0.1150° 5° 9D° 5° 4
0.25	93 💞 🐇	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$
0.50	102 0	
1.0	88,	

Full details and acceptable validation data to support this method are preserved within document M-CA 4, which comple with the EU regulatory requirements outlined within SANCO/3029/99 rev 4 with minor acceptable exceptions only.  $\bigcirc^*$ 

Biologication results:		, ⁶ ⁽¹⁾ , 0'	/
Sublethal Observation	as ( ) 🖄 Ö		
Nominal Test	number with subletha	l effects/number of surv	ivors
Concentration	Hourg	24. Hour	40.11
(mg a.s./L) 🖉		24, Hour 🔊	48 Hour
Control	0/30	0/340 0	0/28
Solvent Control	Q/30 5 65	₩ <b>0</b> /30 ₩	0/30
0.063	Ø/30 ~~ ~	0/290	1/29 (1 OB)
0.125	0/30 0/30	0.30	0/29
0.25	0/30 0	<b>\$</b> \$\$28	0/27
0.50	23/30 (AS, 200B) «	7/30 (7 OB)	15/29 (15 OB)
1.0	30/30 (30 OB)	29/29 (29 OB)	24/24 (24 OB + P)

AS = At surface, OB for bottom, P = Pale color



Mortality	
Nominal test concentration (mg a.s./L)	Percent Dead at 48 hours
Control	6.7 Ø
Solvent Control	0.0
0.063	3.3
0.125	3.3
0.25	
0.50	3.3
1.0	
The final results for the test are based on	III. CONCENSIONS:
<b>48 hour LC</b> ₅₀ (95% C.I.) > 1.0 mg a.s	s./L (SA) Of L A
48 hour LOEC based on 0.25 mg	3 SVL TO LO Q LO DO LO OV
48 hour NOEC based on	
sublethal effects 0.125	asoly is in the second of the
Assessment and conclusion by appli	$\underline{cant}$ : $\underline{\phi}$ $\underline{\phi}$ $\underline{\beta}$ $\underline{\beta}$ $\underline{\phi}$ $\underline{\phi}$ $\underline{\phi}$ $\underline{\phi}$ $\underline{\phi}$
The study is reliable, and the $LC_{50} \approx 1$	.0 mg a.s./Locan be used in the fluopicodde rist assessment.
	Percent Dead at 48 hours 6.7 0.0 3.3 3.3 10 3.3 200 HI. Conset/SIONS: nominal concentrations of fluopicolide in the test system. 5./L (M) 3./L 3./L 3./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4./L 4



#### 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 fluoricolide or the straight formulation fluopicolide SC 486 according to current guidelines, enidance documents or the current understanding of the state-of-the-art of testing.

- Acute oral and contact toxicity to honeybees under laboratory conditions (OECD 2190ECD 214)
- Acute oral and contact toxicity to bumble bees under laboratory conditions (OECD/246/0ECD
- Chronic toxicity to adult honeybees under laboratory conditions (QECD 245)
- Chronic toxicity to honeybee larvae under laboratory conditions (OECD 239)
- Honey bee brood feeding test (Oomen et al., 1992)
- Honeybee brood Semi-Field (OECD GD 75)

For fluopicolide metabolites studies on acuteoral and contact toxicity tophoneybees under laboratory conditions have been conducted.

The studies are summarized below and a full list of the relevant ecotoxicological endpoints for fluopicolide is presented in the following table.

Test	Test species		References
substance	study twoe 🧳	Endpoint Or Q	
	Honeybee, adult, acute, 72 h	$12D_{50} - \text{grad}$ $220 \mu \text{g a.s./bee}$	2012; 20200452-03-1 CA 8.3.1.1.1/01
	Honeybee, adult	Lpg – oral $> 1073 \ \mu g a.s. bee$ Lpg – oral $> 200 \ \mu g a.s. bee$	<u>2015; M-</u> <u>539964-01-1</u> KCA 8.3.1.1.1/02
	Honeybee Qadult, O acute, 72 h		<u>2012;</u> <u>M-200506-03-1</u> KCA 8.3.1.1.2/01
Fluopicellide tech.	Honeybee lorvae, chronic (emergence) after 22 days follow repeated feeding)		<u>2018;</u> <u>M-615695-01-1</u> KCA 8.3.1.3/01
A A A	Bumble bee, adult, acute 48 h	$I_{50}$ - organ $87.3 \ \mu g \ a.s./bumble bee$	<u>2015; M-</u> <u>519981-01-1</u> KCA 8.3.1.1.1/03
L.L.	Bumble bee, adult, acute 48 h	LD - contact > 100 µg a.s./bumble bee	<u>2015; M-</u> <u>511408-01-1</u> KCA 8.3.1.1.2/02
	Bundele bee, adults, 2 acute 48 h. 7 2 acute 48 h. 7 5 acute 4	NOED $\geq 60^{\circ}$ µg a.s./larva $\downarrow 0$ $\geq 60^{\circ}$ µg a.s./larva $\downarrow 0$ $\geq 50 - 0^{\circ}$ $\approx 87.3$ µg a.s./bumble bee $\downarrow 0$ $\geq 100$ µg a.s./bumble bee	

Table 8.3.1-1: Toxicity of fluopicobide (technical and formulated product) to bees



Test substance	Test species/ study type	Endpoint	References
	Honeybee, adult, 10 day feeding test	LDD ₅₀ > 132.68 $\mu$ g a.s./bee/day	2016; M- 552253-01-1 KCA 8.3.1.2/01
Fluopicolide SC 486	Honeybee brood feeding test (Oomen <i>et al.</i> , 1992)	No adverse effects were observed on the development of brood (eggs, young and old larvae) and on pupal mortality. Adult bee mortality in the test item treatment group appeared higher compared to the control group. However, since this observation was not consistent amongst replicates it is considered to be tandom and not of biotogical relevance. Overall, flaopicolide fed at a concentration of 1.33 g as /L sugar solution caused no adverse effects on honev bee corrent performance including no indication for negative impacts on brood reating success.	552253-01-1 KCA 8.3.1.2/01
SC 480	Honeybee Brood Semi-Field (OECD GD 75)	Overall, no adverse effects on brood development adult and pupal mortanty, foraging activity, behaviour, colony development and strength after. application of \$1.6 g product ha (corresponding to 133 g a.s. (ha) onto flowering <i>Chacelia tanacetifolia</i> and bees foraging on the crop.	<u>2020; M-</u>
Metabolite M-0¢ÅE	Senter-Field (OECD GD 75) & Honéybee, again, &	development and strength after application of 133 g a.s./ha anto flowering <i>Phagelia tanacetifoua</i> and bees foraging on the crop. $LD_{50}$ – oral $100 \text{ Hz}$ p.m./bee $LD_{50}$ – contact $80\% \mu \text{g}$ p.m./bee	<u>685049-01-1</u> KCA 8.3.1.3/04 <u>2016;</u> <u>M-571897-01-1</u>
C653711) Metabolite M-02 (AE C657188)	Honeybee, acout,	LD ₅₀ -eral LD ₅₀ -context 710.9 μg p.m./bee	KCA 8.3.1.1.1/04 <u>2016;</u> <u>M-566365-01-1</u> KCA 8.3.1.1.1/05
.m.: pure metab	olite	LD ₅₀ – oral LD ₅₀ – contact – 80% µg p.m./bee LD ₅₀ – oral LD ₅₀ –	



#### CA 8.3.1.1 Acute toxicity to bees

#### CA 8.3.1.1.1 Acute oral toxicity

Data Point:	KCA 8.3.1.1.1/01
Report Author:	
Report Year:	2012
Report Title:	Amendment no. 2 - Oral toxicity (LD50) to honey bees (Apis mellifera L.)
	Substance pure Code: AE C638206 00 1B99 0002 🖉
Report No:	CW00/090
Document No:	<u>M-200452-03-1</u>
Guideline(s) followed in	EPPO Guideline No. 170 (1997)
study:	
Deviations from current	Current Guideline: $OECD 213$ (1998) $\sim$ $0^{\prime} \sim$ $0^{\prime} \sim$
test guideline:	Triazophos was used as reference item @stead of dime@roate as recommended in
	the guideline. Only 3 test item doses were used with a spacing factor of 10 instead
	of 5 with a maximum spacing factor of 2.2 as recommended in the guideline.
	These deviations are not expected to have impacted the study results.
Previous evaluation:	yes, evaluated and accepted y y y y y y y y
	in DAR (200 \$ 47 4 5 5 6 6
GLP/Officially	Yes, conducted under GLPOfficially recognised testing facilities
recognised testing	Yes, conducted under GLP Officially recognised testing secilities
facilities:	
Acceptability/Reliability:	Yes v v v v v v

#### **Executive Summary**

The purpose of this study way to determine the acute of al toxicity of fluopfeolide technical to the honeybee (*A. mellifera* L.) in the laboratory. Mortality of the bees way used as toxic endpoint. Therefore, under laboratory conditions *Apis mellifera* worker bees were exposed by use of sucrose diet paste to mean measured doses of 2.61, 21.04, and 240.74 pg a.s. bee during a 5-hour feeding period. Furthermore, each test consisted of a control and a telerence item group. Each treatment group consisted of 5 replicates (test mits) with 10 bees per replicate. The tests were conducted in darkness, temperature was 24 - 26°C and relative humidity was between 58 and 68%. The number of dead bees in each cage was assessed 24, 48 and 72 hours after application. The oral LD₅₀ value for the reference substance was calculated with the ard of SAS probit analysis. No mortality occurred during the test with the different test doses. Also, no mortality occurred in the control in the test with the reference item the doses of 0.1101, 0.21614 and 0.69278 up product/bee resulted in 5, 16 and 35 dead bees after 72 hours. The LD₅₀ of the reference item was 241 arg a.s./bee. All validity criteria of the test were met. The LD₅₀ (72°F) for honeybees was 241 arg a.s./bee in the oral toxicity test performed with fluopicolide.

## I. MATERIAL AND METHODS:

Test stem: Fluopicofide technicar 99 % w/w, origin batch no.: R001737, Identification code: AE C638206 00 1B99 0002, Certificate of Analysis: C/030/2000 (dated 05 April 2000).

Test organism; Temale worker honerbees (*Tpis mellifera*), obtained from a healthy and queen-right colony.

Under laboratory conditions *Apis mellitera* worker bees were exposed by use of sucrose diet paste to mean measured doses of 2 69, 21.04, and 240.74  $\mu$ g a.s./bee during a 5-hour feeding period. Furthermore, each test consisted of a control and a reference item group. In the control an untreated sucrose diet paste was offered to the bees as food source. In the test AE F002960 00 EC 40 C667 (active ingredient 41.1 % w/w triazophos, Batch no.: C07174065) was used as reference item; the reference item was dested at three different doses (0.1101, 0.21614 and 0.69278  $\mu$ g product/bee).

Each treatment group consisted of 5 replicates (test units) with 10 bees per replicate. Test units were 12-13 cm high cylindrical test cages with a diameter of 5 cm.



For the reference item group 0.4 mL of a 50% sucrose solution containing the reference substance AE F002960 00 EC40 C667 (41.1 % w/w triazophos) in the three different concentrations 0.0006, 0.0012 and 0.0047% product were offered to the bees.

The tests were conducted in darkness, temperature was  $24 - 26^{\circ}$ C and relative humidity was between 58 and 68 %. The number of dead bees in each cage was assessed 24, 48 and 72 hours after application. The oral LD₅₀ value for the reference substance was calculated with the aid of SAS probit analysis.

Dates of experimental work: August 31, 2000 – September 03, 2000

Biological findings

<u>Brone Brown Hindanige</u>		
Test substance	Endpoint State	
Fluopicolide	$24-72 \text{ h LD}_{50} [\text{ug a.s./bee}] \ll 9 > 240 $	
Reference item	72 h LD ₅₀ [μg product/bee]	

II RESULTS AND DISCUSSION:

#### Observations

No mortality occurred during the test with the different test doses. Also, no mortality occurred in the control. In the test with the reference item the doses of 0.1101, 0.21614 and 0.69275 µg product/bee resulted in 5, 15 and 35 dead bees ofter 72 hours.

		ð y
	Total number of dead bees (and mortality in	%) after
Čo (	24 h ~ 0 48 h ~ ~ ~	<i>7</i> 20 h
Control		$\approx 0^{\circ}(0)$
Test item [µg a.s./bee]		<b>S</b>
2.6122	(0) $(0)$ $(0)$ $(0)$ $(0)$ $(0)$ $(0)$	0 (0)
21.038 240.740		0 (0)
240.740		0 (0)
Reference iten ug product/bee		
0.11010	3(6) $3(6)$ $3(6)$ $3(6)$ $3(6)$ $3(6)$ $3(6)$ $3(6)$	5 (10)
0.21614	$15 (30)$ $37$ $0^{2}$ $15 (30)$ $37$	15 (30)
0.692/8	349(68)	35 (70)

Validity criteria: All validity criteria of the test were met

Validity criteria (OECD 249, 1998)	Obtained in this study
Control mortality should not exceed 10 % at test and	Control: 0 %
LD50 of the reference item should be in the specified range	0.162 μg a.s./bee*
(dimethoate: oral test $0.10 - 0.35 \ \mu g a.s./bee)$	(a.s. triazophos)

*0.394 µg product/bee \$41.1 % w/w triazophos

The reference item triazophos confirmed the sensitivity of the bees used in the test.

#### III. CONCLUSIONS:

The  $LD_{50}$  ( $\sqrt{2}$  h) for hone Dees was > 24 Pµg a.s./bee in the oral toxicity test performed with fluopicolide.

#### Assessment and conclusion by applicant:

This study is considered reliable for risk assessment and the endpoint is:

 $LD_{50}$  (72 h) > 241 µg a.s./bee

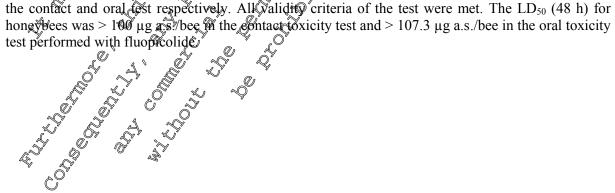


Data Point:	KCA 8.3.1.1.1/02
Report Author:	
Report Year:	2015
Report Title:	Fluopicolide tech.: Effects (Acute contact and oral) on honey bees (Apis melliora L.) in the laboratory
Report No:	99581035
Document No:	<u>M-539964-01-1</u>
Guideline(s) followed in	Regulation (EC) No. 1107/2009
study:	Directive 2003-01 (Canada/PMRA)
State J.	US EPA OCSPP 850.3020, 850.stpp.
	OECD 213 and 214 (1998)
Deviations from current	Current Guidelines: OECD 216 (1998) and OECD 214 (1998)
test guideline:	A 5 µL droplet was chosen if the contact to scity test in departion to the gardeline
	recommendation of a 1 $\mu$ L aroplet, since a higher solume ensured a more reliable.
	dispersion of the test item? This deviation is not expected to have impacted the
	study results.
Previous evaluation:	No, not previously something of the second sec
GLP/Officially	Yes, conducted under ODP/Officially recognised testing facilities
recognised testing facilities:	
Acceptability/Reliability:	Yes in the transformed and the second
Executive Summary	

#### **Executive Summary**

0* Ô The purpose of this study was to determine the actute contact and oral toxicity of fluopicolide technical to the honeybee (A. mellifera L.) in the aboratory. Mortality of the bees was used as the toxic endpoint. Sublethal effects, such as changes in behaviour, were also ossessed.

Therefore under laboratory conditions Apis mellifera worker becowere exposed for 48 hours to a single dose of 100.0  $\mu$ g a sper bee, by topical application of  $\Im \mu L$ , in a contact limit test and to a single dose of 107.3  $\mu$ g a.s. per bee by feeding in an oral limit test (value based on the actual intake of the test item after a feeding period of < 2 hours). Furthermore, each test consisted of a control, solvent control and a reference item group Each treatment group consisted out of Sreplicates (test units) with 10 bees per replicate. The tests were conducted in darkness, temperature was 25°C and humidity was between 50 and 78 % Biological observations, including mortality and behavioural changes were recorded 4, 24 and 48 hafter application. At the end of the contact toxicity test (48 hours after application), there was 6.0 % mortality at 100.0 µg/a.s./bee. There was 2.0 % mortality in the water control group (water + 0.5 % Adhäsit) and no mortality occurred in the solvent control group (acetone). In the oral toxicity test, the actual intake of 107.3 ge a.s. Dee led to no mortality after 28 hours. No mortality occurred in the water control group 50 % v sucrose solution 500 g/sucrose/L tap water) and in the solvent control group (50 % w/v sucrose solution comparing 4% age tone and 1% Tween 80) at the end of the oral toxicity test (after 48 hours). The LD of the reference item was calculated to be 0.23 and 0.14 µg a.s./bee in the contact and oral test respectively. All validity criteria of the test were met. The LD₅₀ (48 h) for





#### I. MATERIAL AND METHODS:

Test item: Fluopicolide technical: 98.8% w/w; origin batch no.: BCHR 1111-2-1, Material: Fluopicolide, technical, Specification No.: 102000016444. Article No.: 06032698, Certificat@ of Analysis No: AZ 20033.

Test organism: female worker honeybees (Apis mellifera), obtained from a healthy and quen-right colony, bred by IBACON.

Under laboratory conditions Apis mellifera worker bees were exposed for 48 hours to a single dose of 100.0  $\mu$ g a.s. per bee, by topical application of 5  $\mu$ L, in a contact limit test and to a Single dose of 107.3 µg a.s. per bee by feeding in an oral limit test (value) based on the actual intake of the test iters). Furthermore, each test consisted of a control, solvent control and a reference item group. In the contact limit test, tap water containing 0.5 % Adhaesit and pure acetone were used as control and solvent control respectively. In the oral limit test a 50 % w/v sucrose solution containing solvent (4 % aceton and 1% Tween) and 50 % w/v sucrose solution were used as solvent control and control, respectively. In both limit tests, BAS 152 11 I (active ingredient 400.9 g/L dimethodae, Batch no, @FRE-000926) was used as reference item. Each treatment group consisted out of replicates (test units) with 90 bees per replicate. Test units were stainless steel cages with 8 cm  $\times$  6 cm  $\times$  4 cm (length  $\times$  height  $\times$  width)? The tests were conducted in darkness, temperature was 25°C and hundidity was between 50 and 78%. The tests were conduct: Biological observations, including mortality and behavioural changes in a polication. The software used to perform the staristical analysis was DoxRat Professional S Dates of experimental work: April 13, 2015 – April 15, 2015 Biological observations, including mortality and behavioural changes were recorded 4, 24 and 48 h after

	Ò	)	<b>OII RES</b>	O SULTS	WND D	ISCUSS.	ION: 🔊	
Biological findings:		A		0			N N N	

Test item		Pluopicolide tech.
Test object		🖓 Apte mellifera 🚕
Test item Test object	Contast (solution in acctone)	Pluopicolide tech. <i>Apte mellifera</i> Oral (sugar/acetone - Tween 80/water solution)
ð á l	(solution in acetome)	(sugar/ acetone # Tween 80/water solution)
Dose [µg a s./bee]	Ø 100.0 X	المراجع
LD ₅₀ [µg x.s./bee]	20100.0 C	> 107.3
Test item       Test object       Exposure       Dose [µg a s./bee]       LD ₅₀ [µg a.s./bee]       LD ₂₀ [µg a.s./bee]	ິ່ງ ¹ 00.0 ບໍ່	≪J [™] _s O [°] > 107.3
$LD_{10}$ [µg a.s./bee]	<i>∞</i> > 1607.0 <i>∞</i>	× ۲۰۰۰ × ۱07.3
NOED [µg a.s./bco]*	j ≥ 200.0 m	$\bigcirc^{\nu} \ge 107.3$
* The NOED was estimated asing Fi	ster's Exact Test (pairwise	comparison, one-sided greater, $\alpha = 0.05$ ).
3 ^v o _s y		1 Contract of the second secon
~9 0 _0		8-
A. Or	\$`4 ^{\$*} \$\$`,	Ű
		J
	×~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	
A A		
	Ó	
	7	
×0×		
$\bigcirc$		(sugar/actione $\neq$ Tween 80/water solution) 107.3 > 107.3 > 107.3 > 107.3 > 107.3 > 2107.3 $\bigcirc \ge 107.3$ comparison, one-sided greater, $\alpha = 0.05$ ).



#### Observations

#### Contact test

At the end of the contact toxicity test (48 hours after application), there was 6.0 % mortality at 100 µg a.s./bee. There was 2.0 % mortality in the water control group (water + 0.5 % Adhäsit) and no mortality occurred in the solvent control group (acetone). During the first 4 hours 42.0 % bees were affected in the test item treated group. Thereafter no more behavioural abnormalities were found by the end of the test (48 hours).

	Afte	r 4 hours	After	24 hours	After	48 hours 🔗
Dosage [µg a.s./bee]	Mortality mean %	Behavioral abnormalities mean %	Mortality	Behavioral abnormalities mean %?	Mortality	Behavioral Johnormaliti g
Testitem	mean 70	mean 70	Magali 70		<u> n</u> ěan % ⁵	mean %
Test item 100.0	4.0	42.0	[♥] 4.0 °		Ø 60	
		*				- y
Water	0.0	0.0	×0.0 č	<b>9</b> .0	2.0 L	. <u>A</u> .0 °
Solvent	0.0	0.0	<u></u> ∞0.0∞	~~0.0 ₄	0.0 °	0.0
Reference item			$\sim$ $\sim$		O' 🔬	~ ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
0.30	10.0	200 6	r 7,2%0 ∘		7 26.0	25
0.20	2.0	20 K.	\$0.0 ×C		\$2.0 Q	0.0
0.15	0.0		°≫ ^y 22.0 [∞]	2.0	\$2.0 \$26.0 \$ 26.0 \$	× 0.0
0.10	0.0	2.0	a 4.0		8.0	°≫ 0.0

m

Results are averages from five replicates (tendees each) per desage / control Water = CO₂/water-treated control Solvent  $\neq$  CO₂/solvent control

#### Oral test

In the oral toxicity test, the maximum normal test level of fluopicolide tech (i.e. 100  $\mu$ g a.i./bee) corresponded to an actual intake of 107.3  $\mu$ g a.i./bee. This dose level left to no mortality after 48 hours. No mortality occurred in the water control group (50 % w/v sucrose solution = 500 g sucrose/L tap water) and in the solven control group (50 % w/v sucrose solution containing 4 % acetone and 1 % Tween 80) at the end of the oral toxicity test (after 48 hours). Notest item induced behavioural effects were observed at any time in the oral toxicity test.

	<u> </u>	<del>ví A Č</del>	» [*] .0.	0 0	-	
pq.	Û Afte	4 houtes	After	24 hours	After	48 hours
Dosage [µg a.s./bee]	Mortafity	Bahaviana	Mortanty	Behavioral abnormalities	Mortality	Behavioral abnormaliti es
Q	mean %.5	mean %	prean %S	mean %	mean %	mean %
Test item 107.3	Q.0 ³		0.05	0.0	0.0	0.0
Water	<u>b</u> .0	D 60 m	<u>م</u> %0	0.0	0.0	0.0
Solven	×0.0	0.0	~0.0°	0.0	0.0	0.0
Reference item						
0.32	5400	32.9	× 100.0	0.0	100.0	0.0
0.16	l 00	32.9 32.9 0 Ø8.0 4	66.0	26.0	78.0	8.0
0.08	A 0.0 &	~~~0.0 ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	2.0	2.0	4.0	0.0
0.16 0.08 0.06		0.0	0.0	0.0	0.0	0.0

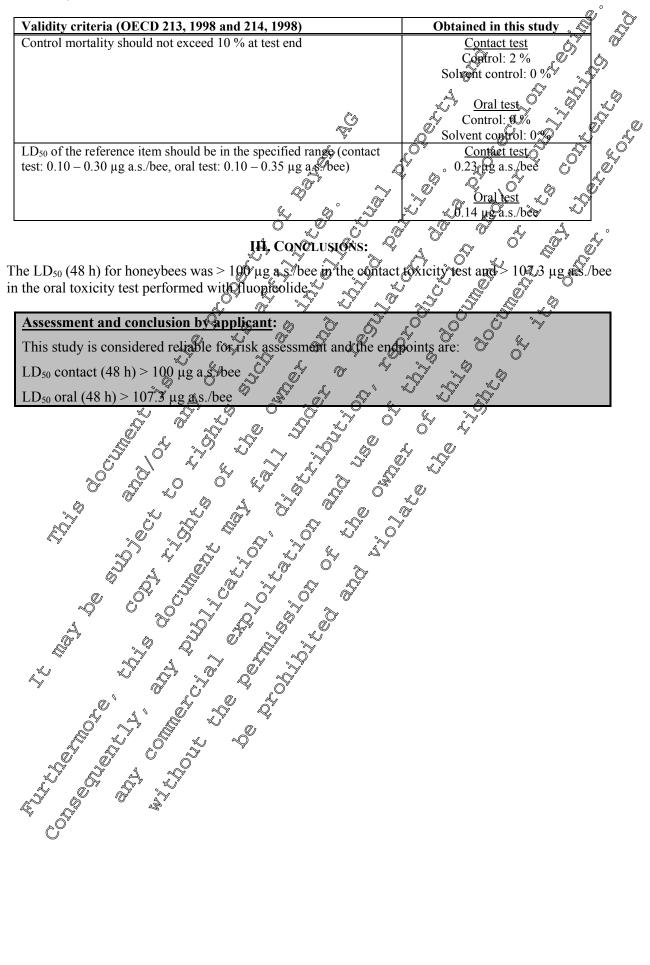
Results are averages from the replicates (ten bees each) per dosage / control

Water = Water control, solvent = solvent control



Validity criteria:

All validity criteria of the test were met.

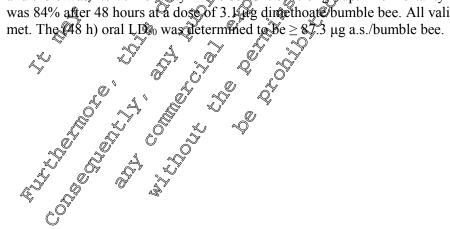




Data Point:	KCA 8.3.1.1.1/03
Report Author:	
Report Year:	2015
Report Title:	Fluopicolide tech.: Effects (Acute oral) on bumblebees (Bombus terrestris L.)
	the laboratory
Report No:	97621105
Document No:	<u>M-519981-01-1</u>
Guideline(s) followed in	(GLP compliant study based on van der Steen (2001) and OECD 214 (1998) with
study:	modifications and adaptions, Ring test bumblebee active contact tox of ty (ICPPR
	non-apis group, 2014))
Deviations from current	Current Guideline: OECD 247 ( $20$ ) 7 $0$ $0$ $0$ $0$ $0$
test guideline:	Analytical determination of the test item was for conducted, but the study was
	conducted before guideline implementation and no analytical dose verification
	was foreseen at that point in time. Moreover, since it is a fimit test with a single
	dosing of the test item this deviation is not expected to have impacted the study
	results. The exposure duration was 6 hours and thus greater than the maximum 4
	hours recommended by the guideline. The test was conducted before
	implementation of the guideline and the opposure duration of the ing test
	discussed at the time was 6 hours. This deviation is not expected to have impacted the study results.
Previous evaluation:	
rievious evaluation.	No, not prevously submitted
GLP/Officially	Yes, conjucted under GLP/Officially recognized testing facilities
recognised testing	Yes, conducted under GLP/Officially recognised testing facilities
facilities:	
Acceptability/Reliability:	Yes & B & a & Y & B

# Executive Summary

0 N A Executive Summary The purpose of this study was to determine the acute oral toxicity of Stiopiconde tech. to the bumble bee (Bombus terrespirs L.) in the laboratory. Mortality of the bomble bees was used as the toxic endpoint. Sublethal effects, such as changes in behaviour, were also assessed. Therefore, under laboratory conditions Bondus terestris worker bumble bees were exposed for 6 hours to a single dose of 87.3 µg a.s. per bumble bee by feeding in an oral limit test. Furthermore, the test consisted of a control, solvent control and reference item group. Each treatment group consisted out of 50 bumble bees with 1 bumble bee per test unit (replicate). The test was conducted in darkness, temperature was 24-25°C and humidity 50-67% during exposure. Biological observations, including mortality and sub-lethal effects were recorded 4, 24 and 48 h after application. The software used to perform the statistical analysis was ToxRat Pro Version 240. At test termination (48 hours) 6.0 % mortality occurred at 87.3  $\mu$ g fluopicolide/bumblebee. No mortality occurred in the water control group (50 % w/v sucrose solution) and there was 2.0 % mortably in the solvent control group. The mortality in the reference item group was 84% after 48 hours at a dose of 3. Leg dimethoate bumble bee. All validity criteria of the test were





#### I. MATERIAL AND METHODS:

Test item: Fluopicolide technical: 100.5 % w/w (analytical), Origin Batch No.: ETFP000273, Customer Order No.: TOX 10747; Material: Fluopicolide, technical; Specification No.: 102000016444-01, Arece No.: 06032698.

Test organism: female worker bumble bees (*B. terrestris*), obtained from a healthy and queen-right colony, bred by a commercial bumble bee breeding company (Biobest Belgium N.V.). Under laboratory conditions *Bombus terrestris* worker bumble bees were exposed for 6 hours to a single dose of 87.3  $\mu$ g a.s. per bumble bee by feeding in an oral limit test (value based on the actual intake of the test item). Furthermore, the test consisted of a control solvent control and a reference item group. In the oral limit test a 50 % w/v sucrose solution containing solvent (5% acetone and 1 %) were 80) and 50% w/v sucrose solution were used as solvent control, respectively. BAS 152 11 I EC (active ingredient 400.9 g/L dimethoate, Batch no.: FREe000926) wascused as reference item. Each treatment group consisted out of 50 bumble bees with 1 Sumble bee per test with (replicate). Test units were cylindrical, latticed plastic cages with a length of approximately 7 cm and a diameter of 2.2 cm at the large and 1.7 cm at the small opening.

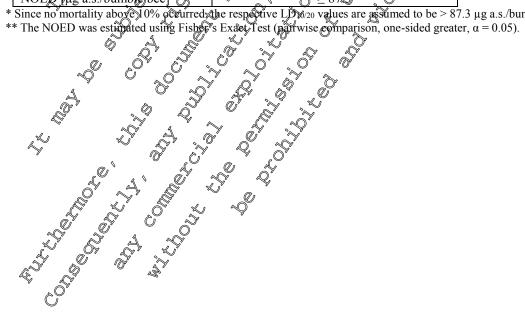
The test was conducted in darkness, temperature was 24-25°C and humdity 50-67% during exposure. Biological observations, including mortality and sub-lethal effects were recorded 4, 24 and 48 h after application. 

The software used to perform the statigical analysis was ToxRat

#### Dates of experimental work: Februar@17, Februar

	🔧 👘 👘 👘 👘 👘 👘	Ô
I	الم المحتوي الله HI RESOLTS AND DISCUSSION:	ý 
	Test item	. 8
	Test object 🖉 🖉 🖉 Bombus terrestris	S.
	Exposure de la construction de l	¥
	Dose [ $\mu$ g a.s./bumple be $\sqrt{2}$	
	based on recorded consumption $\langle \xi \rangle = \langle \xi \rangle^{0/1.3} \langle \xi \rangle \langle \xi \rangle^{1/1.3}$	
	$LD_{50}$ [µg a.s (bumble bee] $O$ $O$ $V$ $O$ > 8 $O$ $V$	
	$LD_{20}$ [µg a s./bumble bee] $2$	
	$LD_{10}$ [µg a.s./bumble bee] * $\sim$ $\sim$ $\sim$ $\sim$ $> 87.3 ~$	
	NOE [2] [4] $\mu$ g a.s./bumble bee] $\pi$ $\sim$	

* Since no mortality above 10% occurred the respective LD1/20 values are assumed to be > 87.3 μg a.s./bumble bee





#### **Observations**

#### Oral test:

At test termination (48 hours) 6.0 % mortality occurred at 87.3 µg fluopicolide/bumble bee. No mortality occurred in the water control group (50 % w/v sucrose solution) and there was 2.0 % mortality in the solvent control group (50 % w/v sucrose solution containing 5 % acetone and 1 % Tween80).

	Afte	r 4 hours	After	· 24 hours	After	· 48 hours
Treatment Group	Mortality mean %	Behavioral abnormalities mean %	Mortality mean 🛠	Behavioral abnormalities mean %	Mortality mean %	Behav@ral abnormalities n@ean %
Test item: 87.3 µg a.s./bumble bee	0.0	0.0	<b>A</b>		6.6	
Control	0.0	0.0	<b>~</b> 0.0	0.0	Q.0	0.0
Solvent Control	0.0	0.0	ي 0.0		2.0 ×	. ≪ ³ 0.0 ≪ ³
Reference item 3.1 µg dimethoate /bumble bee	0	80	\$8.0 \$8.0			

Beh. abnormalities = Behavioural abnormalities. Mean = mean of 50 indoiduals fer treatment group Control = 50 % w/v sucrose solution; solvent control = 50 % w/v sucrose solution containing 5 % acetone 1 % Tween80 Ref. = Reference item

#### Validity criteria:

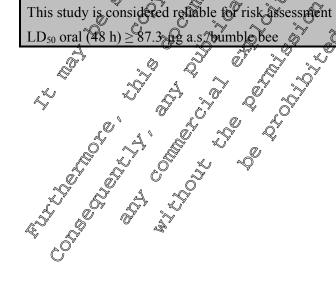
All validity criteria of the test were met.

Validity criteria OECD 247 (	2019)	Obtained in this study 🗸
Control mortality should not ex	eeed 10 % at testend 0	Control: 0%
		Solvent control: 2%
Mortality of the reference item	shoodd be ≥\$0 % aQrest	Reference item: 84 %
end S		$\Phi \phi \Delta \phi$
	Ý &, 🌫 🎝	
	0 H. Coseli	USIONS:

The toxicite of fluopicolide tech was tested in an acute oral toxicity test on bumble bees. The (48 h) oral LD was determined to  $\beta \geq 87 \otimes \mu g$  a.s./bumble bee.

#### Assessment and conclusion by applicant:

This study is considered reliable for risk assessment and the endpoint is:





Data Point:	KCA 8.3.1.1.1/04
Report Author:	
Report Year:	2016
Report Title:	AE C653711: Effects (Acute contact and oral) on honey bees (Apis mellifera )
	in the laboratory - Final report -
Report No:	114821035
Document No:	<u>M-571897-01-1</u>
Guideline(s) followed in	Regulation (EC) No. 1107/2009           Directive 2003-01 (Canada/PMRA)           US EPA OCSPP 850.3020, 850.s@pp.
study:	Directive 2003-01 (Canada/PMRA)
Deviations from current	Current Guidelines: OECD 216 (1998) and OECD 214 (1998)
test guideline:	A 5 $\mu$ L droplet was chosen in the contact to Sicity test in demation to the gapdeline
	recommendation of a 1 µL aroplet, since a higher volume ensured a more reliable
	dispersion of the test item? This deviation is not expected to have impacted the
	study results. $(x, y)^{\circ} = (x, y)^{\circ} = ($
Previous evaluation:	No, not previously submitted a start of the second se
	$\underline{A}  \overline{\mathcal{O}}  \underline{\mathcal{O}}  \overline{\mathcal{Q}}  \underline{\mathcal{O}}  \underline{\mathcal{O}}$
GLP/Officially	Yes, conducted under ODP/Officially recognised testing facilities
recognised testing	
facilities:	
Acceptability/Reliability:	Yes $\mathcal{A}$ $\mathcal{A}$ $\mathcal{A}$ $\mathcal{A}$ $\mathcal{A}$ $\mathcal{A}$ $\mathcal{A}$ $\mathcal{A}$
Exacutiva Summany	

#### **Executive Summary**

The purpose of this study was to determine the agate contact and oral poxicity of M501 (AE C653711) to the honeybee (A. mellifera L.) in the aboratory. Mortality of the bees was used as the toxic endpoint. Sublethal effects, such as changes in behavior, were as assessed. Therefore, under laboratory conditions Apis mellifera worker bees were exposed for A8 house to a single dose of 100.0 µg p.m. per bee, by topical application of 5 µI in a contact limit test and to a single dose of 80.8 µg p.m. per bee by feeding in an oral limit test. Furthermore, each test consisted of a control, solvent control and a reference item group. Each treament group consisted out of 5 replicates (test units) with 10 bees per replicate. The tests wer conducted in darkness, temperature was 24 - 25°C and humidity was between 59 and 70 %. Biological observations, including mortality and behavioral changes were recorded 4, 24 and 48 h after application. The software used to perform the statistical analysis was ToxRat Professional. At the end of the contact toxicity test, no mortality occurred at 100, ug p.m./bee, in the control and in the solvent control. In the oral pxicite test, the actual intake of 80.8 µg p.m./bee led to a mortality of 12.0% after 48 hours. No mortality occurred in the water control group and in the solvent control group at the end of the oral toxicity test. The LD, of the reference item was calculated to be 0.16 and 0.13 µg/bee in the contact and oral test, respectively. All validity criteria of the test were met. The LD50 (48 h) for honeybees was  $> 100 \ \mu g \ pm/be in the contact toxicity test and <math>> 80.8 \ \mu g a.s./bee in the oral toxicity$ test performed with M-O (AE 265372/1).

### . I. MATERIAL AND METHODS

Test item: M-01 (AE C653711), 96.2% w/w; origin batch no.: 08018ET, Material: M-01 (AE C653711), Certificate of Analysis No: AZ 20300.

Test organism: female worker honeybees (Apis mellifera), obtained from a healthy and queen-right colony, bred by BACON.

Under hooratory conditions *pis mellifera* worker bees were exposed for 48 hours to a single dose of 100.0 ug p.m. per bee, by topical application of 5  $\mu$ L, in a contact limit test and to a single dose of 80.8 ug p.m. per bee by teeding in an oral limit test (value based on the actual intake of the test item after a maximum of 6 hours feeding period). Furthermore, each test consisted of a control, solvent control and a reference item group. In the contact limit test, tap water containing 0.5 % Adhaesit and pure acetone were used as control and solvent control, respectively. In the oral limit test a 50 % w/v sucrose solution containing solvent (4 % acetone and 1% Tween) and 50 % w/v sucrose solution were



used as solvent control and control, respectively. In both limit tests, BAS 152 11 I (active ingredient 420.3 g/L dimethoate, Batch no.: FRE-001226) was used as reference item. Each treatment group consisted out of 5 replicates (test units) with 10 bees per replicate. Test units were stainless steel cages with 8 cm  $\times$  6 cm  $\times$  4 cm (length  $\times$  height  $\times$  width).

The tests were conducted in darkness, temperature was 24 - 25°C and humidity was between 39 and 70 %. Biological observations, including mortality and behavioural changes were recorded 4, 24 and 38 h after application. The software used to perform the statistical analysis was ToxRat Professional

II RESULTS AND DISCUSSION:

Dates of experimental work: February 22, 2016 – February 24, 2016

**Biological findings:** 

Test item	l l	Øf-01 (SE C653/11) € 5 5
Test object	0 [°] ,	Aprs mellifera 🖉 🔗 🔬 🔒
Exposure	Contact 🔗	
	(solution in acetone)	Asugar/acetone+Twe@ 80/water solution)
Dose [µg p.m./bee]		0 . ~ 0 80x ~ ~ ~
LD ₅₀ [µg p.m./bee]	Q1000	
LD ₂₀ [µg p.m./bee]	المركز 1000 مركز	
$LD_{10}$ [µg p.m./bee]	$\sqrt{2} > 100.0$	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
NOED [µg p.m./bee]*	<u>₹</u> 100.0 °°	S 0 S < 80.8 S
* The NOED was estimated using F	isper's Exact Test (pairwise)	compatison on excided screater $\alpha = 0.05$

#### **Observations**

#### Contact test

At the end of the confact toxicity test (48 hours after application), there was 0.0% mortality at 100.0  $\mu$ g p.m./bee and nonortably occurred in the water control group (water + 0.5 % Adhäsit) and in the solvent control group (pure acetone), respectively.

Mortality     Behavioral abnormalities     Mortality     Behavioral abnormalities     Mortality     Behavioral abnormalities     Mortality     Behavioral abnormalities     Mortality     Behavioral abnormalities       Test item 100.0 μg p.m./bee     0.0     0.0     0.0     0.0     0.0     0.0     0.0       Water     00     0.0     0.0     0.0     0.0     0.0     0.0     0.0
DosageMortalityDenavioral abnorgalitiesMortalityDenavioral abnorgalitiesMortality abnorgalitiesMortality abnorgalitiesMortality abnorgalitiesMortality abnorgalitiesMortality abnorgalitiesMortality abnorgalitiesMortality abnorgalitiesMortality abnorgalitiesMortality abnorgalitiesMortality abnorgalitiesMortality abnorgalitiesMortality abnorgalitiesMortality abnorgalitiesMortality abnorgalitiesMortality abnorgalitiesMortality abnorgalitiesMortality abnorgalitiesMortality abnorgalitiesMortality abnorgalitiesMortality 
Test item $0.0 \downarrow$
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$
Water $\sqrt{60}$ $\sqrt{90}$ $\sqrt{90}$ $\sqrt{90}$ $\sqrt{00}$ $0.0$ $0.0$ $0.0$
Solvent $(1000)^{\circ}$ $(0.0)^{\circ}$ $(0.0)^{\circ}$ $(0.0)^{\circ}$ $(0.0)^{\circ}$ $(0.0)^{\circ}$ $(0.0)^{\circ}$ $(0.0)^{\circ}$ $(0.0)^{\circ}$
Solvent $0.0$ $0.0$ $0.0$ $0.0$ $0.0$ $0.0$ $0.0$ $0.0$ $0.0$ $0.0$ $0.0$ $0.0$ $0.0$ $0.0$ $0.0$ $0.0$ $0.0$ $0.0$ $0.0$ $0.0$ $0.0$ $0.0$ $0.0$ $0.0$ $0.0$ $0.0$ $0.0$ $0.0$ $0.0$ $0.0$ $0.0$ $0.0$ $0.0$ $0.0$ $0.0$ $0.0$ $0.0$ $0.0$ $0.0$ $0.0$ $0.0$ $0.0$ $0.0$ $0.0$ $0.0$ $0.0$ $0.0$ $0.0$ $0.0$ $0.0$ $0.0$ $0.0$ $0.0$ $0.0$ $0.0$ $0.0$ $0.0$ $0.0$ $0.0$ $0.0$ $0.0$ $0.0$ $0.0$ $0.0$ $0.0$ $0.0$ $0.0$ $0.0$ $0.0$ $0.0$ $0.0$ $0.0$ $0.0$ $0.0$ $0.0$ $0.0$ $0.0$ $0.0$ $0.0$ $0.0$ $0.0$ $0.0$ $0.0$ $0.0$ $0.0$ $0.0$ $0.0$
Reference
$\begin{bmatrix} \mu g \langle a, s./bee \end{bmatrix} = \begin{bmatrix} \mu g \langle a, s./bee \rangle $
$\begin{array}{c c c c c c c c c c c c c c c c c c c $
0.20 0.0 $600$ $68.0$ 0.0 $82.0$ 0.0
0.15 $0.0$ $0.0$ $0.0$ $0.0$ $0.0$
$\begin{array}{c c c c c c c c c c c c c c c c c c c $

Results are averages from five replicates (ten bees each) per dosage / control

Water = @vater-treated control, solvent = acetone treated control



#### Oral test

In the oral toxicity test, the maximum nominal test level of M-01 (AE C653711) (*i.e.* 100 µg p.m./bee) corresponded to an actual intake of 80.8 µg p.m./bee. This dose level led to a mortality of 12.0 % after 48 hours.

No mortality occurred in the water control group (50 % w/v sucrose solution = 500 g sucross L tap water) and in the solvent control group (acetone (4%) and Tween 80 (1%)) at the end of the oral toxic by test (after 48 hours).

	Afte	After 4 hours		After 24 hours		After 48 hours	
Dosage	Mortality	Behavioral abnormalities	Mortality	Behavioral abnormalities	Mortality	apportinationes	
	mean %	mean %	mean	mean %	mean %	Q mean %	
Test item			A	Q' a			
80.8 µg	4.0	72.0	Q2.0		″1⊉.0 ູ©	©_012.0 ©	
p.m./bee				Q X	10 (C)2		
Water	0.0	0.0	° 0.6°	2 0.0 2 0.0	0.6	0.0	
Solvent	0.0	0.0	Ø.0	C BO O	0.0	° L OCO	
Reference		*			<b>A</b> .		
item		S.	N' N				
[µg a.s./bee]					Ý Ô	Q Õ	
0.34	36.0	46.0	.98.0		190.0	¢0.0	
0.17	0.0	22.0	12.0	~6.0	082.0 S	<u></u> ,≪ [*] 4.0	
0.08	0.0	[™] 0.0 Ø	\$ 14.0 [°]	6.0 c	<u>0</u> 20.0 ^C	≫ 4.0	
0.06	0.0	~ 6J '	0.00		0.0	0.0	
Results are avera	iges from five re	plicates (ten bees a	ch) per dosage	e / control		)	
Water = water co	ontrol, solvent =	acetone treated cont	rol 🖓 🖉	c/control	ŝ, ŝ		
		Č Č	Æ s.		NY N		

Validity criteria:

All validity criteria of the test were met.

Validity criteria (DECD 213, 1998 and 214, 1998) Control mortanty should not exceed 60% at test end <u>Contact test</u> Control: 0 %	
Control mortably should not exceed 19% at tost end 5 <u>Contact test</u>	
$\bigcirc$	
Solvent control: 0 %	
Control: 0 %	
LD ₅₀ of the reference item should be in the specified range (contact <u>Contact test</u>	
test: $0.10 - 0.00 \ \mu g \ a^{(3)}/bee, \ oral test 0.10 - 0.35 \ \mu g \ a.s./bec)$ $0.16 \ \mu g \ a.s./bee$	
$\begin{array}{c c} A & O \\ \hline \\$	
<u>Oral test</u> 0.13 μg a.s./bee	

**VIII.** CONCLUSIONS:

The LD₅₀ (48 h) for honeybees was  $\geq$  000 µg p.m./bee in the contact toxicity test performed with M-01 (AE C653711) The LD₅₀ (48 h) for honeybees was  $\geq$  80.8 µg p.m./bee in the oral toxicity test performed with M-01 (SE C653711)  $\approx$ 

Assessment and conclusion by applicant:

This study is considered reliable for risk assessment and the endpoints of M-01 are:

 $LD_{50}$  contact (48 h) > 100 µg p.m./bee

 $LD_{50} \text{ oral } (48 \text{ h}) > 80.8 \ \mu\text{g p.m./bee}$ 



Data Point:	KCA 8.3.1.1.1/05
Report Author:	
Report Year:	2016
Report Title:	AE C657188: Effects (Acute contact and oral) on honey bees (Apis mellifera (
	in the laboratory
Report No:	114811035
Document No:	<u>M-566365-01-1</u>
Guideline(s) followed in	Regulation (EC) No. 1107/2009
study:	Directive 2003-01 (Canada/PMRA)
	US EPA OCSPP 850.3020, 850.s@p. 4 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2
	OECD 213 and 214 (1998)
Deviations from current	Current Guidelines: OECD 216 (1998) and OECD 214 (1998)
test guideline:	A 5 $\mu$ L droplet was chosen in the contact to ficity test in departion to the gapdeline
	recommendation of a 1 $\mu$ L aroplet, since a higher colume allows to test a higher $\sqrt{2}$
	application dose. This degration is not expected to have impacted the study
	results. & & X X X Y
Previous evaluation:	No, not previously submitted a solution of the
GLP/Officially	Yes, conducted under ODP/Officially pecognised testing facilities
recognised testing	
facilities:	
Acceptability/Reliability:	$Yes \qquad \qquad$
Executive Summary	

#### **Executive Summary**

 $\mathbb{O}^{\forall}$ The purpose of this study was to determine the acute contact and oral poxicity of M502 (AE C657188) to the honeybee (A. mellifera L.) in the aboratory. Mortality of the bees was used as the toxic endpoint. Sublethal effects, such as changes in behavior, were the assessed. Therefore under laboratory conditions Apis mellifera worker bees were exposed for 48 bours to a single dose of 100.0 µg p.m. per bee, by topical application of 5 µL in a contact light test and to a single dose of 110.9 µg p.m. per bee by feeding in an oral limit test valuebased on the actual intake of the test item after a reeding period of 1 hour and 35 minutes). Furthermore, each test consisted of a control, solver control and a reference item group. In the contact amit test, tap water containing 0.5% Adhaesit and pure acetone were used as control and solvent control, respectively. In the oral limit pest a 500% w/y sucrose solution containing solvent (5 % acetone) and 50 % w/v sucrose solution were used as solvent control and control, respectively. Each treatment group consisted out of 5 replicates (test phits) with 10 bees per replicate.

a, s

The tests were conducted in darkness, temperature was 27°C (mean) and humidity was between 56 and 64 %. Biological observations, the luding mortality and behavioral changes were recorded 4, 24 and 48 h after application. The software used to perform the statistical analysis was ToxRat Professional. At the end of the contact toxicity test there was 6.9 % mortality at 100.0 µg p.m./bee. 0.0 % mortality occurred in the water control group and 2.0 % in the solvent control group, respectively. In the oral toxicity test, the actual intake of 110. I g pm./bee red to no mortality after 48 hours. 4.0 % mortality occurred in the water control group and 2.0% morality occurred in the solvent group at the end of the oral toxicity test. The LD5 of the reference item was calculated to be 0.22 and 0.14 µg /bee in the contact and oral test respectively All validity criteria of the test were met. The LD50 (48 h) for honeybees was and oral test respectively. All validity criteria of the test were met. The LD₅₀ (48 h) for honeybees was  $> 100 \ \mu g \ p.m./Bee in the contact toxicity test and <math>> 110.9 \ \mu g \ a.s./bee in the oral toxicity test performed with M-02 (Ab C657188).$ 



#### I. MATERIAL AND METHODS

Test item: M-02 (AE C657188): 98.5% w/w; origin batch no.: SES 10250-1-1, Material: M-02 (AE C657188), Certificate of Analysis No: AZ 20206.

Test organism: female worker honeybees (Apis mellifera), obtained from a healthy and queen right colony, bred by IBACON.

Under laboratory conditions Apis mellifera worker bees were exposed for 48 bours to a single dose of 100.0 µg p.m. per bee, by topical application of 5 µL, in a contact limit test and to a single dose of 110.9 μg p.m. per bee by feeding in an oral limit test (value based on the actual intake. Φ the test item? after a feeding period of 1 hour and 35 minutes). Furthermore, each test consisted of a control, solvent control and a reference item group. In the contact limit test, tap water containing 0,5% Achaesic and pure acetone were used as control and solvent control, respectively in the oral print test a 50 % w/w sucrose solution containing solvent (5 % acetone) and 50 % w/v socrose solution were used ac solvent control and control, respectively. In both limit Tests, BAS 452 11 d (active ingredient 420.3 JL dimethoate, Batch no.: FRE-001226) was used as reference item. Each treatment group consisted out of 5 replicates (test units) with 10 bees per replicate. Test units were stainless steel cages with 8 cm  $\times$  6 cm  $\times$  4 cm (length  $\times$  height  $\times$  width).

The tests were conducted in darkness, temperature was 27°C (mean) and hundidity was between 56 and The tests were conducted in darkness, temperature was 27°C (mean) and humdility was between 50 and 64 %. Biological observations, including mortality and behavioral changes were recorded 4, 24 and 48 h after application. The software used to perform the statistical analysis was ToxRat Professional Dates of experimental work: June 21, 2015 – June 23, 2015 II. RESULTS AND DISCUSSION: 5

	<u> v</u>		×, ^y	0 ~~	L'Y
Test item			<u>N</u> -02 (Ag	<u>E C657188)</u>	<i>v</i>
Test object			💙 Apis n	nellifera 🚕	
Exposure	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	Contact A		K KÖr	al
Test item Test object	S _O (soli	Contast Contast Hion ik acetone)	Ö (su	igar/acetone /	water solution)
Dose [µg p.m./bee]		100.0	S (su	ِ 🔊 110	).9
LD ₅₀ [µg p.m./bee]		@100.0 U		>11	0.9
Test item Test object Exposure Dose [µg p.m./bee] LD ₅₀ [µg p.m./bee] LD ₂₀ Aug p.m./bee] LD ₁₀ [µg p.m./bee] NOED [µg p.m./bee] The NOED was estimat m. = pure metabolite		[™] 100.0 Ô		, O″ > 11	0.9
LD10 [µg p.m./bee]		J > 100.0	K. A	>11	0.9
NOED [µg p.m./boe	]* ]*	≥ D00.0 0	0′ 🔈	$\geq 11$	0.9
The NOED was estimat	ed asing Fisher's	Exact Test (pairwise	comparison,	one-sided great	er, $\alpha = 0.05$ ).
.m. = pure metabolite	, ~ ~ ~ ~	$0^{\circ}$ $0^{\prime}$ $0^{\prime}$	°C"		
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	A .%				
6° 9 °0	A Contraction				
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#### Observations

#### Contact test

At the end of the contact toxicity test (48 hours after application), there was 6.0 % mortality at 1000 µg p.m./bee. 0.0 % mortality occurred in the water control group (water + 0.5 % Adhaesit) and 2.0% in the solvent control group (acetone), respectively.

	Afte	r 4 hours	After	24 hours	After	48 hours
Dosage	Mortality	Behavioural abnormalities	Mortality	Behavioural abnormalities	Mortality	Behavioural abnormalities
	mean %	mean %	mean %	mean 🔊	mean %	Anean 🌮 🕺 Ö
Test item 100.0 μg p.m./bee	0.0	0.0				
Water	0.0	0.0	« 0.0°°	2 0.0 v	0.00	^م ري 0.0 م
Solvent	0.0	0.0	O″ <u>0</u> .Ø″		r 2,30°,	c <u>Q</u> 0
Reference item [µg a.s./bee]						
0.30	0.0	96 Q (*	75.0	S ØØ Ö	7 <u>8.0</u>	0.0
0.20	0.0	60.0	¥.0 ≪	× 0.0 ~	Š.0 Š	
0.15	0.0	Q16.0	14.0	\$°0.0	C 22.0	× 0.0
0.10	0.0	0.0	6 [°] 4.0°		<u> </u>	0.0
Results are aver	ages from five r	epticates (ten bees ea	ich) per <b>G</b> osage	Gontrol	Or C	) )
Water = water-t	reated control, s	olvent zacetone area	ted control		Ô.	

8

Oral test In the oral toxicity test, the maximum nominal test evel of M-02 (AE C657188) (*i.e.* 100  $\mu$ g p.m./bee) corresponded to an actual intake of 110.9 kg p.m./bee. This dose level led to no mortality after 48 hours. 4.0% mortality occurred of the water control group. (50% v/v sucrose solution = 500 g sucrose/L tap water) and 2.0 % mortality occurred in the solvent control group at the end of the oral toxicity test (after  $\sqrt{2}$  $\bigcirc$ 6 "¢ 48 hours). 0 M

	×, ×	u an o	· · · ·	0.0		
	Ù Afte	ř 4 hours	After	24 hours	After	r 48 hours
Dosage	Mortality	Behavioural abnormalities	Mortality	Bebavioural abnormalities	Mortality	Behavioural abnormalities
	mean % g	🖉 metan % 🖉	mean %	📎 mean %	mean %	mean %
Test item 110.9 μg p _s bee	0.0 °°			0.0	0.0	0.0
Water	<b>()</b>		2 0 <b>0</b>	0.0	4.0	0.0
Solvent	<u>ک</u> 0.0 ک	) av.o 📎	<u>_</u> ×0.0	0.0	2.0	0.0
Reference item $[\mu g_{a} s./bee]$			20.			
0.38	36.0	ຸ້ 5690 💍	98.0	2.0	98.0	0.0
0.17	0.0 L	Ø0.0 Å	68.0	8.0	74.0	0.0
	^	\$4.0 \$	0.0	4.0	0.0	0.0
0.06	l U.Q.	0.00	0.0	0.0	0.0	0.0

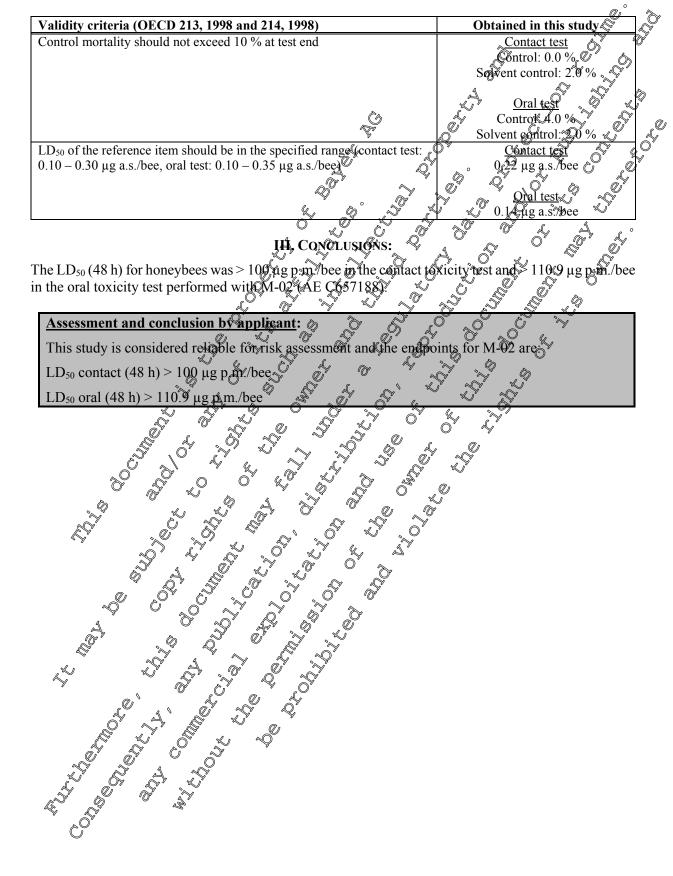
Results are averages from foe replicates (ten bees each) per dosage / control

Water = Water  $c_{ov}$  frol, solvent =  $c_{ov}$  to the treated control



Validity criteria:

All validity criteria of the test were met.





Data Point:	KCA 8.3.1.1.2/01
Report Author:	
Report Year:	
Report Title:	Amendment no. 2 to final study report - Contact toxicity (CD50) to hone bees
	(Apis mellifera L.) - Substance pure Code: AE C638206 00 1B99 0002
Report No:	CW00/065
Document No:	<u>M-200506-03-1</u>
Guideline(s) followed in	EPPO Guideline No. 170 (1992)
study:	
Deviations from current	Current Guideline: OECD 21 (1998)
test guideline:	Triazophos was used as reference item instead of dimethodie as recommended in
	the guideline. The test was conducted at a temperature of 22 – 28.5°C, batside the
	range of $25 \pm 2^{\circ}$ C recommended in the guideline. These deviations are not
	expected to have impacted the study results 2
Previous evaluation:	yes, evaluated and accepted a gradient of the second secon
	in DAR (2005) 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
GLP/Officially	Yes, conducted index GLP/Officially recognized testing facilities
recognised testing	
facilities:	
Acceptability/Reliability:	Yes Q X X X X X
E	
Executive Summarv	

#### Executive Summary

The purpose of this study was to determine the acute contact, toxicity of hopicalide technical to the honeybee (A. mellifera L.) in the laboratory. Mortality of the bees was used as toxic endpoint. Therefore, under laboratory conditions Apis mellifera worker bees were exposed to the test item, reference item or control by topical application of a single cose of 0 µL to the ventral thorax. The solvent control bees were treated with 10 µL dimethy sulfoxide (DMSO). The five dose vates of the test substance prepared in DMSO were \$, 5, 10, 50 and 100 µg a.s bee. Each treatment group consisted of 5 replicates (test units) with 10 bees per replicate. The tests were conducted in tharkness, temperature was 22 – 28.5°C and relative humidity was between 53 and 70 %. The number of dead bees in each cage was assessed 24, 48 and 72 hours after application. Contact LD₅₀ values were calculated with the aid of SAS probit analysis After 72 hours between 3 and 8 dead bees were found in the five test concentrations. In the control 3 dead bees were (found after  $\mathcal{D}^*$  hours? After 72 hours 44 bees were dead in the highest concentration with the reference substance. The LD₅₀ of the reference item was calculated to be 0.113  $\mu$ g /bee. All validity criteria of the test were met. The LD₄ (72 h) for honeybees was > 100  $\mu$ g a.s./bee in the contact foxicity fest performed with Ruopicolide.

# I MATERIAL AND METHODS

Test item: Fluopicotide technical 99.3% w/w origin batch no.: R001737, Identification code: AE C638206 00 1B99 0002, Certificate of Analysis: C/030/2000 (dated 05 April 2000).

Test organism: female worker Honeybees (pis mellifera), obtained from a healthy and queen-right colony.

Under laboratory conditions Apis mellifered worker bees were exposed to the test item, reference item or control by opical application of a single dose of 1.0 µL to the ventral thorax. The solvent control bees were treated with 1.0 µL dimethylsulfoxide (DMSO). The five dose rates of the test substance prepared in DMSO were 1, 5, 10, 50 and 100 µg a.s./bee. The reference item (triazophos 41.1% w/w) prepared in water was tested in 3 dose rates of 0.2, 0.3 and 0.4 µg product/bee. Before application, the bees were slightly avaesthetized with CO₂.

Each treatment group consisted of 5 replicates (test units) with 10 bees per replicate. Test units were 12-13 cm high cylindrical test cages with a diameter of 5 cm.

The tests were conducted in darkness, temperature was  $22 - 28.5^{\circ}$ C and relative humidity was between 53 and 70 %. The number of dead bees in each cage was assessed 24, 48 and 72 hours after application.



Contact LD₅₀ values were calculated with the aid of SAS probit – analysis.

#### Dates of experimental work: August 09, 2000 – August 12, 2000

#### **II. RESULTS AND DISCUSSION:**

#### **Biological findings**

Test substance	Endpoint	Ĉa
Fluopicolide	24-72 h LD ₅₀ [µg a.s./bee]	> 100
Reference item	72 h LD ₅₀ [µg product/bee]	0,274
Reference item	$72 \text{ h LD}_{50} [\mu \text{g product/bee}]$	0,2,/4

#### Observations

Ø One mortality occurred after 24 h in the highest test concentration. After 48 hours between O and 3 dead bees were found in the four highest test concentration. After 72 hours between 3 and 8 dead bees were found in the five-test concentration. In the control no mortality of curred after 48 hours and 3 stead bees were found after 72 hours. The test with the reference itero resulted in high mortalities in the two highest test concentrations after 24 hours. After 72 hours 44 bees were dead in the highest concentration with the reference substance.

Total number of dead brees (and mostal	ity in %) after
	~ 72 m
	Ó
	× 3
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	\$ \$ 8
	∛՝ 4
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	5
	6
Deference it m [u(Greduat/heat)] / m / / a /	
$\begin{array}{c c} \hline \mathbf{Reference} & Referenc$	5
$\begin{bmatrix} 0.2 \\ 0.3 \\ 0.4 \end{bmatrix} \xrightarrow{\sim} \begin{bmatrix} 1 \\ 0 \end{bmatrix} \xrightarrow{\sim} \\ \begin{bmatrix} 1$	30
	44

Validity criteria: All validity criteria of the test  $\mathcal{A}$ 

Validity criteria (OECP 214, 1998) Validity criteria	Obtained in this study
Control mortality should not exceed 10 % at test end	Control: 3 %
$LD_{50}$ of the reference item should be in the specified range	0.113 µg a.s./bee*
(dimethoate: contact test 0.10 0.30 (g a.s./bee)	(a.s. triazophos)

*0.274 µg product/bee × 41 % w/w triazophos

The reference item triazophos confirmed the consitivity of the bees used in the test.

## JII. CONCLUSIONS

for honeybees was  $> 100 \ \mu g$  a.s./bee in the contact toxicity test performed with The LD₅ fluopicolide

#### Assessment and conclusion by applicant:

This study is considered reliable for risk assessment and the endpoint is:

LD₅₀ contact (72 h)  $> 100 \mu g a.s./bee$ 



Data Point:	KCA 8.3.1.1.2/02			
Report Author:				
Report Year:	2015			
Report Title:	Fluopicolide tech.: Effects (Acute contact) on bumblebees (Bombus terrestris			
hepoirt inte.	in the laboratory			
Report No:	88641105			
Document No:	M-511408-01-1			
Guideline(s) followed in	(GLP compliant study based on van der Steen (2001) and OECD 214(1998) with			
study:	modifications and adaptions, Ring test bumblebee acture contact tox only (ICPPR			
2	non-apis group, 2014))			
Deviations from current	Current Guideline: OECD 246 (2017)			
test guideline:	A 5 $\mu$ L droplet was chosen in deviation to the Quideline recommendation of $\sqrt{2}$			
-	μL droplet, since a higher volume ensured a priore reliable dispersion of the fest			
	item and allows a higher application dose. Analytical determination of the test			
	item was not conducted, but the study was conducted before guideline			
	implementation and no analytogal dose werification was foreseen at that point in			
	time. Since it is a limit test with a single dosing of the test derived the deviation is			
	not expected to have impacted the study results.			
Previous evaluation:	No, not previously submitted a contract of the			
GLP/Officially	Yes, conducted under GLP Officially recognised testing facilities			
recognised testing				
facilities:				
Acceptability/Reliability:	Yes a star of			
Executive Summary				
·				

 $\mathbb{O}^{\vee}$ The purpose of this study was to determine the acute contact to xicity of fluopicolice tech. to the bumble bee (Bombus terrestrist.) in the laboratory. Mortal of of the bumble bees was used as the toxic endpoint. Sublethal effects, such as changes in behaviour, were also assessed. L,

Therefore, under laboratory conditions Bombus terrestits worker bumble bees were exposed to 100 µg a.s. per bumble bee by topical application. Furthermore, the test consister of a control, solvent control and a reference tem group. Each treatment group consisted out of 50 bumble bees with 1 bumble bee per test unit (replicate). The test was conducted in darkness, temperature was 24-26°C and humidity was 50-63 %. Biological observations, including mortality and sub-lether effects were recorded 4, 24 and 48 h after application. The NOED was estimated using Fisher Exact Pest. At test termination no mortality occurred at 100 µg fluopicotide tech. a.s per buntste bee. 2.0 % mortality occurred in the water control group and there was no mortality in the solvent control group (acetone). The mortality in the reference item group was 64% after 48 bours at a dose of 12 ug dimethoate/bumble bee. All validity criteria of the test were not. The 48 h) contact  $LD_{50}$  was decrimined to be > 100 µg a.s./bumble bee.

# I. MATERIAL AND METHODS

Test item. Fluopicolide, technical: 100.5 % //w (analytical), Origin Batch No.: ETP000273, Customer Order No.: TOX 10747-06 Material: Europicorde, technical; Specification No.: 102000016444-01, Article No.: 06032698

Article No.: 06032698. Test organism; Cemale worker bumble bees (B. terrestris), obtained from a healthy and queen-right colony, bred by a commercial bumble bee breeding company (Biobest Belgium N.V.).

Under laboratory conditions Bombus torestris worker bumble bees were exposed to 100 µg a.s. per bumble bee by topical application (contact limit test).

Furthermore, the test consisted of a control, solvent control and a reference item group. In the contact limit test tap water containing 0.1% v/v Triton X-100 was used.

BAS 152 PI I EC (active ingredient 400.9 g/L dimethoate, Batch no.: FRE-000926) was used as reference test unit reference test unit for the set of 50 bumble bees with 1 bumble bee per test unit (replicate).

Test units were cylindrical, latticed plastic cages with a length of approximately 7 cm and a diameter of 2.2 cm at the large and 1.7 cm at the small opening.



The test was conducted in darkness, temperature was 24-26°C and humidity was 50-63 %. Biological observations, including mortality and sub-lethal effects were recorded 4, 24 and 48 h after application. The NOED was estimated using Fisher Exact Test (pairwise comparison, one-sided greater,  $\alpha = 0.05$ ).

Dates of experimental w	ork: D	ecember 02, 2	014 – Dece	mber 04, 2014	1 >>	
		U. D	Dec ce		- And	
		II. RESULTS	SAND DISCU	USSION:	2	S S
<b>Biological findings:</b>			ĈA	, d		
T				Fluopicofide		
Test item Test object			~	Bombus terr	tech.	<del>à</del> s
Exposure				Contag		9, 3 <del>7</del> ,
Enposure			and "	(sofațion in@c	etonê) 🗸 🕻	
Dose [µg a.s./bumble bee	2]	(j		.~ 100	JO D	
LD ₅₀ [µg a.s./bumble bee		Ô		<u>لَمْ الْمَارِي الْم</u>	ð Â	
LD ₂₀ [µg a.s./bumble bee			<u> </u>	20×100C	× ~ (	
LD ₁₀ [µg a.s./bumble bee		Ś,		> 100	<u>, Ô^y «, , , , , , , , , , , , , , , , , , ,</u>	
NOED [µg a.s./bumble be	ee]**	in the work of the se		<u>× × × × × × × × × × × × × × × × × × × </u>		
* Since no mortality above 10%	6 occurred	in the test, the re			simed to be $> 10$	ong a.s./bumble
** The NOED was estimated u	using Fishe	er Exact Pest (p	airwise compa	rison with contro	ol, one-sided gre	ater, & # 0.05).
	- (			S L		×
bee ** The NOED was estimated of <b>Observations</b> <u>Contact test:</u> (40.1)	~~~~	× '0'	ð L		Ŭ Õ	×
Contact test:	Ś	«. Š	k m	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	. 6	9
Contact test: At test termination (48 hg	Que effe	Oractempt) no	Ø mortelity o	asterrack 10	A flux	lida taab a a pa
bumble bee. 2.0 % mortal	lity of	real in the aver	tor about 1	Counted at 10	o ug nuopice	10/ y/y Triton V
100) and there was normal	nty occu rtabity in	n the solvent of		(actione)		170 V/V 1111011 A
			Sharon Broat		A.	
	AFF	r 4 hours 🔊	After	324 hours 🛛	After A	48 hours
					~	Behavioral
Treatment Group S N	Aor Pality	Behavioral abnormalities	Mortality	Behavioral abnormatitie	Mortality	abnormalit
Ča.					-5	ies
	nean 🌮	mean %	mean %		mean %	mean %
Test item			, O. 🤝			
100 μg a.s./bumble	0.0		0.0	۵.0 ^{کری} ا	0.0	2.0
bee N	(0/ 1		, 0,			
Water control			2.0	<u> </u>	2.0	0.0
	0.0	0.0	2.0	0.0	2.0	0.0
Solvent Conterl	0.0	0.0	Ô°nn Ø	0.0	2.0 0.0	0.0 0.0
Reference item			Ô°nn Ø			
Reference item		0.0	Ô°nn Ø			
Reference item 12 μg dimethorite/bumble			à la casa da cas	0.0	0.0	0.0
Reference item 12 μg dimethoate/bumble bee			Ô°nn Ø	0.0	0.0	0.0
Reference item 12 μg dimethoate/bumble bee Mean = mean of 50 mdividu Water control = tap water co		Atment group	0,0 0° 28.0 27	0.0	0.0	0.0
Reference item 12 µg dimethoate/bumble bee Mean = mean of 50 individu		Atment group	0,0 0° 28.0 27	0.0	0.0	0.0
Reference item 12 µg dimetheatte/bumble bee Mean = mean of 50 individu Water control = tap water of Solvent control @ acetone		Atment group	0,0 0° 28.0 27	0.0	0.0	0.0
Reference item 12 µg dimetheatie/bumble bee Mean = mean of 50 individu Water control = tap water of Solvent control @ acetone Validity criteria:	0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0	Amenteroup 18.0 Amenteroup 1% Triton X-00	0,0 0° 28.0 27	0.0	0.0	0.0
Reference item 12 µg dimetheatie/bumble bee Mean = mean of 50 individu Water control = tap water of Solvent control @ acetone	0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0	Amenteroup 18.0 Amenteroup 1% Triton X-00	0,0 0° 28.0 27	0.0	0.0	0.0
Reference item 12 µg dimetheatte/bumble bee Mean = mean of 50 individu Water control = tap water of Solvent control @acetone Validity criteria:	0.0 0.0 0.0 0.0 0 0.0 0 0 0 0 0 0 0 0 0	Atment group 1% Trifon X-00 2 e met.	0,0 0° 28.0 27	0.0 72.0	0.0	0.0 36.0

Validuty cruteria (QL/CD 246, 2017)	Obtained in this study
Control mentality should not exceed 10 % at test end	Control: 2.0 %
	Solvent control: 0.0 %
Mortality of the reference item should be $\geq 50$ % at test end	Reference item: 64 %



#### **III. CONCLUSIONS**

The toxicity of fluopicolide tech. was tested in an acute contact toxicity test on bumble bees. The (48 h)contact LD₅₀ value was determined to be  $> 100 \ \mu g$  a.s./bumble bee.

#### Assessment and conclusion by applicant:

Assessment and conc	lusion by applicant:
This study is considered	ed reliable for risk assessment and the endpoint is: $\sqrt[3]{2}$
•	
$LD_{50} \text{ contact } (48 \text{ h}) > 1$	100 μg a.s./bumble bee
~	
CA 8.3.1.2 Chro	Inic toxicity to bees     A     A       KCA 8.3.1.2/01     A     A
Data Point:	KCA 8.3.1.2/01
Report Author:	
Report Year:	
Report Title:	Fluopicolide SC 486 G - Assessment of effects on the adult honey bee, April
	mellifera L., in a To day chronic feeding test under laboratory conditions
Report No:	S15-00366
Document No:	<u>M-552253-0-1</u>
Guideline(s) followed in	No specifi@juidelites avaitable
study:	Based on Kling, A. and Schmitzer, S. (2015)
Deviations from current	
test guideline:	Study. Current Guideline: DECD 245 (2017) The relative humiority was 36.7% for a short period of 2 hours, below the range of
	The relative humidity was 36.7% for a short period of 2 hours, below the range of
~	50 - 70% recommended in the guideline.
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	This deviation is not expected to have impacted the study results.
Previous evaluation:	No port previously Submitted
GLP/Officially	Yes, conducted ander GLP/Qfficially & cognised testing facilities
recognised testing	Yes, conducted inder GLP/Officially @cognised testing facilities
Acceptability/Reliability:	

Executive Summary:

Ő Ŝ ° The purpose of this study was to determine chrome oral toxicity of fluopicolide SC 486 (486 g/L) to the honey bee (A. mellifera L.) for a period of ten days. Mortality of the bees was used as the toxic endpoint. Sublethal effects, such as changes in behaviour, were also assessed.

Therefore, under laboratory conditions 50 newly hatched worker bees divided into 5 replicates, containing 10 test organisms were exposed for 10 days to a concentration of 3000 mg a.s./kg food of the test item treated sugar solutions ad libraum. An untreated control and a reference item with the same number of replicates and bees were included in this study. In addition, 4 additional cages were filled with $\approx 3-4$ mL of pute 50 % (w/v) aqueous sucress solution and weighed daily for the determination of the evaporation. Mortality and behavioural apportanties were assessed every day throughout the 10 days exposure period. The test conditions during the study were 31.7 - 34.6 °C temperature, 36.7 -64.2% relative humidity and 24 h darkness. At test end, 10 days following start of exposure, 2.0 % mortality occurred in the intreated water control. At 132.68 µg a.s./bee/day 4.0 % mortality occurred. This effect/was statistically significant. The reference item at 0.02 µg/bee per day caused 100 % mortality at day 10. All valueity criteria were met in this study. The LDD₅₀ value (10 days) was determined to be > 152.68 ug a.s./bee per day. The LC₅₀ value (10 days) was determined to be > 3000 mg a s./kg freding solution.



I. MATERIAL AND METHODS

Test material: Fluopicolide SC 486 G (486 g/L); Short code: FLC SC 486; Fluopicolide: 40.1 % w/w, 486.5 g/L (analysed); Batch ID.: 2014-014197; Sample description: TOX10894-00, Specification Store and Sto 102000011893; density: 1.213 g/mL.

The chronic effects of the test item Fluopicolide SC 486 on the honey bee, Apis melliferand were assessed in a 10 days continuous oral feeding test in the laboratory (limit test) Under laboratory conditions 50 newly hatched worker bees (Apis mellifera L., 1 to 2 daysold) divided into 5 replicates, containing 10 test organisms were exposed for 10 days to a concentration (3000 mg/ a.s./kg food [ppm]) of the test item treated sugar solutions ad libitum. An untreated control (50 % yev sucrose solution) and a reference item (0.9 mg dimethoate/kg feeding solution [ppm]) with the same number of replicates and bees were included in this study. Mortality and behavioural abnormalities were assessed every day throughout the 10 days exposure period. Q The test conditions during the study were 31.7 - 34.6 °C temperature % Delative humidity

Dates of work: June 09, 2015 to June 23, 2015

II RESULTS

and 24 h darkness.

Analytical results: The actual concentrations of fluorecolide in the feeding solutions were in a range of 95% - 106%. Therefore the concentrations of the test formula of te Therefore, the concentrations of the test them in the dief were confirmed and the endpoints are based on 0 N nominal concentrations.

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

Biological results:

The test item was faily administered to the bees in Sugar Solution at the following concentrations: 3000 mg a.s./kg sugar solution / This concentration fed to a daily frean dose of 132.68 µg a.s./bee per «Ô day after 10 days. ~@ ~~

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At test end 10 days following start of exposure, 2.0 % mortality occurred in the untreated water control (50 % x sucrose solution) At 3000 mg a.s./kg (corresponding to 132.68 µg a.s./bee/day) 4.0 % mortality occurred. This effect was statistically significant (Eisher's Exact Test, $\alpha = 0.05$). In the test item treatment group no remarkable behaviooral altoormalities were observed. The reference item (dimethoate) at a concentration of 0.9 mg dimethoate/kg sugar solution corresponding to 0.02 µg a.s./bee

(dimethoate) at a concentration of 0.9 mg dimethoate/k per day caused 100 % mortabily at day 10.0



Obtained in this study

Chronic oral toxicity of	Fluonicolide SC 486 to	voung honey bees	(laboratory test)

Test Object		Apis mellifera carnica	0
Treatment Group	Concentration [mg a.s./kg]	Dose Level ¹⁾ [µg a.s./bee/day]	Mortality at day 10 ²
Fluopicolide SC 486 (486 g/L)	3000	132.68	
Water control	0.0	0.0	200 (-2.1) ³
Reference Item (dimethoate)	0.9	0.02	
Endpoint at test term	ination (day 10)	A.	
LC50	LDD ₅₀	NOEC 4	
> 3000 mg	> 132.68 µg	2000 mg a.s./kg	102.68 µg a.s./bee/day
a.s./kg	a.s./bee/day		

¹⁾ mean dose per bee per day; dose measured based on consumed feeding solution

 ²⁾ Mortality at study termination 10 days after start of first feeding
 ³⁾ Mortality corrected with the corresponding control morality according to Schneider-Oref ı negati means lower mortality in the test item treatment compared to the control group

⁴⁾ Fisher's Exact Test with Bonferroni Correction (one-sided greater, 2 0.05)

The determination of LC_{10}/LD_{10} and LC_{10}/LD_{20} values has not been possible in this study since it performed as a limit test and due to the low mortality observed in the test item. was

All validity criteria were met in this study

Validity criteria (OECD 245, 2017

Ľ Average mortality at test end in control: <45% 2.0% Ø

Average mortality at lest end in taxic reference item: 100%

III. CONCLUSIONS l Ø

Ş The chronic to acity of luopicolide S 4860(486 g/L) was tested over 10 days on adult honeybees. The LDD₅₀ value (10 days) was determined to be > \$2.68 pg a.s bee per day. The LC₅₀ value (10 days) was determined to be > 3000 mg/a.s./kg/feeding solution.

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Assessment and conclusion by applicant:

This study is considered reliable for risk assessment and the endpoints are:

with the set of the se



CA 0 2 1 2	Effects on honorhood avalanment and other honorhoolife stars
CA 8.3.1.3	Effects on honeybee development and other honeybee life stages

Data Point:	KCA 8.3.1.3/01
Report Author:	. Č
Report Year:	
Report Title:	Fluopicolide - Honey bee (Apis mellifera L.) 22 day larva foxicity test (Repeated)
	exposure) - Final report
Report No:	S17-00177
Document No:	<u>M-615695-01-1</u>
Guideline(s) followed in	Regulation (EC) No 1107/2009 (2009) Directive 2003-01 (Canada/PMRA) US EPA OCSPP 850.SUPP OECD (2016): Series on Testing and Assessment Number 239: Guidance Document on Honey Bee (Apis mellifera) Larval Poxicit Test, Bepeated
study:	Directive 2003-01 (Canada/PMRA)
	US EPA OCSPP 850.SUPP is a construction of the
	OECD (2016): Series on Testing and Assessment Number 239: Guidance
	Document on Honey Bee (Apis mellifera) Larval Poxicit Test, Repeated
Deviations from current	Method: Deviations from current guideline SANCO/3029/99 Sev.4:
test guideline:	Limited sets of validation recoveries were malysed. However, the average
	recoveries were within the acceptable range of 70–110% and the RSD values
	were below 20%. The analytical method can be regarded as fit for purpose.
	Study: Current Guideline: OF&D GD239 (2016)
	The deviations from the recommended humidity ranges recorded for more than 2
Duariana analization.	hours on day 8, 9 and 18 are not expected to have impacted the study results.
Previous evaluation:	No, not previously submitted of 50 00 00 10
CI D/Officially	V & Lunder CLD & Claim and Annual
GLP/Officially	Yes conducted under GLP/Officially recognised aesting facilities
recognised testing facilities:	
Acceptability/Reliability:	Yes A G A G A G A G A G A G A G A G A G A

Executive Summary The purpose of this study was to determine the chronic toxicity (ED10/20/50, EC10/20/50, NOED/NOEC and LOED/LOEC for adultemergence up to day 22) of the test item Muopicolide applied to the honey bee, Apis mellifera L., landae in an in vitro test after repeated oral application. The test item was administered to the larvacat a constant concentration in the diet according to their growth at a range of five increasing doses. Comulative mortalities of honey bee larvae treated with the test item were assessed daily from day 4 to day 8. Cumplative mortalities during the pupation phase were assessed on day 15 and on day 22. All mortalities were compared to the control. The adult emergence rate was assessed on day 22. On day 8, larval mortality was 6.3% in the control group and 9.3% in the solvent control group. Larval mortality in the reference item group was 100 %. The largel mortality was 8.3, 8.3, 12.5, 14.6 and 22.9 % at cumulative doses of 3 06, 7.52, 15.0, 30.0 and 60.1 ug fluopicolide/larva per developmental period, respectively. On day 22, the addifference rate in the control and solvent control group was 83.3 and 79.2 %. Consequently, validit criteria for the control groups and the reference item group were met and the test was considered valid. The adult emergence rates were 81.3, 79.2, 72.9, 70.8 and 60.4 % at cumulative doses of 3.76, 7.52, 5.0, 20.0 and 60.1 µg fluopicolide/larva per developmental period, respectively.

The NOEC for adult emergence on day 22 was determined as \geq 390 mg fluopicolide/kg diet, equivalent to a NOED $g \ge 60$. If g floopicolide/lar a per developmental period. The EC₁₀ for adult emergence on day 22 was determined as 175° mg fluopicolide/kg diet, equivalent to an ED₁₀ of 27.0 µg fuopic@ide/larva peodevelopmental period. The EC20 for adult emergence on day 22 was determined as 16 mg fluppicolide/kg diet, equivalent to an ED₂₀ of 48.7 µg fluppicolide/larva per developmental period. The EC₅₀/ED₅₀ for larval mortality on day 22 could not be calculated due to the lack of inhibition in emergence above 50 %. However, the EC_{50} is empirically considered to be > 390 mg fluopicolide/kg diet, equivalent to an ED₅₀ of > 60.1 μ g fluopicolide/larva per developmental period.



I. MATERIAL AND METHODS

Test item: Fluopicolide; Batch No.: FP805026, Sample description: TOX203703-01, Specification No.: 10200001644-01, Analysed purity a.s.: 98.7 % w/w, Certificate No.: AZ 21472. Test species: Honey bee (*Apis mellifera carnica* (Pollmann)), synchronized first instar (L1, one day old) arvae originating from healthy (free of clinical symptoms of any disease) and queen-right bee (*Apis mellifera carnica* (Pollmann)).

The larvae were taken from hives that had not received treatments with chemical substances for at least one month. The test was conducted at Eurofins Agroscience Services Ecotox GmbH Neulingen-Göbrichen, Nordweg 10, 75245 Neulingen-Göbrichen, Germany.

Test design: Dose response test with a duration of 22 days from grafting on day 1 to the final assessment on day 22. From day 3 until day 6 of the test, five different concentrations of fluopic fide douted in the larval food (aqueous yeast/sugar solution mixed with royal jelly 1:1 (W/w)) were feel to larvae of the test item groups and one single concentration of the reference item dimethoate was ted to the larvae of the reference item group with diet B or C. After the applications, no additional feeding of the harvae took place.

The analysed purity was considered for the calculation of the test item and reference item concentrations; the daily feeding volume increased from 20 μ L to 50 μ L diet per larva over the application period. The cumulative feeding volume from day 3 until days of 140 μ L diet per larva and the density of the diet (1.1 g/cm³) were considered for the calculation of the cumulative doses per larva. A control group was included in the test and exposed for the same period of time under identical exposure conditions to the water treated artificial diet. Each treatment group consisted of 48 larvae from three different colonies (each colony representing a replicate). Assessment of larval mortality was performed out day 15 and day 22. Other observations and, any other adverse effects were qualitatively recorded to aid in the interpretation of mortality in comparison for the solvent control group. Assessment of adult emergence was performed on day 22. The presence of unearen food was qualitatively recorded on day 8.

Test concentrations: One control group; one colvent control group; 5 test item proups with 24.4, 48.8, 97.5, 195 and 390 mg fluopicalide/kg diet, equivalent to cumulative doses of 3.76, 7.52, 15.0, 30.0 and 60.1 µg fluopicolide larva per developmental period; dimethoate reference item group with 48 mg dimethoate/kg diet, equivalent to a cumulative dose of 7.39 µg dimethoate/larvae per developmental period.

Dates of work: August 09, 2000 August 12 3000

II. RESPLTS AND DISCUSSION:

<u>Analytical results</u>: The mean measured concentrations of the test item in the larval diet were within $\pm 20\%$ of nominal for each test item group. Therefore, the concentration of the test item in the larval diet were confirmed and the endpoints are based on nominal concentrations.

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

Biological resoluts:

On day 8, lassal mortality was 6.3 % in the control group and 8.3 % in the solvent control group. Larval mortality in the reference item group was 100%. The larval mortality was 8.3, 8.3, 12.5, 14.6 and 22.9 % in the test item groups of 24.4, 48.8, 97.5, 195 and 390 mg fluopicolide/kg diet, equivalent to cumulative does of 3.76, 4.52, 15.0, 30.0 and 60.1 µg fluopicolide/larva per developmental period, respectively



On day 22, the adult emergence rate in the control and solvent group was 83.3% and 79.2%. The adult emergence rates were 81.3, 79.2, 72.9, 70.8 and 60.4% in the test item groups of 24.4, 48.8, 97.5, 195 and 390 mg fluopicolide/kg diet, equivalent to cumulative doses of 3.76, 7.52, 15.0, 30.0 and 60.1 gg fluopicolide/larva per developmental period, respectively. Compared to the control group the adult emergence rate on day 22 was not statistically significantly different in any test item group (Multiple® Chi²-test with Bonferroni-Holm adjustment, one-sided greater, $\alpha = 0.05$). During the assessments of mortality and emergence no other test item related observations such deviating sizes, appearances and malformations of the test organisms were made. in Juding On day 8, uneaten food was observed in the control groups and all test item groups. Results for larval mortality until day 8, as well as for adult emergence on day corresponding endpoints are presented in the following table.

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Treatment	Conce	entration	Cumu	latiye Dose 🚬 🛛		l Mortality on		Emergence
Group					Day 8		on Da	- 41 .
					[%]Q	Corrected [%]	[%	Inh ib ition ^b
Control			\$,~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~			\$83.3 _×	5
Solvent Control			Ð.		/8.3 🤸		79, 2 🖓	· 0°
	24.4	Â	9.76 <i>i</i>		820	0.0 2 5	<u>8</u>	-2,9
Tractitan	48.8	[mg	7.52	[µg fluopicolide/	S.		7 9.2	× •
Test item (Fluopicolide)		fluopicolide/	15.0	large per developmental	12.5	¥.6 ° °	72.	8.0
(Pruopiconde)	195	kg diet)*	30.0	period ^{C, d}	14.6	6.9	70.8	10.6
	390	, O	60.1		22.9		60.4	23.7
	1	[mg	Č)	[us dimethoate/	× (L			
Reference Item (Dimethoate)	48.00		π^{2}	larva per sy developmental	100 [°]	100 2		
(Dimethoate)		kg diet] 🕻 🔗	Å.	marriad c d	ĮU Į	л. <i>О</i> г		

Mortality and other observations of larvae in the repeated exposure to

Statistical evaluation of non-emergence b

Negative valueSindicate higher emergence compared to the solvent control group. Ø

с Based on the analysed purity

Based on the cumulative feeding volume from day 3 $intil day \sqrt{5}$ of 140 μL diet have and a density of the diet of d 1.1 g/cm8 C

Calculated endpoints of the repeated exposure larvae toxicity test

Treatment	Endpoint: Successful adult emergence	Up to day 22
	ED50 [µg.a.s./larva]	> 60.1 b
	D ₂₀ [ag a.s./]agva]	48.7
Test itemodoses	ED ug a. Marval	27.0
Test itemodoses	LOED [ug/a.s./lanva]	n.d. a
	NOED hug a.s. arva	≥ 60.1
	EC [/[mg a \$//kg food]	> 390 b
	Ke ₂₀ [mg/a.s./kg food]	316
Test item	DEC10 [mg a.s./kg food]	175
Test item	LOOC [mg a.s./kg food]	n.d. a
	NOEC [mg a.s./kg food]	≥ 390
a The LOE LOED could no	be determined due to the lack of statistically	significant effects (Multiple Chi ² -test
with Bonterroni-Holm adjus	tment, one-sided greater, $\alpha = 0.05$	

^b The \mathbb{R}_{V50} /ED₅₀ could not be calculated due to the lack of inhibition in emergence > 50%, but can be regarded as above the highest concentration/dose tested.



<u>Validity criteria:</u> All validity criteria were met in this study.

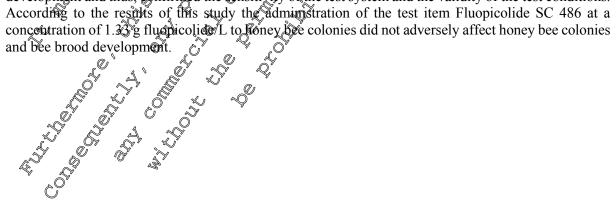
All validity criteria were met in this study.	_ 0
Validity criteria (OECD GD 239, 2016)	Obtained in this study
Cumulative larval mortality from day 3 to 8 in control/solvent control: $\leq 15\%$	6.3% / 8.3%
Mean adult emergence rate on day 22 in control/solvent control: \geq 70%	83 % / 79.2% °
For reference item dimethoate larval mortality at day 8: \geq 50%	100%
III. CONCLUSIONS In a repeated exposure honeybee larval feeding toxicity study with thropidays, the NOED (emergence) was determined to be $\geq 60.1 \ \mu g$ a sharva $\geq 390 \ mg a.s./kg food.$	equivalent to NORC of
Assessment and conclusion by applicant: Assessment and conclusion by applicant: This study is considered reliable for risk assessment and the endpoints at NOED (emergence) ≥ 60.1 µg a.s./kgfood NOEC (emergence) ≥ 390 mg a.s./kgfood Assessment and conclusion by applicant: NOEC (emergence) ≥ 390 mg a.s./kgfood Assessment and conclusion by applicant: Assessment and conclusion by applicant: NOEC (emergence) ≥ 390 mg a.s./kgfood Assessment and conclusion by applicant: Assessment and conclusion by applicant by appli	
	ξ ^Y D



Data Point:	KCA 8.3.1.3/02
Report Author:	
Report Year:	2016
Report Title:	Fluopicolide SC 486 (486 g/L): Effects on honey bee brood (Apis mellifera L
	Brood feeding test
Report No:	98781031
Document No:	<u>M-545732-01-1</u>
Guideline(s) followed in	Oomen P.A., de Ruijter, A. & van der Steen, J., 1992
study:	Regulation (EC) No. 1107/2009
	Directive 2003-01 (Canada/PMR)
	US EPA OCSPP not applicable 🕅 🖉 🖉 🖉
Deviations from current	Current Guideline: Oomen at al. (1992)
test guideline:	No deviations
Previous evaluation:	No, not previously submitted
GLP/Officially	Yes, conducted under GLP/Officially Decognised testing facilities
recognised testing	
facilities:	
Acceptability/Reliability:	Yes the rest of th

Executive Summary

Executive Summary The purpose of the honey bee brood feeding study was to evaluate potential effects of Fluopicolide SC 486 on brood development and mortanty of adult worker boney bees, Apis mellifera L. (Hymenoptera: Apidae). Therefore, a bee brood test was conducted under natural field conditions with a control, 3.23 g test item and the reference item. Hour bee colonies were used per treatment group. Each colony contained two magazines with 12 frames each. The mean strength of the colonies per treatment group, one day before application ranged between 24805 and 16211 adult bes. At the start of the experiment, each colory had 8 - 15 brood combs containing eggs, darvae and capped cells and a sufficient amount of Money and popen. 12-year old healthy sister queens were used. Ontogenesis of a defined number of hones bee eggs, young and old larvae were observed for a period of 21 days following the application for each treatment group and colony. Mortality of adult bees, pupae and larvae was also assessed between 3 Pays before to 21 days after application. Statistical evaluation was done for mortality and brood termination rates using XXRat Professional No effect of the test item on the development of eggs and young larvae was observed. The mean termination rate of eggs and the mean development success of larve was slightly but for significantly higher in the treatment than in the control. The mean termination rate of oldarvae was lower in the test item treatment group than in the control group. Adalt bee mortality in the test it m group was significantly higher when compared to the control group. This waginterpreted as random fluctuation and as not biologically relevant in relation to colony strength. No effects of the test item on honey bee pupae and larvae were observed. The reference item treatment resulted in statistically significant ingrease of unsuccessful egg, young- and old larvae development and thus, confirmed the consistivity of the test system and the validity of the test conditions. According to the results of this study the administration of the test item Fluopicolide SC 486 at a





I. MATERIAL AND METHODS

Test material: Fluopicolide SC 486 (486 g/L), Specification No.: 1020000118893, Supplier Batch No.: 2014-014197, Sample description: TOX10894-00, density: 1.213 g/mL, content of active ingreduent: 40.1% w/w, 486.5 g/L (analysed). 0

Test species: Apis mellifera carnica L. Honey bee colonies, maintained according to normal beekeeping practice, by ibacon's responsible beekeeper. No varroacide has been used in the colonies for at least 4 weeks prior to the experimental starting date. Colonies were well fed and queen-right, each colony occupied two magazines ("Deutsch Normalmaß, DN", with 11 frames each. Ar the start of the experiment, each colony had 8 - 15 brood combs containing eggs, larvae and capped ells and a sufficient amount of honey and pollen. The colonies were assembled at the same time with healthy discens a order. to guarantee uniform bee material in all treatments 2 year-old sister queens were used. The colonies contained a mean of about 14805 - 16211 adult honey bees. All colonies were equipped with a dead bee trap at the entrance.

Test design: The study included three treatments. Control (C, 1 D'ready-to-uss' sugar syrup (Apiin Cert) per colony, Test item (T, 3.23 g test item Fluopicolide SC 486 m 1 L Sugar solution (ready-to-uses) yrup, Apiinvert)), equivalent to an active sobstance concentration of K33 g fluopic Ade/L Sugar solution, Reference item (R, 3.2 g reference item (Insegar) in I L ready-to-use sugar syrup (Apipvert), equivalent to a nominal concentration of 0.8 @fenoxycarb/L.

For all treatments, 4 replicates (colonies) were set up 13 days before reatment (DAT -13). The treatment administration was conducted during the atternoon. The ready propared sugar solutions were offered per colony in a feeding trough. The trough was put into ancempty magazine on top of the populated bee magazines. Food uptake was complete \$2 hours after application. The colonies were monitored from day -3 to day 22 after application.

Test conditions during the experimental phase of this study took place at a settled, constant weather period with survey and moderate warm days. Mean temperatures over the course of the study ranged from 12, °C to 24.7 °C. Rain occurred only on a few occasions and mostly at the end of the experiment.

June 24, 2015 Dates of work: Ma

Validation of the stud

Control Brood Termination Rate

The mean brood terronation rates of eggs young Parvae and old larvae in the control group were: 11.5, 6.8 and 10.2%, respectively. The corresponding historic mean brood termination rates are: 23.8, 17.4 and 7.7%. Thus, the presented control brood termination rates were seen as within normal values compared with historic data A.

Control Mortali and Effects on Brood:

Mean control portality of the adult bees from day 0 after application to day 21 differed between 0.5 and 25.3 dead bees. As the overall mean mortality in the control group after application was low (8.3 dead bees/cology/day), this value can be empirically regarded to be within the range of normal mortality levels of colonies of the employed size under field conditions. In addition, a mean of 1.0 dead pupae per colony per day were found during the 21 days post-application period. This value can be considered to represent a biologically typical number of dead pupae over a period of 21 days.



Reference Item Mortality and Effects on Brood

There was a high number of impacted bee brood, which resulted in 89.5% mean loss of the initial observed cells (98.0% eggs, 94.5% young larvae and 76% old larvae stages, respectively). The termination rates of the different brood stages were statistically significantly higher compared to the control. Thus, the reference item values were on an absolute scale sufficiently high to demonstrate the sensitivity of the test system and the validity of the test conditions.

Effects of Fluopicolide SC 486 G (486 g/L) on honey bee brood

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Effects of Fluopicolide SC 486 G (486 g/L) on hone	y bee brood
Test item	Fluopheolide SC 486 K86 g/L)
Test species	Honey bees (Apis nollifera L.) (complete colonies)
Exposure	vid treated sugar solution
Treatment	Wintreated Fluopiconde SC 286 Reference Itom
	control (486 g/L) (Insegar, 30% = fewoxycarb)
Rate per L sugar solution [product] ¹)	3.32 g Test Item/LD 3.2 g/L
Rate per L sugar solution [a.s.] ¹⁾	1.33 g fluopicobele/L 0.8 ga.s./L
Termination rate of the eggs $[\%]^{2}$	11.5% 015.3% (n.s.) 98.0% (*1)
Termination rate of the young larvae [% γ	6.8% $3.8%$ $(n.s)$ $0.8%$ $0.5%$
Termination rate of the old larvae [%]	(10.2%) $(10%)$ $(1$
Mean brood termination rate over all stages	9.5% 00.5% (*)
Mean mortality of worker bees/colony/dag during	
pre-application phase ³	967 (n.s.) 9.8 (n.s.)
during the entire post-application phase ⁽³⁾	8.3 3 3 0 (*) 3 3 3 3 3 3 3 3 3 3
during the entire post-application phase ³) Mean mortality of pupae/colony/day during pre-application phase ⁴)	
	0.1 0.3 (hrs.) 0.0 (n.d.)
during the entire post-application phase 4	5.2 (*)
Mean number of bees before application ⁵⁾	16211

Statistics: n.s. = not statistically significant compared to the control = statistically significant compared to the control; n.d. = not determined (due to 0" response); Stadent or Welch t-test, a 0.05, parwise comparison, two-sided (before application), one-sided greater (after application)

¹⁾ test and reference item wore mixed in sugar solution
 ²⁾ mean termination rate of 4 colonies per treatment group
 ³⁾ mean number of dead honeybees per day and ealony frond in dead bee gaps

⁴⁾ mean number of dead puper farvae per day and colony found in dead over traps

⁵⁾ mean number of bees per colony

In-hive worker modality (dead bee traps)

The overall daily mean worker bee mortality observed before provisioning of the feeding solutions (DAT -3 to BOAT 0) was low and ranged from 9 1/ to 10.9 amongst the three treatments. No statistically significant differences between weatments were detected. n D

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The overall daily mean worker bee monthlity after provisioning of the feeding solutions (DAT 0 - 21) The overall daily mean worker bee mortality after provisioning of the feeding solutions (DAT 0 - 21) was 8.3, 31.1 and 32.5 in the control, test item and reference item treatment, respectively. Statistically significant differences were detected between control and test item and between control and reference item.



Pupal and larval mortality:

The overall daily mean pupal and larval mortality observed before provisioning of the feeding solutions (DAT - 3 - 0) was 0.1, 0.3 and 0.0 pupae/colony in the control, test item and reference item, respectively. No statistically significant differences between treatments were detected.

The overall daily mean pupal and larval mortality after application (day 0 - 3) of all treatments as 1.0, 1.6 and 5.2 in the control, test item and reference item treatment, respectively. As compared to the control, there was a statistically significant increase of pupae mortality in the reference item treatment throughout the entire post-application phase, but not in the test item treament.

Behaviour:

No behavioural impairments were noted at any time in any of the test or ference item reatment until test end. Also, no behavioural abnormalities were observed in the control group.

Uptake of feeding solutions:

ours in the control and test tem Feeding solutions were fully collected from the feeders with treatment and within 24 hours in the reference treatment.

The mean colony strength before treatment administration DAT (1) bees/colony in the control terror addition (DAT (1)) 4805 and 16211 bees/colony in the control, test item and reference item treatment grespectively.

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Brood Termination Rate

The mean Brood Termination Rate of the control, per item and reference item deatment, respectively, at the last assessment (BFD 2) were 115%, 155% and 98.0% for the second brood termination are of the control, test item and reference tem, respectively at the last assessment were (BFD 22) 6.8%, 9.8% and 94.5% (young larvas). For old larvae the mean brood termination rate at the last assessment (BFD_06) was 10.2%, 6.5% and 76.0%. The mean brood termination rates over all stages were 9.5% for the control, 10,5% for the test item and 8%5% for the reference item. Brood Termination Rates of eggs, young, old tarvae and over all stages did not significantly differ between test item and control replicates.

As compared to the control, in the reference item treatment Brood Termination Rate was statistically significantly higher for eggs young tarvae from BD 22 m wards), as well as for old larvae (both from BFD 16 onwards). Also, the Brood Termination Rate oper all stages was statistically significantly higher in the reference item treatment than in the control. This indicates that the test system was sufficiently sensitive to detect potential effects of plant protection products on honey bee brood.

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III. CONCLUSIONS

No effect on the development of eggs was observed after consumption of the test item treated sugar solution. The mean termination rate of eggs in the test item treatment group was slightly higher with a mean of 15.3 % compared to 11.5 % in the control group. This difference was not statistically significant compared to the control group.

There was also no effect on the development of young larvae after consumption of the test item viace treated sugar solution. The development success of the young larvae in the test item meatment group resulted in a mean termination rate of 9.8 % compared to 6.8 % in the control group. This difference was not statistically significant compared to the control group.

The mean termination rate of old larvae was lower in the test item treatment group (6.5%) when compared to the values of the control group (10.2%). Accordingly, this was not statistically significant different. Thus, there was no effect on the development of old larvae following the consumption of the test item via treated sugar solution.

Adult bee mortality in the test item treatment group was higher (mean of 31.0 dead bees per day) when compared to the control group (8.3 dead bees per day). Although this difference was starstically significantly different to the control, it is interpreted to be a random fluctuation since peaks in mortality occurred in two test item replicates only and since comparable peaks or mortality occurred also in two of the control replicates. Overall this low mortality rate is not biological relevant considering colony strength of about 12000 to 17000 bees per colony.

No effects of the test item on honcy bee pupe and larvae were observed. The reference item treatment (Insegar, a.s. = tenoxycarb) resulted in a statistically significant increase of unsuccessful egg, young- and old farvae development and thus confirmed the sensitivity of the test system and the validity of the test conditions.

Overall, it can be concluded according to the results of this study that the administration of Fluopicolide SC 486 (486 g/C) fortified sugar symp at a concentration of 1.33 g fluopicolide/L to honey bee colonies did not adversely affect honey bee colonies and bee broad development.

Assessment and conclusion by applicant:

This study is considered reliable for tisk assessment and the conclusions are:

No statistically significant effect on the Ovelopment of eggs. Brood termination rate for eggs (BFD 22) of 15.3%.

No statistically significant effect of the divelopment of young larvae. Brood termination rate for young larvae (BFD 22) of 9.8 %

No statistically significant effect on the development of old larvae. Brood termination rate for old larvae (BFD 22) of 6.5 %.

Statistically significant deference on daily dult bee mortality considered as random fluctuation and biologically irrelevant

Fluopicolide SC 486 (486 g/L) applied as fortified sugar syrup at a concentration of 1.33 g fluopicolide L to be be colonies did not adversely affect honey be colonies and be brood development.

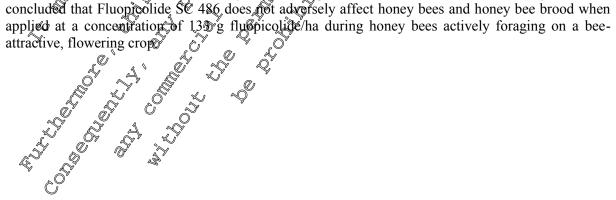
C



Data Point:	KCA 8.3.1.3/03
Report Author:	
Report Year:	2016
Report Title:	Fluopicolide SC 486 (486 g/L): Effects on honey bee brood (Apis melliferate)
	under semi-field conditions - Tunnel Test - Final report 🖉 🔗
Report No:	98781033
Document No:	<u>M-547124-01-1</u>
Guideline(s) followed in	OECD No. 75 (2007) and OEPP/EPPO No. 170 (4)(2010)
study:	Regulation (EC) No. 1107/2009 ©
	Directive 2003-01 (Canada/PMRA)
	Regulation (EC) No. 1107/2009 Directive 2003-01 (Canada/PMRA) US EPA OCSPP not applicable
Deviations from current	Current Guidelines: $OECD_{3}$ (2007) and $BPO 170 (4) (2010)$
test guideline:	No deviations
Previous evaluation:	No, not previously submitted
GLP/Officially	Yes, conducted under GLP/Officially recognised testing facilities
recognised testing	
facilities:	
Acceptability/Reliability:	$Yes \qquad \qquad$

Executive Summary

The purpose of the study was to investigate potential and effects of a opray, application of Fluopicolide SC 486 (486 g/L) on the hopeybee (Apis mellifera L.) under semi-field conditions by following the OECD Guidance Decument No. 75 (2007), with methodological improvements by the AG Bienenschutz (2011), Therefore, turnels were set up on a ca. 75 m² plot of *Phacelia tanacetifolia*. Small bee colonies were introduced to the turnels 3 days before the application one honey bee colony was used per tunnel. Colonies contained 11 combs with honey, willen and brood. The mean strength of the colonies per treatment group, are day before the application, was similar and ranged between 6514 and 6953 adult bees per colony. The preliminary brood check indicated healthy colonies with all brood stages present and a minimum reserve of food. The test item, water and a reference item were applied on the whole poot of plants in two operations, with foraging bees present. The trial was carried out using four tunnels (i.e. replicates) for the test item treatment, the control and the reference item treatment, respectively. The confined exposure plase of the honey bees inside the treated crop was 7 days following the test them application. Ontogenesis of the bees from egg to addit workers, behavioural abnormalities, mortality of adult bees and pupae, the condition of the colonies and the bee brood development were assessed. No biological relevant adverse effects on mortality of pupae were observed. Worker bee mortality, foraging activity, behaviour, nector- and polled storage as well as queen survival was not affected. No effects on colory development, colony strength or bee brood were observed. The observed, characteristic brood effects of the reference item showed that the test conditions allowed a profound detection of effects on immattive honey bee life stages. Based on the results of this study, it can be concluded that Fluopicolide SC 486 does for adversely affect honey bees and honey bee brood when





I. MATERIAL AND METHODS

Test item: Fluopicolide SC 486 (486 g/L): Fluopicolide: 40.1 % w/w (analytical); supplier batch No: 2014-014197; Sample Description: TOX10894-00; Specification No.: 102000011893; deperty: 1.213 g/mL (20°C).

Test species were honeybees (Apis mellifera L.; Hymenoptera, Insecta); small honeybee colonies containing 6514 - 6953 bees each and 5 brood combs containing all brood stages and an appropriate amount of nectar and pollen. Colonies were free of obvious bee diseases and were in a queenright state. A plot of *Phacelia tanacetifolia* with an effective crop area of ca. 75 m² (2) × 36 m²) was prepared for each tunnel (20 m long, 5.5 m wide and 2.5 m high) and cach plot constituted one replicate. For each treatment group (control, test item and reference item), 4 unnels/replicates were set up, resulting in 12 tunnels in total. Per tunnel one honeybee colony was used. The bee colonies were placed into the tonnels with Phacelia (BBCH 65) three days before application. After 7 days of exposure all bee colories were transferred to an area with no pesticide application and main crops flowering. Applications of the test item Fluopicolide SC 486 (486 g/L), control and reference item (hrsegar WG, 250 g/kg fenoxycarb) were conducted by spraying the whole area of plants within the tunnel at full flowering of the crop (BBCH 65) with confined hency bees during bee flight. Confiel tunnels were sprayed with water (400 L/ha), test item tunnels received 33 g.a.s. in 400 L tap water/ha (corresponding to 273 mL product/ha) and reference tem tinnels received 300 g as in 400 Lxtap water/ha (corresponding to nominal 1200 g Insegar/ha)/

- Mortality assessment: Dead worker bees were assessed in dead-bee trap and by collecting dead bees from gauge strips from gauze strips.
- Foraging activity: Numbers of bors for aging of flowering plants were recorded
- Behavioural abnormalities of the bees were recorded.
- Colony conditions The status of the brood (eggs, young and old large, closed brood) and the status of pollen/nectar stores in the colonies was estimated in a quantitative manner (in percentage to the different brood stages on each comb). Ø
- The strength of the colonic was also estimated in a quantitative manner.
- The development of beg brood (ontogenesis of eggs) was evaluated for appropriate amount of eggs (250) from each colory

A

Statistical evaluation was done for mortality, foraging activity, colony strength, brood termination rate and brood indices using Shaping-Wilk's test Levene's test check for homogeneity of variance), Student or Welch t-test (pairwise comparison); software, TOX Rat Professional, Version 2.10.05, ®ToxRat Solutions GmbH).

Dates of experimental work: June 10, 2015 June 11, 2015



II. RESULTS AND DISCUSSION:

Mortality of the adult bees (worker bees)

Mean worker bee mortality in dead-bee traps was comparable between all treatment groups during preexposure, i.e. 32, 32.6 and 23.9 dead bees/colony/day in the control, test item treatment and the reference item group, respectively.

During the exposure phase the average control mortality (53.1 dead bees/colony/day) was higher than the average mortality in the test item group (38.8 dead bees/colony/day) and the average mortality in the group with the reference item (43.6 dead bees/colony/day).

During the post-exposure phase the mortality in the test item group and the reference item group (40 dead bees/colony/day for both groups) was slightly Wigher than in the control (2.8 Dead bees colony/day).

There was no significant difference (p >0.05) in the mean worker bee mortality between control and test item and control and reference item considering the pre-application phase (day -2 to0), the exposite phase (day 0 - 7) and the post-exposure phase (day 8 to 27). D

Mortality of pupae

0 No dead pupae were found in the control treatment before application. In the test and reference item group 0.25 dead pupae/day/colony and 1,33 dead pupae/day/colony were found, respectively. Mean pupae mortality during exposure phase in the control, test item and reference group was 0, 19, 0.41 K) and 0.16 dead pupae/day/colony, respectively?

During the post-exposure phase 0.05 dead pupat day/colony were gound in the control 0.41 dead pupae/day/colony in the test item group and 2.17 dead pupae/day/colony were found in the group with the reference item. Both figure during post-application, i d for the test ftem group and the reference item group were statistically different from the control.

Parameter	10 14 14 10	🏷 Tratment gro	up?
Parameter		Sunophyriae SC 400	Reference Item
N 0 N X	🖉 Constrol 🦻	(486 g/L)	Insegar
		[133 g g s./ha]	[300 g a.s./ha]
Mean mortality of Forker pees /			
colony / day [n] during		V a. W	
pre-application phase 2^{2}	32.0 ± 26.1	32.6 ± 17.6 (n.s.)	23.9 ± 13.3 (n.s.)
exposure phase in the cannels	53,1°± 1,7.0°	38.8 ± 19.4 (n.s.)	43.6 ± 18.5 (n.s.)
phase outside the transfels $3\chi^{3}$	$\hat{2}.8 \pm 2, \hat{1}$	≪ 4.0 ± 4.4 (n.s.)	4.0 ± 2.3 (n.s.)
overall after application	17.1 ±024.8	0^{*} 13.9 ± 17.6 (n.s.)	15.3 ± 20.6 (n.s.)
Mean mortality of larvae and pupae	×,	-S	
[n] during		*U.	
pre-application phase 4)	0.00 ± 0.00	0.25 ± 0.43 (n.s.)	1.33 ± 2.31 (n.s.)
exposure phase in the tunnels ⁴	0.19,₽0.29≼	0.41 ± 0.61 (n.s.)	0.16 ± 0.23 (n.s.)
phase \mathfrak{S}^{atside} the turne is 5) \mathfrak{S}^{b}	0.05 ± 0.10	0.35 ± 0.71 (*) ^a	2.98 ± 3.58 (*)
overall after application	9 ,09 ± 0,18	0.37 ± 0.67 (*) ^a	2.17 ± 3.27 (*)
Mean foraging activity mar / colony /			
day [n] during	Å		
day [n] during pre-application phase	15.4 ± 8.1	12.6 ± 9.2 (n.s.)	14.7 ± 5.3 (n.s.)
exposure phase in the tunnels	24.8 ± 9.4	23.7 ± 11.6 (n.s.)	23.9 ± 9.1 (n.s.)
Mean brood termination rate [%] ⁶⁾	^ø 13.2 (n.s.)	24.2 (n.s.)	63.4 (*)

Ŵ Effects of Fluopicolide SC 486 G (486 g/Lipon haney bee brood under semi-field conditions

1) each with four punnels (replicate)

2) mean number of dead money bees per day and colony found in dead bee traps and on gauze strips in the tunnels

3) noan number of dead honey bees per day and colony found in dead bee traps, only

4) bean number of dead pupae/larvae per day and colony found in dead bee traps and on gauze strips in the tunnels

5)

n.s. = not statistically significant compared to the control; * = statistically significant compared to the control

the statistically significant higher mortalities was caused by 1 out of the 4 colonies only.

⁶⁾ at BFD23Statistic: Student or Welch t-test, α =0.05, pairwise; before application: two-sided; after application: one-sided greater (mortality and termination rate), one-sided smaller (foraging activity).



Behaviour

No test item related behavioural abnormalities occurred at any time during the whole assessment period (up to day 27). No behavioural abnormalities were observed in the control group and in the reference item group.

Foraging activity

Overall daily mean foraging activity observed before application was similar in all treatments 12.6 and 14.7 bees in the control, test item and reference item group, respectively. During the exposure phase on average 24.8, 23.7 and 23.9 were regorded for control, test item and reference item, respectively. No statistically significant differences were observed between control an test item and between control and reference item.

		00.	\sim	w v	
		Control	Test Item	Reference Item	
	Mean foraging activity / m ² / day during	. 15.4 🔊	126 ^{n.s.}	14.78	
	pre-application phase		\mathcal{Q}		
	Mean foraging activity / m ² / day during	24.8	3 7 n.s	23.9 n.s. O &	
	exposure phase in the tunnels				
n.s	= not significant compared to the control	E C			Ŭ,
		de la companya de la comp			Š. L
		d'a	d B		°~~
C	olony condition	The second se		~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	a

Colony condition

At the beginning of the trial all colonies to be used for the test were very simplar, all queens (or eggs) and brood stages (eggs, laterae and closed brood was found in all colonies as an indication of healthy colonies. Moreover, the amount of food reserves (upcontarphated nectar and pollen) was sufficient to ensure colony viability and brood status but also allowed that enough space was available for exposure of the brood to new food sources. A sufficient presence of eggs were found on the test iter treated colonies during all

following brood checks indicating that the queens were alive and sealthy

At the end of the 7th day after appleation, the hives were relocated from their tunnels. In general, the test item treated colonies developed in the same manner as the controk colonies. Compared to the control, a similar anyount of brood could be found during the assessments with no indication of a test item related effect. At test item treated colonies remained yigh with increasing bee numbers and healthy brood. There was no indication of any effect of the test item on the condition of the bee colonies.

In contrast to this, the development of the larger and suparin the reference item colonies was

distinctly decreased after the application.

Brood termination rate

The mean brood termination rates (BPR) on BFD 23 were 13.2%, 24.2% and 63.4% for control, test item and reference item colonies, respectively. The statistical analysis showed no significant difference in the brood termination rates of colonies exposed to the control and test item (p >0.05). In contrast, a statistically significant effect of the brood termination rate was detected in the comparison between the control and reference item convines (p < 0.0).

	Contro P	Test Item	Reference Item
Brood Termination A Rate (BTR) [%]	13.2	24.2 ^{n.s.}	63.4*

n.s. \neq not significant compared \neq the control, * = significant compared to the control (p < 0.05)



Brood index

Following the labelling of the egg stages, mean brood indices of the test item group indicated_a continuous brood development with values slightly lower when compared to the control group between BFD +5 to BFD +23. The mean brood indices in the test item group were 2.5, 3.1, 3.0 and 3.8 at BFD +5, BFD +9, BFD +15 and BFD +23, respectively compared with 2.8, 3.6, 3.5 and 4.3 in the control group. This was not statistically significantly different compared to the control (p > 0.05). Accordingly, no adverse effects of the test item on brood development have been observed throughout the study following the labelling of the egg stage up to day 22 after application (BED + 23). After peatment with the reference item Insegar (a.s.: fenoxycarb) following the belling of the eggs, the mean Brood Indices were statistically significant lower compared to the control indices (>0.05).

Free a time and Creasers	DED 15		BFD +15	BFD 423	
Freatment Group	BFD +5	BFD +9	BLP +12	BFD #25	
Control	2.8	3.6	305	4.3	
Гest Item	2.5 ^{n.s.}	3.1 ^{n.s.}	3.0 ^{n.s.} ©°	3.5 n.s. ~	
Reference Item	1.4*	1.6 ^{n.s.} C	1.5*	ČY.8* 🔗 泠	Ø Ø & A
not significant compa	red to the contr	rol, * = significa	int compared	ϕ the control (p < 0	
		Â.	\sim \sim	$\partial \mathcal{L}$	0.05) 5 5 5 6 6 7 7 7 7 7 7 7 7 7 7 7 7 7
		Q' q'	Y N		

+9, BFD +15 and BFD +23, respectively compared with 2.8, 9.6, 35 and 4 in the control. This slight difference was not statistically significant compared to the control (p > 0.05). Accordingly, no adverse effects of the test item on brood development have been observed throughout the study, following the labelling of the egg stage op to day 22 after application (BFD +23). The bigh termination rate of the marked cells after treatment with the reference iten (insegor (a.s. fenoxycarb) & also reflected by the statistically significantly lower Brood Compensation Indices in the reference item group when compared to the control (p > 0.05). Brood Compensation Indices for the reference item were 1.6, 1.7, 2.0, and 3.2 at BFD +5, BFD 3, BFD + 15 and BFD +23.

	ð -	× . @	~ ~	¥
Treatment Group	₿FD,95	BFD #9	B FD +1.5	BF€ +23 ⊘
Control	2,8, 3	3.6-	3.5	A 4
Test Item	Q.5 n.s.	20 n.s.	3.14	4.1 ^{n.s.}
Reference Item	1.6**	1.7 ^{n.s.}	2:9*	3.2**

n.s. = not significant compared to the control. significant compared to the control (p < 0.05)

Validation of the

Foraging activity:

Mean flight densities in all experimental groups shortly before application were 22.4 bees per m² in the control tunnels, 19.8 bees per m² in the cest item tunnels and 20.3 bees per m² in the reference item tunnels.

Control Mortality

Control Mortality: Mean control mortality from day 0 after application to day 7 (gauze strips + traps) varied between 25.0 and 77,0 dead bees per tunner. As the overall mean mortality after application until day 7 (53.1 dead bees/tunnel/day) was comparable to the level before the application (32.0 dead bees/tunnel/day), it can be regarded to be within regular mortality levels under semi-field conditions. In particular, the majority of the daad bees was found on the gauze strips (i.e. older foragers), reflecting the natural dynamics of the colony. Mortality at the hive (in the traps) was consistently low, indicating that colonies were healthy and adapted to the tunnel conditions.



Reference Item Mortality and Effects on Brood:

There was a high number of impacted bee brood, which resulted in 63.4 % loss of the initial observed eggs. This was statistically significant compared to the control.

Sufficient exposure was additionally verified by a high number of dead pupae mainly found from day 13 onwards. In total, 243 dead pupae were found in all 4-reference item treated colonies following the application of the reference item (24 times higher compared to the control value). The mean bumber of dead pupae found in the reference item treatment was statistically significantly higher compared to the control. The considerable effects on honey bee brood as observed in the reference in the re demonstrated the sensitivity of the test system to detect effects on immature honey bed life stages.

III. CONCLUSIONS

To assess the potential effects of Fluopicolide SC 486 (486 g/L) on honey bee cotonies including brood development, 0.273 L (331.6 g) product in 400 L ap water/ha (corresponding to 133 g fluoppeolide ha), tap water for the control and a reference item were applied to a full-flowering and highly bee-attractive crop (i.e. Phacelia tanacetifolia) under semi-field (turnel) conditions during beerlight No biological relevant adverse effects on mortality of pupae were observed. Worker beginortality, foraging activity, behaviour, nectar- and poller storage as well as queen survival was not affected No effects on colony development, colony strength of bee brood were observed. Based on the results of this study, if can be concluded that Fluppicolide SC \$6 (486 g/L) does not

adversely affect honey bees and honey bee brood when applied at a rate 0.273 L (391.6 g) product in 400 L tap water/ha (corresponding to 133 g fluopicolige/ha) ouring honex bees actively foraging on a bee-attractive, flowering crop L

The observed, characteristic brood effects of the reference item Insegar (a.s. fenoxycarb) in terms of typicality, time of occurrence and extent, showed that the prevailing test conditions allowed for a profound detection of effects on immature honey bestife stages. Ô

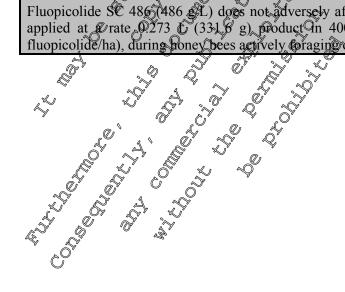
\$ 1 Assessment and conclusion by applicant:

This study is considered reliable for risk assessment and the conclusions are:

No statistically significanceffect on the development of bee brood. Brood termination rate for eggs S. (BFD 23) of 24.2%. \diamond

No biological relevant adverse effects on mortality of preae were observed. Worker bee mortality, foraging activity, behaviour, nectar- and pollen storage as well as queen survival was not affected. No effects on colony development, colony strength or bee brood were observed.

Fluopicolide SC 486 (486 gL) does not adversely affect honey bees and honey bee brood when applied at wrate 0.273 (331, 8 g) product in 400°L tap water/ha (corresponding to 133 g fluopicolide/ha), during boney bees actively for aging on a bee-attractive, flowering crop.





Data Point:	KCA 8.3.1.3/04
Report Author:	
Report Year:	2020
Report Title:	Assessment of side effects of fluopicolide SC 480 G on the honeybee (Apis
-	mellifera L.) in the semi-field after one application on Phacelia tanacetifolia tanacetifolia
Report No:	B180AMS
Document No:	<u>M-685049-01-1</u>
Guideline(s) followed in	OECD Guidance Document No. 75 (2007)
study:	and current recommendations of the AG Bienenschutz (Pistorius Let al. 2012)
	OEPP/EPPO Guideline No. 170 (\mathfrak{F}) (2010)
Deviations from current	Current Guidelines: OECD 75((2007) and EP20/170 (4) (2010)
test guideline:	No deviations
Previous evaluation:	No, not previously submitted
GLP/Officially	Yes, conducted under GLP/Officially recognised testing facilities in the second
recognised testing	
facilities:	
Acceptability/Reliability:	Yes N N N A O K

Executive Summary

Executive Summary The purpose of the study was to investigate potential fide effects of a spray application of Fluopicolide SC 480 G (482.8 gL) on the honeybee Apis mellifera L.) under semi-field conditions by following the OECD Guidance Document No. 75 (2007), with methodological improvements by the AG Bienenschutz (2012). The study included three treatment groups with four replicates (tunnels) each; one tap water treated composition group (C), one test item group (T) and one reference item group (R). For the test item 3 additional replicates were used for analytical verification. Therefore, tunnels were set up on an area of 100 pr, covered by abour 85.5 pr Phaselia tanacettolia. Small bee colonies were introduced to the tomels 4 days of fore the application After application the bees were exposed for 8 days. The post-exposure period was 21 days. One boney bee county was used per tunnel. Colonies contained 10 combs with honey, polien and broad. The mean strength of the colonies per treatment group, one day before the application, was similar and anged between 6338 and 6468 adult bees per colony. The preliminary brood check indicated healthy colonies with all brood stages present and a minimum reserve of food. All beatments were applied at full-flowering (BBCH 64-65) with honeybees actively foraging on the cropy. The target application rate of the test item Fluopicolide SC 480 G was 133 g a.s./ha (actual average rate applied 131.80 g a.s. ha). Tap water was applied in the control group and Insegar 25 WG was applied at a target rate of 300 g fep xycarb/ha in the reference item group. The spray volume was 300 E/ha in all treatment groups

Ontogenesis of the bees from egg to adult workers, behavioural abnormalities, flight intensity, mortality of adult bees and pupae, the condition of the coronies and the bee brood development were assessed. No test item related adverse effects on mortality of pupae were observed. Worker bee mortality, foraging activity, behaviour, mectar-and pollen storage were not affected. No effects on colony development, colory strength or bee brood development were observed. Based on the results of this study, it can be concluded that Eluopicolide SC 480 does not adversely affect honey bees and honey bee brood when applied at a nominal concentration of 133 g fluopicolide/ha during honey bees actively foraging on a bee-attractive flowering crop.

Analytical verification confirmed that the spray solutions were prepared correctly and confirmed that the honeybees were adequately exposed to the test item.



I. MATERIAL AND METHODS

Test item: Fluopicolide SC 480 (482.8 g/L analysed): Fluopicolide: 39.8 % w/w (analytical); supplier batch No: 2015-008261; Sample Description: TOX20609-00; Specification No.: 102000011(993; density: 1.213 g/mL).

The aim of the study was to evaluate potential side effects of a spray application of Fluopicolide SC 480 G on honeybee (*Apis mellifera* L.) brood under confined semi-field conditions by following the OECD guidance document No. 75 (2007), with methodological improvements by the AG Bienenschutz (Pistorius J. *et al.*, 2012). The crop used was full-flowering *Phacelia tanacetifolia*. The study was conducted in a site near Mézin, Lot-et-Garonne, SW-France.

The study included three treatment groups with four replicates (tunneds) each: one tap water treated control group (C), one test item group (T) and one reference item group (R). For the test item 3 additional replicates were used for analytical verification.

All treatments were applied at full-flowering (BBCH 64-65) with honeybees actively foraging on the crop. The target application rate of the test item Fluopicolide SC 480 G was 103 g as ha (actual average rate applied 131.80 g a.s./ha). Tap water was applied in the control group and insegar 25 WG was applied at a target rate of 300 g fenoxycarb/ha in the reference item group. The spray folume was 300 L/ha in all treatment groups.

The initial mean colony size assessed during bee flight per treatment group was in the range of 6338 to 6468 bees. The bee colonies were placed in the tunnels on the 22ⁿ⁴ of April 2018 in the face evening and remained in the tunnels for 12 consecutive days. On the 4th of May 2018 the colonies were relocated to the monitoring site. Thus, there was a pre-exposure period of 4th days on exposure period of 8 days and a post-exposure period of 21 days. The colonies were assessed twice during the confined phase, and four times after the end of the confined phase.

The following endpoints were assessed

- Total and mean number of drad bees (worker and pupae + larvae separately) on linen sheets in tunnels and in the dead bee traps before as well as after the start of exposure in C. T and R.
- Flight intensity (mean number of forager bees/m²) phacelia) before and after the start of exposure in C, T and R.
- Behaviour of the bees in the grop and around the lave.
- Condition of the colonies colony strength and area of the different brood stages and food storage per colony at each assessment date.
- Development of the bee bood assessed in individual brood cells. For this particular assessment, 200 individually marked egg cells for column were selected.
- Determination of residue loads in pollen and neotar from collected forager bees as well as the active ingredient contents in the test item solutions

The data from all treatment proups were tested for normality using the Shapiro-Wilks Test ($\alpha = 0.05$) and for equality of error of variances using Levene's Test. The data of mortality of worker bees were compared to the control pooled by period with ANOVA analysis followed by Dunnett's t-Test, $\alpha = 0.05$. In case ANOVA assumptions were not met, analysis was done with a non-parametric test (Mann-Whitney U-test, $\alpha = 0.05$). Addit mortality at each assessment moment and mortality of pupae + larvae were analysed with the Mann-Whitney U-test, $\alpha = 0.05$.

Flight intensity was statistically compared using Dunnett's t-Test ($\alpha = 0.05$) with the data pooled for the pre-exposure period, the exposure day and the whole exposure period. Data from each assessment day was compared to the control group with the Mann-Whitney U-test, $\alpha = 0.05$.



Brood indices, compensation indices and termination rates from the different treatment groups were statistically compared to the control group using Dunnett's t-Test, α =0.05. Colony strength and number of cells containing food were compared between control and treatment groups using the Dunnett's 't-Test ($\alpha = 0.05$). Cells containing brood were compared to the control using with the Mann-Whitney Utest, $\alpha = 0.05$. All statistical analyses and data representation with box plots were conducted using IBM² SPSS statistics Version 23 for Windows. Data calculations and graphical representation of the findings were performed with Excel 2016.

Dates of experimental work: April 22, 2018 – May 25, 2018

II. RESULTS AND DISCUSSION

Mortality of adult bees (worker bees)

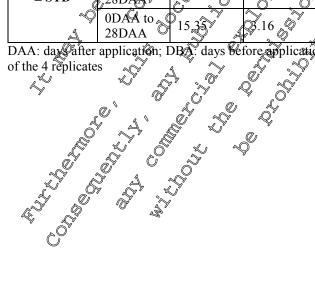
Throughout the period before exposure, mortality of adult bees acress all future treatment groups was similar indicating comparable acclimatization of the colonies to restricted conditions in the turnels. On the application day and during the entire exposure period from day 0 until day 7 after application, mortality of adult bees across all treatment groups was similar, indicating no effect of the test oftem Ø) (Dunnett's t-Test, pooled data, $\alpha = 0.05$) \bigcirc

The number of dead adult bees in the test item treatment did not differ statisfically from the control treatment group in the monitoring period 8DAA to 28DAA and over the entire post application period 0-28DAA, Dunnett's t-Test, pooled data, $\alpha = 0.05$). The number of adult bees in the R group did not show statistically significant differences with the control group over these periods.

mortanty (auti			0 8		5 6 ·		
Treatment gro	a a a a a a a a a a a a a a a a a a a	Mean	STD S	Mean	STD	Mean	STD
Treatment gro	up & K	Control		Fest item	- L	Reference it	tem
		(Q) × ×		(T)	ũ S	(R)	
Ő	OADBAGOO ODBAX ×	Ø7.70 O	\$\$ 9 91	33.65	12.40	19.45	7.93
Daily mean mortaGity	0DAA	10,500	2.16	15.75	5.44	10.25	3.40
(dead worker	0DAA to 7DAA	20.04	0.82 J	22 ,42	8.11	21.91	10.88
bees/colony) ± STD	SDAA to 28DAA	13,556	3.97	12.74	5.41	23.39	12.07
- A	0DAA to 28DAA	15.35	3.16	Ø ^{3.39}	3.69	22.98	6.35

Mortality (adult worker bees)

DAA: days after application; DBA: days before application; STD: standard deviation of the daily mean mortality





Mortality of pupae

The number of dead pupae and larvae observed before start of the exposure was similar between the control and the T group and lower in the R group in comparison to the control (P=0.020, Mann-Whitney U-test, pooled data). However, on 1DBA to 0DBA no statistically significant differences were observed for pupae mortality between the C and the R group, which ensured comparable starting conditions. During the exposure period (0 to 7DAA) in the tunnels the number of dead pupae and larvae in the T and R treatments did not show a statistically significant difference compared to the control treatment (Mann-Whitney U-test, pooled data, $\alpha = 0.05$). Data analysis of the mortality from each assessment. moment showed a statically significant increase of the number of dead gupae+larvae in the Treatment group on 4DAA in comparison with the C group (P = 0.047). This was considered as transient without biological relevance and the mortality values between the T treatment group and the S group were therefore comparable during this period. Q

Over the monitoring period after the exposure phase of the study (8-28DAA) and over the entire post application period (0-28DAA), the test item treatment & did not show statistically significant differences in comparison with the control (P = 0.773 and P = $\mathcal{Y}.000$ despectively, Mann-Whitney U-test pooled Ì data).

Pupae and larvae mortality in the reference item treatment was statistically significant over the period of 8DAA to 28DAA (P = 0.021, ManneWhitney U-Test pooled data) and over the entire post application period of 0-28DAA (P = 0.021). These effects are expected after exposure of bees to this reference substance confirming exposure and sensitivity of the test system to detect harmful effects. Overall, no test-item related adverse effects on adult bee of pupal mortativy were observed.

Mortality (larva	e & pupae)	A	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	ý _Í r v		2 S	
	, Cr	Mean	STD ~	Mêan O	STD ?	Mean	STD
Treatment grou	ıp of A		y S	Fest item	0 4	Reference i	tem
	<u>\$.0</u>		× ~ ~	Q(T) ©	<u> </u>	(R)	
() ()	GADBA to 0DBA				0.93 0.93	0.701)	0.62
Daily mean mortality	0DAA	0.00 4	0.00	Q.50	10-58	0.25	0.50
(dead larvae & pupae	0DAQto 7DAA	0.70	Q.65 ~~~		1.91	0.75	0.79
/colony) ± STD	80AA to 28DAO		0.78	0.62	0.85	26.40 ²⁾	20.71
	0DAA to 28DAA	0.80		8,96 8,	0.66	19.33 ²⁾	16.02

DAA: days after application; DBA: days before application; SCD: standard deviation of the daily mean mortality of the 4 ¹⁾ Statistically significantly ower than the Ogroup p = 0.029, Mann-Whitney U-test, pooled data) ²⁾ Statistically significantly in the transformation of the transformati

¹⁾ Statistically significantly lower than the C group (P = 0.92), Mann-Whitney U-test, pooled data) ²⁾ Statistically significantly higher than the C group (P = 0.92), Mann-Whitney U-test, pooled data)



Flight intensity

The observed foraging intensity was similar across all treatment groups before exposure (4DBA and 0DBA). No significant differences were found between future treatment groups and the control group (Dunnett's t-Test, pooled data, $\alpha = 0.05$). On the application day just before the water application, the average number of foraging bees was 11.5 in the C, 10.0 in the T treatment and 1.3 in the R treatment. Individual tunnels with less than 10 bees/m² at the initial assessment moments were reassessed before the application and fulfilled the criterion of ≥ 10 bees/m².

On the day of application (0DAA) no statistically significant differences of the mean number of forager bees were observed between the test item group and the control group (P = 0.388) Dunnet's t-Test. pooled data). The R group showed a statistically significant reduction of the number of foraging bees on the application day (P = 0.022). \bigcirc

Over the entire exposure period from 0DAA to TAA foraging activity was similar in all treatment groups and no test item and reference item related effects occurred (P= 0.667 for and P= 0.458 for R, Dunnett's t-Test, pooled data).

were Thus, no relevant test-item related adverse affects on flight intensity observed.

Flight intensity	
Treatment group	Control C Fest item C Reference Item (R)
Daily mean flight interview $(h \cos(m^2))$	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$
intensity (bees/m ²) ± STD	$\begin{array}{c c c c c c c c c c c c c c c c c c c $

DAA: days after application; DRA: days before application; STD standard deviation of the daily mean flight intensity of the 4 replicates;

¹⁾ Assessment before the application:

²⁾ Statistically significantly lower than the C group (V = 0.022, Duncet's t-Test, pooled data)

Behaviour of the bee

During the assessments of flight intensity and once the colonies were in the monitoring site the behaviour of the honeybees in the ctop and around the hive was assessed. No abnormal behaviour was observed at any of the assessment moments during the study period. No behavioural abnormalities related to the test

the stady period. I



Development of honeybee brood in individual cells

In the control group C, successful development was observed in the majority of the marked brood cells, indicating a healthy development of the brood. The mean termination rate at the end of the observation period (BFD +22) was at 20%. Throughout the study period the mean values of the brood and compensation indices in the C group increased from 1 to 4.10 and 4.52 respectively.

In the test item treatment group T the observed mean termination rate at the end of the observation period (BFD +22) was 17%. This termination rate did not result on a statistically significant differences compared to the control group (P = 0.900, Dunnett's t-Test). The mean brood and compensation indices were 4.27 and 4.64 respectively at BFD+22. There were not statistically significant differences with the control group (P= 0.841 and P = 0.710 respectively).

The mean termination rate in the reference item group R was 22% at BFD +22. This was not statistically significantly different than the control group (P = 0.917 Dunnett's t-Test). The mean values of the brood and compensation indices at the end of the observation period were comparable to those of the C group and no statistically significant differences were observed (P = 0.844 and P = 0.994 respectively). These findings show that exposure to the reference item did not show the strong negative effect observable on individually marked cell level, in contrast to what was seen in pupae mortality. However, historical data show that replicates with low brood termination rate often display atvincreased pupal martality, which is the case in this study, indicating that here was sufficient exposure of the honey bees and therefore the test system was suitable to detect potential effects on the bee brood (Pistorius ef al. 2012).

In summary, the brood development of oggs, followed on individually marked cells indicated similar mean brood and compensation indices and a similar mean termination rate in comparison with the control group and these differences were not statistically significantly different in any case.

Strenght of the colonies

The overall development of colony strength (number of bees per hive) of all treatment groups showed fluctuations in a typical and normal range. At the sourt of the test the colony strength of all future treatment groups was comparable to the control group and no statistically significant differences were observed between the freatment groups and the control group (Dunnett's t-Test, $\alpha = 0.05$). The mean number of bees in the control and the treatment groups I and R showed a similar trend throughout the study period and no statistically significant differences in comparison with the control group were observed at any of the sessment points.

Development of the Brood Area

The mean amount of prood of the colonies (sum of cells containing eggs, larvae, and pupae) was assessed. At the start of the test the brood area of all future treatment groups was comparable to the control group.

The mean number of brood stages in the control and the test item treatment group showed a similar trend from the first to the last assessment. No statistically significant differences between the test item treatment and the control were detected in the post-application assessments (Mann-Whitney U-Test, α = 0.05). No effects were observed in the R group.

Developmen of the Food Storage Area

The mean amount of food stores in the colonies (sum of cells containing nectar and pollen) was assessed. No statistically significant treatment-related effects on the food storage area were observed in the T group in comparison with the C (Dunnett t-test, $\alpha = 0.05$). No effects were observed in the R group. Thus, no treatment related adverse effects on the development of the food storage area were detected.



Residue analysis

Analysed concentration of fluopicolide, M-01 (AE C653711) and M-02 (AE C657188) in samples of nectar and pollen collected by honeybees are presented in the table below.

Residues of fluopicolide, M-01 (AE C653711) and M-02 (AE C657188) in samples of nectar and pollen

					"O"	a v
Sample ID	Matrix	Treatment group	Timing	Fluopicolide = [mg/kg]	M-01 (AE C653711) [mg/kg]	M-02(AE C659188) [mg/kg]
B180AMS-1-NB-Te-D0E		Те		0.37 Q	<0.01	\$0.01
B180AMS-1-NB-Tf-D0E	Nectar	Tf	0DAA	0.35	<0.01 2	<0.05 \$
B180AMS-1-NB-Tg-D0E		Tg	A	0.31 @ 0	<0.0	<001
		Mean 🔊	¢″	0.34 0	< 6 ,01 \0"	50.01
B180AMS-1-NB-Te-D1E		Te	\$ °	Q@29 ~~~	<i>⊗</i> 0.01 ≫	×0.01 \$
B180AMS-1-NB-Tf-D1E	Nectar	Tf 🔗	1DAA 🔬	JØ.23 🏑 🎽	/<0.04	<0.01
B180AMS-1-NB-Tg-D1E		Tg	N Û	0.0840	<0.09	< 0,0 1 (°
		Mean 📎		0.20	40 .01	&0.01 V
B180AMS-1-PL-Te-D0E		Tel N	Ň		0.013	<0.01
B180AMS-1-PL-Tf-D0E	Pollen	JY X	QDAA ≽	24 24	0.01	<0.0
B180AMS-1-PL-Tg-D0E	e ⁽	ŎĬġ 📡 🔪	§ ,§	18 0 2	0.600	⊗9 .01
		Mean		22,7 0	0.0 12 , N .	≪0.01
B180AMS-1-NB-Te-D1E	×	TO S		0)9 L A	0.01	×<0.01
B180AMS-1-NB-Tf-D1E	Pohen	xJf	DAA L	6.0	<0.01	< 0.01
B180AMS-1-NB-Tg-D1E	w w	Ťg 🌮 "	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	4.5	39.01	< 0.01
LOO – Limit of Quantification		Mean O		6.1	₩0.01 Q	<0.01

LOQ = Limit of Quantification = 0.01 mg/kg (= 10 µg/kg = 10 ppb) for fluopicolde, M-91 (AF C653711) and M-02 (AE C657188) C Ő

LOD = Limit of Detection ~ 0.003 mg/ppb(for fluopicolide)M-01 (AE C6537/1) and M-02 (AE C657188) DAA = Days after application

Analysed concentration of fluopicolide in spray solutions are presented in the table below.

Sample U	Mateôx	Treatment	Timing	Puopicolide mg/kg	Fluopicolide [%]**	Mean Fluopicolide [%]**
B180AMS-1-SO-T	¥ // ۲		S &	444	100	
B180AMS-1-SO-JB-	A Q	K	* 0	(1)	104	
B180AMS-1-SQ-Tc-			ô ^s a	448	101	
B180AMS-№90-Td-	Spray Splution	$\frac{\mathcal{O}}{\mathcal{T}} \sim \frac{\mathcal{O}}{\mathcal{O}}$	ØDAA	448	101	<u>102</u>
B180AMS-1-SO-Te-	Garnion.			449	101	
B180AX5-1-SO-Tf-				463	105	
B180AMS-1-SO-Tg-	A.	y" Q"	, y	443	100	
Mean Eluopiaclido pomir@looptont:	ð S		e ^c	<u>451</u>		

Fluopicolide nominal content: 443 mg/kg ** Concentration [mg/kg]) / (Concentration nominal [mg/kg])) *100; unrounded values were used. DAA = Days after application



III. CONCLUSIONS

Fluopicolide SC 480 G was applied nominally at 133 g a.s./ha at full-flowering *Phacelia tanacetifolia*, during daily honeybee foraging activity. The effects on honeybee colonies under confined conditions considering mortality, flight intensity, behaviour, colony strength, amount of brood and food, and brood cell development were evaluated.

No test item related adverse effects on mortality were observed during the exposure phase and once the colonies were placed in the monitoring site (0DAA to 28DAA).

Over the entire exposure period from 0DAA to 7DAA the foraging activity was similar beween ontrol and test item group. Thus, no test item related adverse effects on flight intensity were observed? No unusual behavioural was observed during the study period in the test item treated group The colony strength in the control and the treatment group T showed Similar trengthroughout the stud period and no differences between the control and test item groups were observed. The test item did not show any difference compated to the control on the amount obbrood and food, measured as mean number of cells covered with the different brood stages and of the food storages The mean brood and compensation indexes and the brood termination rate in the test item freated group showed similar values in comparison to control group.

Analytical verification confirmed that the spray solutions were prepated correctly and confirmed that the honeybees were adequately exposed to the test item.

Assessment and conclusion by applicant: 🤗

This study is considered reliable for risk assessment and the conclusions are;

No statistically significant effection the development of bee brood Brood permination rate for eggs of 17 % (BFD 22).

No test item related adverse effects on mortality of pupae were observed. Worker bee mortality, foraging activity, behaviour, notar- and pollen storage were not affected. No effects on colony development, colony strength of bee brood development were observed

Fluopicolide SC 486 (482.8 g/L) does not adversely affect here y bees and honey bee brood when applied at a rate of 0.30 L (352.8 g) product in 300 L tap water ha (corresponding to 131.80 g fluopicolide/ha), during hone bees actively braging on a bee-attractive, flowering crop.

> \bigcirc A

Sub-lethal effects CA 8.3.1.4

There is no particular study design / test guideline to assess sub-lethal effects" in honeybees. However,

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, there study, sub-lethal effects, if occurring, are described and reported.



CA 8.3.2 Effects on non-target arthropods other than bees

Studies on non-target arthropods have been performed with the representative formulation and are presented in the respective Document MCP, Section 10.3.2. Additionally, studies on non-target arthropods have been conducted with the solo formulation fluopicolide SC 480 and are presented in this document, MCA, Section 8.3.2.1.

CA 8.3.2.1 Effects on Aphidius rhopalosiphi

Data Point:	KCA 8.3.2.1/01
Report Author:	
Report Year:	
Report Title:	Acute dose-response toxicity (LR50) of AE Covered SC40 A2 to the cereal
	aphid parasitoid Aphroius rhopalosiphi (Desterani-Perez) under laboratory
	conditions
Report No:	
Document No:	$\underline{M-218217-01}_{\mathbf{M}} \xrightarrow{\sim} \xrightarrow{\sim} \xrightarrow{\sim} \xrightarrow{\sim} \xrightarrow{\sim} \xrightarrow{\sim} \xrightarrow{\sim} $
Guideline(s) followed in	IOBC: Mead Briggs et al 2000
study:	
Deviations from current	M-218217-01-4 IOBC: Mead Briggs et al 2000 Current Graideline: Mead-Briggs et al. (2000) No deviations
test guideline:	No deviations ϕ ϕ ϕ ϕ ϕ ϕ
Previous evaluation:	yes evaluated and accepted 2 Q
	DAR (2005)
GLP/Officially	Yes, conducted under GP/Officially recognised testing facilities
recognised testing	
facilities:	
Acceptability/Reliability:	Y & J J J J J J J J J J J J J J J J J J
Ű	

Executive Summary 0

The objective of this laboratory study was to investigate the lethal and sublethal toxicity of Fluopicolide SC 480 G on the parasitoid wasp. *Aphidius rhopalosipis* when exposed on a treated glass surface. The test item was applied on glass plates at rates of 170, 255, 383, 574° and 861-mL product/ha in 200 L deionised water/ha. The effects of the test item of the parasitoid wasp. *Aphidius rhopalosipis* when exposed on a treated glass surface. The test item was applied on glass plates at rates of 170, 255, 383, 574° and 861-mL product/ha in 200 L deionised water/ha. The effects of the test item of the parasitoid wasp. *Aphidius rhopalosiphi* were compared to those of a deionised water treated control. Dimethoate EC 400 (0.3 ml product/ha in 200 L/ha of water) was used as toxic reference treatment. Mortality of 30 adult wasps, not older than 48 h at study start (3 replicates with 16 wasp) (7 female and 3 mate wasps) per test group, was assessed 2, 24 and 48 h after exposute. For the assessment of the effects on the relative fecundity of surviving wasps, if possible, a minimum of 9 females per treatment group were taken from the test units after 48 h. They were individually confined to an acrylic cylinder contaming untreated aphid-infested wheat plants. After 24 hours, the wasps were removed, and the plants were maintained under controlled conditions for further 10 days before the number of aphid maximise was assessed (for each individual wasp). All validity criteria were med. The hR_{50} (median ethal rate) was estimated to be > 861 mL product/ha (equivalent to > 419 g a.s./ha).

product/ha (equivalent to 41 g a.s./ha) (No signification of the second second



I. MATERIAL AND METHODS:

The fungicide Fluopicolide SC 480 G (AE C63820600 SC40 A2), purity: 487 g/L fluopicolide, specification: Lot No.: OP220823) was tested under laboratory conditions after residual contact exposure of adults of the cereal parasitoid Aphidius rhopalosiphi to spray residues with rates of 70 255 – 383 – 574 and 861 ml product/ha in 200 L deionized water/ha applied onto glass places. The control was treated with deionized water (200 L/ha) only and Dimethoate EC 400 (0.3 ml product/ha m 200 L/ha of water) was used as toxic reference treatment.

The sprayed and for one-hour dried glass plates were assembled with an auminium frame to build a cage. Afterwards 7 females and 3 males of Aphidius rlopalosiphi were added to each cage. Three replicate cages for the control and each treatment, and one cage for the reference item were prepared. During the mortality test, the wasps were fed with aqueous fructose solution (25 % w/v). For the assessment of the effects on the relative fecundity of survoving wasps, it possible, a rominuf of 15 females per treatment group were taken from the test units after 48 h. They were individually confined to an acrylic cylinder containing untreated aphid-intested wheat wants after 24 hours the wasps were removed, and the plants were maintained under controlled conditions for further, 10 days before the number of aphid mummies was assessed

The number of surviving wasps and the number of parasitised aphids (mumples) were recorded over a period of 14 days. From these data the endpoints mortality after 48 hours and feeundity over a 24 h oviposition period were calculated.

ND **Di**&CUSSIÒ

– July 01 Dates of experimental work: June 17,

ummary of effects	of Fluopicolide SC 48			S S
Test item	Fluopicolide SC 480 G	(AE @38206,00 S	SC40 A2) \$	-
Test object	Aphidius rhopalosiphi	(Destefani-Berez)		
Exposure	Doed spray depositis onto glass plates			
Treatment	Wortality after 48 (7) hours [%]	mean number of mammies female	Celative to control	Reduction relative to control [%]
Control	Corrected martality	15.9	- 0	-
Application rate	Corrected montality $\begin{bmatrix} 9 \\ 9 \end{bmatrix}$		Â ^Y	
170 🗐	9.3 C 0	¥6.1	101.3	0 (+ 1.3)
255 🖉 🔊	03.3 pr 2 0	15.40 0	96.9	3.1
383 ~ 🖓 🖤		14.9 %	93.7	6.3
574 🕰	3.3 2 4	¢5.6 Ø	98.1	1.9
861		M3.4 °	84.3	15.7
LR50 [CŁ 95 %]	not determinable (above the 861 ml/na)			
Reférence item Dimethoate EC 400 0.3 ml product ha		not assessed	-	-

In the test item groups, no significant difference in mortality compared to the control group was observed. Because of no or negligible mortality in all test item treatment groups, a calculation of the LRso was not possible. The LR₅₀ has to be regarded being above the highest tested application rate of the test of m (861 ml product/ha equivalent to 419 g a.s./ha). No statistically significant difference in reproduction (mean number of mummies/female) was observed in all test item groups, when compared to the control group. The toxic reference treatment resulted in 100% corrected mortality within 24 hours.



Validity criteria:

Validity Criteria (Mead-Briggs et al., 2000)	Recommended	Obtained				
Control Mortality	≤13%					
Toxic reference mortality (according to study protocol)	> 50%					
Reproduction rate in control:	\geq 5 mumples per female, \leq 2 females producing 0 mumples	15.9 munimies per females				
III. CONCLUSIONS:						

The LR₅₀ (median lethal rate) of fluopicolide SC 4800G (AE C638206 00 SC40 \times 2) to the cereal aphid parasitoid *Aphidius rhopalosiphi* was > 861 ml product/havequivalent to \times 419 g a.s./ha). No significant sublethal effects were observed up to the sate of 861 ml product/ha (equivalent to 419 g a.s./ha). Assessment and conclusion by pipplicant: This study is considered religible for fisk assessment and the endpoint is Rso > 861 mL product/ha Concerned and the endpoint is Rso > 861 mL product/ha Concerned and the endpoint is Concerned and the



CA 8.3.2.2 Effects on Typhlodromus pyri

Data Point:	KCA 8.3.2.2/01
Report Author:	
Report Year:	
Report Title:	Acute dose-response toxicity (LR50) of AE C638206 00 SC40 A2 to predatory
	mite Typhlodromus pyri (Scheuten) under laboratory conditions
Report No:	C035109
Document No:	<u>M-218216-01-1</u>
Guideline(s) followed in	IOBC: Bluemel et al 2000
study:	
Deviations from current	Current Guideline: Bluemel @ al. (2000)
test guideline:	No deviations
Previous evaluation:	yes, evaluated and accepted
	DAR (2005) $(\chi_{1}, \chi_{2})^{\circ} (\chi_{2})^{\circ} (\chi_{2})^{\circ$
GLP/Officially	Yes, conducted und GLP/Officially recognised terring facilities
recognised testing	
facilities:	
Acceptability/Reliability:	Yes y y y y y y y

Executive Summary

The objective of this laboratory study was to investigate the ternal and subbithal toxicity of fluopicolide SC 480 G to the predatory mite *Typhlodromus pyri* when exposed to a treated glass curface. The test item was applied onto glass plates at rates of 170 255, 383, 574 and 861 product/hain 200 L deionised water/ha. The effects of the test item on the predatory mite *Typhlodromus pyri* were compared to those of a deionised water treated control. Dimethoate EC 400 (03 ml product/ha in 200 L/ha of water) was used as toxic reference treatment. Pfotonymphs of *T. pyri* were exposed in 5 replicates of 20 mites (per treatment group) to the spray residues of the test item reference item and control, respectively. During the assessments the predatory mites were fed with pollen (*Pinus negra* and *betula pendula*). The number of surviving, dead and escaped predatory mites and the number of eggs laid per viable female per evaluation period wate recorded over a period of 14 days. Allovalidity criteria were met. The LR₅₀ of fluopicolide SC 480 G to *Typhlodromus pyri* was 642 mL product/ha with 95% confidence limits ranging from 591 to 698 mL product/ha.

T. MATERIAL AND METHODS:

The fungicide fluopicofide SC 480 \bigcirc (AE C638206 00 \bigcirc C40 A2) (purity: 487 g/L AE C638206; specification: Cot NO OP220823) was costed under laboratory conditions on protonymphs of the predatory mite *T. pyri* (Scheuten) with rates of 470 - 255 - 383 - 574- and 861-mL product/ha in 200 L deionized water/ha applied onto glass plates. The control was treated with deionized water (200 L/ha). Dimethode EC 400 (40 mL product/ha in 200 L/ha) of water) was used as a toxic reference treatment.

Protonymphs of *T. pyri* were exposed in 5 replicates of 20 mites (per treatment group) to the spray residues of the test item, reference item and control, respectively. During the assessments the predatory mites were fed with pollen *Pinus regra* and *Betula pendula*). The number of surviving, dead and escaped predatory notes and the number of eggs laid per viable female per evaluation period were recorded over a period of 14 days. From these data the endpoints mortality and effect on reproduction were calculated.

Dates of experimental work: June 16, 2003 – June 30, 2003



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II. RESULTS AND DISCUSSION:

Summary of effects of Fluopicolide SC 480 G on Typhlodromus pyri

·	•	•••		<i>a</i> . •			
Test item	Fluopi	colide SC 480 G (AE C6.	38206 00 SC40	A2)			
Test object	Typhlodromus pyri (Scheuten)						
Exposure		Dried spray deposits onto glass plater					
Treatment]	Reproduction	× , Ç			
	Mortality after 7 days	mean number of eggs/	Relative to	Reduction relative			
	[%]	female	control	to controp [%]			
		<u>Č</u>	× [%]				
Control	2	3.15	0 0				
Application rate	Corrected mortality						
[ml product/ha]	[%]	A Q	~ ~ ~				
170	1	5.75 🧹	0, 111:Q	0^{\vee} 0 (+ 4.4) 0^{\vee}			
255	1	\$5.05	y 98gal న	, ≪ [°] 1.9 , S			
383	14.3*	5.47	106.2	0 (+ 6.2)			
574	52.0*	× 4,97 O	96.5 °				
861	65.3*	× 4 07 7 Notassessed**					
LR50	642 ml product/ha						
[CL 95 %]	(lower CL: \$91)		× ô ^y				
	(upper Ch. 698)						
Reference item							
Dimethoate EC 400	¥00 Q	Notassessort**		× -			
10 ml product/ha				\$			
Statistically significantly di	fferent from the control Or <	0.05)	Č,	U			

* Statistically significantly different from the control $0^{\circ} < 0.05$) ** Reproduction was not assessed because the corrected modulity was not $\leq 50\%$ Ô

There were statistically significant differences in mortality caused by a repellence effect a high number of mites escaped) in the 383, \$74 and 861 mL product/ha/fest iterDtreatment groups compared to control group. No statistically significant differences in reproduction, were found in the test item treatment groups, which were tested, compared to the control group.

Validity criteria:	
Validite criteria (Bluemel e al., 2000) Recommended	Obtained
Control Mortality	2%
Toxic reference mortality (according to study protocol)	100%
Reproduction rate in control: 2 24 eggs per female	5.15 eggs per female

MII. CONCLUSIONS:

The LR₅₀ (median lethal rate) of flugpicolide SC 480 G (AE C638206 00 SC40 A2) to Typhlodromus pyri was 642 pL product/ha with 95% confidence limits ranging from 591 to 698 mL product/ha.

sment and conclusion by applicant: This stud Vis considered reliable for risk assessment and the endpoint is: 50 = 642 mL product/ha



CA 8.4 Effects on non-target soil meso and macrofauna

Test substance	test design	C	n studies with active substance
Fluopicolide	<i>Eisenia fetida</i> reproduction 56 d, mixed	NOEC 31.25 mg æs./kg dws* EC ₁₀ calculation not possible	0218270-01-1 KCA 8.4.1/09
M-01 (AE C653711)	<i>Eisenia</i> <i>fetida</i> reproduction 56 d, mixed	NOEC 250 mg p.m./kg dws EC ₁₀ calculation nor	KCA 8.4.1/06
M-02 (AEC657188)	<i>Eisenia</i> <i>fetida</i> reproduction 56 d, mixed	$NOFC \geq 100 \text{ mg pan./kg dws}$ $C_{10} \sim Calculation pot$ $C_{10} \sim Calculation pot$	2016; M-58329-04 KCA 8.4.1/98
M-03 (AE0608000)	Eisenia fetida reproduction 56 d maxed *	$\begin{array}{c} \text{NOEC} \geq 50^{\circ} \text{mg p.m./kg tws*} \\ \text{C}_{10} \qquad \qquad \text{calculation oft} \\ \text{PC}_{10} \qquad \qquad \text{possible} \end{array}$	557750-01-1 KCA8.4.1%7
		p.m. pure métabolite ophärte substance (log Pow > 2)	



Acute earthworm studies

Data Point:	KCA 8.4.1/01
Report Author:	
Report Year:	
Report Title:	Acute Toxicity of AE C638206 Technical to the Earthworn, Eisenia fetida
Report No:	B003665
Document No:	<u>M-240680-01-1</u>
Guideline(s) followed in	OECD 207 (1984)
study:	OECD 207 (1984)
Deviations from current	Current Guideline: OECD 207 (1984)
test guideline:	
Previous evaluation:	yes, evaluated and accepted in DAR (2005)
	in DAR (2005)
GLP/Officially	Yes, conducted under GLP/Officially recognized testing facilities
recognised testing	
facilities:	
Acceptability/Reliability:	Yes V A V

Executive Summary

The purpose of this study was to assess the effect of the purpose of the Conical AE Co38206 on survival of the earthworm Eisenia fetida during exposure to an artificial soil at 5 different application rates. Adult earthworms (Eisenia fetida), between 364 539 g/worm were exposed for 14 days in groups of 40 (four replicates of 10 worms per concentration) to control, solvent control, 62.5, 129, 250, 500 and 1000 mg test item/kg dry weight mixed into artificial soil. The assessment of the number of surviving earthworms and observations were made of day 7 and day 14 After 1 days of exposure to luopicolide technical (AE C638206) the mortality was 0% in the control treatments and 2.5% for the vehicle control treatments. The control and vehicle control sorvival were within the acceptability range specified by the protocol. Mortality in the treated soils was 0% in treatments 250 mg/kg and 2.5% in both the 500 and 1000 mg/kg treatments. Theo-day and 14 day Low values for earthworm survival are both > 1000 mg/kg of dry soil.

LA MATERIAL AND METHODS

Test item: Fluopicol de (AF C638206), Batch No: 2050190//PP241024/2, Sample No: 12671, purity: 97.1%. Adult earthworms (Eisenja fetjaa), between 364 - 539 g/worm were exposed for 14 days in groups of 40 (four replicates of 10 worms per concentration) to 62.5, 125, 250, 500 and 1000 mg test item/kg dry weight model into artifical soi Containing 70% U.S. silica sand, 20% kaolin clay and 10% sphagnum peat. Calcium carbonate was added to the soil to adjust the pH to a range of approximately 6.0 ± 0.5 The assessment of the number of surviving earthworms and observations were made on day Dates of work: December 14, 2001 – December 28, 2001 7 and day 14. Earthworms were weighed ontially and at end of the test. Control: untreated, solvent



II. RESULTS AND DISCUSSION:

E	iological find	lings:							
E	ffects on mor	tality and grow	th of the e	arthworm	ns are show	n in the fo	ollowing	tables.	
							U	~	R R
	Test item	Fluopicolide (A	AE C63820)6)			d	Ç ^o r	
	Test object	Eisenia fetida					Ő	ř.	
	Exposure	Artificial soil					st.	~	
		Mortality			Č,		Ő.	Š	
	LC	[mg test item/k]	g soil d.w.]		×	Ć	\$ \	×,	J A O
	LC ₅₀	> 1000			Ú.		~ °		
				ал <i>(</i> ⁴	Fluopicoli	de î sa E C	6 328 206) [î	mg test iten	n/k@ soil
			Control	Solvent ^{&} control	d.w.lo	S. ×	<u>×</u> ø		
	0/ Mortality	f a dult warma		0		<u>,725</u>	250		
	% Mortality of after 14 days	of adult worms	0	2,5, ~	Ø _v	0 ~	0.0	2.5 0	
	Biomass char	nge in %				à à			
	(change in fre	esh weight after	6.15	0.86	£6.09 ~	-9.52		₽ -15.70 [°]	-10.7
	14 days relati fresh weight)		Å	O .	$\mathbb{A}_{\mathbb{A}}^{\times}$			Y S	K ²
	nesh weight)					$\hat{\rho}$			Ay
V	alidity criteri	a. 🗳	Ş 'N		O' L		<i>b</i> .	Ö Ö	
	•				D guideline	207 3	e fulfilled		
1	ne validity cr	iteria of the test	according	g to UEC		207 wen			
			<u>çi q</u>	Ő		<u> </u>	 %, ``	, O	
		eria (OECD 207		Recomm		btamed	\$* \$,	
		he advits in the c		210%	* , *				
	Average loss	of biomask in the	e-sontroi	<u>≤20,%</u>	<u> </u>	<u>15 % 0°</u>	- K		
	0					Ő "	Ø		
	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	×,	~~~ (n>	, «	NCLUSIONS		<i>y</i>		
F	luopiçôlide sl	nowed no effect	Son sut viv	valofthe	earthworm	Eişe@ia fe	<i>etida</i> in ar	tificial soil	at the highest
		00 mg test item						l to earthwo	orms ( <i>Eisenia</i>
		n nominal test o	$\sim$	~ ~ ~ ~	C	5			
	Assessment	and conclusio	n by annl	icant: »	O' O'				
	The endpoir	nt from this stud	ly is concl	aded to a	e LC@> 10	)00 mg/kg	g dry wei	ght soil.	
		Č,	5 8		, L			-	
	However, ac	cute earthworm	studies are	e notiong	a data req	uirement	and are th	nerefore no	t considered
	in the risk as	ssessment.			Ý				
	V	a, [\]		× °.					
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	Û,	Ê Č		v					
	Nº 6		1						
		T I I I I I I I I I I I I I I I I I I I							
		And conclusion the from this stuce cute earthworm ssessment.							
	Ű								



Data Point:	KCA 8.4.1/02	
Report Author:		
Report Year:	2001	
Report Title:	2,6-dichlorobenzamide (BAM): Acute toxicity to earthworms (Eisenia foetida)	Õ
Report No:	C034061	ř
Document No:	<u>M-234309-01-1</u>	
Guideline(s) followed in	OECD 207 (1984)	
study:		6
Deviations from current	Current Guideline: OECD 207 (1984)	2
test guideline:	No deviations	C
Previous evaluation:	yes, evaluated and accepted V Q Q Q	Å
	in DAR (2005)	0
GLP/Officially	Yes, conducted under GLP/Officially recognised testing facilities	/
recognised testing		
facilities:		
Acceptability/Reliability:	Yes & & & & & & &	

#### **Executive Summary**

The purpose of this study was to asses the effect of M-01 (2,6-dicplorobenzamide) on survival of the earthworm Eisenia fetida during an exposure into an artificial soibat 5 different application rates. Earthworms were exposed for 14 days in groups of 40 (four replicate of 10 worms per concentration) to control, solvent control and concentrations of 200, 180, 320560 and 1000 mg test item/kg dry weight mixed into artificial soil, containing 69% industrial quartz sand, 29% kaolin clag, 10% sphagnum peat and 1% calcium carbonate. The assessment of the number of surviving earthworms and observations were made on day 7 and day 14. The mortality of earthworms was 6% in the control and 100% in the highest treatments. The weight change of the carthworms ranged between 12.2 of -2.5% in the treated groups and was -9.52% in the control. The 94-da@LC5% for the test material to earthworms (Eisenia fetida) based on nominal test concentrations is 750 mg/kg soil Q.w. The No Observed Effect Concentration is 320 mg/kg soil d.w.

## 9. MAPERIAGAND AFETHODS:

Test item: M-01 (2,6-dichlorobenzamide), Baten No.: FUX@1000/FUN81G02C, purity: 99.5%. Earthworkins were exposed for 4 days in groups of 40 (four replocates of 10 worms per concentration) to concentrations of 100, 180, 320, 560 and 1000 mg test item kg dry weight mixed into artificial soil, containing 69% industrial quartz and, 20% kaoin clag 10% sphagnum peat and 1% calcium carbonate. The assessment of the number of surviving earthworms and observations were made on day 7 and day 14. Earthwornes wer weighed initially and at and of the test. Toxic standard: 2-chloroacetamide, separate study at concentrations of 5.6, 40, 18, 32 and 56 mg/kg soil d.w.; control: untreated, solvent Dates of work: Apr/24, 2001 – May 8, 2001



#### **II. RESULTS AND DISCUSSION:**

**Biological findings:** 

Effects on mortality and growth of the earthworms are shown in the following tables. M-01 (2,6-dichlorobenzamide) Test item Test object Eisenia fetida Exposure Artificial soil Mortality [mg test item/kg soil d.w.] NOEC 320 LOEC 560 LC50 750 X M-01 (2,6-dichloropenzamide) [mg/test item/kg_soil 0 Ù Q d.w Control **56**0 1000 100 180 % Mortality of adult worms after 100 14 days Biomass change in % (change in fresh weight after 14 days relative to initial fresh weight)

*Not determinable due to 100% mortality after

The mortality of earthworms was 0% in the control and 60% in the highest the atments. The weight change of the earthworms ranged between 12.2 to -22.5% in the treated groups and was -9.52% in the

Validity criteria

The validity criteria of the test according to OEC D guideline 7 were fulfilled.

**	Š	× 4	Å.	N U	
Validity criteria	<b>GOECD</b>	207, 1984)	Recon	mended	Qbtained
Mortality of the	dults in th	ne control	$\% \leq 10^{9}$		
Average loss of t	piopoass in	the control	ľ _≲©0 %	6. O'	Ø9.52 %
	<u> </u>		- 7		

To verify the sensitivity of the test system; the reference item 2-chloroacetamide was tested at concentrations of 5.6, 10, 18, 52 and 56 mg/kg soil d.w. The result of this positive control study gave a 14-day DC₅₀ for 2-chloroacetamide of 23 mg/kg.soll d.w.

# **IIE**CONCLUSIONS:

M-01 (2,6-di Morosbenzaro de) showed effects on survival of the earthworm Eisenia fetida in artificial soil at the highest reatmont at 1000 motest item/kg soil d.w. The 14-day LC₅₀ for the test material to earthworks (Eignia fetida) based on nominal test concentrations was 750 mg/kg soil d.w. The No Observed Effect Concentration was 320 mg/kg soil d.w.

### Assessment and conclusion by applicant:

The chdpoint from this study is concluded to be  $LC_{50} = 750 \text{ mg/kg}$  dry weight soil.

However, acute earthworm studies are no longer a data requirement and are therefore not considered in the risk assessment.



Data Point:	KCA 8.4.1/03	
Report Author:		
Report Year:	2003	
Report Title:	Acute toxicity (14 days) of AE C657188 to the earthworm Eisenia fetida in artificial soil	, N N
Report No:	C035115	
Document No:	<u>M-218222-01-1</u>	
Guideline(s) followed in	ISO: 11268 part 1 (1993); OECD: 207 (1984)	
study:		V
Deviations from current	Current Guideline: OECD 207 (1084)	a
test guideline:	No deviations	Å
Previous evaluation:	yes, evaluated and accepted $\mathcal{K}$ $O^{\mathcal{V}}$ $\mathcal{K}$ $O^{\mathcal{V}}$	),
	DAR (2005)	
GLP/Officially	Yes, conducted under GLB Officially recognised esting acilities	
recognised testing		
facilities:		
Acceptability/Reliability:	Yes O' V A A A	

#### **Executive Summary**

The purpose of this study was to assess the effect of M-02 (AE Ch 57188) on superival of the earthworm Eisenia fetida during an exposure into an afficial soil at 5 different application rates Earthworms were exposed for 14 days in groups of 40 (four replicates of 0 worms per concentration) to control, solvent and concentrations of 63, 125, \$20, 500 and 1000 me test item/kg ary weight mored into artificial soil, containing 69.5% fine quartz/sand 20% kaofin clay, 10% sphagnum peat and 0.5% calcium carbonate. The assessment of the number of surviving earthworms and observations were made on day 7 and day 14. The mortality of earthworms was 0% in the control and all tested treatments. The weight change of the earthworms ranged between -9.5 @ -13.9% in the treated groups and was 2% in the control. M-02 (AE C657188) showed no effects on survival of the carthworm Eisonia fetida in artificial soil at the highest treatment at 1000 by test nem/kg soil d.w. The 14-day LC₅₀ for the test material to earthworms (Eisenia fetida) based on nominal test concentrations is > 1000 mg/kg soil d.w. The No Observed Effect Concentration 1000 mg/kg soil do Ì

## I MATERIAL AND METHODS

Test item: M-02 (AE \$657,188), Batch Nov. RAW244055/1, purity: 97.2%. Earthworms were exposed for 14 days in groups of 40 (four peplicates of 16 wornes per concentration) to concentrations of 63, 125, 320, 500 and 1000 mg test item kg dry weight mixed into artificial soil, containing 69.5% fine quartz sand, 20% kaokin clayO10% sphagnum peaO and 0.5% calcum carbonate. The assessment of the number of surviving earthworms and observations were made on day 7 and day 14. Earthworms were weighed initially and at end of the test. Toxic standard 2-chloroacetamide, control: untreated, solvent control: none.

Dates of work: June 04, 2003 – June 23, 2003



#### **II. RESULTS AND DISCUSSION:**

**Biological findings:** 

Test item	M-02 (AE C657	188)	hworms are shown in the following tables.
Test object	Eisenia fetida		
Exposure	Artificial soil		
	Mortality		
	[mg test item/kg	soil d.w.]	
NOEC	≥ 1000		
LOEC	> 1000		
LC ₅₀	> 1000		
		Control 🛓	M-02 (AP C657488) [mg test item/kg soil d.x]
		L.	<u>163 123 250 500 11000</u>
% Mortality after 14 days	of adult worms		
in fresh weig	nge in % (change ht after 14 days tial fresh weight)©		-1 h g g 9.5 g -10 2 G 2.8 G -12.5

The mortality of earthworms was 0% on the control and all ested treatments. The weight change of the earthworms ranged between \$3.5 to \$13.5% in the treated groups and was -8.2% in the control.

#### Validity crite

The validity criteria of the test according to OFCD guideline 207 were fulfilled. ñ

	Ŭ Ŝ	E.	S '	
Validity criteria (OR	CD 207, 1984	4) Recom	mended	Obtained
Mortality of the adpits	in the control	1 10 %		Q %
Average loss of bioma	st in the cont	$\operatorname{rol}_{\mathcal{O}} \leq 20\%$	<u>C</u>	§.2 %
			O ^v (	×*

To verify the sensitivity of the test system, the reference item 2-chloroacetamide was tested. In the most recent test with the toxic standard compound 2 chlorescetamide the LC50 after 14 days was determined as 26.8 mg/kg. III. COOCLUSION:

M-02 (AE C657188) showed no effects on survival of the earthworm *Eisenia fetida* in artificial soil at the highest treatment at 1000 mg tost item kg soil d.w. The 14-day LC₅₀ for the test material to earthworms (Eisenia fetida) Based on nominal test concentrations was > 1000 mg/kg soil d.w. The No Observed Effect Concentration was 1000 mg/kg soil d.w.

### Assessment and conclusion by applicant:

X)

The endpoint from this study is concluded to be  $LC_{50} > 1000 \text{ mg/kg}$  dry weight soil.

However, acute earthworm studies are no longer a data requirement and are therefore not considered in the risk assessment.



Data Point:	KCA 8.4.1/04	
Report Author:		
Report Year:	2003	
Report Title:	Acute toxicity (14 days) of AE 0608000 to the earthworm Eisenia fetida in artificial soil	
Report No:	C035116	
Document No:	<u>M-218223-01-1</u>	
Guideline(s) followed in study:	ISO: 11268 part 1 (1993); OECD: 207 (1984)	Ê,
Deviations from current test guideline:	Current Guideline: OECD 207 (1984) No deviations	
Previous evaluation:	yes, evaluated and accepted DAR (2005)	
GLP/Officially recognised testing facilities:	Yes, conducted under GLP Officially recognised festing facilities	
Acceptability/Reliability:	Yes O L & A .	,
		_

#### **Executive Summary**

The purpose of this study was to assess the effect of M-03 (AE 0608000) on survival of the earthworm Eisenia fetida during an exposure into an artificial soil at 5 different application rates Earthworms were exposed for 14 days in groups of 40 (four replicates of 10 ovorms per concentration) to control and concentrations of 63, 125, 320, 500 and 1000 mg best item/kg gev weight mixed into artificial soil, containing 69.5% fine quartz/sand 20% kaolin clay, 10% sphagnum peat and 0.5% calcium carbonate. The assessment of the number of surviving earthworms and observations were made on day 7 and day 14. The mortality of earthworms was 0% in the control and all tested treatments. The weight change of the earthworms ranged between -5.4 @ -10.9% in the treated groups and was 33% in the control. M-03 (AE 0608000) showed no effects on survival of the carthworm Eisonia fetida in artificial soil at the highest treatment at 1000 tog test nem/kg soil d.w. The 14-day LC₅₀ for the test material to earthworms (Eisenia fetida) based on nominal test concentrations was 1000 mg/kg soil d.w. The No Observed Effect Concentration was 1000 mg/kg soil d.w.



Test item: M-03 (AP 0608000), patch No.: Mor 4622M, purity: 96.9%. Earthworms were exposed for 14 days in groups of 40 four replicates of 10 worms per concentration) to control and concentrations of 63, 125, 320, 500 and 0000 mg test fem/k odry weight mixed into artificial soil, containing 69.5% fine quartz sand, 20% kaolin day, 10% sphagnum peat and 0.5% calcium carbonate. The assessment of the number of surviving earthworms and observations were made on day 7 and day 14. Earthworms were weighed initially and a end of the test. Toxic standard: 2-chloroacetamide, control: untreated, solvent control; none.

Dates of work: May 20, 2003 + June 06, 2005



#### **II. RESULTS AND DISCUSSION:**

**Biological findings:** 

Test item	M-03 (AE 06080	)00)	worms are shown in the following tables.
Test object	Eisenia fetida		
Exposure	Artificial soil		
	Mortality		
	[mg test item/kg	soil d.w.]	
NOEC	≥ 1000		
LOEC	> 1000		
LC ₅₀	> 1000		
		Control	M-03 (AP 0608900) [me test i@m/kg soil d.w. ) [
		L. K.	$63 \sqrt{1423}$ $250 \sqrt{500}$ $1000$
% Mortality after 14 days	of adult worms		$\begin{array}{c ccccccccccccccccccccccccccccccccccc$
	nge in % (change ht after 14 days itial fresh weight)§	Q - 5.3 x 3 ~ ~ ~	

The mortality of earthworms was 0% on the control and all ested treatments. The weight change of the earthworms ranged between \$.4 to \$10.3% in the reated groups and was -5.3% in the control.

#### Validity crite

1

The validity criteria of the test according to OECD guideline 207 were fulfilled. Ø)

	0 29	Å.	S 1	
Validity criteria (OR	CD 207, 198	4) Recom	mended	Obtained
Mortality of the addits	in the contro	1 10 %		Q %
Average loss of bioma	is in the cont	$\operatorname{rol}_{\mathcal{O}} \leq 20\%$	,C?	§.3 %
<u> </u>				

To verify the sensitivity of the test system, the reference item 2-chloroacetamide was tested. In the most recent test with the toxic standard compound 2 chlorescetamide the LC₅₀ after 14 days was determined as 26.8 mg/kg.

### **ÎII. CONCLUSION:**

M-03 (AE 0608000) showed no effects on survival of the earthworm Eisenia fetida in artificial soil at the highest treatment at 1000 mg test iten kg soil d.w. The 14-day LC₅₀ for the test material to earthworms (*Elsenic fetida*) based on nominal test concentrations was > 1000 mg/kg soil d.w. The No Observed Effect Concentration was 1000 mg/kg soil d.w. Õ

### Assessment and conclusion by applicant:

K)

The endpoint from this study is concluded to be  $LC_{50} > 1000 \text{ mg/kg dry weight soil}$ .

However, acute earthworm studies are no longer a data requirement and are therefore not considered in the risk assessment.



Data Point:	KCA 8.4.1/05
Report Author:	
Report Year:	2003
Report Title:	AE C638206 technical: Effects on survival, growth and reproduction on the
	earthworm Eisenia fetida tested with 5 percent peat in the test substrate
Report No:	C035163
Document No:	<u>M-218270-01-1</u>
Guideline(s) followed in	BBA: VI, 2-2 (1994); ISO: 11268-2 E (1998)
study:	
Deviations from current	Current Guideline: OECD 222 (2004)
test guideline:	The pH was greater than $6.0 \pm 0.3$ on day 56 (6 $\frac{1}{20}$ ). The number of representation in $\frac{1}{20}$
	the control was 4 instead of 8. $\sqrt{2}$
	These deviations are not expected to have impacted the stud Presults.
Previous evaluation:	yes, evaluated and accepted
	DAR (2005)
GLP/Officially	Yes, conducted under GLP/Othcially accognised testing facilities
recognised testing	
facilities:	
Acceptability/Reliability:	Yes v v v A O v v

#### **Executive Summary**

The purpose of this study was to assess the effect of floopicofide technical (AE C638206) on survival, growth, and reproduction of the earth form *Elsenia fetida* during an exposure into an artificial soil at 5 different application rates. A0 adult earth worms *Eisenia fetida* (9, months old, 4  $\stackrel{\circ}{}$  10 animals per control and test item group) were exposed in an artificial soil to the application rates of 0 (control), 15.63, 31.25, 62.50, 125 and 250 mg a.s kg weight sol. After 28 days, the number of surviving animals and their weight alteration was determined. After further 28 days, the numbers of juveniles were determined. All validity criteria were met. No statistically significant effects (p <0.05) are observed in mortality and

All validity criteria were met. No statistically significant effects (p < 0.05) are observed in mortality and reproduction of treated earthworms and hence the NOEC based of mortality (28 days) and the NOEC based on reproduction 56 day) is 250 mg as /kg dry soil. The NOEC for growth (28 days) is 62.5 mg a.s./kg dry soil based on statistically significant bromass changes seen at higher treatment rates.

## S I. MATERIAL OND METHODS:

Test substance: Fluppicolide (AFC 638206 technical; batch AEC 638206 00 1C96 0001; OP2050046 of purity 96.1%). 240 adult earth forms *Etsenid fetida* (9 months old,  $4 \times 10$  animals per control and test item group) were exposed in an artificial sol to the application rates of 0 (control), 15.63, 31.25, 62.50, 125 and 250 mg a.s. kg weight sol. Artificial sol composition was 73-74% quartz sand, 20% kaolin clay, 5% sphagnum peat, 4% diffed organism manure and 0.2-1% calcium carbonate. The artificial sol was prepared by mixing the do components intensely in a laboratory mixer. The test vessels were kept in a temperature-controlled room at 20 ± 2°C under a 16-hour light to 8-hour darkness photoperiod and a light intensity at light period between approximately 400-800 lux. The test item was mixed into the soil.

After 28 days, the number of surviving animals and their weight alteration was determined. They adult earthworms were then removed from the artificial soil. After further 28 days, the numbers of juveniles were determined in the artificial soil. After further 28 days, the numbers of juveniles

The most recent reference test with carbendazim (360 g a.s./L; trade name "Derosal flüssig") was performed from October to December 2002 with the following 3 application rates: 1.25, 2.50 and 5.00 mg a.s./kg fly weight soil. The test ensured that the laboratory test conditions were adequate and verified that the response of the test organisms did not change significantly over time. No statistically significant effects (p < 0.05) were observed in mortality and reproduction of treated earthworms and hence the NOEC based on mortality (28 days) and the NOEC based on reproduction (56 day) is 250 mg a.s./kg



dry soil. The NOEC for growth (28 days) was 62.5 mg a.s./kg dry soil based on statistically significant biomass changes seen at higher treatment rates.

#### Dates of experimental work: January 09, 2003 – March 06, 2003

#### **II. RESULTS AND DISCUSSION:**

No mortality of adult earthworms was observed at any application rate of the test item in the study

The growth of the adult worms was significantly different to the control at the application rates of 125 and 250 mg a.s./kg dry weight soil. No significantly different values of the growth to the control were observed at the application rates of 15.63, 31.25 and 62.50 mg a.s./kg dry weight soil.

The number of juveniles was not significantly reduced at any application rate.

Summary of effects of fluopicolide on mortality and changes in body weight of the adults after an exposure period of 28 days and the number of juveniles after 56 days.

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Control	0 . 1	🔬 Fluopic	olide 🏟 C	6 <b>38</b> 206) నో	
	ne co	× 21 75	20 50	r 1.70	× 250
	10.03		 		چې 250
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1 + 07 2		758			+ 75.5
	0,10,40		$2 + 70.9^{\circ}$	$+ \mathcal{O}_{4.0}$	+73.3 $\pm 8.4$
	±18.4	₩2.4 (	D = 12.4	, 1.0	± 0.4
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~			(n c	, ,	
	,			J S.	S.
10.4	<i>∞</i> ⁷ 11.6 <i>γ</i>	10.2	S 12.8 ·	11.4	13.8
±₽.4 [©]	∀ ± 205	₽ 1.6 Å	⁵ ° ± 2⊘0	± 0.6	± 3.0
Q A		The second second			
	₩.S.	11.SQ	\sim $^{\prime On.S.}$	11.S.	n.s.
	$ \begin{array}{c} $	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$

* Result of a Dunnett's multiple t-test one sided smaller, $\alpha = 005$

**Result of a Williams multiple sequential t-test, and sided smaller, $\alpha = 0.05$

s.: mean value statistically significantly different compared to the control (p < 0.05)

n.s.: mean value not statistically significantly different compared to the control ($p \ge 0.05$)

The calculation of an ECx curve for reproduction was not possible due to the lack of a significant doseresponse relationship.

Results of the most recent test with the reference substance (carbendazim 360 g a.s./L): The growth of adult earthworms was significantly reduced at the application rate of 5 mg a.s./kg dry substrate. The reproduction rate was significantly reduced at the application rates of 2.5 and 5 mg a.s./kg dry substrate. The survival of the adults was not affected.

Validity criteria:

Validity critecta OECD 222 (2004)	Recommended	Obtained
Adult control mortanty	≤10%	0 % (after 4 weeks)
Number of juveniles per control replicate	\geq 30	109, 90, 121, 96
Coefficient of variation of reproduction in the control	≤ 30%	13.3 %



III. CONCLUSION:

No statistically significant effects (p < 0.05) were observed in mortality and reproduction of treated earthworms and hence the NOEC based on mortality (28 days) and the NOEC based on reproduction (56 day) is 250 mg a.s./kg dry soil. The NOEC for growth (28 days) was 62.5 mg a.s./kg dry soil. Dased on statistically significant biomass changes seen at higher treatment rates.

Assessment and conclusion by applicant:

This study is considered reliable for risk assessment and the endpoint is

NOEC = 62.5 mg a.s./kg dws

Data Point:	$ KCA 8.4.1/06 \qquad \bigcirc $
Report Author:	
Report Year:	
Report Title:	Effect of AE C653,711 on reproduction and growth of earthworms Eisenig tetida in
-	artificial soil
Report No:	C035112 5 5 6 5 5 5 5
Document No:	$\underbrace{M-218219-0}_{M-2} \underbrace{M}_{\mathcal{A}} \underbrace{\chi}_{\mathcal{A}} \underbrace{\chi}_{$
Guideline(s) followed in	M-218219-064 BBA: VI 2Q, 1995 ISO: 19268 part 2 (1998)
study:	
Deviations from current	Current Guidefine: OF CD 222 2004) The number of replicates in the control was 4 instead of 8 to recommended by the
test guideline:	The number of replicates in the control was a instead of 8 as recommended by the
	This deviation is not expected to have impacted the study results.
Previous evaluation:	yes, evaluated and accepted by the second se
- La	DAR/2005a
GLP/Officially recognised testing facilities:	Yes, conducted under GLC/Officially recognised testing facilities
recognised testing	
Acceptability/Reliability;	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$
T S	

Executive Summary

The purpose of this study was to investigate the effects of M-D1 (2:6 dichlorobenzamide (AE C653711)) on survival, growth, and reproduction of the earthworm *Eisenia fetida* during an exposure into an artificial soil at 5 different application rates. Under laboratory conditions *Eisenia fetida* (40 worms per treatment group and control) were exposed to the following concentrations of AE C653711: 0, 31, 63, 125, 250 and 500 mg/kg artificial soil After 28 days, the number of surviving animals and their weight alteration was determined. After further 28 days, the number of juveniles was determined. Endpoints were mortality, growth and reproduction

were mortality, growth and reproduction. All validity criteria were met During the 4 weeks of exposure one adult worm died at the test item concentration of 31 mg/kg soil dry weight and two worms died at 250 mg/kg soil dry weight. The body weights in the test item treated group increased by 38.9 % to 50.8 %. The reproduction ranged from 205 to 274 juvenile worms in the groups treated with test item. M-01 (AE C653711) did not show significant effects on mortality and growth of the earth or *Eisenia fetida* up to the concentration of 500 mg/kg soil dry weight, *i.e.* the highest concentration tested. Reproduction of *Eisenia fetida* was not significantly affected up to and including the concentration of 250 mg/kg soil dry weight but at 500 mg/kg soil dry weight a statistically significant reduction of reproduction was observed. The no-observed-effectconcentration (NOEC) determined in this study is 250 mg/kg dry artificial soil.



I. MATERIAL AND METHODS:

Test substance: M-01 (2,6-dichlorobenzamide (AE C653711 technical; batch No: 8808018; purity 97.0%)) was tested in a laboratory study lasting eight weeks. The control was untreated and moistered with deionised water. Under laboratory conditions *Eisenia fetida* (40 worms per treatment group and control) were exposed to the following concentrations of M-01 (AE C653711): 0, 31, 63, 125, 230 and 500 mg/kg artificial soil. Endpoints were mortality, growth and reproduction. Artificial soil composition was approximately 69.5% fine quartz sand, 20% kaolin clay, 10% sphagnum peat and approximately 0.5% calcium carbonate to adjust the pH. The test vessels were kept in a temperature-controlled room at 18 – 21°C under a 16-hour light to 8-hour darkness photoperiod and a light intensity between approximately 422 – 527 lux. The test item was mixed into the soil. After 28 days, the number of surviving animals and their weight alteration was determined. The most recent reference test with Derosal SC 360 (active ingredient: carbendazim) was performed from August to October 2002. The test ensure that the laboratory test conditions were adequate and verified that the response of the test organisms and not change significantly over time.

Dates of experimental work: March 18, 2003 - May 16

IS RESELTS AND DISCUSSION

Observations:

During the 4 weeks of exposure one adult worm died at the cest item concentration of 3 K mg/kg soil dry weight and two worms died at 250 mg/kg soll dry weight which was not significantly different compared to the control (Fisher Exact test, a 0.05)

The body weights in the test from treated group increased by 38.9% to 50.8%. None of the weight changes was significantly different compared to the control group where weight increase was 37.4% (Dunnett-test, a = 405).

The reproduction ranged from 205 to 274 invenile worms in the groups treated with test item. Up to and including the concentration of 250 mg/kg soil dry weight the reproduction was not significantly different compared to the control where 281 invenile worms were found (Bonferroni-t test, a = 0.05). At the concentration of 500 mg/kg soil dry weight the number of jovenile earthworms was statistically significantly reduced compared to the control (Bonferroni-t test, a = 0.05).

	.0	O 🔈				
Test item		Å.	M-01 (AI	E C653711)	
Test species		~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	Eisent	ia fetida		
Exposure & & &		Te:	st item mix	ked into the	e soil	
	Sontral	31	63	125	250	500
	Control	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
Mean Mortality 🛠 St. Dex 🚧 👘 🖉		2.5 ± 5	0 ± 0	0 ± 0	5.0 ± 5.8	0 ± 0
Mean body weight change + St. Dev.	_0//.4 ±	$50.8 \pm$	$44.8 \pm$	$44.9 \pm$	$44.9 \pm$	$38.9 \pm$
	<i>∱</i> 7.7	8.9	6.2	7.9	8.9	4.7
Mean reproduction of juveniles + St.	≫ 281 ±	259 ±	$239 \pm$	274 ±	257	$205 \pm$
[%] ¹ Mean reproduction of juveniles +St. Dev.	23	25	21	37	± 69	25*

St. Dev. = Standard deviation *significantly different compared to the control

A calculation of a valid ECx curve for reproduction was not possible due to the lack of a significant dose-response relationship.

The most recent toxic standard test showed statistically significant effects on reproduction at a concentration of 1.6 mg carbendazim/kg artificial soil (dry weight); the EC₅₀ for reproduction was calculated as 1.9 mg carbendazim/kg soil dry weight.



Validity criteria:

Validity criteria OECD 222 (2004)	Recommended	Obtained
Adult control mortality	$\leq 10\%$	0 % (after 4 weeks)
Number of juveniles per control replicate	\geq 30	281 (mean)
Coefficient of variation of reproduction in the control	≤ 30%	8.00

III. CONCLUSION:

M-01 (AE C653711) did not show significant effects on mortality and prowth of the earthworm *Eisenia fetida* up to the concentration of 500 mg/kg soil dry weight, *i.e.* the highest concentration dested. Reproduction of *Eisenia fetida* was not significantly affected up to and including the concentration of 250 mg/kg soil dry weight but at 500 mg/kg soil dry weight a statistically significant reduction in number of juveniles was observed. The no-observed-effect-concentration (NOEC) determined in this study was 250 mg/kg dry artificial soil.

Assessment and cor	clusion by applicant: N N N A A
This study is conside	red reliable for fisk assessment and the endpoint is
NOEC = 250 mg/kg	red reliable for fisk assessment and the endpoint is 5 5 5 5
Data Point:	KQX 8.4.1/07
Report Author:	
Report Year:	2016
Report Title	A E ATO 8000 (BCS X 860 %): Effects or survival growth and reproduction of

Report Litle:	AE 0008000 (BCS X86048): Effects on survival, growto and reproduction of
L. C.	the earth worm Eisenia fected tested in artificial soil
Report Title:	E 312 0 864-8 2 2 0
Document No: 5	<u>M-5577757-014</u> ~ ~ ~
Guideline(s) followed in	International Standards ISO 1268-2: 1998 E): "Soil quality - Effects of
study:	pollutants on earthworms (Eisenia fetida) - Part 2: Determination of effects on
<i>w</i>	reproduction" July 1998.
	VOECD 222: opril 13, 2004: "OECD Guideline for the Testing of Chemicals -
	Earthworn Reproduction Jest (Eisenia fatida / Eisenia andrei)"
Deviations from current	Corrent Guideling. OECD 222 (2004)
test guideline:	The temperature increased up to 22.7 °C, out of the range of 20 °C ± 2 °C
Q A	, recommended by the guideline. This deviation is not expected to have impacted
	the study results.
Previous evalQation:	No, not previously submitted
a fi	
GLP/Officially	Yes conducted under GLP Officially recognised testing facilities
recognised testing	
facilities:	A C' N'
Acceptability/Reliability:	

Executive Summary

The purpose of this study was to investigate the effects of M-03 (AE0608000) on survival, growth and reproduction of the earthworn *Eisenia fetida* during exposure to an artificial soil at one application rate. Under Jaboratory conditions *Eisenia fetida* (80 worms per treatment group) were exposed to the following concentration of M-03 (AE0608000) which was mixed into the soil: 100 mg/kg dry weight artificial soil. Endpoints were mortality, growth and reproduction.

All validity criteria were met. After 28 days, the number of surviving animals and their weight alteration was determined. The no-observed-effect-concentration (NOEC) determined in this study is \geq 100 mg/kg soil dry weight.



I. Material and Methods:

Test substance: M-03 (AE0608000 (BCS-AX86048)); batch No: SES 12767-19-2; purity 99.4%) was tested in a laboratory study lasting eight weeks. The control was untreated and moistened with deion water. Under laboratory conditions Eisenia fetida (80 worms per treatment group) were exposed to the following concentration of M-03 (AE0608000) which was mixed into the soil: 400 mg/kg draweight artificial soil. Endpoints were mortality, growth and reproduction. Artificial soil composition was approximately 70% industrial quartz sand, 20% kaolin clay and 10% sphagnum peat. Calcium carbonate for the adjustment to pH 6.0 \pm 0.5 was added. The test vessels were kept in a temperature-controlled room at $20 \pm 2^{\circ}$ C under a 16-hour light to 8-hour darkness photoperiod and a light intensity between 400 - 800 lux. The test item was mixed into the soil.

After 28 days, the number of surviving animals and their weight alteration was determined. The were then removed from the artificial soil. After further 28 days, the nomber of juveniles was determined The most recent reference test with the reference test item (360 g carbendazing) was performed from August 25 to November 19. The test ensured that the laboratory test conditions were adequate and verified that the response of the test organisms did not change significantly over the.

Dates of experimental work: January 1& ∽March√2

Observations:

ontrol and the treatment with 100 mg During the 4 weeks of exposure go adult earthworm died in the /kg dry weight artificial soil.

AND DISCU

The body weights in the test item meated group becreased in average by 65 2%. In the control group the increase was in average 61%. The weight change in the treatment was not significantly different from the control (STUDEN kt-test wo-sided, a = 0.05) O

The mean reproduction was approximated 190 worms per test vessel in the control. In the treatment group a slightly lover reproduction was found i.e. 170 worms perfest vessel. No statistically significant differences concerning the number of juveniles relative to the control was observed (STUDENT to est, one-sided smaller, $\alpha = 0.05$).

				A O	
Test item				<u>M</u> 03 (AE060800	0)
Test species		ý ô		🖇 Eisenia fetida	
Exposure	S A Q		Tes O	st item mixed into the	he soil
<i>.</i> .			Control		100 mg/kg
Mean Mortality	U . O .	$\gamma \sim$	× × 0		0
[%] 🛇	body fresh weigh 0 to day 28 + St.		€1.03 ± 6.9	9	65.0 ± 6.59
after 56 days + S)r vesser	190.4 ± 24.	5	169.5 ± 26.5
St. Dev. = Standard de					

Qurve for reproduction was not possible as only one concentration was tested. A calculation of an

The nost recent toxic standard test showed statistically significant effects on reproduction at the test concentrations of 208 and 5.0 mg carbendazim/kg artificial soil dry weight.



Validity criteria:

Validity criteria (OECD 222, 2004)	Recommended	Obtained
Adult control mortality	$\leq 10\%$	0 %
Number of juveniles per control replicate	\geq 30	157 to 234
Coefficient of variation of reproduction in the control	≤ 30%	12.90%

III. CONCLUSIONS:

M-03 (AE0608000) did not show significant effects on mortality, growth and reproduction of the earthworm *Eisenia fetida* at the limit concentration of 100 mg/kg sold dry weight. The no-observed-effect-concentration (NOEC) determined in this stude was therefore ≥ 100 mg/kg soil dry weight.

Assessment and concl	usion by applicant:
This study is considere	d reliable for risk assessment and the endpoint is
This study is considered	
NOEC \geq 100 mg/kg dv	vs A or A or A or A
	usion by applicant: od reliable for risk assessment and the endpoint is vs KCA 8.4.1/08 2046 Byridyl@arboxyDrc acid@BCS-AB43478): Effects on survival, growth and
Data Point:	KCA 8.4.1/08 2 2 0 4 0 4
Report Author:	
Report Year:	2646 6 27 6 6
Report Title:	Byridyl@arboxyDrc acid@BCS-AB43478): Effects on survival, growth and
	reproduction of the earthworth Eisenia fetida tested in artificial soil
Report No:	EBACN0560 O & O & O
Document No:	<u>M0558320-01-1</u> 0, 27 20 07 47
Document No: Guideline(s) followed in study:	M055832901-1 (DECDQ22 (2004), ISO 112682 (1998); US EPA OGSPP not applicable
Deviations from current	Current Grideline OECD 222 (2004)
test guideline.	No deviations 2 2 5 0
Previous evaluation:	No, norpreviously separated of
GLP/Ottrcially	Yes conducted under GLO Officially recognised testing facilities
recognised testing	
facilities:	
Acceptability/Reliability	Yess or s
Acceptability/Reffability	Yes conducted under GL® Officially recognised testing facilities

Executive Summary

Executive Summary The purpose of this study was to investigate the effects of M-02 (AEC657188) on survival, growth and Ť reproduction of the earth worm Eisenia fetida churing an exposure into an artificial soil at five different application rates. Under aboratory condition Eisenia fetida (80 worms for the control and 40 worms per treatment group) were exposed in an additical soil to the application rates of 0, 10, 18, 32, 56 and 100 mg/kg d. . artitical soft. After 28 days, the number of surviving animals and their weight alteration was determined. After further 28 days, the number of juveniles was determined.

All validity criteria were met. No-Observed-Effect-Concentration (NOEC) was determined to be ≥ 100 mg metabolite kg soll dry weight, and the Lowest-Observed-Effect-Concentration (LOEC) was determined to be > 000 mg kg soil dry weight.



I. MATERIAL AND METHODS:

Test substance: M-02 (AEC657188 (BCS-AB43478)); batch code: AE C657188-PU-01, SES 10250-1-1; purity 98.5%) was tested in a laboratory study lasting eight weeks. The control was untreated and moistened with deionised water. Under laboratory conditions Eisenia fetida (80 worms for the control and 40 worms per treatment group) were exposed in an artificial soil to the application rates 20, 10, 18, 32, 56 and 100 mg/kg d.w. artificial soil. Endpoints were mortality, growth and reproduction. Artificial soil composition was approximately 69.5% industrial quartz sand, 20% kaolin, clay 10% sphagnum peat and 0.5% calcium carbonate. The test vessels were kept in a temperature controller room? at 18 – 21.9 °C under a 16-hour light to 8-hour darkness (photoperiod and a light intensity of 380 kg The test item was mixed into the soil.

After 28 days, the number of surviving animals and their weight alteration was determined. They were then removed from the artificial soil. After further 28 days, the number of juvenfles was determined. As a reference item Maypon Flow (Carbendazim, SC 500) was tested in a seprate study at concentrations of 5 and 10 mg product/kg soil dry weight.

Dates of experimental work: March 23, 2016

Observations:

During the 4 weeks of exposure one adult worm died at the test item concentration of 10 mg/kg soil dry weight and two worms died at 56 mg/kg soil dry weight which was not significantly different compared to the control (Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm, $\alpha = 0.05$).

II RESULTS

The weight change of the adult worms ranged between 23.6 and 26.5% in the treated groups and 25.0% in the control group. The test item caused no statistically significant change in bomass compared to the control groups at any concentration tested \mathcal{Q} will an s-t-test, $\alpha = 0.05$, one-sided smaller).

 $\tilde{\alpha} = 0.05$, operideal smaller) on the number of No statistically significant effect (Williams, t-test juveniles was found for any concentration tested. Ø

<i>iteration</i>	<i>'0' ~</i>	<i>Č</i> n 4						
Test item	Å.		» °°°		M-02 (XE	C657188)		
Test species				Ô ^y Vy	D senic	-		
Exposure	~0 4	2 E	O V	🖉 🌾 Test	t item mixe	ed into the	soil	
(à A	С. С.	Control		Treatment	nt (mg/kg s	soil d.w.)	
<i>a</i> n				S 10	18	32	56	100
Mortality 🂖]			× 1.3 ×	25	0	0	5.0	0
Mean body wei	ght change + 9		[≸] 25.0€	24.4±	$25.6 \pm$	$23.6 \pm$	$26.5 \pm$	$24.9 \pm$
			&4	4.8 آمریک	2.5	2.4	4.4	2.4
Number of juve	eni les per repl	icate	\$ 86.9 \$	141.0 ±	$130.3 \pm$	144.0±	$138.5 \pm$	$132.8 \pm$
after 8 weeks		N 4	20.90	24.9	24.3	28.1	11.5	10.0
Reproduction	4 ° _0	ntrol (%)	° _0	103.0	95.2	105.2	101.2	97.0

A calculation of a valid EOx curve for reproduction was not possible due to the lack of a significant doseresponse relationship.

In the most steen stridy with Maypon Flow (Carbendazim, SC 500) the number of juveniles was reduced $b\sqrt{39}$ and 96% at concentrations of 5 and 10 mg product/kg soil dry weight after 8 weeks of test duration when compared to control (mean number of juveniles = 148).



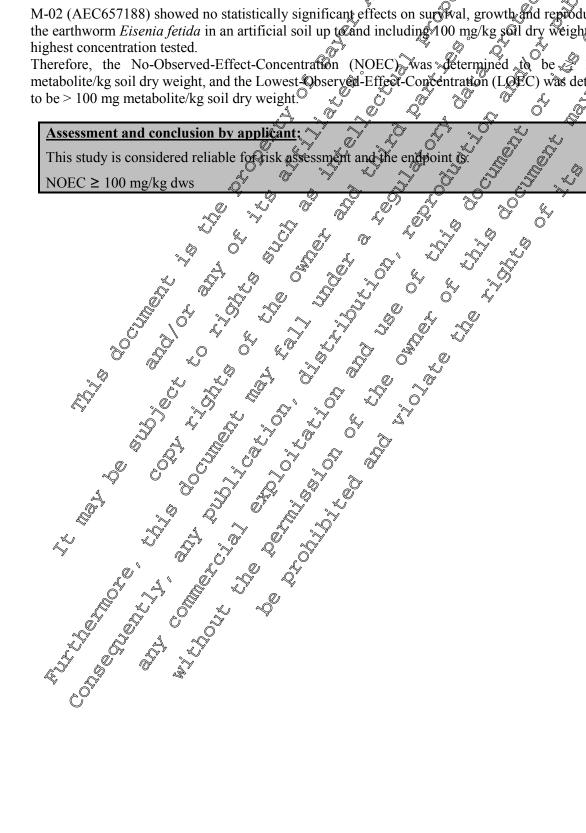
Validity criteria:

Validity criteria (OECD 222, 2004)	Recommended	Obtained	
Adult control mortality	$\leq 10\%$	1.3 %	
Number of juveniles per control replicate	\geq 30	109 to 168	N A
Coefficient of variation of reproduction in the control	≤ 30%	15.20%	

III. CONCLUSION:

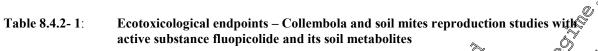
M-02 (AEC657188) showed no statistically significant effects on survival, growth and reproduction of the earthworm Eisenia fetida in an artificial soil up to and including 100 mg/kg soil dry weight 9.e. the

100 mg metabolite/kg soil dry weight, and the Lowest Observed Effect Concentration (LOPC) was determined to be > 100 mg metabolite/kg soil dry weight. A A

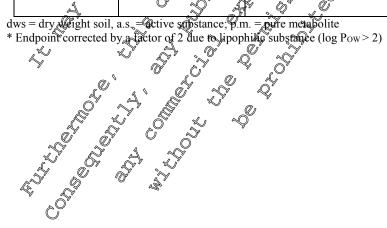




CA 8.4.2 Effects on non-target soil mesoand macrofauna (other than earthworms)



	eartiiworiiis)		0
Table 8.4.2- 1 :		oints – Collembola and soil mites 1 icolide and its soil metabolites	reproduction studies with
Test substance	Test species, test design	Ecotoxicological Endpoint	Reference S
Collembola, rep	roduction		
Fluopicolide	<i>Folsomia candida</i> reproduction 28 d, mixed	NOEC 31,25 mg a.s./kg dws* EC ₁₀ 44 mg a.s./kg dws*	<u>M-241194-91-1</u> KCA 8:4.2.1/01 EC ₁₀ carculation. <u>01-1</u> 2620: M-679537
M-01 (AE C653711)	Folsomia candida reproduction 28 d, mixed	NOFC 25 mgp/m./kgdws EC10 calculation notpossible	
M-02 (AE C657188)	Folsomia candida reproduction 28 d, mixed	$\sqrt{NOEC} \geq 100 \text{ mg p-m./kg shws}$ FC_{10} calculation not possible	KCA 8.4.2.1994
M-03 (AE 0608000)	Folsomia candiday reproduction	$ \begin{array}{c} \text{NOEC} & \geq 50 \text{ mg p.m./kg dys}^{\ast} \\ \text{EC} & \text{cateulation not possible} \\ \end{array} $	<u>2016; M-</u> <u>CO8337-C1-1</u> KCA&A.2.1/Q3
Soil mites, repro	duction		
Fluopicolide	Hypoaspis acuQifer reproduction 1440, mixeQ	NOFC $\geq 500 \text{ mg ass./kg/dws*}$ EC10 Galculaton not possible	2016;20-548042-01-1 KCA 8.4.2.1/05
M-01 (AE C653711) کُ	Hypoaspis acut fer	NOEC > 500 mg @m./kg dws EC10 carculation not @ssible	2015; M-538626-01-1 KCA 8.4.2.1/06
M-02 (AE COV/188)	Hypoaspis achdeifer 4 reproduction 142d, mixed	NOBC $\geq 100 \text{ mgp.m./kgdws}$ EC ₁₀ Scalculation not possible	<u>2016; M-</u> <u>557987-01-1</u> KCA 8.4.2.1/07
M-03 (AE 0608006)	Hypouspis aculeifer reproduction 14 d, apped	ŎŎEÇ Ő≥ 50 mg p.m./kg dws*#	[#] Calculated endpoint, assuming a 10-fold higher toxicity than the active substance fluopicolide





CA 8.4.2.1 **Species level testing**

Data Point:	KCA 8.4.2.1/01
Report Author:	
Report Year:	
Report Title:	Effects of AE C638206 on Reproduction of the Collembora Folsomia candida in
	Artificial Soil
Report No:	15021016
Document No:	<u>M-241194-01-1</u>
Guideline(s) followed in	ISO 11267 ISO Soil Quality - Introduction of reproduction of Collembolar Folsonia
study:	Candida) by soil pollutants, 1999
Deviations from current	Current Guideline: OECD 22 (2016)
test guideline:	The number of replicates in the control were 5 instead of S as recommended by &
	the guideline.
	The number of replicates in the control were 5 instead of S as recommended by the guideline. This deviation is not expected to have impacted the study results.
Previous evaluation:	yes, evaluated and accepted a set of the set
	DAR (2005)
GLP/Officially	Yes, conducted under GLP/Officially recognised testing facilities
recognised testing	
facilities:	
Acceptability/Reliability:	Yes O ^v Y Y Y V A S S Q
Data Point:	KCA&8.4.2.108
Report Author:	
Report Year:	
Report Title:	Statistical re-evaluation (non-glp) of the Folsomia capdida reproduction study
4 n	with Ruopicolide (Rein, 2003; M-041194201-1), using the Probit analysis
Report No:	M2679535-01-1
Document No:	<u>M-67987-01-05 ~ 2 0 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1</u>
Guideline(s) followed in	
study:	
Deviations from current	Not applieable & & & @
test guideline:	
Previous evaluation:	No not prevously submitted
L. C	
GLP/Officially	por apprecable of the second sec
recognised testing	prot applicable of the structure of the
facilities:	No not preventily submitted
Acceptability/Reliability.	
ExecutiveSummary	

Executive Summary

The purpose of the study was to determine the effects of fluopicolide technical (AE C638206) on reproduction of the Collerobola Folsomia candida in artificial soil. 10 (age of 10-12 days) collembolans per replicate were exposed to control (water treated), solvent control (acetone) and treatments with 62.5, 125, 250, 500 and 1000 mg/kg dry soil in a first assay. The assay was conducted under controlled environment of conditions, Fest containers were reusable glass vessels (volume: 100 mL; diameter: 5 cm), closed tightly by plastic-life to avoid water evaporation from the artificial soil. Due to unusually high reproduction recorded to controls in the first assay a second assay was conducted at nominal concentrations of 15, 5, 31.3 and 62.5 mg/kg dry soil. Mortality and reproduction were determined after Š 28 days.

All validity criteria were met. Based on the effects observed on reproduction, it is concluded, that the overall NOEC for the study is determined to be 62.5 mg a.s./kg artificial soil dry weight. Thus, the overall LOEC is determined to be 125 mg a.s./kg artificial soil dry weight. The probit analysis revealed an EC₁₀ = 32.88 mg a.s./kg dws, an EC₂₀ = 134.60 mg a.s./kg dws, and an EC₅₀ = 1995.40 mg a.s./kg.



I. MATERIAL AND METHODS:

Test item: Fluopicolide (AE C638206 00 1C99 0005, Batch No: 2050190//PP241024/2). Test organisms: Folsomia candida (Collembola: Isotomidae), age of 10-12 days. 10 collembolans@per replicate were exposed to control (water treated), solvent control (acetone) and treatments with 52.5 125, 250, 500 and 1000 mg/kg dry soil in a first assay. Due to unusually high reproduction repoded in controls in the first assay a second assay was conducted at nominal concentrations of 15.6, 31 \$ and 62.5 mg/kg dry soil. Both assays were conducted under controlled environmental conditions at 19-21 27 and 590-660 lux in the 1st experiment and 18-21°C and 430-550 lux in the second experiment with light regime of 16 h light and 8 hours dark. There was one additional container per test itera, and control for measurement of pH and humidity. Test containers were reusable glass vessels (polume) 100 mL; diameter: 5 cm), closed tightly by plastic-lids to avoid water evaporation from the artificial soil filled with approximately 30 g \pm 5% wet weight artificial soil. Artificial soil contained, 10% Sphagnum peak 20% Kaolin clay, approximately 0.5% Calcium cathonate and approximately 69.5% the quartz-sand. During the study, the test organisms were fed with granulated dry yeast. Mortality and reproduction were determined after 28 days. The pH and water content were measured at start and finish of the study for each concentration. The software used to perform the statistical analysis was Systa Wersion 9.0.

	Dates of experimental work: November 13, $2002 - February 04, 2003$											
υ	Dates of experimental work: November 13, 2002 – February 04, 2003											
A												
1	^{at} experiment	Ô	o s									
ſ	Test item		H.	. Water a	ntept (%) 👋		max ²					
Ī	Test item concentration ¹		H X, End	Water of	ntent (%)	Start	max ² End					
		Start 5.6		Start 36%	ntent (%) 🔍							
	concentration ¹	Start S	H	Start's 36% 36%	ntent (%) [%] DEnd &	Start	End					
	concentration ¹ Control	Start 5.6	H	Start 🔊	ntent (%) [%] <u> </u>	WHC Start 55%	End 52%					
	concentration1 Control Solvent control &	Start Start 5.6 5.7 5.7 5.7 5.7 5.7	H	Start's 36% 36%	ntent (%) [%] <u> </u>	Start 55% 54%	End 52% 51%					
•	concentration ¹ Control Solvent control 62.5	Start 5.6	H 	Start 7 36% 36% 36% 36% 36% 36% 36% 36%	ntent (%) [%] <u> </u>	WHC Start 55% 54% 54%	End 52% 51% 52%					
	concentration ¹ Control Solvent control 62.5 125	Start Start 5.6 5.7 5.7 5.7 5.7 5.7	H 	Start 36% 36% 36% 3 36% 3 36% 3	ntent (%) [%] <u> </u>	WHC Start 55% 54% 54% 54%	End 52% 51% 52% 53%					

¹ mg a.s./kg soil dry weight ² % WHC_{max} = percent @ maximum watchholding capa

2nd experiment

	S O		<u>, 0 </u>			
Test item	pH Start		Watercont	ent (%)	WHCmax ²	
concentration ¹	Start 🔊	Lnd 🖓 , ኛ	Start	End	Start	End
Control	62 Q	End 5.8 5.7 5.7	36%	33%	54%	51%
Solvent control	x 2 1	57	~36%	33%	54%	51%
15%6	6.1	5.7 🖓 👸	36%	33%	55%	51%
31.3	6.0	5.00	36%	33%	55%	51%
62.5	60	\$6	36%	34%	54%	51%
mg a.s./kg soft dry we	night 🖉 🖉		•			
% WHCmax > percent	of maximum wat	er holding capaci	ty			
15%6 31.3 62.5 % WHCmag & percent						
Č ^O						



Biological results:

1st experiment

experiment								
	Water	Solvent	Pooled	AE C6382	06 [mg/kg a	rtificial soil	dry weight]	
	control	control	control	62.5	125	250 🏷	500	9000 <u> </u>
Mortality ¹	4%	6%	5%	6%	12%	2%	10% 🖏	6%
	± 5.5%	± 5.5%	± 5.3%	± 5.5%	± 4.5%	±4.5%	± 10%	*5%
Statistical analysis	-	n.s. ²	-	n.s. ²	n.s. ²	(n.s. ²	n.s.?	n.s. ²
NOEC	1000 mg/	/kg		× L	R		, v z	
LOEC	> 1000 m	ng/kg		4ÚV	Å	, Ő	Q.	
LC ₅₀	Data not	appropriate	for calculation	and the second sec				
Reproduction ¹	916	909	912	753 。	700 2	647	598 🔊	565
	45	128	97	97, *	33 L	84 5	48 🗇	81
% of (pooled) control	-	99	- 4	083 V	77 Q	91	60	62
Statistical analysis	-	n.s. ⁴		*3		*3		
NOEC	< 62.5	,Ô				5 .4)
LOEC	62.5	<u> </u>	*0·	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~		ř Ö		
EC ₅₀	Data not	appropriate	forcalculation	on 🖉	Ô L			
Mean ± standard de	eviation (SD)) 🖧 🛸	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~			. °°	×	

Mean \pm standard devia		\mathbb{Y}		, _{(Å-} () [*]	Ő
² Fisher Exact-Test, α =	= 0.05, two-stded				0
³ Williams Test, $\alpha = 0.0$	05, one-sided smaller	S Ó	× 10× 1		
⁴ Student-t test, $\alpha = 0.0$	5, one-sided smaller	Q Q	~~~```		
- = not applicable			~ ⁽¹⁾ () ⁽¹⁾ ().		
² Fisher Exact-Test, $\alpha =$ ³ Williams Test, $\alpha = 0.0$ ⁴ Student-t test, $\alpha = 0.0$ - = not applicable n.s. = not significantly differently differently differently differently.	different compared to p	ooled water ar	dacetone controls		
* = significantly differe	ent compared to pooled	water and ace	one controls	0' 4	
Å				(, @	
2 nd experiment				¹ ² ³	
	Water Solven	Pooled o	Fluojecolide	C638206 [mg/kg ar	tificial soil dry])
Ď	control control	control	15.6	\$ 31.3	62.5
Mortabity	8%	Ø 6%		20%	6%
	± \$4% \$5.5%	* ±2%0% %	0 ± 8.4% ~ 0	± 7.1%	± 5.5%
Statistical	9 - <u>3</u> p ²			#2	n.s. ²
analysis 🖗			S S		
NOAEC			62.5 mg/kg		
LOEÇ 👻			062.5 mg/kg ot appropriate for ca		
LC		Data n	ot appropriate for ca	alculation	
Reproduction ¹	755 4645	700	م م ۲04	606	684
×.,	£84 ± 107,×	£108 >	¥ ± 71	± 89	± 65
% of (pooled)		Q 29	101	07	0.9
control 🖉	- 0 85		101	87	98
Statistical	n.s.4	_ ₽	3		3
analysis	n.s.	~~ -	n.s. ³	n.s. ³	n.s. ³
NOABC		¥	< 62.5 mg/kg soil		
LOEC S	4		> 62.5 mg/kg soil		
EC50 OF	A V	Data n	ot appropriate for ca		

¹ Mean humber of juve thes per replicate \pm standard deviation (SD) ² Fisher Exact Test, $\alpha = 0.05$, two-sided

³ Williams est, $\alpha = 0.05$, one-sided smaller

⁴ Student test, $\alpha = 0.05$, one-sided smaller

- = not applicable

n.s. = not significantly different compared to pooled water and acetone controls

Significantly different compared to pooled water and acetone controls



ECx evaluation for reproduction

ECx were statistically re-evaluated based on the number of juveniles of *F. candida* provided in this study report (Table 7 and Table 8, original study report (M-241194-01-1), page 25). Details are given in a separate report (M-2020; M-679537-01-1). From each run separately, all mean data referring to the number of juveniles at day 28 in a test item treatment group was corrected as a percentage of the mean pooled control (water and solvent control were pooled). Both runs were then combined and a probit analysis was performed in order to derive EC_{10} , EC_{20} and EC_{50} -values for the % effect on the number of juveniles of *F. candida*. The concentration of 62.5 mg/kg was tested in both runs, hence, the average of both means was used at 62.5 mg/kg (overall mean of 90.14 % as a mean of 97.74 % and 82.57 % at 62.5 mg/kg). This re-analysis was performed using the software ToxRatPto 3.2.5

Percentage of the mean number of juveniles of F. candida transformed from the results presented in the study report at different test item treatment concentration of the test item in soil mg a.s. Bg dws at day 28.

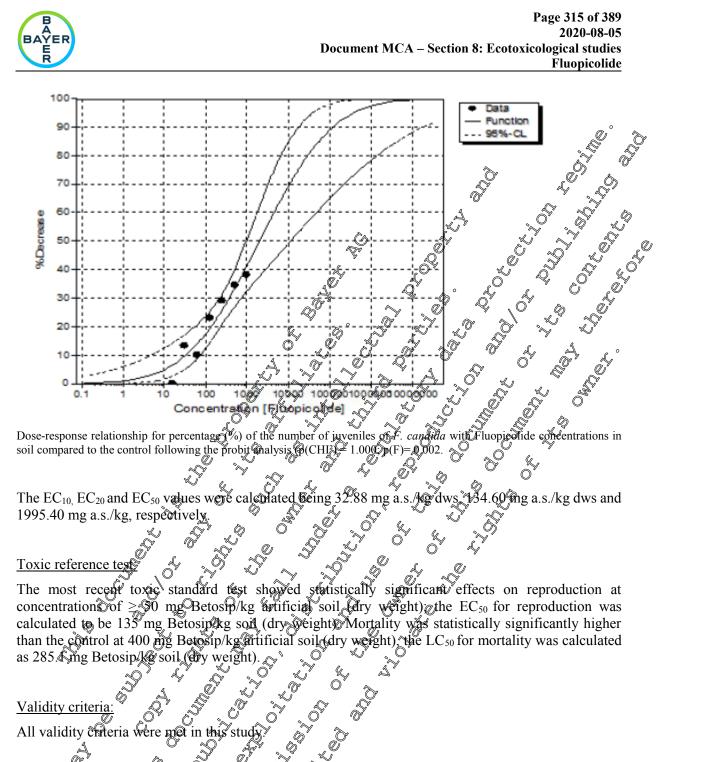
			Q	× · · ·		5 6 6	
Test item treatment	Pooled Control	15.6 mg/kg	31.3 52 52 mg/kg mg/kg	T25 C mg/kg	239	500 × mg/kg	1000 Ang/kg •
% of control	100 %	100.57 %	86.57% 90.14%			69.57 %	61.55%
		~					Č2

The probit analysis revealed an $EC_{10} = 32.88$ mg a.s./g dws an $EC_{20} = 132.60$ mg a.s./kg dws, and an $EC_{50} = 1995.40$ mg a.s./kg. The dose-response curve from the problemalysis doesn't to the most relevant data; the calculated $EC_{10} = 32.88$ mg a.s./kg dws is considered reliable. This is supported by $p(CHI^2) = 1.000$ and p(F) = 0.002 indicating osufficiently robust EC_x -calculation.

EC10, EC20, and	d EC 🔊 😽	alues	with con	nfidenke	limits	for %	ffect on	nundbe	r of fuver	iles compare	ed to the
control	<i>L</i>	L'	, Ô	~~	~ ~ /		, O	C	a.		

control						
		م الم		S [×] ECOV	ČZ	EC50
Value [mg a.s.%	g dway	32.883		130.604	V	1995.397
Lower 95%-cl	×.	4.908	S N	\$54.28		947.186
Upper 95%-cl	• 3	73.228		227, 296		11301.761
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than the control at 400 mg Betosip kg artificial soil (dry weight) the LC₅₀ for mortality was calculated as 285 king Betosip/kg soil (dry weight). <u>Validity criteria:</u> All validity criteria were met in this study.

Valjdity criteria (QECD 232, 2016)	Obtained in this study
Mean adult mortality $\leq 20\%$ $c^{4}$	< 20% in 1 st and 2 nd experiment
Mean number of juveniles/replicates 2100	> 100 in 1 st and 2 nd experiment
Coefficient of variation calculated for the number of juvenil oper replicate $\leq 30\%$	< 30% in 1 st and 2 nd experiment



#### **III. CONCLUSION:**

Based on the effects observed on reproduction, it is concluded, that the overall NOEC for the study is determined to be 62.5 mg a.s./kg artificial soil dry weight. Thus, the overall LOEC is determined to be 125 mg a.s./kg artificial soil dry weight. The  $EC_{10}$ ,  $EC_{20}$  and  $EC_{50}$  values for reproduction were calculated being 32.88 mg a.s./kg dws, 134.60 mg a.s./kg dws and 1995.40 mg a.s./kg, respectively.

Assessment and concl	usion by applicant:
This study is considered	ed reliable for risk assessment. A calculated $DC_{10} = 32.88 \text{ mg} \text{ a.s.} \text{ g dws is}$
considered as the relev	ant endpoint for the risk assessment.
Data Point:	KCA 8.4.2.1/02
Report Author:	
Report Year:	
Report Title:	Effects of AE 653711 on Reproduction of the Collembola Folsomia candida in Artificial Son
Report No:	$150310160^{\circ} \xrightarrow{\sim} \overline{\checkmark} \xrightarrow{\sim} \overline{\rightarrow} $
Document No:	<u>M-241167-01-1</u>
Guideline(s) followed in	ISO 11267 ISO Soil Smality, inhibition of reproduction of Collembola (Folsomia
study:	Candida) by soil pollutants, (1999 2 2 2
Deviations from current	Current Guideline OECD 232 (2016)
test guideline:	The number of plicates in the control were Sistead of 8 as recommended by
	the guideline. I for the state of the state
	This deviation is not expected to have impacted the study results.
Previous evaluation:	yes, evaluated and accepted
GLP/Officially	DAR (2005)
GLP/Officially	Yes, conducted under GLP Officially recognised testing facilities
recognised testing	
facilities: O S Acceptability/Reliability:	
Acceptability/Reliability:	Yes Q A V V Q Q Q Q Q Q Q Q Q Q Q Q Q Q Q Q
Â	Yes Q A Y Q A
Executive Summary	

The purpose of the study was to determine the effects of No.01 (AE C653711) on reproduction of the Collembola *Folsomia candida* in efficial soil. Test organisms: *Folsomia candida* (Collembola: Isotomidae), age of 10-12 days. 10 collembolans per replicate were exposed to control (water treated), solvent control (acetone) and treatments with 6.25, 92.5, 25, 50 and 100 mg/kg artificial soil (dry weight). The assay was conducted under controlled environmental conditions. Test containers were reusable glass vessels volume: 100 mL; drameter. 5 cm) and contained artificial soil. Mortality and reproduction were determined after 28 days.

reproduction were determined after 28 days. All validity criteria were net. In this study, 60-01 (AE C653711) causes no mortality and no adverse effect on reproduction of *Folsomia andido* at concentrations up to and including 25 mg/kg artificial soil dry weigh (NQEC). The LOEO for both endpoints is 50 mg/kg artificial soil dry weight.

soil dry weight (NOEC). The LOEO for both endpoints is 50 mg/kg artificial soil dry weight.



#### I. MATERIAL AND METHODS:

Test item: M-01 (AE C653711 00 1B97 0001, Batch No: 8808018; purity: 97.0% w/w). Test organisms: Folsomia candida (Collembola: Isotomidae), age of 10-12 days. 10 collembolans per replicate sere exposed to control (water treated), solvent control (acetone) and treatments with 6.25, 12.5, 25, 50 and 100 mg/kg artificial soil (dry weight). The assay was conducted under controlled environmental conditions at 19-21°C and 480-650 lux with a light regime of 16 h light and Shours dark. There was one additional container per test item and control for measurement of pH. Test containers were reusable glass vessels (volume: 100 mL; diameter: 5 cm), closed tightly by plastic-lids to avoid water evaporation? from the artificial soil, filled with approximately  $30 \text{ g} \pm 1.5 \text{ g}$  fresh weight artificial soil. Artificial soil contained 10% Sphagnum peat, 20% Kaolin clay, approximately 0.5% Calcium carbonate and approximately 69.5% fine quartz-sand.

Feeding: After the introduction of the test organisms, approximately 2 mg (half of a small spatula) of granulated dry yeast (Dr. Oetker) were spread over the soil surface at test start. After 14 days, granulated dry yeast was added ad libitum. Ò

Mortality and reproduction were determined after 28 day At day 28 after application, water content and pH of one additional test container (prepared at test start and without Collembola) per treatment group and control were determined according to DIN 19683 and DIN 19684 (CaCh). The software used to perform the statistical analysis was Systat Version 9.0.

### Dates of experimental work: November 18, 2002 - December 17,

o perform the stati	ates of experimental work: November 18, 2002 – December 17, 2002						
<b>.</b>	4 - 1 <b>-</b> 1 NT		≫' [©] ⁄an D ² a1a			N V	
Dates of experiment	ntal work: No	vemberoi 8, 20	02 – Decembe				
	<i>S</i>	KII. RESULT	'S AND DISCUS	SKON N			
Analytical results:			ý k a				
	A A			<u> </u>			
Test item	Start 2	Find a	~wv ater‱oonten	₱,( ~0)~			
concentration ¹	Start 2	Luu 🔨	Start 🔊	End S	Start	End	
Control	509.0	5.8° &	35% 0 ⁵ 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	33%	54%	51%	
Solvent control	5.8 4	5.9 4	35% 0	33%	54%	51%	
6.25	5.80 55	<u>5.9</u>	35% 2	340%	54%	52%	
12.5		$\mathcal{Z}_1  \mathcal{O}^{\vee}$	35%	34%	54%	52%	
25 Ø		5.8 5.1 5.8 5.4 5.4 5.4 5.4 5.4 5.4 5.4 5.4 5.4 5.4	35%	34%	54%	52%	
50	ES C	357 ~ U	×35%	34%	54%	51%	
100 🔺	5.8 0 3	5.7	35%	34%	54%	51%	
minintem	5.8 5.8 5.8 S	<u>5.7</u>	35%	33%	54%	51%	
maximum	5.8 2 Q	<u>5.7</u> <u>76.1</u>	×35%	34%	54%	52%	

¹ mg a.s./kg artificial soil dry weight ²

 2  % WHC_{max} = per Cent of maximum water hading capacity (WHC_{max} = 65.47% dry weight)

² % WHC_{max} = percent of maximum water helding capacity (WHC_{max}
 ³ the results represent rounded values calculated on the exact raw data



#### **Biological results:**

mg/kg soil dry weight nominal conc.	Adult mortality (%)	Mean number of juveniles/test vessel ± SD	Reproduction (% of pooled controls)	Significance
Control	4	$677 \pm 92$	-	
Solvent control	4	$769 \pm 124$	114	as.1
6.25	6	$834 \pm 144$	115	$h.s.^2$
12.5	6	$679 \pm 83$	24	n.s
25	8	$683 \pm 46$	× 94 %	O no v
50	16	548 ± 100 💍	A 76 🔬	
100	6	571 ± 128 🚿	⁰ 79	\$9 ^{*2} \$
¹ Student t-Test $\alpha = 0$	05 one-sided greater		08 47	<u>~~</u> 0'

¹ Student t-Test,  $\alpha = 0.05$ , one-sided greater ² Williams Test,  $\alpha = 0.05$ , one-sided smaller

A valid ECx curve for reproduction could not be calculated due to the lack of a significant dose-response relationship.

#### Toxic reference test:

The most recent toxic standard test showed statistically significant effects on reproduction at concentrations > 50 mg Betosip/kg artificial soil dry weight; the ECG for reproduction was calculated as 135 mg Betosip/kg soil dry weight.

Mortality was statistically significantly higher than the control at 400 tog Betosip/kg artificial soil dry weight, the  $LC_{50}$  for mortality was calculated as 285.1 mg Betosip/kg soil dry weight.

#### Validity criteria:

All validity criteria were the in this study.	
Validity criteria (OFCD 232, 2016)	Obtained in this study
v  =  v  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u  =  u	2 4% ±0.5% Ø
Mean number of juveniles/replacate 100 0	$0$ > $7 \pm 92$
Coefficient of variation calculated for the num juveniles per replicate $\leq 30\%$	be of \$16%
juveniles per replicate $\leq 30\%$ .	
	SELUSION:
	DECLUSION:

In this study, M-01 (AE C6537) caused no mortality and no adverse effect on reproduction of *Folsomia candida* at concentrations we to and including 25 mg/kg artificial soil dry weight (NOEC). The LOEC for both endpoints was 50 mg/kg artificial soil dry weight.

### Assessment and conclusion by applicant

This study is considered retrable for risk assessment. The NOEC of 25 mg/kg for reproduction is the relevant endpoint for the risk assessment.



Data Point:	KCA 8.4.2.1/03
Report Author:	
Report Year:	2016
Report Title:	AE 0608000 (BCS-AX86048): Effects on mortality and reproduction of the
	collembolan species Folsomia candida tested in artificial soil
Report No:	EBACN060
Document No:	<u>M-558337-01-1</u>
Guideline(s) followed in	OECD 232 (2009): OECD Guideline for testing of chemicals No. 232 (adopted 7
study:	September 2009): Collembolan reproduction test in soil; US EPA QCSPP not
	applicable
Deviations from current	Current Guideline: OECD 232 (2016)
test guideline:	The illumination was 12 h light and 12 h dark and not 16 h light and sh dark as
	recommended by the guideline.
	This deviation is not expected to have impacted the study results
Previous evaluation:	No, not previously submitted
GLP/Officially	Yes, conducted und GLP/Officially recognised testing facilities
recognised testing	$A  \mathcal{T}  T$
facilities:	
Acceptability/Reliability:	Yes a way of the second

#### **Executive Summary**

The purpose of the study was to determine the effects of M 33 (AE 0608000) on reproduction of the Collembola Folsomia candida n artificial soil. Folsomia & andida Collembola. Isotopidae), age of 9-12 days. 10 collembolans per replicate were exposed to control (water treated), and treatments with 10, 18, 32, 56 and 100 mg/kg dry Weight soil. The assay was conducted under controlled environmental conditions in glass vessels (volume: 150 mts) covored with a lid. Mortality and reproduction were determined after 28 days. All validity criteria were met, in this study M-03 (AE 0608000) caused no mortality and no adverse effect of reproduction of Folsomia candida at concentrations up to and including 100 mg/kg soil dry weight (NOEC). The LOEC for both endpoints are > 100 mg/kg soil dry weight weight.

I. MATERIAL AND METHODS:

Test item: M-03 (AE 60800 (BCS-AX86048)), Batch Code: AE 0608000-01-01; purity: 99.4% w/w. Test organisms: For omia candida (Collembola, Isotomidae), age of 9-12 days. 10 collembolans per replicate were exposed to control (water treated), and treatments with 10, 18, 32, 56 and 100 mg p.m./kg dry weight soil, The asay was conducted inder controlled environmental conditions at 18.9 -21.6°C and 520 lux with a light regime of 12h light and 12 hours dark. Test containers were reusable glass vessels (volume: 150 mL) covered with a lid; surface area of soil: 18.9 cm², filled with 30 g wet weight artificial foil. Artificial foil contained 5% sphagnum peat, 20% kaolin clay, 74.7% industrial quartz sand und 0.3% calcium consonate. Feeding: After the introduction of the test organisms and after 14 days, approximately 2 mg of granulated dry yeast were spread over the soil surface.

Mortality and reproduction were determined after 28 days. The pH and water content of the test substrate

were determined at the start and at the end of the test in the control and each treatment.



#### Dates of experimental work: February 26, 2016 – March 25, 2016

II.	<b>RESULTS</b>	AND	<b>DISCUSSION:</b>
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. . .

<u>nalytical resu</u> ater content,		ling capacity	(WHC) and	pH in the	control and	treatment	8		Ĉ
Water conter					p				
Start	End		Start	End	l St	tart 🛋	End	S S	, Ô
25.0 - 25.1	24.3 - 24	.8	59.1 - :	59.3 57	<b>-</b> 58.6 6.	04-6.13	5.84	× 5.93 ×	ļ
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Test obj			ll.		somia candi		~		
Exposu			<u> </u>		rtificial soil		<u> </u>	× ×	_
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dry weight arti			Signifi <u>a</u> nce		number of	Reprod		Significance	
(nomin		mortality		Juvenies	per test vess	$\mathbf{e}$ (%) $\mathbf{f}$ c	<u> </u>		
concentrat	,	(%)			rd deviation			<del>) 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0</del>	_
Contro	ol	1.3		<u>1166</u>	$\neq$ $\pm$ 104		<u>,                                    </u>	, Ôj	
10		2.5	- <i>6</i> .	🔊 1135		<u>~</u>	7 🔊	<u> </u>	
18		5.0 👋	\$ - \$	1083	J₽			°~ -	
32		2 <i>5</i> 0	K. O	<b>\$169</b>	$\mathcal{O}$ $\pm$ $\mathcal{O}$ $2$	1 0 196			
56		5,0	÷Q	1177	× ± \$82		$\mathfrak{I}_1 \mathbb{O}^{\mathbf{I}}$	-	
100		5.0		1149	± 12			-	
	¢	× 4	Q	Å		Mort	ality	Reproduction	
NOEC (mg pu	re metabøli	te/kg dry wei	ght artifoial	sojil 🔍	D ^v 🥾	2 <b>(</b>	<b>Ø</b> 0	$\geq 100$	
NOEC (mg pur LOEC (mg pur ne calculations w ) = Williams-t-te	e metaboli	te/k@dry wei	ght artificial	(1) J	° Oʻ		00	> 100	

(*) = Williams-t-test, one-side Osmaller,  $\alpha = 0.05$ , + = significant, - = not significant (**) = Multiple Sequentially-rejective Fisher Test after Bonfertoni-Holm, one-sided greater,  $\alpha = 0.05$ , + = significant, - = not significant «° significant. O Ľ

The calculation of an EG& curve for reproduction was not possible due to the lack of a significant doseresponse relationshi Š

#### Toxic reference tes

In the most recent study with the reference tem boic acid the EC₅₀ for reproduction was determined to be 103 mg/kg dry weight soil. The LC₅₀ was determined to be 162 mg /kg dry weight soil. The NOEC for mortality and for reproduction was determined to be 67 and 44 mg/kg soil dry weight, respectively. The EC value for reproduction was close to the value of 100 mg/kg soil dry weight as stated in OECD 232 (2009). The ECS therefore showed that the test system was sensitive.

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V	O,	C, V	× .0″
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Validity criteria:			$\mathcal{O}'$
validity criteria.	Aid		¥
0		· · · ·	<i>a</i> ,
All validity criteri	a were met	in this stud	V.
A C	× P	.~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	
U D	/ 0	ñ	

Validity criteria according to OECD 232 (2016)	Obtained in this study
Mean adult mortality $\leq 20\%$	1.3%
Mean number of juveniles/replicate $\geq 100$	1166
Coefficient of variation calculated for the mean number of juveniles < 30%	9.0%



#### **III. CONCLUSION:**

In this study, M-03 (AE 0608000) caused no mortality and no adverse effect on reproduction of Folsomia candida at concentrations up to and including 100 mg/kg soil dry weight (NOEC). The LOEC for both endpoints was > 100 mg/kg soil dry weight.

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#### Assessment and conclusion by applicant:

05 This study is considered reliable for risk assessment. The NOEC of  $\geq 100$  mg/kg for reproduct the relevant endpoint for the risk assessment.

	KCA 84 21/04
Data Point:	KCA 8.4.2.1/04
Report Author:	$\begin{array}{ c c c c c c c c c c c c c c c c c c c$
Report Year:	KCA 8.4.2.1/04
Report Title:	Pyridyl carboxylicacid (BSS-ABC3478): Effects on mortality and reproduction of
	the collembolan species Polsonia candida tested in artificial soil
Report No:	EBACN056 QY , Y QY , Y QY , Y
Document No:	<u>M-558332-921</u>
Guideline(s) followed in	OECD 232 2009 OECD Guideline for testing of chemicals No. 232 (adopted 7
study:	September 2009): Collegnbola reproduction test in sort, US PBA OCSPP not
	applicable with the applicable with the second
Deviations from current	Current Guideline: QECD 232 (2016)
test guideline:	The illumination was 12 h light and 12 h dark and not 16 h light and 8 h dark as
*	recommended by the goddeline.
	This deviation is not expected to have impacted the study results.
Previous evaluation:	No not previously submitted in the second second
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GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
recognised testing	
facilities:	
Acceptability/Reliability:	Nes A A A
Summany	

### Executive Summary @

The purpose of the study was to determine the effects of M-02 (AE C657188) on reproduction of the Collembola Folsemia candida in artificial soll. Folsomia candida (Collembola: Isotomidae), age of 9-12 days. 10 collembolans per replicate were exposed to control (water treated), and treatments with 10, 18, 32, 56 and 00 mg p.m. (bg dry weight soil. The assay was conducted under controlled environmental conditions in glass vessels (volume: 450 mla) covered with a lid. Mortality and reproduction were determined after 28 days.

All validity criteria vere met. In this study, M-Q2 (AE C657188) caused no mortality and no adverse effect on reproduction of *polsonia candida* at concentrations up to and including 100 mg p.m. /kg soil dry weight (NOEC). The LOEC for both endpoints is > 100 mg p.m./kg soil dry weight.

... LOEC for both e



#### I. MATERIAL AND METHODS:

Test item: M-02 (AE C657188 (BCS-AB43478)), Batch Code: AE C657188-PU-01, Origin Batch No.: SES 10250-1-1, CAS No.: 80194-68-9, LIMS No.: 1518969, purity: 98.5 % w/w. Test organisms: Folsomia candida (Collembola: Isotomidae), age of 9-12 days. 10 collembolaos per replicate were exposed to control (water treated), and treatments with 10, 18, 32, 56 and 100 mg m./kg dry weight soil. The assay was conducted under controlled environmental conditions at 189 -21, 200 and 620 lux with a light regime of 12h light and 12 hours dark. Test containers were reusable plass vessels (volume: 150 mL) covered with a lid; surface area of soil: 18.9 cm² filled with  $3^{\circ}$  wet weight artificial soil. Artificial soil contained 5% sphagnum peat, 20% kaolin clar, 74.7% industrial quartz sand und 0.3% calcium carbonate. Feeding: After the introduction of the test organisms and after 14 days, approximately 2 mg of granulated dry yeast were spread over the soi Surface. 21 Mortality and reproduction were determined after 28 trays. The pH and water content of the test substrate were determined at the start and at the end of the test in the control and each treatment on this study M-02 (AE C657188) caused no mortality and no adverse effect on reproduction of Folsomia candida at concentrations up to and including 100 mg m. /kg soil dry weight (NOEC) The LOEC for both endpoints was > 100 mg p.m./kg soil dry weight. 

#### Dates of experimental work: April

1	. •	1	1.	

Analytical results:

#### Water content, water holding capacity@WHC and ph in the control and treatment

Water content (g	g/109 g soil c	ry weight)	ØVHC.∢		PØ 4	
Start	End 🔗	Ľ,	Stant	<b>\$ O End</b>	{Start @	End
24.9 - 25.0	24.4 24.8		and		8.6 6.05 6.09	5.79 - 5.84
	Â, Ĉ	) Oʻ			Š O.	

Riological

Biologicar esuits:	~~~~			4	$\searrow$				
$\sim 100$ $\sim 100$ $\sim 100$ $\sim 100$									
Test object		Contra candida							
Exposure		17 0	O Antii	ficia	l soil				
mg pure metabolite/kg			A Mean nu	mbe	r of				
dry weight artoficial sol	Adult 👡	Signif@ance	Guveniles per	tes	t vessel	Reproduction	Significance		
(nominal	~ mortality/		$\neq $ standard	dev	iation	(% of control)	(*)		
concentrations)	° (%)		Ű						
Control 🔊	205		مَرْ 808	±	79	-			
10	A 2.5		S 814	Ŧ	46	101	-		
18	\$\$V0.0	Q ~~	826	Ŧ	94	102	-		
32	° 2,\$	a, - 2,	828	Ħ	81	102	-		
56	Q.5 ~		806	Ħ	41	100	-		
100 ~	2.5	@-	816	Ŧ	134	101	-		
					Mortality	Reproduction			
NOEC (hug pure netabolite/kg dry weight artificial soil)						$\geq 100$	$\geq 100$		
LOEQ mg pure metabolite/kg dry weight artificial soil)						> 100	> 100		

The calculations were performed with unrounded values

(*) William t-test, one-sided smaller,  $\alpha = 0.05$ , + = significant, - = not significant (**) = Multiple Sequentially-rejective Fisher Test After Bonferroni-Holm, one-sided greater,  $\alpha = 0.05$ , + = significant, - = not significant,



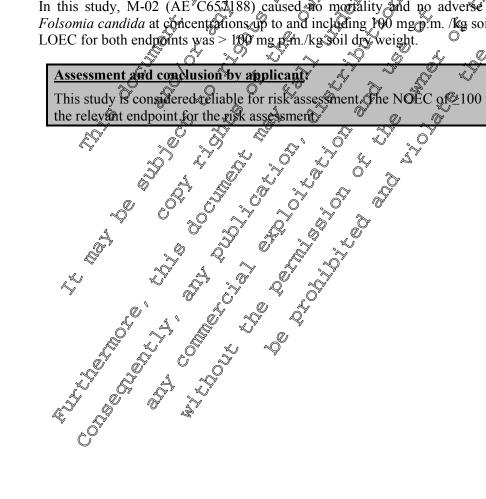
The calculation of an ECx curve for reproduction was not possible due to the lack of a significant doseresponse relationship. 

<u>Toxic reference test:</u> In the most recent study with the reference item boric acid the  $EC_{50}$  for reproduction was determined to be 103 mg/kg dry weight soil. The LC₅₀ was determined to be 162 mg /kg dry weight soil. The NOEC for mortality and for reproduction was determined to be 67 and 44 mg/kg soil dry weigh Prespectively for mortality and for reproduction was determined to be 67 and 44 mg/kg soil dry weight respectively. The EC₅₀ value for reproduction was close to the value of 000 mg/kg soil dry weight as stated in OECD 232 (2009). The EC₅₀ therefore showed that the test system was sensitive. Validity criteria: All validity criteria (OECD 232, 2016) Mean adult mortality < 20%

-		~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~		
Validity criteria (OECD 232, 2016)	o Abtai	ned in this st	hdy	
Mean adult mortality $\leq 20\%$	2.5%		2	
Mean number of juveniles/replicate 100	× 268		20 0	
Coefficient of variation calculated for the	mean 9 8%		Ľ.	. 2
number of juveniles < 30% 👋 👸 🧔				°
	Ô'L'		8 X	1
w w. Cox	CLUSIONS:	s.	¢ ~	
	, - U	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	i O	

In this study, M-02 (AE C65,2188) caused no mortality and no adverse effect on reproduction of Folsomia candida at concentrations up to and including 100 mg p.m. /kg soil dry weight (NOEC). The

This study is considered reliable for risk assessment. The NOEC of 2100 mg/kg for reproduction is the relevant endpoint for the risk assessment.





Data Point:	KCA 8.4.2.1/05
Report Author:	
Report Year:	2016
Report Title:	Fluopicolide a.s. (BCS-AM59797): Influence on mortality and reproduction of
	the soil mite species Hypoaspis aculeifer tested in artificial soil
Report No:	E 428 4833-2
Document No:	<u>M-548042-01-1</u>
Guideline(s) followed in	OECD 226 from October 03, 2008: OECD guideline for the Testing of Chemicals
study:	- Predatory mite (Hypoaspis (Geolaelaps) aculeifer) reproduction tee in soit
Deviations from current	Current Guideline: OECD 226 (2006)
test guideline:	No deviations
Previous evaluation:	No, not previously submitted 🔬 🖉 🖉
GLP/Officially	Yes, conducted under GLP Officially recognised esting a cilities
recognised testing	
facilities:	
Acceptability/Reliability:	Yes O Q Q Q Q Q Q Q Q Q Q Q Q Q Q Q Q Q Q

#### **Executive Summary**

The purpose of this study was to assess the effect of fluopiconide as (BCS-AM59797) on mortality and reproduction of the soil mite species *Hypoaspis aculetter* tested during an exposure of 4 days in artificial soil comparing control and treatment. Ten adults, fortilized, female *Hypoaspis aculeifer* per replicate (8 replicates for the control group and 4 replicates for each treatment group) were exposed to control and treatments. Concentrations of 78, 32, 56, 100, 178, \$16, 562 and 1000 mg a.s./kg artificial soil dry weight were tested. After operior of 14 days, the surviving adults and the hying juveniles were extracted by applying a temperature gradient using a MacFadyen-apparatus. Af *Hypoaspis aculeifer* were counted under a binocular.

Were counted under a priocupal. All validity criteria were met. The NOE  $C_{reproduction}$  and  $COE C_{eproduction}$  of fluopicolide were determined to be  $\geq 1000$  mg a.s./kg artificial soil dry weight and > 4000 mg a.s./kg artificial soil dry weight, respectively. Since there are no adverse effects on mortality and poproduction, no EC₁₀/EC₂₀ calculation was possible.

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Fluopicolide a.s. (PCS-AM59777) (analytical Indings: 98% % w/w; batch code: AE C638206-01-26; customer order no.: TOX 10889-00; specification no.: 102000016444; origin batch no.: BCHR 1111-2-1; certificate fo.: AZ200339, purity: 98.8%. Ten adults, fertilized, female *Hypoaspis aculeifer* per replicate (8 replicates for the control group and 4 replicates for each treatment group) were exposed to control and treatments. Conceptrations of 18, 32, 56, 100, 178, 316, 562 and 1000 mg a.s./kg artificial soil dry weight were tested. During the test, the *Hypoaspis aculeifer* were fed with nematodes bred on watered oat flakes. During the study a temperature of  $20 \pm 2$  °C and light regime of 400 - 800 Lux, 16 h light: 8 h dark was applied. The artificial soil was prepared according to the guideline with the following construents (percentage distribution on dry weight basis): 75 % fine quartz sand, 5 % Sphagnum pear, 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; 26 detargent/k fixing solution were added). All *Hypoaspis aculeifer* was observed.

Concerning the number of juveniles statistical analysis (William's-t test, one-sided smaller,  $\alpha = 0.05$ ) revealed to significant difference between control and any treatment group. Since there were no adverse effects on mortality and reproduction, no EC₁₀/EC₂₀ calculation was possible.

**Dates of experimental work:** November 20, 2015 – December 11, 2015



#### Experimental conditions:

All values were within the range recommended by the guideline.

[mg a.s./ kg dry weight	рН		% water content			So of WHC mag	
artificial soil]	Start	End	Start	End	% deviation	Start	And a
Control	5.65	5.55	19.52	19.45	0.4	47.46	47.25
18	5.63	5.56	19.25	19.20	00	46.64	4630
32	5.60	5.68	19.31	18,81	<u>0</u> 2.7	46.82	46.50 46.50 46.50
56	5.59	5.55	19.36	¥9.20	Q 0.8 °	46.99	¥46.5
100	5.56	5.57	19.02	° 19.30 ∧	<u>10</u>	\$45.980	46,80
178	5.56	5.57	19.22	12.02 5	x1.1 x )	46.06	¥5.96 √
316	5.51	5.56	19.64°	¥9.02	3.30	<b>4</b> 7.83 L	45.96
562	5.51	5.53	19.22	19.00	× 1.2 ¢	≫46.56 ^{©°}	45,90
1000	5.51	5.58	48.98	19:60	3.2 3	45 85	47.71 S

<u>Diological results</u>: In the control group no mortality of adult *Hypoaspis acyleifer* was observed. Concerning the number of juveniles statistical analysis (Witham's) test, one-sided smaller,  $\alpha = 0.05$ ) revealed no significant difference between control and any treatment group. Since there were no adverse effects on mortality and reproduction, no  $C_{10}/E_{20}$  calculation was possible

Test item		6 6 Fluopicoli	De a.s. &	. 6	
Test Object		💭 💦 Hyppoaspis a	culeifer 🚿		
Exposure	osure of a start and a source of a start start and a start				
Exposure [mg a.s./kg dry weight	% mortality	Mean number of	Reproduction	Significance	
	(adults)	🌾 juveniles per rest 🔪	(% of control)	(*)	
artificial <b>Sof</b> []	Ş ,O	y juveniles per test Vessekt standard de			
Control		₫ 233.0 €/31.6		-	
18	210.0	$\sqrt[6]{234.5 \pm 32.1}$	\$ 400.6	-	
\$32	6,70	[∞] 21,7%7 ± 407 ≪	× 093.4	-	
56	<u>)</u> 2 ³ 2 4	230.3 ₹ 2.1 %	107.4 🖉	-	
100 🔊	5.0	, [*] ∕282.3⁄€ 27.6 © [*]	121.1	-	
178 🔍	ð 7.5 Å	262,5 ± 48,6	112.7	-	
316	5.0 5.0 5.0 5.0	209.8 ± 29.8	120.1	-	
562 🌾		289.0 \$22.3	124.0	-	
1000	2.5	260,0°±15,0°	111.6	-	
NOECoproduction [mg	a s /kg dry weig	sht artification soil 2/1000			
LOEC _{reproduction} [mg	§s./kg.dry weig	ht artificial soil > 1000			

LOFC reproduction [mg  $\eta_{rs}$ ]/ $\kappa_{s}$ / $\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/\kappa_{s}/$ 

<u>The calculation of an ECx curve for reproduction was not possible due to the lack of a significant dose-</u> response relationship.



Validity criteria:

All validity criteria were met.

Validity criteria (OECD 226, 2016)	Obtained in this study	
Mean adult mortality should not exceed 20 % at the end of the test	0 %	
Mean number of juveniles per replicate should be at least 50 (with 10 mites introduced)	233	
Coefficient of variation calculated for the number of juveniles per replicate should not be higher than %	13.6 %	
, And	JOY X	

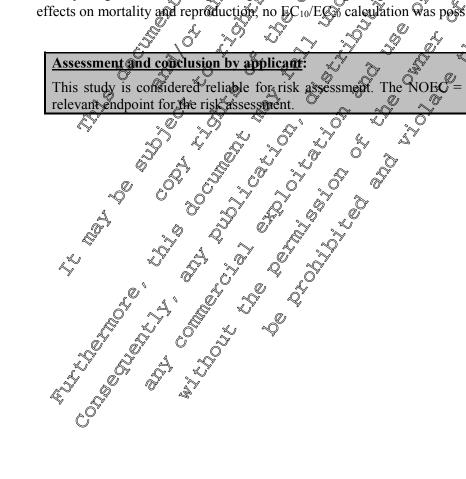
#### Toxic reference test:

In a separate study (Maria Ivonne Larnaudie Loper LAR/HR-O-21/15 November 09 2015) performed with the reference item dimethoate at test concentrations 1.0, U.8, 3,2, 5.6 and 100 mg dimethoate/kg dry weight artificial soil, the LC₅₀ was calculated to be 1. King a s/kg for mortality. The NQEC was calculated to be 3.2 mg a.s./kg and the LQEC was 5.6 mg a.s./kg. Since variances of the fata were homogenous Williams-t test  $\alpha = 0.05$ , one-sided smaller was used. Dimethodie EC 400 O showed an EC50 of 5.36 mg a. s./kg (99 % confidence limits from 405 mg a. s./kg to 568 mg a. s./kg for reproduction. This is in the recommended range of the guideline and demonstrates the sensitivity of the test system. 

1 The NOEC reproduction and LOEC reproduction of Iluopicolide were determined to be 1000 mg a.s./kg artificial soil dry weight and > 1000 mg a. ./kg ortificial soil de weight, respectively. Since there were no adverse effects on mortality and reproduction, no EC10/ECT calculation was possible 2

III. Conceusios:

This study is considered reliable for risk assessment. The NOEC = 1000 mg a.s./kg dws is the relevant endpoint for the risk assessment.





Data Point:	KCA 8.4.2.1/06
Report Author:	
Report Year:	2015
Report Title:	AE C653711 (BCS-AA65784): Influence on mortality and reproduction of the
	soil mite species Hypoaspis aculeifer tested in artificial soil
Report No:	E 428 4712-8
Document No:	<u>M-538626-01-1</u>
Guideline(s) followed in	EU Directive 91/414/EEC
study:	Regulation (EC) No. 1107/2009
	US EPA OCSPP: not applicable &
Deviations from current	Current Guideline: OECD 22¢(2016)
test guideline:	No deviations
Previous evaluation:	No, not previously submitted
GLP/Officially	Yes, conducted under GLP/Officially Decognised testing facilities in the second
recognised testing	
facilities:	
Acceptability/Reliability:	Yes N N N A O L

The purpose of this study was to assess the effect of M-01 (AE 6537 H (BCS AA65784)) on mortality and reproduction of the soil mite species *Hypoaspis oculeifs* tested during an ecosure of 14 days in artificial soil comparing control and treatment. A concentration of 100 mg/kg artificial soil dry weight was tested. After a period of 14 days, the urviving adults and the living juveniles were extracted by applying a temperature gradient using a MacFadyen-apparatus. All *Hypoaspis aculeifer* were counted under a binocular. In the control group 3.8 % of the adult *Hypoaspis aculeifer* and which is below the allowed maximum of 20 % protabily.

All validity criteria were met. Concerning the number of juveniles statistical analysis (Student-t test, one-sided smaller, a = 0.6) revealed no significant difference between control and any treatment group. The NOEC_{reproduction} and LOEC_{reproduction} of fluopicolide were determined to be  $\geq 100$  mg/kg artificial soil dry weight and  $\geq 100$  mg/kg artificial soil dry weight, respectively.

# KMATERIAL AND METHODS?

M-01 (AE C65371) (BCS-AA65784)) (analytical findings 96.2 % w/w; batch code: M-01 (AE C653711 001B96 0001; origin batch no.: 08018ET, certificate no.: AZ17535)), purity: 96.2%. Ten adults, fertilized, female *Hypotspis aduleifer* per replicate 8 replicates for the control and the treatment group) were exposed to control and treatments. A concentration of 100 mg/kg artificial soil dry weight was tested. During the test, *Hypotspis aduleifer* were fed with nematodes bred on watered oat flakes. During the study a temperature of  $20 \pm 2$  °C and light/regime of 400 - 800 Lux, 16 h light: 8 h dark was applied. After a period of 14 days, the surviving adults and the living juveniles were extracted by applying a temperature gradient using a MacFadyen-apparatus. Extracted mites were collected in a fixing solution (20% ethylene giveol, 80% deionised water; 2 g detergent/L fixing solution were added). All *Hypoaspis aculeifer* were counted under a binocular.

All validity criteria were met. In the control group 3.8 % of the adult *Hypoaspis aculeifer* died which is below the allowed maximum of  $\leq 20$  % mortality. Concerning the number of juveniles statistical analysis (Student-triest, one-sided smaller,  $\alpha = 0.05$ ) revealed no significant difference between control and any treatment group. The NOEC production and LOEC_{reproduction} of fluopicolide were determined to be  $\geq 100$  mg/kg artificial soil dry weight and > 100 mg/kg artificial soil dry weight, respectively.

Dates of experimental work: February 20, 2015 – March 12, 2015



#### Experimental conditions:

All values were within the range recommended by the guideline.

g dry pH		% water content		Ĩ	So of WHC mar		
Start	End	Start	End	% deviation	Start	Find S	
5.64	5.54	19.06	18.78	1.5	49.88 🔊	48.92	
5.75	5.58	19.06	18.25	38	49.86	42,00 0 0	
	<b>Start</b> 5.64	Start         End           5.64         5.54	Start         End         Start           5.64         5.54         19.06	Start         End         Start         End           5.64         5.54         19.06         18.78	Start         End         Start         End         % deviation           5.64         5.54         19.06         18.78         1.5           5.75         5.58         19.06         18.25         333	Start         End         Start         End         % deviation         Start           5.64         5.54         19.06         18.78         1.5         49.88         49.88	

**Biological results:** 

In the control group 3.8 % of the adult *Hypoaspis of uleifer* died which follow the allowed maximum of  $\leq 20$  % mortality.

Concerning the number of juveniles statistical analysis (Student-t test, one-sided smaller,  $\alpha \stackrel{=}{=} 0.05$  revealed no significant difference between control and any reatment group.

		S.
Test item	M-01 (AE C653/11) S	
Test Object	🖉 🥵 Hypeaspis actileifer 🖉 🖉 🖉	
Exposure	O' Artificial Soil O S &	Ô
[mg/kg	% mortality Mean number of Reproduction Significance	J
dry weight	(adults) juveniles per test 0% of control	
artificial soil]	(adults) juveniles per test (%) of control (*)	
Control	$3.8^{\circ}$ ( $24.9.9 \pm 29.0$ ( $4.7 - 5.7$ ) ( $-$	
100	$5.0^{\circ}$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$ $(-2.1)$	
NOEC _{reproduction} [mg	kg dry weight artificial soil 2100 y y	

 $LOEC_{reproduction}$  [mg/kg/dry weight artificial sof] > 100

Calculations were done with un-rounded values. (*)=Welch-t-test for inhumogeneous variances, one sided smaller; (*)=0.05; (2): non-significant; (*+": significant

The calculation of an Rex curve for reproduction was not possible as only one concentration was tested.

### Validity criferia:

All validity criteria were met,

	/
Validity criteria (DECD 226, 2016) NOT	Obtained in this study
Mean adult mortality should not exceed 20 % at the end of the test	3.8 %
Mean number of juveniles per replicate should be at least 30 (with 10 mites introduced)	241.9
Coefficient of variation calculated for the number of juveniles for replicate should not be higher than 30 %	12.0 %

### Toxic reference dest:

In a separate Study (Maria bonned arnaudie Lopez, LAR/HR-O-16/14, January 05, 2015) performed with the reference item dimethoate at test concentrations 1.0, 1.8, 3.2, 5.6 and 10.0 mg dimethoate/kg dry weight artificial soit the LO₅₀ was calculated to be 2.51 mg/kg (95 % confidence limits from 0.85 mg/kg to 3.30 mg/kg) for motality. The NOEC was calculated to be 3.2 mg/kg and the LOEC was 5.6 mg/kg. Since variatees of the data were homogenous Williams-t test  $\alpha = 0.05$ , one-sided smaller. Dimethoate EC 400E G showed an EC₅₀ of 5.47 mg/kg (95 % confidence limits from 4.09 mg/kg to 7.30 mg/kg) for reproduction. This is in the recommended range of the guideline and demonstrates the sensitivity of the test system.



#### **III.** CONCLUSION:

Assessment and concl	lusion by applicant:
This study is consider	red reliable for risk assessment. The NOEC $\geq$ 100 mg/kg is the represent.
endpoint for the risk as	ssessment.
-	
Data Point:	KCA 8.4.2.1/07
Report Author:	
Report Year:	
Report Title:	Pyridyl carboxylic acid (BCS7AB43478): Effects on mortality and reproduction
-	of the predatory mite Hypgaspis a veleifer ested in artificial soil
Report No:	EBACN057 C C C
Document No:	M-557987-01 4 5 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6
Guideline(s) followed in	OECD 226 (2008); Predatory mite (Hypoaspis (Geolaelaps) aculeiter)
study:	reproduction test in soil; US EPA, OCSPPonot applicable
Deviations from current	Current Guideline. OECD 226 (2016) Star Contract Contract Star
test guideline:	No deviations ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ?
Previous evaluation:	No not prexiously submitted of the submi
GLP/Officially	Xes, conducted under @P/Officially recognized testing facilities
recognised testing	
facilities:	Yes 2 a d y y y y

### Executive Summary

**Executive Summary** The purpose of this study was to assess the offect of MI-02 (AE Cost7188) on mortality and reproduction of the soil mile species Hypoaspis aculetfer tested doring an exposure of 14 days in artificial soil comparing control and treatment. Ten adult soil mites (females) Hypoaspis aculeifer per replicate (8 replicates for the control and the treatment) were exposed to control and treatments. A concentration of 100 mg/kg artificial soll dry weight was tested. After a period of 14 days, the surviving adults and the living juveniles were extracted by applying a temperature gradient using a MacFadyen-apparatus. Extracted mites were collected in a fixing solution and courted.

All validity criteria were met in the control group 7.3 % of the adult Hypoaspis aculeifer died which is below the allowed maximum of  $\leq 20\%$  mortality. Concerning the number of juveniles statistical analysis revealed no significant difference between control and any treatment group.

# 71. Materian And Methods:

M-02 (AE C657188), (batch code: C657188-PU-01; origin batch no.: SES 10250-1-1, certificate AZ 20206, CAS No.: 80194-680, LIMS No. 1518969, purity: 98.5%. Ten adult soil mites (females) Hypoaspis achleifer per replicates (8 replicates for the control and the treatment) were exposed to control and treatments. A concentration of 100 mg/kg artificial soil dry weight was tested. During the test, Hypoasses acuteifer were fed every 2-3 days with cheese mites Tyrophagus putrescentiae. During the study temperature of 19.8 20.4 °C and light regime of 450 Lux, 16 h light: 8 h dark was applied. The artificial sel was prepared according to the guideline with the following constituents (percentage distribution on dry weight basis): 74.7 % fine quartz sand, 5 % Sphagnum peat, air dried and finely ground, 20 % Kaolin clay and 0.2% calcium carbonate. After a period of 14 days, the surviving adults and the living juveniles were extracted by applying a temperature gradient using a MacFadyenapparatus. Extracted mites were collected in a fixing solution and counted. The NOEC_{reproduction} and



LOEC_{reproduction} of fluopicolide were determined to be  $\geq 100$  mg /kg artificial soil dry weight and > 100mg/kg artificial soil dry weight, respectively.

#### **II. RESULTS AND DISCUSSION:**

m								
D	Dates of experimental work: March 18, 2016 – April 20, 2016							
	II. RESULTS AND DISCUSSION:							
	II. RESULTS AND DISCUSSION:       Image: Conditions:         Experimental conditions:       Image: Condition of the superimental conditis of the superimental condition of the superimental condition of							
	[mg/ kg dry weight pH % water content % & WHOmax							
	artificial soil]	Start	End	Start End Meviation Start Eng				
Γ	Control	5.8	5.4	18.37 k P7.77 2 2 2 2 47.79 48.10				
	100	5.8	5.4	$18.49 \odot^{*}$ $C^{*}$ $18.05$ $C^{*}$ $2.2 \odot^{*}$ $46.24$ $47.04$				
_								

#### **Biological results:**

In the control group 1.3 % of the adult Hypogspis activities died which is below the allowed maximum of  $\leq 20$  % mortality. Ø

of  $\leq 20$  % mortality. Concerning the number of juvenites statistical analysis (Fisher's Boact Binominal Test one-sided greater,  $\alpha = 0.05$ ) revealed no significant difference between control and any treatment group.  $\mathcal{O}$ 

	A CT	ĭ∼y	'0' L		0 .8
Test item	l v k		M-02 (AE_C	6571 <b>88</b> ) 🔌	Ô,
Test Object	<u></u> \$ 0		Hypoaspis ac	culeifer 🚿 🍶	Ž, Ž,
Exposure	× A		Artificial	Soil 🗸 🗸	Z,
[mg/kg	‰mortaΩity	_√ ⊘Mean¶	umber of 📎	Reproduction	Significance
dry weight	🖇 (adults)		es per test 🖉	(% of control)	 
artificial soil] 🔬		vessel ± st	andard dev.	A A. O	
Control	1.3 4	324	×± 38 °√ ۵	5° 0° 59	
100 0		۵ <b>۵۵</b> ی 🐔	$\pm 10^{\circ}$	104	-
	a.s./kg <dry th="" weig<=""><th></th><th></th><th>0 _0</th><th></th></dry>			0 _0	
LOEC _{reproduction} [mg	a s /kg dry werg	ht artificial s	ล์ที่1>100		

reproduction [1118 ·/& 5 Calculations were done with un-rounded value

+ = significant - = not significant(*)= Fisker's Exact Binomial Test one-sided greater, or 0 A

s not possible as only one concentration was tested. The calculation of an

### Validity criteria:

All validity criteria were met

Validity criteria (OECD 226, 2016)	Obtained in this study
Mean adult mortality should not exceed 20 % at the end of the test	1.3 %
Mean number of javeniles for replicate should be at least 50 (with 10 miles infroduced)	324.3
Coefficient of cariation calculated for the number of justification replicate should not be higher than 30 %	11.8 %



Toxic reference test:

In a separate study (L. Schulz, BioChem project No. R151048001S, February 24, 2015) performed with the reference item dimethoate at a test concentration 100 mg dimethoate/kg dry weight artificial soil, the  $EC_{50}$  was calculated to be 6.7 mg/kg soil d.w. The results of the reference test demonstrate the sensitivity of the test item.

#### **III. CONCLUSION:**

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The second secon Assessment and conclusion by applicant: This study is considered reliable for risk assessment. The NOE  $\ge 100$  mg/s is the relevant endpoint for the risk assessment. the performance of the other o



#### CA 8.5 Effects on nitrogen transformation



Data Point:	KCA 8.5/01
Report Author:	
Report Year:	2003
Report Title:	AE C638206 technical ingredient: Determination of effects on nitrogen transformation in soil
Report No:	C031645
Document No:	<u>M-230023-01-1</u>
Guideline(s) followed in study:	OECD 216 (2000)
Deviations from current test guideline:	Current Guideline: OECD 216 (2009) The sand content was 78% and not between 50 and 75% as recommended by the guideline. This deviation is not expected to have impacted the study results.
Previous evaluation:	yes, evaluated and accepter in DAR (2005)
GLP/Officially	Yes, conducted under GLP/Officially occognised testing facilities in the second
recognised testing	
facilities:	
Acceptability/Reliability:	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$

Executive Summary The purpose of this study was to determine the effects of flugpicoline technical of the activity of soil microflora with regard to nitrogen transformation in Mabor Mory test. A sifty sand soil was exposed for 28 days to concentrations of Q18 and 1.84 ne test item/kg dry weight soa and a control. Each treatment consisted of 3 replicates. Application rates were equivalent to 0.138 kg test item/ha/corresponding to 0.133 kg test item/ha) and 1.38 kg test item/ha (corresponding to 1.33 kg test item/ha). The nitrogen transformation was determined in soil enriched with lucerne meal/(concentration in soil 0.5 %). NH4nitrogen, NO₃- and NO₂-nitrogen were determined by an autoanalyzer of different sampling intervals (0, 7, 14 and 28 days after treatment). A reference item is not required by the guideline. Nevertheless, sodium chloride was used as a reference. The test item fluopeolid dechnical caused no effect > 25% on nitrogen transformation at the test doses of 0018 mg test item/kg dry weight soil and 1.84 mg test item/kg dry weight soff. Flugpicolide technical causes no adverse effects (difference to control < 25 %, OECD dry weight soil. Fluopicolite technical causes to adverse effects (difference to control < 25 %, OECD 216) on the soil nitrogen transformation (expressed as NO₃-N-production rate) at the end of the 28-day incubation period. Test item: Fluopicolite technical ingredient, batch No.: (P2050046, development No.: 3000312102,

analysed purity: 96. 9% w/w. A silty sand soil was exposed for 28 days to concentrations of 0.18 and 1.84 mg test item/kg dry weight Soil and a control. Each treatment consisted of 3 replicates. Application rates were equivalent to 0.138 kg test nem/ha (corresponding to 0.133 kg test item/ha) and 1.38 kg test item/ha (corresponding to 1.33 kg test item/ha). The nitrogen transformation was determined in soil enriched with lucerne meat (concentration in soil 0.5 %). NH₄-nitrogen, NO₃- and NO₂-nitrogen were determined by an autoanalyzer at different sampling intervals (0, 7, 14 and 28 days after treatment). A reference item is not require by the guide the. Nevertheless, sodium chloride was used as a reference. The test conditions were: Soil held in the dark at  $20 \pm 2$  °C, about 40% water capacity and pH values of 5.5 - 5.8. The pH values of the soil used in the test were measured at test start (after application) and at the final sampling on day 28 Homogeneity of variances was determined by F-test (significance level 5%). Depending on the results of the F-test, the appropriate T-tests were performed.



ped in this study

#### **II. RESULTS AND DISCUSSION:**

The test item fluopicolide technical caused no effect > 25% on nitrogen transformation at the test doses of 0.18 mg test item/kg dry weight soil and 1.84 mg test item/kg dry weight soil.

Time interval	erval equivalent to 0.138 kg test item/ha						u woigh	4 mg nt equ	aivalen	m/kg soft dry t to 1.38 kg test /ha			
(days)	Nitr	ate-	•N ¹	Nitr	rate-N ¹ % difference to control		e Niti	rate-l	N ¹	o difference to control			
0-7	- 2.25	±	0.07	-2.23	±	0.01		, 1	-296	±	0.0		Ø
7-14	1.11	±	0.03	1.05	Ħ	0.02	Å	5	Őľ.17	Ŧ	<b>Q.16</b>	0 55° 40	Ÿ
14-28	1.02	±	0.02	1.07	Ŧ	0.03	S ^O	5	Q 1.09 °	± "	0.09	V O O	

Rate: Nitrate-N in mg/kg soil dry weight/day, mean of 3 replicates and standard deviation

Ż In a separate study (non-GLP) with the same agricultural soft as used for this study, 16 g NaCl/kg dry skinfluefee on facrobial mineralization of fittrogen." weight soil had a distinct and long-term (> 28 day

Ø

#### Validity criteria:

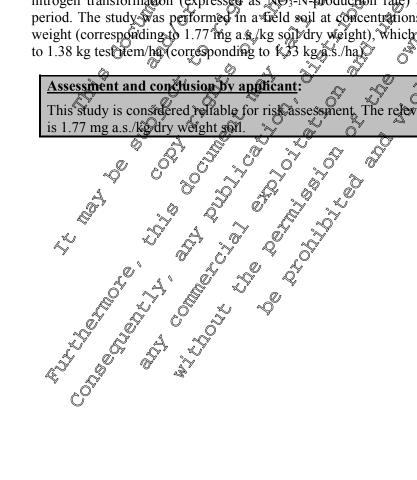
All validity criteria were met in this stod

Validity criteria (OECD 216, 200	Ô	ġ.	ð	Obtai
The coefficient of variation in the con	tray for	NON	0 × 0/	

The coefficient of variation in the control for

### III. CONCLUSION Fluopicolide technical caused no adverse effects (difference to control \$25 %, DECD 216) on the soil nitrogen transformation (expressed as NØ3-N-production rate) at the end of the 28-day incubation period. The study was performed in a field soil at concentrations up to 1984 mg test item/kg soil dry weight (corresponding to 1.77 mg a & /kg soil/dry weight), which are equivalent to application rates up

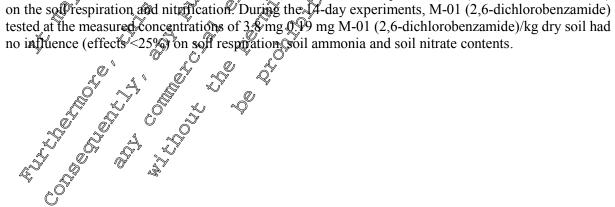
This study is considered reliable for risk assessment. The relevant endpoint for the risk assessment is 1.77 mg a.s./kgdry weight soil.





Data Point:	KCA 8.5/02
Report Author:	
Report Year:	1996
Report Title:	Effect of 2,6-dichlorobenzamide on the activity of soil microflora: Short torm respiration and nitrogen minieralization
Report No:	C034063
Document No:	<u>M-234312-01-1</u>
Guideline(s) followed in study:	SETAC: Chapter 4, 1995
Deviations from current test guideline:	Current Guideline: OECD 216(2000) The exposure period was only 14 days instead of 28 days as Pecommended by the guideline. The sand content was 88.2 % and not between 60 and 5%. Also, no finformation on variation in the control was provided.
Previous evaluation:	yes, evaluated and accepted a star of the
GLP/Officially recognised testing facilities:	Yes, conducted under GLP Officially recognised testing facilities
Acceptability/Reliability:	Supportive Boly ( Star Star Star Star Star Star Star Star

Executive Summary The purpose of this study was to determine the effects of M-01 (26-dictorobenzamide (BAM)) on the activity of soil microflam with the effects of M-01 (26-dictorobenzamide (BAM)) on the activity of soil microflora with regard to carbon and nitrogen transformation in a laboratory test. A sandy soil, with a low organic carbon content (<1%), provided by the "Landwirtschaftlicher Untersuchungsanstalt" (Speyer Germany) was used. The effects of the test compound M-01 (2,6dichlorobenzamide) vere investigated at two nominal concentrations: 6 and 0.12 mg/kg dry weight soil. The measured test concentrations of 2,6-Dichlorobenzamide were 3,8 mg and 0.19 mg 2,6-Dichlorobenzamide/kg dy soil Dinoseb acetate was used as reference compound. This standard was tested at a dose date of mg dinoseb acetated 100 g dry soil. The test substance and reference compound were weighed and dissolved in acetone. These acetonic solutions were mixed with sand, whereafter the acetone was evaporated slowly under reduced pressure. The addition of M-01 (2,6-dichlorobenzamide), coated on the sand was cerifically expection with methanol and analysis by HPLC. As nitrogen source, 0.5 g Lucerne meal was added. The reference compound dinoses acetate showed an increased level of soil respiration (51% deviation versus control after 2 weeks). Dinoseb acetate also showed a significant effect (> 25%) on the phrogen mineralisation (after two yeeks a 962% increase of NH4⁺ and a 45% reduction of NQ3- compared to the control) The test compound M-01 (2,6-dichlorobenzamide) showed no effect at both test concentrations on the soil respiration (< 25% effect). The study was terminated after two weeks as no noticeable offect was found for the test compound M-01 (2,6-dichlorobenzamide) on the softrespiration and nitrofication. During the A-day experiments, M-01 (2,6-dichlorobenzamide)





#### I. MATERIAL AND METHODS:

Test item: M-01 (2,6-dichlorobenzamide), batch No.: FUX00100/FUN81G02C, purity: 99%.

A sandy soil, with a low organic carbon content (<1%), provided by the "Landwirtschaftlicher" Untersuchungsanstalt" (Speyer, Germany) was used. The effects of the test compound M 90 (2.6dichlorobenzamide) were investigated at two nominal concentrations: 6 and 0.12 mg/kg dry weight soil. Dinoseb acetate was used as reference compound. This standard was tested at a dose rate of 2 mg dinoseb acetate/100 g dry soil. The test substance and reference compound were weighed and dis-solved in acetone. These acetonic solutions were mixed with sand, whereafter the acetone was evaporated slowly under reduced pressure.

The addition of M-01 (2,6-dichlorobenzamide) coated on the sand was verified by extraction with methanol and analysis by HPLC. The recoveries were 61% and 55% of the high and low nominal addition level, which resulted in the measured concentrations of 0.58 mg and 10 µg M-01 (2,6-dichlorobenzamide)/0.5 g sand respectively. The low recovery at the high addition level (61%) may have been caused by partial volatilization of the test compound during the coating process. The high recovery at the low addition level (155%) is most likely the result of cross contamination, as extended first.

Half a gram sand coated with either M-01 (2,6-dichlorobenzamide) (high and low level), dinoseb acetate or acetone only (control) was mixed with 100 g pre-fucubation soil. The final test concentrations of M-01 (2,6-dichlorobenzamide) were, therefore, 6.0 mg (measured 3.8 mg) and 0.12 mg (measured 0.19 mg) M-01 (2,6-dichlorobenzamide) kg dry soil.

As nitrogen source, 0.5 g Lucerne meal was added. The soll samples were incubated in a thermostatic room at  $20 + 1^{\circ}$ C in the dark. Within 6 hours and after 2 weeks, respiration and nitrogen mineralisation were determined in three soil samples per test condition.

Dates of work: October 15, 1996, October 30, 1996

### O. RESULTS AND DISCUSSION:

The reference compound dinose acetate showed an increased level of soil respiration (51% deviation versus control after 2 weeks). Dinose acetate also showed a significant effect (> 25%) on the nitrogen minerafisation (after two weeks a 962% increase of NH4 and a 45% reduction of NO3- compared to the control).

The test compound M-01 (2,6-dichlorobenzamide) showed to effect at both test concentrations on the soil respiration (< 25) effect). The study was terminated after two weeks as no noticeable effect was found for the test compound M-01 (2,6-dichlorobenzamide) on the soil respiration and nitrification.

	<u>Ď[*].</u> Ū	
	🗴 🖓 Éffects on soil respi	ration as a function of time
Test substance (conceptrations)	Percentage deviation	compared to the control (%)
	After 6 hours incubation	After 14 days incubation
Control		
M-01 (2,6-dichlorobénzamiek) (0.12 mg/kg)	0.2	1.8
M-01 (2,6-techlorobenzandde) (3,8 mg/kg)	-3.3	3.4
Reference (20 mg/kg)	-7.1	51.2



Test substance	Effects on nitrogen transformation as a function of time									
(concentrations)	Percentage deviation compared to the control (%)									
	After	6 hours incu	bation	Aft	er 14 days ir	ncubation 🔊 🦉	F			
	NH4 ⁺	NO ₃ -	NO ₂ -	NH4 ⁺	NO	NØ2 6				
Control					<u>o</u>	<u> </u>				
M-01 (2,6- dichlorobenzamide) (0.19 mg/kg)	-2.7	-7.8	8.4	-18	A.7 (					
M-01 (2,6- dichlorobenzamide) (3.8 mg/kg)	4.7	-5.8	5%6	-46 2 2 2	-5.4 °		,0 ,¥			
Reference (20 mg/kg)	30	1.2 🌭	<u>(</u> )	\$ 962	≪-45.4°	°>> 4296				
<b>C C</b> /					<u>g</u> ø					

From the results on nitrogen transformation, it is clear that MOI (2, Frichloodbenzamide) tested at the two nominal concentrations of 3.8 and 0219 mg/kg dry soil, has no effect on the soil contents of ammonia and nitrate (<25%) after 6 hours and 4 days of incobation with regard to soil nitrite contents the absolute levels of nitrite in the controls were stry low compared and nitrate (<25%) after 6 hours and days of incobation

to the nitrate content (<0.2%). Due to these low levels the variation in the percentage deviation from the controls is relatively high, after 2 weeks incubation, the effect was -25 and -26% for both M-01 (2,6-dichlorobenzamide) doses. Compared with the effects of the reference compound and taking into account the high variation this effort is considered to be not significant.

 $\bigcirc$ During the 14-day experiments M. (2,6-dichlorobenzamide) tested at the measured dose concentrations of \$.8 mg and 019 mg M-01/(2,6 d) chlorobenzamide)/kg dry soil had no influence (effects <25%) on soil respiration, soil ammonia and soil nitrate contents.

IIP.Conclusion:

The study design deviates significantly from the standard OFCD 2016 test protocol, i.e. study duration. Therefore, the study is not further considered in the risk assessment.

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Data Point:	KCA 8.5/03
Report Author:	
Report Year:	2004
Report Title:	Metabolite AE C653711 (AE C653711 00 1B96 0001): Determination of effects on nitrogen trandformation in soil
Report No:	C044264
Document No:	<u>M-235991-01-1</u>
Guideline(s) followed in	OECD 216 (2000)
study:	
Deviations from current	Current Guideline: OECD 216 (2000)
test guideline:	The sand content was 78.1 % and not between $\mathfrak{O}$ and 75% as recommended by the $\mathfrak{O}$
	guideline.
	This deviation is not expected to have impacted the study results
Previous evaluation:	yes, evaluated and accepted
	in DAR (2005)
GLP/Officially	Yes, conducted under GLP. Officially recognised testing facilities a A
recognised testing	
facilities:	
Acceptability/Reliability:	Yes a y a y y y y

The purpose of this study was to determine the effects of MSOI (AEC653011) of the activity of soil microflora with regard to nitrogen transformation in a laboratory est. A loamy and soil was exposed for 28 days to concentrations of 0.09 and 0.92 mg test item/kg dry weight soil and a control.

Each treatment consisted of 3 replicates. Application rates were equivalent to 0.069/kg test item/ha and 0.69 kg test item/ha. The nitrogen transformation was determined in soil enriched with lucerne meal (concentration in soik 0.5 %). NHa-nitrogen, NO₃- and NO₂-nitrogen were determined by an autoanalyzer at different sampling intervals (0, 7, 14 and 28 days after treatment). A reference item is not required by the guideline. Nevertheless, sodium chloride was used as a reference. The test item M-01 (AE C653711) caused no effect > 25% on nitrogen transformation at the test doses of 0.09 mg test item/kg dry weight soil and 0.92 mg test item/kg dry weight soil of 0.0653711) causes no adverse effects (difference to control < 25%, OECD 216) on the soil nitrogen transformation (expressed as NO₃-N-production rate) at the end of the 28 day incubation period.

Test item: M-01 (AE C653711), batch No.: 68018ET, analysed purity: 96.2% w/w. A loamy sand soil was exposed for 28 days to concentrations of 0.09 and 0.92 mg test item/kg dry weight soil and a control. Each treatment consisted ocd replicates Application rates were equivalent to 0.069 kg test item/ha and 0.69 kg test item/ha. The nitrogen transformation was determined in soil enriched with lucerne meal (concentration in soil 0.5 %). NH4-nitrogen, NO₃- and NO₂-nitrogen were determined by an autoanalyzer at different sampling intervals (0, 7, 14 and 28 days after treatment). A reference item is not required by the guideline. Nevertheless, sodum chloride was used as a reference. The test conditions were: Soil held in the dark at  $20 \pm 2$  %, about 40% water capacity and pH values of 5.5 - 5.9. The pH-values in the soil used in the cest were measured at test start (after application) and at the final sampling on day 28. Homogeneity of variances was determined by F-test (significance level 5%). Depending on the results of the F-test, the appropriate T-tests were performed.

the results of the Fitest, the appropriate Fitests were performed.



The test item M-01 (AE C653711) caused no effect > 25% on nitrogen transformation at the test doses of 0.09 mg test item/kg dry weight soil and 0.92 mg test item/kg dry weight soil. Ö

Time interval	Control						soil dry weight kg test item/ha	0.92mg equiva	g test alent	jtem/k to 0.69	g soil dry weight kg test item/fra	
(days)	Nitra	nte-I	N ¹	Nitr	Nitrate-N ¹		% difference to control	Niț	ate-l	N ¹	% difference	
0-7	-1.59	±	0.06	-1.64	±	0.05	(C)	-1.40	±	0.07		
7-14	0.79	±	0.12	0.72	±	0.03	₹9	<b>B</b> 68	ŧ	0.06		Ç
14-28	1.01	±	0.02	0.99	±	0.05	L 2	0.97	±	ØA 5	Q. 45° W	) [°]

Rate: Nitrate-N in mg/kg soil dry weight/day, mean of 3 replicates and standard deviation .

In a separate study with the same agricultural soil as used for this study, 16 Nackkg dry weight soil had a distinct and long-term (> 28 days) influence on microbial mineralization of mitrogen.

#### Validity criteria:

All validity criteria were met in this stud

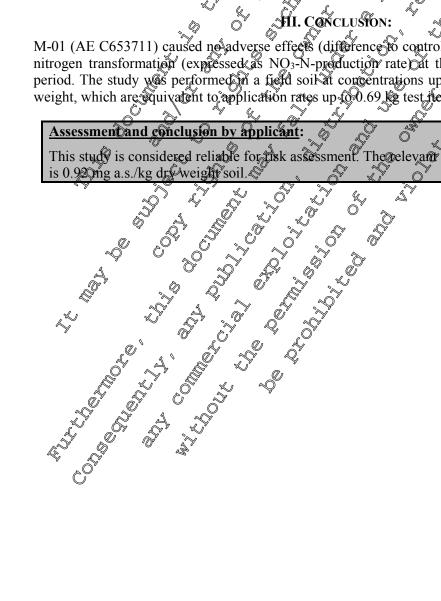
Validity criteria (OECD 216,	2000			J.	Obtained	h this study
	0	<b>^</b>	8	·		

The coefficient of variation in the control for NQ  $N \le 1.5\%$ 

M-01 (AE C653711) caused no adverse effects (difference to control < 25%, @ECD 216) on the soil nitrogen transformation (expressed as NO3-N-production rate) at the end of the 28-day incubation period. The study was performed in a field soil at concentrations up to 0.92 mg test item/kg soil dry weight, which are equivatent to application rates up 100.69 kg test tem/ha

TII. CONCLUSION:

# This study is considered reliable for the assessment. The gelevant endpoint for the risk assessment





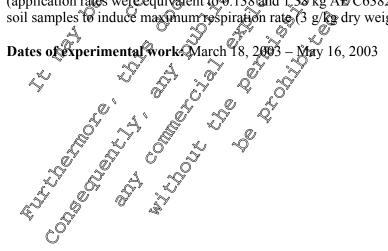
Data Point:	KCA 8.5/04
Report Author:	
Report Year:	2003
Report Title:	AE C638206 technical ingredient: Determination of effects on carbon
	transformation in soil
Report No:	C031644
Document No:	<u>M-230021-01-1</u>
Guideline(s) followed in	OECD 217 (2000)
study:	
Deviations from current	Current Guideline: OECD 217 (2009)
test guideline:	Not evaluated
Previous evaluation:	yes, evaluated and accepted $\mathcal{K}$ $\mathcal{O}^{\vee}$ $\mathcal{K}$ $\mathcal{O}^{\vee}$
	in DAR (2005)
GLP/Officially	Yes, conducted under GLP Officially recognised festing facilities
recognised testing	
facilities:	
Acceptability/Reliability:	Yes O C C A C

The purpose of this study was to determine the effects of floopicotide on the activity of soil meroflora with regard to carbon transformation in a laboratory test. A silty soil was exposed for 28 d to concentrations of 0.18 and 1.84 mg/luopicolide/kg dry weight soil (application rates were equivalent to 0.138 and 1.38 kg AE C638206 tech./ba. Glucose was added to the soil complecto induce maximum respiration rate (3 g/kg dry, weight soil). No adverse effects of fluppicolide technical on carbon transformation in soil were observed at both test concentrations (0.18 mg test item/kg dry weight soil and 1.84 mg test item/kg soil dry weight soil) after 28 days. Differences from the control of 8.35% (test concentration 0.18 mg test item/kg dry weight soil) and 5.75% (test concentration 1.84 mg test item/kg dry weight soil) are measured at the end of the 28 day increasing period. Flugpicolide technical causes no adverse effects (deviation from control < 25 %, OECD 217) on the soil carbon transformation at the end of the 28-day incubation period. The study is performed in a field soil at concentrations up to 1.84 mg test item/kg soil dro weight, which are equivalent to application rates up to 1.38 kg test item/ha.

# I MATERIAL AND MECHODS

Test item: Fluopicolide technical (AE C6\$8206 00 1C96 000), development No.: 3000312102, batch No.: OP2050046), analytical findings: 96.1% www.

A silty soil was exposed for 28 to concentrations of 0.18 and 1.84 mg fluopicolide/kg dry weight soil (application rates were equivalent to 9.138 and 1.38 kg A& C638206 tech./ha. Glucose was added to the soil samples to induce maximum respiration rate (3 g/log dry weight soil).





No adverse effects of fluopicolide technical on carbon transformation in soil were observed at both test concentrations (0.18 mg test item/kg dry weight soil and 1.84 mg test item/kg soil dry weight soil). After 28 days. Differences from the control of 8.35% (test concentration 0.18 mg test item/kg dry weight soil) and 5.75% (test concentration 1.84 mg test item/kg dry weight soil) were measured at the end of the 28 day incubation period.

Sampling	Control	0.18 mg test item 0.138 kg test item	/kg dws equiv. to n/ha	equiv. to 1.38 kg test item/ha				
date	[mg CO2 / hour / kg dws]	[mg CO ₂ / hour / kg dws]	% difference to control [#]	fing CO ₂ / hour	% configure 4			
0	192.71	180.16	6.51	190,28	Ş1.26			
7	197.91	181.72	8.18	187.69	5.16			
14	187.49	173.38	7.59 N	187.42	4.84			
28	147.02	134.74*	*8.35 C	138057	8.75 × × °			

* Significant difference between treated and untreated soil samples (Nest with 5% probability of error) # Exact Values not given in study report; calculated on the basis of the mg  $O_2$  / how / kg two values given in this table.

dws = dry weight soil

In a separate study the reference item sodium chloride was used as a efference standard. In tests (non-GLP) with the agricultural soil described above, 16 c NaCl kg dry weight soil had distinct and longterm (> 28 days) influences or microbial mineralization of earbord

Validity criteria:

The validity criteria of the test according to OECD guideline 219 were fulfilled.

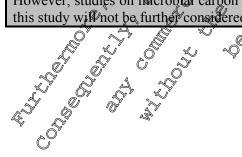
4	Š, Č	۶Ŷ	s C	y .?		Å.	Ø
Validity criter	ia (OECD	2Î7, 20	19) <b>R</b>	ecommen	ded	Obtained	×.
CoefficientSof	variation it	the con	trol $\leq \leq$	150%	O Ó	2.2 <u>~</u> 2.4 %	6
	- U - U	L.	A C	S &	, W	Ň	
jan and a start and a start a		8	.» ايلا	Conclus	NON:	0 [×]	

Fluopicolide technical caused no adverse effects (deviation from control < 25 %, OECD 217) on the soil carbon transformation at the end of the 28-day incubation period. The study was performed in a field soil at concentrations of to 1.84 mg fest item/kg foil dry weight, which are equivalent to application rates up to 1.85 kg test item/ha.

### Assessment and conclusion by applicant

This study is considered feliable. The codpoint is 1.84 mg/kg dry weight soil.

However, studies on microbial carbon transformation are no longer a data requirement. Therefore, this study will not be further considered in the risk assessment.





Data Point:	KCA 8.5/05	1
Report Author:		ĺ
Report Year:	2004	
Report Title:	Metabolite AE C653711 (AE C653711 00 1B96 0001): Determination of effects on carbon transformation in soil	
Report No:	C044266	-
Document No:	<u>M-235993-01-1</u>	
Guideline(s) followed in	OECD 217 (2000)	in a
study:		
Deviations from current	Current Guideline: OECD 217 (2009)	0
test guideline:	Not evaluated	L.
Previous evaluation:	yes, evaluated and accepted $\mathcal{K}$ $\mathcal{O}^{\vee}$ $\mathcal{K}$ $\mathcal{K}$	0
	in DAR (2005)	×.
GLP/Officially	Yes, conducted under GLP Officially recognised festing facilities	ĺ
recognised testing		ĺ
facilities:		
Acceptability/Reliability:	Yes O' V A A	

The purpose of this study was to determine the effects of M-01 AE C63371 Fon the activity of soil microflora with regard to carbon transformation in a laboratory test. Advamy sand soft was exposed for 28 d to concentrations of 0.09 and 0.92 mg M 1 (AEC653) 1)/kg dry weight soll (application rates were equivalent to 0.069 and 0.69 kg M-01 (AE C653711) ha. Glocose was added to the soil samples (2 g/kg dry weight soil) to induce maximum respiration rate. No adverse effects of fluopicolide technical on carbon transformation in soil were observed at both test concentrations to .09, the test item/kg soil d.w. and 0.92 mg test item/kg dry weight soil) after 28 days. Differences from the control of 8.09% (test concentration 0.09 mg test item/kg drg weight soil and 6.90% (test concentration 0.92 mg test item/kg soil dry weight) were measured at the end of the 28-day mcubation period. M 01 (AE C653711) causes no adverse effects reviation from control < 25 %, OFCD 21%) on the soil carbon transformation at the end of the 28-dap incubation period The study is performed in a field soft at concentrations up to 0.92 mg test item/kg soil dry weight, which are equivalent to application rates up to 0.69 kg test item/ha.



Test item: M-01 (AD C653711, batch NO. 08018ET) analytical findings: 96.2% w/w. A loamy sand soil was exposed for 28 d to concentrations of 0.09 and 0.92 mg M-01 (AE C653711)/kg dry weight soil (application rates were equivalent to 0.069 and 0.69 kg M=01 (AE C653711)/ha. Glucose was added to the soil samples (2 gdig dry weight soil) to induce maximum respiration rate.

Dates of work: July 28, 2004 August 25, 2904



No adverse effects of M-01 (AE C653711) technical on carbon transformation in soil were observed at both test concentrations (0.09 mg test item/kg soil d.w. and 0.92 mg test item/kg dry weight soil) and 6.90% (test concentration 0.92 mg test item/kg soil dry weight) were measured at the end of the 28 day incubation period.

Sampling	Control	0.09 mg test item equiv. to 0.069 kg	/kg soil d.w. g test item/ha	0.92 mg test item equiv. to 0.69 kg	/kg soil d.w. rest item/ha
date	[mg CO2 / hour / kg dws]	[mg CO ₂ / hour / kg dws]	% difference to control [#]	Ang CO ₂ / hou	% difference to control#
0	351.2	324.9*	7.49 ^Q	3287	<b>46.41</b>
7	398.8	361.9	7.16	-251.7	9.72 ~~~
14	343.0	307.4	10,378	320.9	6.44
28	286.9	263.7	\$.09	26701	6.90 ~ <

* Significant difference between treated and untreated soil samples (Nest with 5% probability of error) #Exact Values not given in study report; calculated on the basis of the mg  $\mathcal{O}_2$  / how, kg dws value given in this table.

#### Validity criteria:

The validity criteria of the test coording to OBCD gyrdeline 217 were fulfilled

### Validity criteria (OECO 217, 2000) 🖉 Recommended 🔒 Obtained

Coefficients of variation in the control 5%

M-01 (AE C653 11) caused no advetse effects (deviation from control \$25 %, OECD 217) on the soil carbon transformation at the end of the 28 day incubation period. The study was performed in a field soil at concentrations up to 0.92 mg test item kg soil dry weight, which are equivalent to application rates up to 0.69 kg test item/ha

JII, CONCLOSION;

 $\bigcirc$ 

### Assessment and conclusion by applicant:

This study is considered retable. The endpoint 60.92 mg/kg dry weight soil. However, studies on microbial carbon transformation are no longer a data requirement. Therefore, this study will not be further considered in the risk assessment.

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KCA 8.5/06
2016
Pyridyl carboxylic acid (BCS-AB43478): Effects on the activity of soil microftora
(Nitrogen transformation test)
EBACN059
<u>M-557910-01-1</u>
OECD 216; adopted January 21, 2000, OECD Guideline for the Testing of
Chemicals, Soil Microorganisms: Nitrogen Transformation.
Current Guideline: OECD 216 (2009)
No deviations
No, not previously submitted $\mathcal{K}$ $\mathcal{O}^{\vee}$ $\mathcal{K}$ $\mathcal{A}^{\vee}$
Yes, conducted under GLP Officially recognised festing facilities
Yes O L L A

ÉŚ

The purpose of this study was to determine the effects of M²02 (AE C657188) on the activity of soil microflora with regard to nitrogen transformation in a laboratory test. A loans sand soil (DIN 4220) was exposed for 28 days to 0.33 and 1.89 mg/kg soil dry weight and a control Each treatment consisted of 3 replicates. Application rates were equivalent to 0.248 kg/m and 0.421 kg/m. The nitrogen transformation was determined in soil enriched with lucerne mead concentration in soil 0.5%). NH₄-nitrogen, NO₃- and NO₂-nitrogen were determined by an autoanalyzer at different sampling intervals (0, 7, 14 and 28 days after treatment). A reference item is not required by the guideline. Nevertheless, Dinoterb was used as a reference. The test item M-02 (AE C657188) causes a temporary stimulation of the daily nitrate rate at the tested concentrations of 0.33 and 1.89 mg/kg soil dry weight at time interval 7-14 days after application. However, no adverse effect of AE C657188 on fur ogen transformation in soil could be observed at both tested concentrations at the end of the test, 28/days after application (time interval 14-28). M-02 (AE C65/188) causes in adverse effects (difference to control < 25%, OECD 216) on the soil nitrogen transformation (expressed as NO₃-N-production rate) at the end of the 28-day incubation period. The study is performed in a field soil at concentrations up to 1.89 mg /kg soil dry weight, which are equivalent to application rates up to 1.421 kg/ma.

I. MATERIAL AND METHODS:

Test item: M-02 (AE 6657188, batch No.: XE C657188 DU-01, origin batch code: SES 10250-1-1, LIMS No.: 15 (8969, OAS No.: 80194-68-0) Certificate No.: AZ 20206, analysed purity: 98.5%).

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A loamy sand soil (DIN 4220) was exposed for 28 days to 0.33 and 1.89 mg/kg soil dry weight. and a control. Each treatment consists of of 3 replicates. Application rates were equivalent to 0.248 kg/ha and 1.421 kg/ha. The autogen transformation was determined in soil enriched with lucerne meal (concentration in \$61 0.5%). H4-nitrogen, NO₃- and NO₂-nitrogen were determined by an autoanalyzer at different simpling intervals (0%), 14 and 28 days after treatment). A reference item is not required by the guideline. Nevertheless, dinoterb was used as a reference. The test conditions were: Soil held in the dark at 19.6 21.6%, with 44.92 to 48.30% water capacity and pH values of 6.0 - 6.1. The pH-values in the soil used in the test were measured at test start (after application) and at the final sampling on day 28. A statistical evaluation of the test results was performed by means of a 2-sided Student test (tor homogeneous variances at 5% significance level).

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The test item M-02 (AE C657188) caused a temporary stimulation of the daily nitrate rate at the tested concentrations of 0.33 and 1.89 mg /kg soil dry weight at time interval 7-14 days after application. However, no adverse effect of AE C657188 on nitrogen transformation in soil could be observed aboth tested concentrations at the end of the test, 28 days after application (time interval 14-28).

ſ	Time interval	Сог	ntro	1	0.33 m equivale	dry weight g /ha				l dry weight	ÊQ.		
	(days)	Nitra	te-ľ	N ¹	Nitr	ate-	N ¹	% difference to control	Nit	rate-N	N ¹ Č	% difference tocontrof	Ļ
	0-7	3.31	±	0.04	3.16	±	0.23	- 4.6	<b>Ø</b> .14	±	<b>Q</b> .47	-5.3	$O^{r}$
	7-14	1.01	±	0.23	1.44	±	0.29	≤ + 42.9 ¢	1.56 。	±	0.42	5∉,7©	×,
	14-28	1.06	±	0.13	0.82	±	0.060	-22.1 ^{s.}	001	Ľ	@9.0	©13.5 🖉	

Rate: Nitrate-N in mg/kg soil dry weight/day, mean of 3 replicates and standard deviation The calculations were performed with unrounded values

s. = statistically significantly different to control (Student-t-test for homogenous mariances 2-side  $p \le 0.45$ ) Ù

In a separate study with the same agricultural soil as used for this study the reference item Directerb caused an effect of - 37.0% and + 37,6% on the nitrogen transformation in a field soil at the tested concentrations of 6.8 and 27.0 mg Dimiterb per kg sol dry, weight respectively, 28 days after application (time interval 14-28) and thus demonstrates the sensitivity of the test system (

Validity criteria:

All validity criteria were met in this study

		a Car	P	4	17	_~(^'_	<u> </u>
Validity criteria (OECD 216 🍂	00)	No.		<b>A</b> Ob	tained i	in [%] this s	stady

The coefficient of variation in the control for NO3-15 %

0 M-02 (AE C657188) caused no adverse effects difference to Control < 25 %, OECD 216) on the soil nitrogen transformation (expressed as NO3-N production gate) a(the end of the 28-day incubation period. The study was performed in a field soil at concentrations up to 1.89 mg/kg soil dry weight, which are equivalent to application rates up to 1, \$21 kg/ha. S

IH. CONCLUSION

This study considered reliable for risk assessment. The relevant endpoint for the risk assessment is 1.89 mg a.s./kg dry weight soil



Data Point:	KCA 8.5/07	
Report Author:		
Report Year:	2016	
Report Title:	AE 0608000 (BCS-AX86048): Effects on the activity of soil microflora (nitrogen	Ő
	transformation test)	5
Report No:	16 10 48 022 N	
Document No:	<u>M-555852-01-1</u>	
Guideline(s) followed in	OECD 216; adopted January 21, 2000, OECD Guideline for the Testing of	R.
study:	Chemicals, Soil Microorganisms: Nitrogen Transformation; US EPAOCSPO not	<i>Q</i>
	applicable (3) A A A	a
Deviations from current	Current Guideline: OECD 216 (2000) $\mathcal{O}$	Ľ
test guideline:	No deviations	0″
Previous evaluation:	No, not previously submitted	Í
GLP/Officially	Yes, conducted under GBP/Officially recognised testing facilities	
recognised testing		
facilities:		
Acceptability/Reliability:	Yes $\Delta$ $\partial$ $Q$ $Q$ $\partial$ $O'$ $\partial'$	

The purpose of this study was to determine the effects of M-03 AE 06080000 on the activity of soil microflora with regard to nitrogen transformation in a laboratory test A loamy sand soil was exposed for 28 days to concentrations of 0.56 and 2.78 mg/kg/dry weight foil an Da control. Each treatment consisted of 3 replicates. Application rates were equivalent to 0.417 kg/ha and 2.093 kg/ha. The nitrogen transformation was determined in soil enriched with lucerne meat (concentration in soil 0.5 %). NH4nitrogen, NO₃- and NO₂-nitrogen were determined by an autoanalyzet at different sampling intervals (0, 7, 14 and 28 days after treatment). A reference item is not required by the guideline. Nevertheless, Dinoterb was used as reference. The test item at 0608000 caused no effect > 25% on nitrogen transformation at the rest doses of 0.56 ng/kg dry weight soil and 208 mg/kg dry weight soil. M-03 (AE C0608000 (BCS-AX\$6048)) Dauses no adverse effects (Affference to control < 25 %, OECD 216) on the soil nitrogen transformation (expressed as NO3-N production take) at the end of the 28-day incubation period. The study is performed in a field soft at concentrations up to 2.78 mg/kg soil dry weight, which are equivalent to application rates up to 2083 kg ha.

I. MATERIAL AND METHODS:

Test item: M-03 (AP0608000), bach Nov: SES 2767 9-2, analysed purity: 99.4% w/w. A loamy sand soil was exposed for 28 days to concentrations of 0.56 and 2.78 mg/kg dry weight soil and a control. Each treatment consided of Freplicates. Application rates were equivalent to 0.417 kg/ha and 2.083 kg/ha. The nitrogen transformation was determined in goil enriched with lucerne meal (concentration in soil 0.5 %, NH4-nitrogen, NOS and NO2-nitrogen were determined by an autoanalyzer at different sampling intervals (0, 9, 14 and 28 days after treatment). A reference item is not required by the guideline. Nevertheless, dinoterb was used as a reference. The test conditions were: Soil held in the dark at 18,8 – 20.7 °C, with 46,92 to 49.54% water apacity and pH values of 5.7 - 5.8. The pH-values in the soil used in the test were measured at test start (after application) and at the final sampling on day 28. Homogeneity of variances was determined by means of a 2-sided Student-t-test (significance level 5%). A statistical evaluation of the test results was performed by means of a 2-sided Student-t-test.

The second secon



The test item M-03 (AE 0608000) caused no effect > 25% on nitrogen transformation at the test doses of 0.56 mg/kg dry weight soil and 2.78 mg/kg dry weight soil. Ő

Time interval	Co	ntro	1				dry weight 417 kg/ha	2.7 eq	'8 mg uiva	/kg soil ent to 2	dry weight 2.083 kg/ha
(days)	Nitra	te-ľ	N ¹	Nitr	ate-1	N ¹	% difference to control	Nit	rate-l	N ¹	% difference Oto control
0-7	4.30	±	0.18	4.35	±	0.14	(d₅2	4.3	±	0.32	
7-14	2.23	±	0.15	2.17	±	0.08	∕₹3.0	1099	Ŧ	0.28	\$11.1\$ A
14-28	1.42	±	0.26	1.39	±	0.20	-2.0	Q.36	Ŧ	<b>Ø</b> .24	

Rate: Nitrate-N in mg/kg soil dry weight/day, mean of 3 replicates and standard deviation . The calculations were performed with unrounded values

In a separate study with the same agricultural soil as used for this study the reference item dinoterb caused a stimulation of nitrogen transformation of #37.0% and +37.6% and 6.8 and 27 mg Dimeterb per kg soil dry weight, respectively determined 28 days after application (time interval 14-28).

#### Validity criteria:

All validity criteria were met in this study

Validity criteria (OECD 216,	<b>20</b> 00)	O,	-	Obtained in	n Oris study	Š.
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The coefficient of variation in the control for  $MO_3-N \le 15$ 

# IIDCONFUSIO

M-03 (AE C060800@(BCŞ-AX86048)) caused to adverse effects (difference to control < 25 %, OECD 216) on the soil nitrogen fransformation (expressed as NO₃-b)-production rate) at the end of the 28-day incubation period. The study was performed in a field soil at concentrations up to 2.78 mg kg soil dry weight, which are equivalence application rates pr to 2083 kg ta. Ø

This study is considered reliable for risk assessment. The relevant endpoint for the risk assessment is 2.78 mg/kg dtg weight soil 3



#### CA 8.6 Effects on terrestrial non-target higher plants

#### CA 8.6.1 Summary of screening data

Not necessary as guideline GLP studies conducted with the representative formulation for fluorizolida for terrestrial non-target plants are available (see Point KCP 10.6.2). A study with technical fluppicolide formulated as a WP has been conducted and is presented in section 8.7/02. R

#### CA 8.6.2 Testing on non-target plants

Studies on terrestrial non-target plants (seedling emergence and vegetative vigour) conducted with the representative formulation for fluopicolide are presented under Point KCP 10.6.20Studies on terrestria non-target plants conducted with the solo formulation Fluopicolide SC 40 and with the fluopicolide metabolite M-01 (2,6-dichlorobenzamide) are presented below

	KCA 8.6.2/01
Data Point:	KCA 8.6.2/01
Report Author:	
Report Year:	
Report Title:	2004 Tier 1 seedling emergence and vegetative vigor nontarget phytotospetty study using AE (2220) C (10)
	using AE (038206 SC40 2 2 2 2 2 2 2 2
Report No:	
Document No:	M-227139-04- 7 & A A
Guideline(s) followed in	$\frac{M-22}{M}\frac{39-04}{M} = \frac{1}{2} \frac{1}{M} = \frac{1}$
study:	
Deviations from current	Gurrent Guidelines: OFCD 208 (2006) and OFCD 227 (2006)
test guideline:	The temperature ranged from 14 to 39°C. This is outside the proposed range of
L.	$22^{\circ}$ $\mathbb{C}^{\pm}$ 10 $\mathbb{C}$ . The hamidic ranged from $\mathbb{C}^2 - 103^{\circ}$ . This $\mathbb{R}$ outside the range of
Pravious avaluation:	$70\% \pm 25\%$ . The dight intensity was not reported Planting density was higher than
	tecommended by the current gardeline (more plants per pot, smaller pots).
	These deviations are not expected to have impacted the study results.
Previous evaluation:	yes, evaluated analytic copied.
	in DAR (2005) 6 6 0
GLP/Uniciziany	Yes, conducted under CLP/Officially recognised testing facilities
recognised testing	
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	
Acceptability/Reliability:	$\chi ds \sim 0' \chi' \ll \Delta'$
Executive Summery	

Executive Summary 2

Ô Ô The purpose of this specific study is to evaluate the effect of fluopicolide SC 40 at an application rate of 133 g as /ha on the seedling emergence and vegetative vigour of ten plant species representing 8 different plant families In the seedling energence and vegetative vigour test for all test plants, 4 replicates with 10 plants per replicate were used giving a total of 40 plants per treatment and control. Assessments were made for effergence, plant survival, growth, shoot dry weight and visual

phytotoxicity. The analysis of Quopicolide in the initial test item stock solution revealed a measured concentration of approximately 100% of neminal.

There were no significant adverse effects in the seedling emergence and vegetative vigour test for any of the ten crops tested after the treatment with Fluopicolide SC 40 at an application rate of 133 g a.s./ha.



I. MATERIAL AND METHODS:

Test item: Fluopicolide SC40, CAS number: 239110-15-7, Batch No.: AE C638206 SC40: OP220233, analysed content of active ingredient: 485 g/L.

0 Test species: Six dicotyledonous and four monocotyledonous species representing 8 different plant families (EPPO code): Fagopyrum esculentum (FAGES), Helianthus annus (HSLAN), Cucumis satisfies (CUMSA), Brassica rapa (BRSRR), Glycine max (GLXMA), Lycopersicon esculentum (LyPES)/Zea mays (ZEAMA), Allium cepa (ALLCE), Lolium perenne (LOLPE), Triticum aestivum (TRZAVE).

Seedling emergence

For the six dicotyledonous plants, two seeds per pot with five pots per replicate were planted. For the four monocotyledonous plants, five seeds per pot with two pots perceplicate were planted. Per treatment level for all test plants 4 replicates were used, giving a total of 40 plants, Growing poo were filled with a sandy loam. For the seeding of the monocotyledonous and dicotyledonous plants pots with and 10.5 cm in diameter were used, respectively. Prior to spray application, each pot was randomly assigned to treatment and replicate. After spray application of 133 & a.s./he to the soil surface, fots were placed on a capillary irrigation mat in the green bourse.

Following application, the pots were maintained under greenhouse conditions. The photoperiod throughout the study was approximatedy 16 hours light and hours dark

The measured minimum and maximum greenhouse temperatures were \$7 and \$9°C. The minimum and maximum relative humidity was 12 and 103%. °

The number of emerged seedlings, the number of surviving plants and the phytotoxicity ratings were recorded on day 8, 14 and 21. At day 21 after treatment, an seedings were cut at soiDlevel for height and weight measurement.

<u>Vegetative vigour</u>

Ô In order to reach the 24 leaf rage with start of testing the selected non-target terrestrial plant species were sown in a sandy loant and were grown in plastic pets. Planting conditions were the same as in the seedling emergence study described above. After the application of 123 g a.s./ha with a laboratory spraying chamber to caropy height of the test plants, the pots were impartially arranged, by replicate in the greenhous?

In the greenbouse the plants were kept ander 16 hours light and 8 hours dark. The measured minimum and maximum greenhouse temperatures were 14 and 42°C, the minimum and maximum relative humidity was 12 and 101%. After the 21-day survival and phytotoxicity observations, all seedlings were cut at soil level for gright and weight measurements.

Statistical evaluation

Ì Means were calculated for all parameters in the seedling emergence and vegetative vigour test at study termination. A significant adverse effect was defined as greater than or equal to 25% inhibition or 25% damage for any endpoint as compared to the controls. Computerized spreadsheets (Excel, 1997) were

damage for any endpoint as compared to the controls. Computerized spreadsheets (Excel, 1997) were used to calculate the percent effect for seeding energence, seedling survival, visual phytotoxicity, plant height and dry weight.



Validity criteria:

The germination rate of the seeds used in this study was \geq 70%. Emergence was only reported for the seedling emergence part of the study but is assumed to be similar for the plants used for the vegetative vigour part of the study.

The validity criterion of at least 90% survival of the plants during the study perfod was achieved for the untreated controls for all species tested.

The control seedlings of each species did not exhibit visible phytotoxic effects (e.g. chlorosis wilting, leaf and stem deformations).

itting, four und storif der	ormations).		T.	"Q"	Č .	° × °
nalvtical findings:			L.	Ő¥		
he analysis of fluopico	olide conten ¹	t in the sprax	solutions r	eoealed me	easured concer	ntrations
pproximately 100% of n	ominal		, » » » » » » » » » » » » » » » » » » »		Q. O	là Â
	ommun.			N 6	* ^ ×	j , S
iological findings:		×				~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
he ten plant species treat	ed pre- and p	ost-emergence	at a dose rate	Sf13 26 a	s /ha showed n	otherbicit
fects $> 25\%$ Detailed re	sults are she	wn in the forli	owing tables	201 135 gu.		
				Nº N		A A A A A A A A A A A A A A A A A A A
eedling emergence:	ź	Q, 1,4, 1,5		~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	L' Â	0
<u> </u>	L ^O)' 🖓 🦻			S. S.	, Ô
ffects of the test item o	n emerg@nce	e and surviva		o so		»
nalytical findings: he analysis of fluopico oproximately 100% of n iological findings: he ten plant species treat ffects ≥ 25%. Detailed regender: ffects of the test item o Species Fagopyrum esculentum,	∧ Mean [™]	🖌 👋 😚 🌾	av 21	Mean	% Survivation	dav 21
			I tookihition			uuy 21 Inhihiti
Species	Cont Col	133 g a.s.4ha		Control	133 g a.s./ha	$\frac{111110100}{250}$
			$2 23 20^{\circ}$			$ll \ge 23\%$
Fagopyrum esculentum	93 95			100		no
Zeu muys	95 1000		_~©110	UV ·	× <i>1</i>	110
Cucumis sativus		× 100	no no	100 V 100 V	100	no
Glycine max 6	400	100	/ 190 [/]		100	no
Helianthus condus	0 93 0	<u> </u>	Ono is	100	100	no
Lycopersicon esculentum	80	<u>A</u> 80 X	o ^y no	×100	100	no
Allium cepa		0 90 ⁻	- 1965	100	100	no
Lolium, perenne	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	95 0	MO N	100	100	no
Brassica rapa 🔬 "	100		Sy no A	100	100	no
Triticum aestivum	, la anticia de la companya de la compan		nô	100	100	no
a sõs			The second se			
	\sim \sim	<i>0</i> , <i>6</i>	ð			
A			, Ø			
	Ą į		2			
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N n		Q 33				
	í Lí L					
A A		~~				
	\$ 4,	~0				
) N	8				
	~~~~					
	<i>S</i> ^v					
Le g 'a s	. <i>Y</i>					
× "Ô [¥]						
Glycine max Glycine max Helianthus angus Lycopersicon esculentum Allium copa Lolium perenne Brassica rapa Triticum aestivum A C C C C C C C C C C						



	Mean sh	oot height (cm)	on day 21	Mean dry weight (g) on day $21_{m_0}$			
Species	Control	133 g a.s./ha	Inhibition ≥25%	Control	133 g a.s./ha	Inhibitio n 325%	
Fagopyrum esculentum	53.4	49.9	no	17.788	£ 16.169	L no D	
Zea mays	81.3	78.0	no	22.655	22.563	noy	
Cucumis sativus	23.9	23.6	no	16.753	16.681 0		
Glycine max	25.9	26.0	_ ÛD	9.04	9.18	no 🖓	
Helianthus annus	32.5	36.1	Nno	140726	18570	9 not	
Lycopersicon esculentum	19.9	19.0	no no	<u>\$</u> .763	3.300 Q	Do &	
Allium cepa	14.5	15.7	no	©;0.1123°	~0.162C	ono L	
Lolium perenne	31.1	28.9	no 🚿	1.441	1.399	a no	
Brassica rapa	15.5	15.4	no پُ مَرْثُ no مُ	x5.363 x	5 <b>9</b> 628 ~	160°	
Triticum aestivum	31.2	319.0 🔬	no i	° 3.024°	Ø3.297L	Ano o	
ytotoxicity rating of th	e test item						

#### Effects of the test item on shoot height and dry weight

			× _ O		
hytotoxicity rating of th				on $dat 21$ dat 21 dat 25% dat and $dat 25%$	
	, d		y rating (%)	on day 21	
Species	Con	trol 🖉 13	3 g a.s./ha	Damage≥ 25%	
Fagopyrum esculentum		L 6	Î	🕈 🔬 no 🔗	
Zea mays	j ý ů	Y ala	03 K	inos nos	
Cucumis sativus			\$ 0 @	N mo	Ŝ b
Glycine max	<i>,</i> 1		R O	no 🖓	
Helianthus annus	2 3	òÔ	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	🕵 no	
Lycopersicon esculentum	Ô SO	Q .	Ş 1 L	° by	Ś
Allium cepa	ې ۲ رو کې			e s ^{no} e	
Allium cepa	~~~~ Q			no S	
Drussicu rupa	o đ	KO I			
1 riticum destivum 🔍	[©]			no no	

Effects of the test item on s	survival		Č,
	Ncal	n %Survivation	day 21
Species A	Control	9133 ga.s./ha	<pre> F Inhibition ≥ 25% </pre>
Fagopyrum esculentum	[™] 100	10Q	no
Zeckmays Z		Ľ 100	no
Ċučumis sativus 🔊	000	لاً [™] روم	no
Glycine max $\mathbb{Q}^{\circ}$	× 100	Q ⁹ 100	no
Helianthus Oinus 🧹	100	100	no
Lycopersiçon esculentun	×400 ×	<b>Q</b> 100	no
Allium cepa	0100	100	no
Lolian perenne	> 100	100	no
Brossica rapa 🔿 💦	100	100	no
Triticutoaestivum	100	100	no

Vegetative vigour Eff



	Mean sho	ot height (cm)	on day 21	Mean dry weight (g) on day 21 🧷			
Species	Control	133 g a.s./ha	Inhibition $\geq 25\%$	Control	133 g a.s./ha	Inhibitio n 325%	
Fagopyrum esculentum	105.1	104.8	no	54.709	\$ 54.295	no 💭	
Zea mays	90.6	89.7	no	46.280	46.227	no	
Cucumis sativus	44.4	46.7	no	63.429	62.624 0		
Glycine max	37.2	37.3	_ ÛMO	27.895	27.43)	no S	
Helianthus annus	52.9	52.9	no	30,255	40,38		
Lycopersicon esculentum	39.2	39.4	no no	44.013	24.300 Q	<u> </u>	
Allium cepa	18.9	19.6	no	°¢ 0.7 <b>9</b> 5°	~ 0.89 <b>0</b>	Ono 2	
Lolium perenne	34.9	33.4	no 🕎	<u>6</u> 273	5.843	s no	
Brassica rapa	13.0	13,0	no چ° no	×1.434	<b>8</b> .150 ×	ħø	
Triticum aestivum	31.5	39.5 🔬	p po	õ 7.1 <b>40</b>	\$5.86X	A no o	

#### Effects of the test item on shoot height and dry weight

nytotoxicity rating of th	Q Phytot	$\frac{133 \text{ gyrs./hg}}{2}$	on day 2	
Species	Control	0133 ggs.s./ha	Damage ≥ 25%	
Fagopyrum esculentum			~ <b>\$</b> \$\$	
Zea mays			a no	
Cucumis sativus		A A A	🖉 no 🖑	
Glycine max	S S		O ney >>	
Helianthus annus 🦉			no	
Lycopersicon es Mentur			no s	
Allium cona			no [®]	
Lolium perenhe			60	
Brassica Papa 🔬			no no	
Triticativaestivum	S OF		no	

In a Tier 1 sædling @mergence and veg@ative @igour@tudy fluopicolide SC 40 was tested under greenhouse conditions for frects on emergence survival, growth and shoot dry weight of ten non-target terrestrial plant species. There were no significant adverse effects greater than 25% in the seedling emergence and vegetative vigour test for any of the ten crops tested after the treatment with fluopicolide SC 40 at an application rate of 133 g a.s./ha. 

### Assessment and conclusion by applicants

In this Tight seedling emergence and vegetative vigour study, no adverse effects > 50% on emergence, survival, gowth and show dry weight of ten non-target terrestrial plant species after the treatment with fluopieolide SC 40 at an application rate of 133 g a.s./ha were detected. The study is considered reliable

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Data Point:	KCA 8.6.2/02
Report Author:	
Report Year:	2004
Report Title:	Effects of BAM (2.6-dichlorobenzamide, AE C653711) on non-target terrestrict plants: seedling emergence and seedling growth test (Tier 2) Test item: BAK (2.6-dichlorobenzamide) AE C653711, substance pure Code: AE F653711, 90 1B96 0001
Report No:	M-225892-01-2
Document No:	<u>M-225892-01-2</u>
Guideline(s) followed in study:	OECD: 208A (draft, 2000)
Deviations from current	Method: Deviations from current guideline SANCO/3029/99/rev.4:
test guideline:	No details regarding the line with is reported Nevertheless, Data on accuracy and
	precision are presented to confirm the method validation. The accuracy of the
	method was proved by analysing a reference solution with a singular
	concentration. Recoveries were within the acceptable range $\mathcal{O}70-110\%$ and the
	RSD values were below 20%. The analytical method can be regarded as fit for
	RSD values were below 20%. The analytical method can be regarded as fit for purpose.; Study: Gurrent Guideline: OECO 208 (2006)
	For oilseed rape the seedling emergence, was $65\%$ instead $\ge 70\%$ . No information
	on humidity and light intensity was reported The plant density was seeds per
	pot and not D & S & S & C & C
	These deviations are not expected to have impacted the study results.
	The temperature ranged from 14 to 39°C. This is outside the proposed range of
	$22^{\circ}C \neq 10^{\circ}C$ . The hypridity ranged from $12 \neq 103^{\circ}$ . This is outside the range of
	$70\% \neq 25\%$ The light intensity was not reparted. Planting density was higher
	than recommended by the current guideline (more plants per pot, smaller pots).
Previous evaluation:	ages, evaluated, and accepted
	$VDAR_{4}(2005)$
GLP/Officially	
recognised testing	Test conducted under OEIOOmetany recognised testing ravinties
facilities:	
Acceptability/Reliability	
Acceptaolity/Kenaolity	$\Psi_{\text{Yes}} \xrightarrow{\gamma} \xrightarrow{\gamma} \xrightarrow{\gamma} \xrightarrow{\gamma} \xrightarrow{\gamma} \xrightarrow{\gamma} \xrightarrow{\gamma} $
xecutive Summary	

The purpose of this specific study is to evaluate the effect of MAY (AE C653711) on the seedling emergence and seedling grow for of eight plant species representing 6 different plant families. Seeds of eight plant species were sown into soil in which various concentrations were incorporated. Eight pots per treatment group with 5 seeds per pot were used for testing.

Plants were grown and set under greenhouse conditions. Ossessments for the respective plants were made 7, 14 and 21 days after emergence of 50% seeds in the control treatments. Assessments were made for plant surveral, visual phytotoxicity, plant growth stage and shoot dry weight.

The validity criteria of the study were fulfilled for all species except for oilseed rape. For this species, emergence in the control was 55% only, failing to reach 70%. It should be noted that the emergence in all treatment rates of Miseed rape was between 879 and 97.5%. The analysis of M-01 (AE C653711) in the initial test item stock solution revealed a measured concentration of 95.5% of nominal.

There were no visible symptoms of phytotoxicity from any treatment concentration in any of the species tested apart from some minor growth stage delays. The maximum rate of 12.1 µg/kg did not result in

adverse effects on emergence, survival or biomass of any of the 8 species tested in this study.



#### I. MATERIAL AND METHODS:

Test item: The study was conducted using M-01 (AE C653711); Code: AE C653711 00 1B96 0001, Batch No.: 08018ET, purity: 96.2%, appearance: beige powder.

Test species: Seeds of eight plant species, i.e. corn (Zea mays), cucumber (Cucumis sativa), oat sativa), oilseed rape (Brassica napus), onion (Allium cepa), pea (Pisum sativum) soybean (Glycine max) and sugar beet (*Beta vulgaris*) were sown into soil in which various concentrations of the fluopic lide metabolite M-01 (AE C653711) were incorporated.

Seeds used on the study had not been treated with pesticides or repellents prior to test initiation. Eight pots per treatment group with 5 seeds per pot were used for testing. Standard soil (silty logm) sieved to 2 mm with an organic carbon content of 1.19% and ApH value of 74 was used for testing. The test itera was dissolved in methanol and added to soil and thoroughly mixed to provide the maximum concentration of 12.1 µg/kg. An aliquot of this soil (dry weight) was then subjected to serial dilution to provide the 5 other test concentrations. Details of the range of application rates are summarized in the following table:

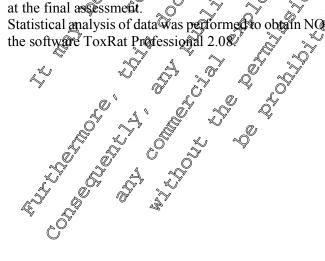
### Application rates during the study

		Q	u S			×,	õ a		Ş.
Test item	rates in μg/kg soil	Å.	0011	0.045	ي 0.18 ي	y 0.75	3.0	12	
Species								Ŭ	°~y
ZEAMA	Zea mays		X O	X	X	XQ″	X		1
CUMSA	Cucumis sativus	(a	X	X	X		ÔX 🔊	X U	
AVESA	Avena sativa 🔊		× .	í X	ØX	'X 🔊	X	ХÔ	
BRSNN	Brassica nàppis 🔬		X	X	X	X∜Ľ	X	×	
ALLCE	Allium cepa	Ô	XÕ	X	"XO"	×	X _	)X	
PIBSX	Pisum stivum 🔊		X _e	, A	Ň	O _X [™]	X X	X	
GLXMA	Glycine max (	S X	X	××	X N	X	X ,	Х	
BEAVX	Beta vulgar		X	X	X	X	N.	Х	
		×,		.~		, C	Ś		•

Plants were grown and secunder glasshouse, conditions with a temperature of  $23^{\circ}C \pm 5^{\circ}C$  and a photoperiod of approximately 16 hours light and 8 hours dark.

Daily checks were made to identify the date when 50% of the seedlings emerge in the controls for each species. Number of plants emerged were then counted after 7, 14 and 21 days. Visual phytotoxicity ratings were made 1/14 and 21 days after emergence of 50% of seeds in the control treatments. Growth stages as the final assessment were reported according to BBCH-Monograph. Number of plants that died after application vere recorded af the end of the assessment period. The dry weight was determined at the final assessment. Õ

Statistical analysis of data was performed to obtain NOFC/LOEC values and EC₅₀, where possible, using





Validity criteria:

The germination rate of the seeds used in this study was  $\geq$  70% for all species except for oilseed rape. For this species, emergence in the control was 65% only, failing to reach 70%. It should be noted that the emergence in all treatment rates of oilseed rape was between 87.5 and 97.5%. The validity criterion of at least 90% survival of the plants during the study period was achieved for the untreated controls for all species tested. No visible phytotoxic effects were observed in the control.

#### Analytical findings:

The analysis of M-01 (AE C653711) in the initial test item stock solution revealed a measured concentration of 95.5% of nominal.

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

#### **Biological findings:**

The EC₅₀ values for emergence and survival for the final assessment are expressed in  $\mu g/kg$  soil and presented in the following table. In this table are also presented the results of the final assessments for shoot dry weight, i.e. the EC₅₀ values the ne observed effect rate NOEC and the lowest effect rate (LOEC).

	Emergence	🖌 Survival 🗡	TO SI	woot dry weight	
Species	EC50 V	EC30	ÆC ₅₀	NOEC ~	LOEC
Zea mays	> 12.1	\$12.1 ° °	> 12 9	Z 12.1 S	> 12.1
Cucumis sativa	£12.1	> 12.	> 2.1	D≥ 12.1√	> 12.1
Avena sativa	\$ 12.5° ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	> 2.1 ~	Q12.10 K	> 10/1	> 12.1
Brassica napus	> 12,1 ~	\$ 12.1 × K	> 12.1	₹Î2.1	> 12.1
Allium cepa	$\approx$ 12.1 $\odot$	P>12.₩ 6	>2.1	> 12.1	> 12.1
Pisum satõgum	> 12.1	> 12,1	®12.1	> 12.1	> 12.1
Glycine hax	> 101 ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	£12.1 \$	r > 12,¶ ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	> 12.1	> 12.1
Beta vulgaris	× 2.1 × ×	> 12 4 3	≥ 12.1	> 12.1	> 12.1
	Ş. X ₆ ç	N W	Ő "		

## Effects of the test item on emergence, survival and shoot dry weight

There were no visible sometons of phytotoxicity from any freatment concentration in any of the species tested apart from some minor growth stage delays

Growth stages of the non-target terrestrial plant species at the application rate at the final assessment

Grøwth stage (BB	CH) Xin-M	ax at app	ication	ates (in µş	g/kg soil)	at the fina	al
assessment	. 4		Å				
Species	Control	Q. 011	0.045	0.18	0.75	3.0	12.1
Zea mays 🖉 🔬		16	15-16	15-16	16	16	15-16
Cucumis sativa 🖓	<b>1</b> 3-14	¥12-14	13-14	13-14	13-14	13-14	10-14
Avena Sativa S	<u>4</u> 14-21	14-21	14-21	14	14	14-15	14-22
Brassica napus	√14-¥6	15-16	14-16	14-16	14-16	14-16	14-16
Album cepa 🛛 🖉	1,1,1/3	12-13	11-13	11-13	11-13	11-13	11-13
Pisum sativum	16-18	16-18	16-18	16-18	16-18	10-18	14-18
Glycine max	14-16	14-15	12-16	14-15	14-15	14-15	12-15
Beta vulgaris	14-16	16	14-16	14-16	16	10-16	16



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#### **III. CONCLUSION:**

In a Tier 2 seedling emergence and growth test the metabolite M-01 (AE C653711) was tested under greenhouse conditions for effects on the survival, visual phytotoxicity, growth and shoot dry weight of 8 non-target terrestrial plant species. The seeds of the test species were sown into soil in which various concentrations of the test item were incorporated. The maximum rate of 12.1 mg/kg did not result in adverse effects on emergence, survival or biomass of any of the 8 species in the study.

#### Assessment and conclusion by applicant:

In this Tier 2 seedling emergence and growth study, no adverse effects > 50% or survival, visual phytotoxicity, growth and shoot dry weight of eight non-targe terrestrial plant species after incorporation in soil of the metabolite M-01 (AE (\$53711) were detected. The study is considered reliable.

# CA 8.7 Effects on other terrestrial organisms (flora and fauna)

Data Point:	KCA 8.7/01 2 2 0 4 6 2 2 2
	KCA 8.7/01 0 2 2 2 2
Report Author:	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$
Report Year:	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$
Report Title:	Laboratory screening trials to determine insectividal activity Code: AE C638206
Report No:	$C014350$ $d^{2}$ $d^{2}$ $d^{2}$ $d^{2}$ $d^{2}$ $d^{2}$ $d^{2}$ $d^{2}$
Document No:	M-296449-01-1
Guideline(s) followed in	
study:	
Deviations from current	Not applicable, as no guideline is available
test guideline:	
GLP/Officially	yes, evaluated and accepted
	$A B_{A} (9005)$ $A = 100000000000000000000000000000000000$
GLP/Officially	No. hot conducted under GLP/Officially recognised testing facilities
recognised testing	
facilities:	
Acceptability/Reliability:	Ves
	$\begin{array}{cccccccccccccccccccccccccccccccccccc$
R ^A .	
Executive Summary	

In the screening assessment the test item was used at dose tates of 1 and 100 ppm to determine whether it exhibits insecticidal activity on relevant entomological (arget species. Therefore, it was used against seven different insect species (*Spotoptero exigita*, *Heliothis virescens*, *Aphis fabae*, *Nilaparvata lugens*, *Diabrotica undecimpunctata*, *Meloidogyne incognita*, *Tetranychus urticae*). The test models that were used represent the standard characterization screen of the insecticide research department of Aventis CropScience in Frankfurt. All treated species were held in a climate chamber (25°C, 40-60% RH). Assessment was done 6 days after application in percentage of efficacy in comparison to the untreated control. The activity of the test compound is expressed as the concentration (ppm) required to cause 90% effect ( $fC_{90}$ ). The fungicide AE C638206 tech. showed no efficacy on the seven treated entomological species. As the test methods have been designed to optimize the chances of detecting new insecticidal lead compounds, it is concluded that AE C638206 tech. has no potential for causing insecticidal lead compounds, it is concluded that AE C638206 tech. has no potential for causing insecticidal effects at concentrations below approximately 100 ppm.



#### I. MATERIALS AND METHODS

Test item: AE C638206 tech., name: Fluopicolide; content of a.s. analysed: > 95%. Material was dissolved in a solvent mixture containing 0.2 mL DMSO + 0.3 mL Acetone + 1.4 mL Methanol@nd then diluted in deionized water in order to obtain a dose rate of 100 ppm.

Test design: In the screening assessment the test item was used to determine whether illexhibits insecticidal activity on relevant entomological target species. Therefore, it was used against seven different insect species. The test models that were used represent the standard characterization section of the insecticide research department of Aventis CropScience in Frankfurt. The screening methods are described in the following paragraphs:

-The test compound solution is carefully rinsed over 10 second instal larvae of Spodoptera exigua and dropped onto an artificial diet.

- Heliothis virescens eggs on filter paper are dipped into the test compound solution for 5 seconds and carefully placed in a Petri dish with the eggs an wards Additional compound solution is dropped onto S. Ŵ diet. õ n

- Seeds of field beans (Vicia faba) infested by Appris fabge are dripped into the test compound solution for 5 seconds.

- Rice plants (Oryza sativa) are dipped into the test compound solution for 5 seconds. Icomediately after the plants are dipped, they are laid into Petridishes. After drying, the rice plants are infested with approx. 20 planthopper larvae (Nilaparvata lugens). Ô Ŵ Ø

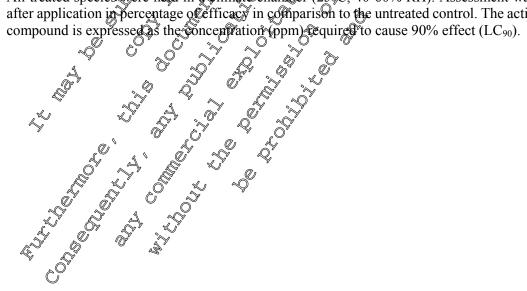
-The test compound solution is dropped onto eggs of Dickrotic and egimpunctata and on a sprouted maize seed. Ø m  $\bigcirc$ 

-The test compound solution is pipetted into a nematode (Meloidog he incognited suspension (dilution factor 1:100). If immobility 80% coccurs after 6 d, the compound solution with the pre-treated nematodes is drench into soil.  $\cap$ 

-Seeds of French beans (Chaseorins vulgaris) infested with Letranychus urlicae are dipped into the test compound solution for \$ seconds. Ø

-Roots of intact seedings of field beans (Vicia faba) are inserted into glass bottles. The test compound solution is propeted into the bottles (dilution factor 1:10). Afterwards, the plants are infested with Aphis fabae. 🔊  $\bigcirc$ L)

All treated species were had in a climat Chamber (25%C, 40-50% RH). Assessment was done 6 days after application in percentage of efficacy in comparison to the untreated control. The activity of the test





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#### **II. RESULTS AND DISCUSSION**

#### Characterization of screening results and calculated LC90

Treated stage	% effect in test	LC90 (ppm)
Larvae	0 at 100 ppm	
Eggs	0 at 100 ppm 🖉	
Mixed population	0 at 100 ppm	, 100 Å
Larvae	0 at 100 pp	
Eggs	0 at 100 ppm	2 D100 2 0
Larvae	0 at ppm °	
Mixed population	. 0. 0. 0. 0. 0. 0. 0. 0. 0. 0. 0. 0. 0.	>400
Mixed population		
	Larvae Eggs Mixed population Larvae Eggs Larvae Mixed population	Larvae0 at 100 ppmEggs0 at 100 ppmMixed population0 at 100 ppmLarvae0 at 100 ppmEggs0 at 100 ppmLarvae0 at 100 ppmLarvae0 at 100 ppmMixed population0 at 100 ppm

No insecticidal activity at the tested rates on the treated target species was observed. test methods have been designed to optimize the chances of detecting new insecticidal lead compounds, it is concluded that AE C698206 toch. hav no potential for causing insecticidal effects at concentrations

Assessment and conclusion by applicant: The information it provides is sopplemental as it is not relevant for

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KCA 8.7/02
2001
Glasshouse screening trials to determine herbicidal activity AE C638206
C013880
<u>M-205444-01-1</u>
Not applicable, as no guideline is available for NTTP screening studies
yes, evaluated and accepted 🕅
yes, evaluated and accepted V
No, not conducted under GL Officially recognised testing facilities
Yes & & X & X & X

The purpose of this screening study was to determine whether the furgicide fluopicolide formulated in a typical screening formulation (fluopicolide WP20) exhibits hetbicidar activity. Therefore, 27 plant species were selected to cover important grass and broad leaved weeds and include globally important arable crop. Two screening stages were performed, and 8 plant species tested at screening Stage I and 24 plant species at screening stage II. For both screening stages, plant seeds were sown in a sandy loam soil in 4 cm diameter peat pots

The spray solution of 1000 g a.s./be was applied at a spray volume of 800 L/ba in the first stage. In the second stage the spray solutions of 20, 80, 320 and 1280 g a.s./ha were applied at a spray volume of 600 L/ha. The pots were maintained under glasshouse conditions.

The treated plants were monifored daily for symptoms of herbicidal injury in comparison with untreated control plants. The treated plants showed no herbicidal effect at the Screening Stage 1 and 2.

## E-MATERIAL AND METHORS:

Test item: Fluopicolide WP20: Fluopicolide was supplied as a 95% pure technical grade sample. This material was formulated as a 20% wettable powder (standard WP20) screening formulation).

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Test species: 27 species selected to cover important grass and broad-leaved weeds and include globally important arable corps (EPPO code): Zea mays (ZEAMA), Tricitium aestivium (TRZAS), Glycine max (GLXMA), Beta vulgaris (BEAVA), Oriza sativa (ORYSA), Abutilon theophrasti (ABUTH), Agropyron repens (ACRRE), Alopecturus invosuroides (ALOMY), Amaranthus retroflexus (AMARE), Avena fatua (AVEFA), Asena sativa (AVESA), Cheropodium album (CHEAL), Cyperus esculentus (CYPES), Cyperus iria (CYPIR), Digitaria sanguinalis (DIGSA), Echinochloa crus-galli (ECHCG), Galium sparine (GALAP), Eolium multiforum (OLMU). Matricaria inodora (MATIN), Pharbits purpurea (PHBPU), Fallonia convolvutus (POECO), Sesbania exaltata (SEBEX), Setaria viridis (SETWI), Sorghum haleponse (SORHA), Stelfaria media (STEME), Sinapis alba (SINAL), Veronica persica (VERPE).

Two screening stages were performed and 8 plant species tested at screening Stage I and 24 plant species at screening stages. II. For both screening stages, plant seeds were sown in a sandy loam soil in 4 cm diameter pear pots. In order to investigate the soil and foliar activity of fluopicolide, the pots were either treated before the seedlings emerged from the soil (pre-emergence) or after the seedlings had produced the first 2-4 leaves (post-emergence). The pots were placed in a glasshouse at 22°C day and 18°C night.

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The Fluopicolide WP 20 screening formulation was diluted in deionized water (containing 0.2% of the wetting agent Agrotin 390) in order to obtain dose rates of 20, 80, 320, 1000 and 1280 g a.s./ha. The spray solution of 1000 g a.s./ha was applied at a spray volume of 800 L/ha in the first stage. In the second stage the spray solutions of 20, 80, 320 and 1280 g a.s./ha were applied at a spray volume of 600 %/ha. After application the pots were returned to the glasshouse.

The treated plants were monitored daily for symptoms of herbicidal injury in comparison with intreated control plants. The plants treated post-emergence were formally scored for injury 7 and 14 days after treatment. The pre-emergence plants were assessed 21 days after treatment. The assessments were made on a scale of 0 (= no effect) to 100 (= complete kill) for visual effect.

### **II. RESULTS AND DISCUSSION:**

**Biological findings:** 

The 8 plant species treated pre- or post-emergence at a dose rate of 1000 g a.s. ha showed no herbiogral effect at stage I. At Stage II also no herbicidal effect was observed in the 24 planespecies treated preor post-emergence with dose rates of 20, 80, 320 and 280 g technical sample/has

### In a screening test Fluopicolide WP20 was tested under greenhouse Conditions for herbierdal effects on 8 species at stage I (test rate = 1000 g/a.s. 4a) and 24 species at stage 4 (test states = 20, 80, 320 and 1280 g a.s./ha) to cover important grass and broad leaved weeds and including important global arable crops. No herbicidal effects were observed at both stages at the given rates

II. Conclusions:

### Assessment and conclusion by applicant:

No herbicidal effects on 25 weed and a rop species were observed in this screening test for the active substance fluopicolide formulated as a WP20 screening formulation up to a test rate of 1000 g

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Data Point:	KCA 8.7/03	
Report Author:		
Report Year:	2003	
Report Title:	AE C638206 technical ingredient: Determination of effects on growth of pure cultures of a soil fungus, Mucor circinelloides (Zygomycetes), on nutrient medium	Ş
Report No:	LKC-SF-01/03	
Document No:	<u>M-235058-01-1</u>	
Guideline(s) followed in		)
study:		a
Deviations from current	Not applicable, as no agreed guideline is available	Š
test guideline:		, ,
Previous evaluation:	yes, evaluated and accepted accepted and acc	
GLP/Officially	Yes, conducted under CKP/Officially recognised testing facilities	
recognised testing		
facilities:		
Acceptability/Reliability:	Yes A & Q Q A O' Q' A	

The study investigated the influence of the test item on growth of pure cultures of a soil fungus, *Mucor circinelloides* (Zygomycetes), on malt agar mixed with steril, silty sand soil. The soil, which served as a carrier for the test substance and a source of micro-nutrients for the fungus, was treated with the test item to receive concentrations of (3, 1.5, 3.0, 7.5, 15.0) and 30.0 mg as /kg dry weight soil. Controls contained malt agar and untreated, sterile soil, Colonies were grown in the dark at  $20 \pm 2$  °C. Three replicate dishes were prepared per test concentration. The percent of inhibition has determined after 3 days. Significant differences in growth between treated and control samples were less than 25 %. Therefore, the NOEC was  $\geq$  30.0 mg a S/kg dry weight soil and the C₅₀ was > 30.0 mg a S/kg dry weight soil.



Test iten AE C63820 Ctech, name: Fluopicolide Datch/FC no. OP2050046; content of a.s. analysed: 96.1 %; development code of a.s.: AE C638206; development no.: 3000315241.

Test design: Small pieces of ager (4 mm in diameter) containing mycelium from an actively growing *Mucor circinelloides* calture were used to inoculate the centers of petri dishes containing nutrient medium prepared by mixing malt agar with sterile, AE C638206-treated, silty sand soil (0.6 % org. C, pH 5.3).

The soil, which served as a capier for the test substance, and a source of micro-nutrients for the fungus, was treated with the test item to receive concentrations of 0.3, 1.5, 3.0, 7.5, 15.0 and 30.0 mg a.s./kg dry weight soil. Controls contained malt agap and untreated, sterile soil. Colonies were grown in the dark at  $20 \pm 2$  °C. Three replicate dishes were prepared per test concentration. Percent inhibition of growth was determined after 3 days by comparison of mean diameters (± std. deviation) of treated colonies to mean diameters of the unneated controls.



Concentration tested (mg a.s./kg dry wt soil)	Mean Colony diameters ± standard deviation (cm) from 3 replicates	Inhibition of growth in % of untreated control
0	7.3±0.1	
0.3	7.1 ± 0.1	
1.5	7.1 ± 0.3	
3.0	7.3 ± 0.1	
7.5	7.1 ± 0.2 ( ) ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~	
15.0	$7.0 \pm 0.1$	
30.0	$6.8\pm$	A ST A ST

Significant differences in growth between meated and control samples were detected only in the highest test treatment (30 mg a.s./kg dry weight soil). The efforts were less than 25%. Therefore, the NOEC was  $\geq$  30.0 mg a.s./ kg dry weight soil and the  $\mathcal{E}C_{50}$  was > 30.0 mg a.s./ kg@dry weight soil.

Validity criteria: Internationally agreed and accepted guidelmes for determining the influence of test items on pure cultures of soil fungic ould not be found in the literature. Therefore, vandity criteria were not defined. The endpoints are: NOEC 200.0 mg a.s./bg dry weight soil EC₅₀ > 30.0 mg a.s./bg dry weight soil

NOEC 
$$\gtrsim$$
 \$0.0 mg a.s.  $\swarrow$  dry  $\bigotimes$  eight soil  
EC₅₀ > 30.0 mg a.s.  $\bigotimes$  dry  $\bigotimes$  eight soil  $\sim$ 

#### conclusion by applicant: Assessment

F The study is considered reliable. However, as this study is not a data requirement it will not be further considered in the risk assessment.

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Data Point:	KCA 8.7/04
Report Author:	
Report Year:	2003
Report Title:	AE C638206 technical ingredient: Determination of effects on growth of pure cultures of a soil fungus, Phytophthora nicotianae (Oomycetes), on nutrient medium
Report No:	LKC-SF-03/03
Document No:	<u>M-235061-01-1</u>
Guideline(s) followed in	
study:	
Deviations from current	Not applicable, as no agreed guideline is available
test guideline:	
Previous evaluation:	yes, evaluated and accepted DAR (2005)
GLP/Officially	Yes, conducted under GCP/Officially recognised testing facilities
recognised testing	
facilities:	
Acceptability/Reliability:	Yes $\underline{A}$ $\underline{\partial}^{\vee}$ $\underline{\partial}^{\vee}$ $\underline{Q}^{\vee}$ $\underline{Q}^{\vee}$ $\underline{\partial}^{\vee}$ $\underline{\partial}^{\vee}$ $\underline{\partial}^{\vee}$

The study investigated the influence of the test item on growth of pure cultures of a soil fungus, *Phytophthora nicotianae* (Oomycetes), vegetable juice agar with sterile,  $\Delta E$  C638206 tech. – treated, silty sand soil. The soil, which served as a carrier for the test substance, and a source of micro-nutrients for the fungus, was treated with the test item to receive concentrations of 0.3, 12, 3.0, 7.5, 15.0 and 30.0 mg a.s./kg dry weight soil. Colones were grown in the dark at 20  $\pm 2^{\circ}$  C. Three replicate dishes were prepared per test concentration. Percent inhibition of growth was determined after 6 days. Significant differences in growth between treated and control samples were detected in the test concentrations with 1.5, 3.0, 7.5, 15 and 30 mg a.s./kg dry weight soil. The EC₅₀ is 1.2 ing a.s./kg dry weight soil.



Test item AE C63820 tech. Frame: Fluopicolide Frach/FF no. OP2050046; content of a.s. analysed: 96.1 %; development code of a.s.: AE C638206; development ro.: 3000315241.

Test design: Small pieces of ager (4 mm in diameter) containing mycelium from an actively growing *Phytophthora nicotiante* culture were used to incertate the centers of petri dishes containing nutrient medium prepared by mixing vegetable juice agar with sterile, AE C638206 tech. – treated, silty sand soil (0.6% org. C, pH 5.3) The foil, which served as a carrier for the test substance, and a source of micro-nutrients for the fungus was treated with enough AE C638206 tech. to give concentrations of 0.3, 1.5, 3.0, 7.5, 150 and 30 mg a.s./kg dry weight soil. Controls contained vegetable juice agar and untreated, sterile soil. Colonies were grown in the dark at  $20 \pm 2^{\circ}$ C. Three replicate dishes were prepared per test concentration. Perfect inhibition of growth was determined after 6 days by comparison of mean diameters (± std. deviation) of treated colonies to mean diameters of the untreated controls.

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#### **II. RESULTS AND DISCUSSION**

Effect of fluopicolide on growth of pure cultures of *Phytophthora nicotianae* on nutrient medium

	standard deviation (cm) from 3 replicates	Inhibition of growth in so of untreated control
0	$5.6 \pm 0.2$	
0.3	6.0±0.1	
1.5	2.2 ± 0.3	
3.0	$1.5 \pm 0.2$	
7.5	1.3 ± 0.2	
15.0		$\begin{array}{cccccccccccccccccccccccccccccccccccc$
30.0		898 8 A A

Significant differences in growth between treated and control samples were detected in the test concentrations with 1.5, 3.0, 7.5, 15 and 30 mg a sike dry weight soil. At these rates the differences were > 25%. Therefore, the NOE6 is 0.3 mg a.s./kg.dry weight soil The EC 50 is 3.2 mg/a.s./kg dry weight soil.

#### Validity criteria:

Internationally agreed and accepted guidelines for determining the influence of test items on pure cultures of soil fungi could not be found in the literature. Therefore, validity criteria were not defined.

LEP. CONFLUSION

The endpoints are:

NOEC = 03 mg a.s./kg dry weight so

weight soil  $EC_{50} = 1$ .2 mg a.s./kg dry

Assessment and conclusion by applicant. The study of considered eliable However, as this study is not a data requirement it will not be further considered in the risk ossessment.

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Data Point:	KCA 8.7/05
Report Author:	
Report Year:	2003
Report Title:	AE C638206 technical ingredient: Determination of effects on growth of pure cultures of a soil fungus, Cladorrhinum foesundissimum (Deuteromycetes), on nutrient medium
Report No:	LKC-SF-07/03
Document No:	<u>M-235063-01-1</u>
Guideline(s) followed in	
study:	
Deviations from current test guideline:	Not applicable, as no agreed guideline is available
Previous evaluation:	yes, evaluated and accepted DAR (2005)
GLP/Officially	Yes, conducted under GCP/Officially recognised testing facilities
recognised testing facilities:	
Acceptability/Reliability:	Yes $A$ $\partial^{\prime}$ $Q$ $Q$ $Q$ $O^{\prime}$ $\partial^{\prime}$ $\partial^{\prime}$

The study investigated the influence of the test item on growth of pure cultures of a soil fungus, *Cladorrhinum foesundissimum* (Deuteromycetes), or onalt agar mixed with sterile, silty sand soil. The soil, which served as a carrier for the test substance, and a source of micro-nutrients for the fungus, was treated with the test item to receive, concentrations of 0.3, 1.5, 3.0, 7.5, 15 (Cand 30.0 mg a.s./kg dry weight soil. Colonies were grown in the dark at  $20 \pm 2$ , °C. Three replicate dashes were prepared per test concentration. Percent inhibition of growth was determined after 7 days. No significant differences in growth between treated and control samples were detected. The effects were less than 25 %. Therefore, the NOEC was  $\geq 300^{\circ}$  mg a.s./kg dry weight soil and the EC₅₀ was > 30.0 mg a.s./kg dry weight soil.

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# 9. MATERIALS AND WETHODS

Test item: AE C638206.tech., name: Friopicobile; batch/FL no.: OP2050046; content of a.s. analysed: 96.1 %; development code of as.: AE C638206; development no 3000315241.

Test design: Suspensions of spores from *Oladorrhinum foesundissimum* cultures were used to inoculate the cultures of petri dishes containing nutricat medium prepared by mixing malt agar with sterile, AE C638206 tech. – treated, sitty sand soil (0.6% of C, pH 5.3). The soil, which served as a carrier for the test substance and a source of micro-nutrients for the fungus, was treated with enough AE C638206 tech. to give concentrations of 0.3, 1.5, 3.0, 7.5, 15, 9 and 30.0 mg a.s./kg dry weight soil. Controls contained malt agar and untreated, sterile soil. Colonies were grown in the dark at  $20 \pm 2$  °C. Three replicate dishes were prepared per test concentration. Percent inhibition of growth was determined after 7 days by comparison of mean diameters 4 std deviation) of treated colonies to mean diameters of the untreated controls

replicate alishes were prepared per test concentration. Percent inhibition of growth was determined after 7 days by comparison of mean diameters (# std deviation) of treated colonies to mean diameters of the untreated controls.



Effect of fluopicolide on growth of pure cultures of *Cladorrhinum foesundissimum* on nutrient medium

Concentration tested (mg a.s./kg dry wt soil)	Mean colony diameters ± standard deviation (cm) from 3 replicates	Inhibition of growth in 25 of untreated control
0	$6.1 \pm 0.2$	
0.3	6.1 ± 0.2	
1.5	6.0 ± 0.1	
3.0	$6.0 \pm 0.1$	
7.5	6.3 ± 0.2	
15.0		TO DE LE LE
30.0	6.1 ± 0.1 0 0 0 0	

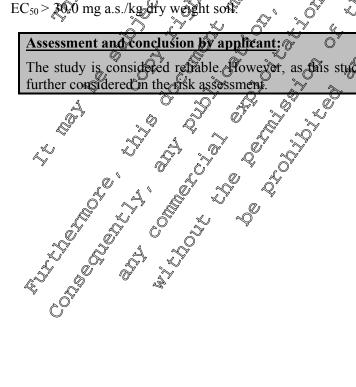
No significant differences in growth between treated and control samples were detected. The effects weight soil and the EC 50 was > were less than 25 %. Therefore, the OOEC was  $\ge 90.0$  mg a.s. /k@g dr 30.0 mg a.s./kg dry weight soil.

#### Validity criteria:

Internationally agreed and accepted guidelings for determining the influence of test items on pure cultures of soil fungi could not be found in the literature. Therefore, validity criteria were not defined.

The endpoints are NOEC  $\geq$  3000 mg a.s./kg,dry weight soil g dry weight soft  $EC_{50} >$ **30**0 mg a.s.

The study is considered repable flowever, as this study is not a data requirement it will not be





Data Point:	KCA 8.7/06	
Report Author:		
Report Year:	2003	
Report Title:	AE C638206 technical ingredient: Determination of effects on growth of pure cultures of a soil fungus, Penicillium janthinellum (simplicissimum) (Ascomycetes), on nutrient medium	Ĵ,
Report No:	LKC-SF-11/03	
Document No:	<u>M-235065-01-1</u>	
Guideline(s) followed in		)
study:		0
Deviations from current	Not applicable, as no agreed guideline is available	Å
test guideline:		9
Previous evaluation:	yes, evaluated and accepted	
GLP/Officially	Yes, conducted under CKP/Officially recognised testing facilities	
recognised testing		
facilities:		
Acceptability/Reliability:	Yes $\Delta$ $\phi$ $Q$ $Q$ $\phi$ $O'$ $\phi'$ $\phi'$	

The study investigated the influence of the test item on growth of pure culture of a soil fungus, *Penicillium janthinellum* (Asconycetes), on malt agar nixed with sterile, stry sandsoil. The soil, which served as a carrier for the test substance, and a source of micro-nutrients for the fungus, was treated with the test item to receive concentrations of 0.3, 1.5, 3.0, 7.5, 15.0 and 30.0 mg a.s./kg dry weight soil. Colonies were grown in the dark at  $20 \pm 2^{\circ}$  °C. Three replicate diskes were prepared per test concentration. Percent inhibition of growth was determined after 11 days. No significant differences in growth between treated and control samples were detected. The effects were less than 25 %. Therefore, the NOEC was  $\geq 300^{\circ}$  mg a.s./kg dry weight soil and the EC₅₀ was > 30.0 mg a.s./kg dry weight soil.

# 9. MATERIALS AND WETHODS

Test item: AE C638206.tech., name: Friopicobile; batch/FL2no.: OP2050046; content of a.s. analysed: 96.1 %; development code of As.: AE C638206; development no 3000315241.

Test design: Suspensions at spore from *Penicillium fanthinelium* cultures were used to inoculate the cultures of petri dishes containing nutrient medium prepared by mixing malt agar with sterile, AE C638206 tech. – treated silty and solv (0.6% org. C pH 53). The soil, which served as a carrier for the test substance and a source of micro-nutrients for the fungus, was treated with enough AE C638206 tech. to give concentrations of 63, 1.503.0, 73, 15, and 30.0 mg a.s./kg dry weight soil. Controls contained malt agar and untreated, sterile soil. Colonies were grown in the dark at  $20 \pm 2$  °C. Three replicated dishes were prepared per test concentration. Percent inhibition of growth was determined after 11 days by comparison of mean diameters ( $\pm$  side deviation) of treated colonies to mean diameters of the untreated controls.



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#### **II. RESULTS AND DISCUSSION**

Effect of fluopicolide on growth of pure cultures of *Penicillium janthinellum* on nutrient medium

Concentration tested (mg a.s./kg dry wt soil)	Mean colony diameters ± standard deviation (cm) from 3 replicates	Inhibition of growth in the second se
0	$6.0 \pm 0.5$	
0.3	5.7±0.6	5
1.5	5.5 ± 04	
3.0	$5.2 \pm 0.3$	
7.5	6.4 ± 0.1	
15.0	6.4 ± 0.2 ( ) ( ) ( ) ( ) ( ) ( ) ( ) ( ) ( ) (	7 . 9 . 5
30.0	$6.3 \pm 0.1$ $0^{2}$ $0^{3}$ $0^{3}$ $0^{3}$	125° ° & A

No significant differences in growth between treated and control samples were detected. The effects weight soil and the EC 50 was > were less than 25 %. Therefore, the OOEC was  $\ge 90.0$  mg a.s. /k@g dr 30.0 mg a.s./kg dry weight soil.

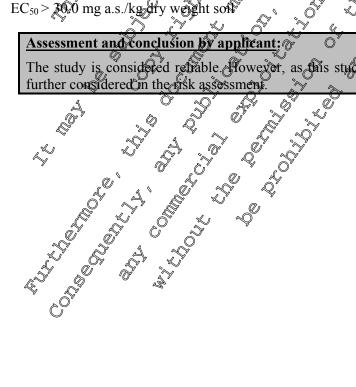
#### Validity criteria:

Internationally agreed and accepted guidelings for determining the influence of test items on pure cultures of soil fungi could not be found in the literature. Therefore, validity criteria were not defined.

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The endpoints are NOEC  $\geq$  3000 mg a.s./kg,dry weight soil g dry weight soft  $EC_{50} >$ **30**0 mg a.s.

The study is considered repable flowever, as this study is not a data requirement it will not be





Data Point:	KCA 8.7/07
Report Author:	
Report Year:	2003
Report Title:	AE C638206 technical ingredient: Determination of effects on growth of pure
	culture of a soil fungus, Suillus granulatus (Basidiomycetes), on nutrient medium
Report No:	LKC-SF-05/03
Document No:	<u>M-218467-01-1</u>
Guideline(s) followed in	
study:	
Deviations from current	Not applicable, as no agreed guid tine is available of a start of a
test guideline:	
Previous evaluation:	yes, evaluated and accepted $\mathcal{K}$ $\mathcal{O}^{\vee}$ $\mathcal{K}^{\vee}$ $\mathcal{O}^{\vee}$
	DAR (2005)
GLP/Officially	Yes, conducted under GLF Officially recognised asting facilities
recognised testing	
facilities:	
Acceptability/Reliability:	Yes O' V A A
Executive Summary	
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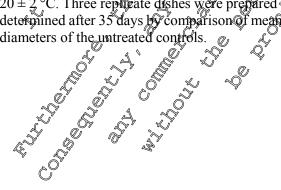
The study investigated the influence of the jest item on growth of pure cultures of a set funges, Suillus granulatus (Basidiomycetes), on roalt agar mixed with sterile silty sand soil The soil, which served as a carrier for the test substance, and a source of microgrutrients for the fungers, was treated with the test item to receive concentrations of 0.3, 1.5, 3.0, 7.5, 15.0 and 30, 2 mg ags/kg dry weight soil. Controls contained malt agar and untreated sterile soil. Golonies were grown in the gark at  $20 \pm 2$  °C. Three replicate dishes were prepared per test concentration. The percent of inhibition was determined after 35 days. No significant differences, in growth between treated and control samples were detected. The effects were less than \$5 %. Therefore, the NOE was \$30.0 rog a.s As dry weight soil and the EC 50 was > 30.0 mg a.s.// dry weight soil.

## 9. MAYERIALS AND METHODS

Test item: SE C638206 tech., name: FinopicoDde; batch/Fi@no.: OD2050046; content of a.s. analysed: 96.1 %; development code of as.: AE C638206; development no 3000315241.

Test design: Small pieces of agar 4 mmon diameter containing mycelium from an actively growing Suillus granulatus eulture were used to inoculate the centers of petri dishes containing nutrient medium prepared by mixing math agar with sterile, AE C638206-treated, silty sand soil (0.6 % org. C, pH 5.3).

The soil, which served as a Carrier for the test substance, and a source of micro-nutrients for the fungus, was treated with the test item to receive concentrations of 0.3, 1.5, 3.0, 7.5, 15.0 and 30.0 mg a.s./kg dry weight soff. Controls contained malt agar and untreated, sterile soil. Colonies were grown in the dark at  $20 \pm 2$  °C. Three replicate dishes were prepared per test concentration. Percent inhibition of growth was determined after 35 days by comparison of mean diameters (± std. deviation) of treated colonies to mean diameters of the untreated controls.





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#### **II. RESULTS AND DISCUSSION**

Effect of fluopicolide on growth of pure cultures of Suillus granulatus on nutrient medium

Concentration tested (mg a.s./kg dry wt soil)	Mean colony diameters ± standard deviation (cm) from 3 replicates	Inhibition of growth in so of untreated control
0	$2.7 \pm 0.3$	
0.3	2.4±0.1	
1.5	2.4 ± 0.1	
3.0	$2.7 \pm 0.2$	
7.5	2.4 ± 0.1	
15.0	2.5 ± 0.1 ( )	
30.0	$2.6 \pm 0.2$ $\bigcirc$ $\checkmark$ $\checkmark$ $\checkmark$ $\checkmark$	4 8 8 4 A

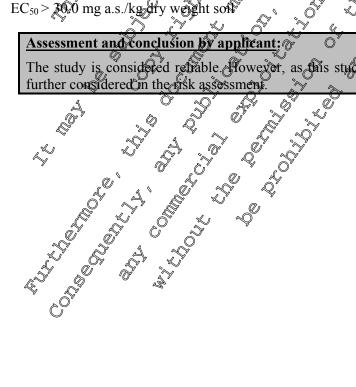
No significant differences in growth between treated and control samples were detected. The effects weight soil and the EC 50 was > were less than 25 %. Therefore, the OOEC was  $\ge 90.0$  mg a.s. /k@g dr 30.0 mg a.s./kg dry weight soil.

#### Validity criteria:

Internationally agreed and accepted guidelings for determining the influence of test items on pure cultures of soil fungi could not be found in the literature. Therefore, validity criteria were not defined.

The endpoints are NOEC  $\geq$  3000 mg a.s./kg,dry weight soil g dry weight soft  $EC_{50} >$ **30**0 mg a.s.

The study is considered repable flowever, as this study is not a data requirement it will not be





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Data Point:	KCA 8.7/08
Report Author:	
Report Year:	2003
Report Title:	AE C653711 (AE C653711 00 1B97 0001): Determination of effects on growth of pure cultures of a soil fungus, Mucor circinelloides (Zygomycetes), on nutrient medium
Report No:	LKC-SF-02/03
Document No:	<u>M-235067-01-1</u>
Guideline(s) followed in	
study:	
Deviations from current test guideline:	Not applicable, as no agreed guideline is available
Previous evaluation:	yes, evaluated and accepted DAR (2005)
GLP/Officially	Yes, conducted under GEP/Officially recognised testing facilities
recognised testing	
facilities:	
Acceptability/Reliability:	Yes A & Q Q O' Q' A

### **Executive Summary**

The study investigated the influence of the test item M-01 (AF C653/11,  $\frac{1}{2}$ 6-dichoroben zamide) on growth of pure cultures of a soil fungus. *Mucor circinelloides* (Zygomycetes) on malt agar mixed with sterile, silty sand soil. The soil, which served as a carrier for the test substance and a source of micro-nutrients for the fungus, was treated with the test item to receive concentrations of 0.3, 1.5, 3.0, 7.5, 15.0 and 30.0 mg p.m./kg dry weight soil. Controls contained malt agar and thereated, sterile soil. Colonies were grown in the dark at 20  $\frac{1}{2}$  °C. Three replicate dishes were prepared per test concentration. The percent of inhibition was determined after days. No significant differences in growth between treated and control samples were detected. The effects were less than 25 %. Therefore, the NOEC was  $\geq$  30.0 mg p.m./kg dry weight soil and the EC₃₀ was  $\geq$  30.0 mg p.m./kg dry weight soil.

## I. Materials and methods

Test item M-01 (AE C69371), chemical name: 2,6 dichlorobenzamide; batch/FL no.:8808018; content of a.s. analysed: 97 0%; development code of a.s.: AE C653711 00 1B97 0001; development no.: 3000325964.

Test design: Small pieces of near (4 pm in diameter) containing mycelium from an actively growing *Mucor circinetoider*, culture were used to inoculate the centers of petri dishes containing nutrient medium prepared by mixing managar with sterile, AF C653711-treated, silty sand soil (0.6 % org. C, pH 5.3).

The soil, which served as a carrier for the fest substance, and a source of micro-nutrients for the fungus, was treated with the test item to receive concentrations of 0.3, 1.5, 3.0, 7.5, 15.0 and 30.0 mg p.m./kg dry weight soil. Controls contained malt agar and untreated, sterile soil. Colonies were grown in the dark at  $20 \pm 2$ ° C. Three reflected fishes were prepared per test concentration. Percent inhibition of growth was determined after 3 days by comparison of mean diameters (+ std. deviation) of treated colonies to mean diameters of the untreated controls.

colonies to mean diameters of the untreated controls.



Concentration tested (mg p.m./kg dry wt soil)	Mean Ccolony diameters + standard deviation (cm) from 3 replicates	Inhibition of growth in % of untreated control
0	7.0±0.2	
0.3	7.0 ± 0.3	
1.5	6.9 ± 0.1	
3.0	6.9 ± 0.1	
7.5	$7.0\pm0.2$ ( $^{\circ}$ $^{\circ}$ $^{\circ}$	
15.0	$7.0 \pm 0.1$ 0 * $$ $$ $$	
30.0	$6.8\pm 0.2$	

No significant differences in growth between treated and control samples were detected. The effects were less than 25 %. Therefore, the NOEC was 30. Ong p.p./kg doy veight soil and the EC50 was > 30.0 mg p.m./kg dry weight soi

Validity criteria: Internationally agreed and accepted guidelines for determining the influence of test items on pure cultures of soil fungicould not be bund in the literature. Therefore, validity criteria were not defined.

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The endpoints are:  
NOEC 
$$\gtrsim$$
 90.0 mg p.m kg dry weight soil  
EC₅₀ > 30.0 mg p.m kg dry weight soil

#### Assessment conclusion by applicant:

R The study is considered reliable. However, as this study is not a data requirement it will not be further considered in the risk assessment.

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Data Point:	KCA 8.7/09	
Report Author:		
Report Year:	2003	~
Report Title:	AE C643711 (AE C653711 00 1B97 0001): Determination of effects on growth of pure cultures of a soil fungus, Phytophthora nicotianae (Oomycetes), on	0°
D ()I	nutrient medium	
Report No:	LKC-SF-04/03	
Document No:	<u>M-235068-01-1</u>	
Guideline(s) followed in		
study:		0
Deviations from current	Not applicable, as no agreed guideline is available	Ľ
test guideline:		)"
Previous evaluation:	yes, evaluated and accepted	
	DAR (2005)	
GLP/Officially	Yes, conducted under GRP/Officially recognised testing facilities	
recognised testing		
facilities:		
Acceptability/Reliability:	Yes A & Q Q A O Q' A	

The study investigated the influence of the test item M-01 (AF C653/11, C6-dichorobenzamide) on growth of pure cultures of a soil fungus *Phytophthoronicotiquae* (Gomycees), or vegetable juice agar mixed with sterile, silty sand soil. The soil, which served as a carrier for the test substance, and a source of micro-nutrients for the fungus, was treated with the test item to receive concentrations of 0.3, 1.5, 3.0, 7.5, 15.0 and 30.0 mg p.m./kg dry weight soil. Colonies were grown in the darkat  $20 \pm 2$  °C. Three replicate dishes were prepared per test concentration? Percent inhibition of growth was determined after 6 days. No significant differences in growth between treated and control samples were detected. The effects were less than 25 %. Therefore, the NOEC was 30.0 mg p.m./kg dry weight soil.

## Ø I. MATERIALS AND METHODS

Test item M-01 (AE C69371), chengeal name: 2,6 dichlorobenzamide; batch/FL no.:8808018; content of a.s. analysed: 97.6 %; development code of a.s.: AE C653711 00 1B97 0001; development no.: 3000325964.

Test design: Small pieces of agar (4 mm in diameter) containing mycelium from an actively growing *Phytophthora ficotionae* culture were used to inoculate the centers of petri dishes containing nutrient medium prepared by mixing vegetable joice agar with sterile, AE C653711-treated, silty sand soil (0.6 % org. C and 5.3).

The soil, which served as a carrier for the test substance, and a source of micro-nutrients for the fungus, was dicated with the test item to receive concentrations of 0.3, 1.5, 3.0, 7.5, 15.0 and 30.0 mg p.m./kg dry weight soil. Controls contained vegetable nice agar and untreated, sterile soil. Colonies were grown in the dark at  $20 \pm 2$  °C. Three replicate dishes were prepared per test concentration. Percent inhibition of growth was determined after 6 days by comparison of mean diameters (+ std. deviation) of treated colonies to mean diameters of the untreated controls.

colonies to mean drameters of the untreated controls.



Concentration tested (mg p.m./kg dry wt soil)	Mean colony diameters + standard deviation (cm) from 3 replicates	Inhibition of growth in % of untreated control
0	5.8±0.1	
0.3	5.6 ± 0.2	
1.5	5.5 ± 0.1	
3.0	5.2 ± 0.3	
7.5		
15.0	$6.4 \pm 0.2$ 0 $\sqrt{2}$ $\sqrt{2}$ $\sqrt{2}$	
30.0		

No significant differences in growth between treated and control samples were detected. The effects were less than 25 %. Therefore, the NOEC was 30. Ong p.p./kg doy veight soil and the EC50 was > 30.0 mg p.m./kg dry weight soi

Validity criteria: Internationally agreed and accepted guidelines for determining the influence of test items on pure cultures of soil fungicould not be bund in the literature. Therefore, validity criteria were not defined.

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The endpoints are:  
NOEC 
$$\gtrsim$$
 90.0 mg p.m.kg dry weight soil  
EC₅₀ > 30.0 mg p.m.kg dry weight soil

#### Assessment conclusion by applicant:

R The study is considered reliable. However, as this study is not a data requirement it will not be further considered in the risk assessment.

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Data Point:	KCA 8.7/10
Report Author:	
Report Year:	2003
Report Title:	AE C653711 (AE C653711 00 1B97 0001): Determination of effects on growth
	of pure cultures of a soil fungus, Cladorrhinum foesundissimum
	(Deuteromycetes), on nutrient medium
Report No:	LKC-SF-08/03
Document No:	<u>M-235070-01-1</u>
Guideline(s) followed in	
study:	
Deviations from current	Not applicable, as no agreed guideline is available
test guideline:	
Previous evaluation:	yes, evaluated and accepted v v v v v v v v v v v v v v v v v v v
	DAR (2005)
GLP/Officially	Yes, conducted under GCP/Officially recognised testing facilities
recognised testing	
facilities:	
Acceptability/Reliability:	Yes $A$ $\partial^{*}$ $Q$ $Q$ $\partial^{*}$ $\partial^{*}$ $\partial^{*}$

The study investigated the influence of the test item M-01 (AF C653/11, 56-dichoroben zamide) on growth of pure cultures of a soil fungus, *Clador hinung foesurdissingum* (Deuterony cetes), on malt agar mixed with sterile, silty sand soil. The soil, which served as a carrier for the test substance, and a source of micro-nutrients for the fungus, was treated with the test item to receive concentrations of 0.3, 1.5, 3.0, 7.5, 15.0 and 30.0 mg p.m./kg dry weight soil. Colonies were grown in the dark at  $20 \pm 2$  °C. Three replicate dishes were prepared per test concentration Percent inhibition of growth was determined after 7 days. No significant differences in growth between treated and control samples were detected. The effects were less than 25 %. Therefore, the NOEC was 30.0 mg p.m./kg dry weight soil.

## 👳 I. Materials and methods 🖉

Test item M-01 (AE C63371 ), chemical name: 2,6 dichlorobenzamide; batch/FL no.:8808018; content of a.s. analysed: 97.6 %; development code of a.s.: AE C653711 00 1B97 0001; development no.: 3000325964.

Test design: Suspensions of spores from *Cladorrhigum foesundissimum* cultures were used to inoculate the centers of petri dishes containing nutrient medium prepared by mixing malt agar with sterile, AE C653711-treated, silty said soil 4.6 % or C, pH 5.3 P

The soil which served as a capier for the test substance, and a source of micro-nutrients for the fungus, was treated with the test item to receive concentrations of 0.3, 1.5, 3.0, 7.5, 15.0 and 30.0 mg p.m./kg dry weight soil. Controls contained map agar and untreated, sterile soil. Colonies were grown in the dark at  $20 \pm 2$  °C. Three replicate dishes were prepared per test concentration. Percent inhibition of growth was datermined after 7 days by comparison of mean diameters (+ std. deviation) of treated colonies to mean diameters of the untreated controls.



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#### **II. RESULTS AND DISCUSSION**

Effect of M-01 (AE C653711) on growth of pure cultures of Cladorrhinum	foesundissimum	on nutrient
medium	~	

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Concentration tested (mg p.m./kg dry wt soil)	Mean colony diameters + standard deviation (cm) from 3 replicates	Inhibition of growth in % of untreated control
0	6.2 ± 0.2	
0.3	6.3 ± 0.2	
1.5	6.2 ± 0.1	
3.0	6.3 ± 0.3 ( ° ° °	
7.5	6.2 ± 0.2 0 0 0 0	
15.0		
30.0	6.3 00.1 2 y 2 y 2 y	

No significant differences in growth between treated and control samples were detected. The effects were less than 25 %. Therefore, the NOEC was  $\geq$  300 mg p.m./kg dry weight soil and the EC₅₀ was >30.0 mg p.m./kg dry weight soil.

Validity criteria:

Internationally agreed and accepted guidelines for determining the influence of test items on pure cultures of soil fungi could not be found in the literature. Therefore walidity criteria were not defined. Ŵ

ACONCLOSION

The endpoints are: weight soil NOEC  $\geq$  30.0 mg pm./kg dry  $EC_{50} > 30.0 \text{ mg pm}./kg dry weight soil.}$ 

Assessment and conclusion by applicant: The study is considered reliable. However, as this study is not a data requirement it will not be further considered in the risk assessment



Data Point:	KCA 8.7/11	
Report Author:		
Report Year:	2003	
Report Title:	AE C653711 (AE C653711 00 1B97 0001: Determination of effects on growth of	Ŷ
	pure cultures of a soil fungus, Penicillium janthinellum (simplicissimum)	1
	(Ascomycetes), on nutrient medium	
Report No:	LKC-SF-12/03	
Document No:	<u>M-235072-01-1</u>	
Guideline(s) followed in		
study:		a
Deviations from current	Not applicable, as no agreed guideline is available	Ś
test guideline:		)`
Previous evaluation:	yes, evaluated and accepted	
	DAR (2005)	
GLP/Officially	Yes, conducted under GCP/Officially recognised testing facilities	
recognised testing		
facilities:		
Acceptability/Reliability:	Yes A & Q Q O' Q' Y	

The study investigated the influence of the test item M-01 (AF C653/11,  $\frac{1}{2}$ 6-dichoroben zamide) on growth of pure cultures of a soil funges, *Penicillium anthindlum* (Asconycetes), on vegetable juice agar mixed with sterile, silty and soft. The soil, which served as a carrier for the test substance, and a source of micro-nutrients for the fungus, was treated with the test item to receive concentrations of 0.3, 1.5, 3.0, 7.5, 15.0 and 30.0 mg p.m./kg dry weight soil. Colonies were grown in the dark at  $20 \pm 2$  °C. Three replicate dishes were prepared per test concentration. Percent inhibition of growth was determined after 11 days. No significant differences in growth between treated and control samples were detected. The effects were less than 25 %. Therefore, the NOEC was  $\geq$  30.0 mg p.m./kg dry weight soil and the EC₅₀ was  $\geq$  30.0 mg p.m./kg dry weight soil.

## 🎾 I. MATERIALS AND METHODS 🖉

Test item M-01 (AE C69371), chemical name: 2,6 dichlorobenzamide; batch/FL no.:8808018; content of a.s. analysed: 97:0 %; development code of a.s.: AE C653711 00 1B97 0001; development no.: 3000325964.

Test design: Suspensions of spores from *Penicillium jantfunellum* cultures were used to inoculate the centers of peth dishes containing nutrient medium prepared by mixing malt agar with sterile, AE C653711-treated, silty safe soil 6.6 % org. C, SH 5.3

The soil, which served as a capier for the test substance, and a source of micro-nutrients for the fungus, was treated with the test item to receive concentrations of 0.3, 1.5, 3.0, 7.5, 15.0 and 30.0 mg p.m./kg dry weight soil. Controls contained vegetable piece agar and untreated, sterile soil. Colonies were grown in the dark at  $20 \pm 2$  °C. Three replicate dishes were prepared per test concentration. Percent inhibition of growth was determined after 11 days by comparison of mean diameters (+ std. deviation) of treated colonies to mean diameters of the untreated controls.



#### Effect of M-01 (AE C653711) on growth of pure cultures of *Penicillium janthinellum* on nutrient medium

Concentration tested (mg a.s./kg dry wt Soil)	Mean colony diameters + standard deviation (cm) from 3 replicates	Inhibition of growth in % of untreated control		
0	$6.3 \pm 0.2$			
0.3	6.0±0.1			
1.5	5.9 ± 0.6 V			
3.0	$5.8\pm0.6$			
7.5	6,200.4			
15.0	(5.7 ± 0,5° ,5 ×			
30.0		5		

No significant differences in growth between treated and control samples were detected. The effects /log dry weight soil and the EC 50 was > were less than 25 %. Therefore, the OOEC was  $\ge 30.0$  mg/p.m 30.0 mg p.m./kg dry weight soil.

#### Validity criteria:

Internationally agreed and accepted guidelings for determining the influence of test items on pure cultures of soil fungi could not be found in the literature. Therefore, validity criteria were not defined.

The endpoints are NOEC  $\geq$  3000 mg p.m./kg dry weight soil kg, drv weight soll  $EC_{50} >$ **30.0** mg p.m./

The study is considered repable flowever, as this study is not a data requirement it will not be

However, as this assessment.



Data Point:	KCA 8.7/12
Report Author:	
Report Year:	2003
Report Title:	AE C653711 (AE C653711 00 1B97 0001): Determination of effects on growth of pure culture of a soil fungus, Suillus granulatus (Basidiomycetes), on nutrient medium
Report No:	LKC-SF-06/03
Document No:	<u>M-218466-01-1</u>
Guideline(s) followed in	
study:	
Deviations from current test guideline:	Not applicable, as no agreed guideline is available
Previous evaluation:	yes, evaluated and accepted DAR (2005)
GLP/Officially	Yes, conducted under GRP/Officially recognised testing facilities
recognised testing	
facilities:	
Acceptability/Reliability:	Yes A & Q Q A O Q A

The study investigated the influence of the test item M-01 (AF C65-711, 26-dichorobenzamide) on growth of pure cultures of a soil fungus, *Suillus granufatus* (Basidionycetes), on malt agar mixed with sterile, silty sand soil. The soil, which served as a carrier for the test substance, and a source of micronutrients for the fungus, was treated with the test item to receive concentrations of 0.3, 1.5, 3.0, 7.5, 15.0 and 30.0 mg p.m./kg dry, weight soil. Controls contained malt agar and untreated sterile soil. Colonies were grown in the dark at 20  $\pm$ 2 °C. Three replicate dishes were prepared per lest concentration. The percent of inhibition was determined after 35 days. No significant differences in growth between treated and control samples were detected. The effects were less than 25 %. Therefore, the NOEC was  $\geq$  30.0 mg p.m./kg dry weight soil and the EC5 was > 30.0 mg p.m./kg dry weight soil.

👳 I. MATERIALS AND METHODS 🖉

Test item M-01 (AE C69371), chemical name: 2,6 dichlorobenzamide; batch/FL no.:8808018; content of a.s. analysed: 97:0 %; development code of a.s.: AE C653711 00 1B97 0001; development no.: 3000325964.

Test design: Small pieces of agar (4 mm in diameter) containing mycelium from an actively growing *Suillus granuletus* currure were used to inoculate the centers of petri dishes containing nutrient medium prepared by mixing malt grar with sterile, AE (65371)² treated, silty sand soil (0.6 % org. C, pH 5.3).

The soil which served as a capier for the test substance, and a source of micro-nutrients for the fungus, was treated with the test item to receive concentrations of 0.3, 1.5, 3.0, 7.5, 15.0 and 30.0 mg p.m./kg dry weight soil. Controls contained map agar and untreated, sterile soil. Colonies were grown in the dark at  $20 \pm 2$  °C. Three replicate dishes were prepared per test concentration. Percent inhibition of growth was determined after 35 days by comparison of mean diameters (+ std. deviation) of treated colonies to mean diameters of the untreated controls.



Concentration tested (mg p.m./kg dry wt soil)	Mean colony diameters + standard deviation (cm) from 3 replicates	Inhibition of growth in % of untreated control
0	2.6 ± 0.1°	
0.3	2.5 ± 0.2	
1.5	$2.5 \pm 0.2$	
3.0	2.5 ± 0.0	$4\hat{q}^{\circ}$ , $\hat{Q}^{\circ}$ , $\hat{O}^{\circ}$ , $\hat{q}^{\circ}$
7.5	2.6 ± 0.1 ( ) ( ) ( ) ( ) ( ) ( ) ( ) ( ) ( ) (	
15.0	$2.6 \pm 0.0$ $^{\circ}$ $^{\circ}$ $^{\circ}$ $^{\circ}$ $^{\circ}$ $^{\circ}$	0 8 8 4 4
30.0		

No significant differences in growth between treated and control samples were detected. The effects veight soil and the EC50 was > were less than 25 %. Therefore, the NOEC was  $\geq$  30. (Ong p m)/kg dov 30.0 mg p.m./kg dry weight soi

Validity criteria: Internationally agreed and accepted guidelines for determining the influence of test items on pure cultures of soil fungicould not be bund in the literature. Therefore, validity criteria were not defined.

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The endpoints are:  
NOEC 
$$\gtrsim$$
 90.0 mg p.m. kg dry weight soil  
EC₅₀ > 30.0 mg p.m. kg dry weight soil

#### Assessment conclusion by applicant:

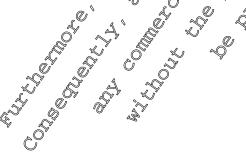
R. The study is considered reliable. However, as this study is not a data requirement it will not be further considered in the risk assessment.

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Data Point:	KCA 8.7/13
Report Author:	
Report Year:	2003
Report Title:	AE C638206 SC 480: Effects on soil litter degradation
Report No:	C038792
Document No:	<u>M-225764-01-1</u>
Guideline(s) followed in	
study:	
Deviations from current	Method: none
test guideline:	Study: Current Guideline: OECD Guidance Document No 56 (2006)
	No deviations
Previous evaluation:	yes, evaluated and accepted $\mathcal{A}$ $\mathcal{O}^{\vee}$ $\mathcal{A}^{\vee}$ $\mathcal{O}^{\vee}$
	in DAR (2005)
GLP/Officially	Yes, conducted under GLP Officially recognised resting facilities
recognised testing	
facilities:	
Acceptability/Reliability:	Yes O a a a
Executive Summary	
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The study investigated the influence of the test item Fluopical de SC480 on soil latter degradation. Therefore, six plots in the field An Hohenseh 410 of Bayer, Experimental Farm Hofchen Burscheid, Germany were treated at 24th of March at an application rate of 196.8 g test iter ha corresponding to 42.61 g a.s./ha. Six plots served as untreated control plots. All plots measured  $9 \times 10^{10}$  m = 81 m². By careful harrowing the test item was incorporated into the upper 10 cm soil layer to achieve a plateau concentration of 28.4 µg a 9/kg son dry weight in 0-10 cm soil depth. On the same day untreated summer barley, variety 'Scarlett', was sown onto all flots. On the F of April 2003, 40 fter bags (12 cm × 22 cm, mesh size 8 mm, filled with 4 g of dry straw each were bured per plot. In igation with 10 mm of water was performed on the 9th of April 2003. Soil samples were taken on the 25th of March 2003 one day after application and incorporation of the plateau concentration. The application of the rate representing the plateau concentration of fluopicolide resulted in soil residues of 185.5  $\mu$ g fluopicolide/kg/dry soft, which is 114.7% of the nominal amount of 28 & µg/kg dry soil. The degradation of the straw was determined for the time periods of  $0^{2}$  29, 0 - 92 and 0 - 184 days by recording the Objective: This study was designed to evaluate the onfluence of Fluopicolide SC 480 on soil litter degradation. weight of undegraded straw. The results of this study show that at no sampling time (29, 92 and 184 days after introduction of litter-bags into the soil) a statistically significant difference in proportion of straw degradation could be observed between untreated control plots and the plots treated with Fluopicolide SC 430. From the results of this study it can be concluded that residues of Fluopicolide SC 480 in soil even after Dong form use (plateau concentration) have no influence on organic matter





#### I. MATERIAL AND METHODS

Test item: The study was conducted using Fluopicolide SC 480, Batch-No.: OP220233, development No.: 3000315241, TADS-No.: TADS14510, density: 1.216 g/cm³, content of fluopicolide: 485 g/L, purity of fluopicolide: 99.3%. Six plots in the field Am Hohenseh 410 of Bayer Experimental Farm Höfchen Burscheid, Germany were treated at 24th of March at an application rate of 106.8 g test item/ha corresponding to 42.64 g a.s. that Six plots served as untreated control plots. All plots measured  $9 \times 9$  m = 81 m². By careful harrowing the test item was incorporated into the upper 10 cm soil layer to achieve a strateau concervation of 28,4% µg a.s./kg soil dry weight in 0-10 cm soil depth. On the same day untreated summer barley, variety 'Scarlett', was sown onto all plots. The seed rate was 160 kg/ha. On the 7th of April 2003, 40 litter bags (12 cm × 22 cm, mesh size 8 mm) filled with 4 g of dry straw each were buried per plot. In gation with 10 mm of water was performed on the 9th of April 2003. Soil samples were taken on the 25th of March 2003 one day after application and incorporation of the plateau concentration? The application of the rate representing the plateau concentration of fluopicolide resulted in soil residues of 1855 µg fluopicolide/kg dry soil, which is 114.7% of the nominal amount of 28.4 µg/kg dry soil. The degradation of the straw was determined for the time periods  $\partial t^0 - 29, 0 - 92$  and 0 - 184 days by recording the weight of undegraded straw. Day 0 was set on April 07, 2003 when little bags had been buyed. Calculating the difference of the weight of straw at the start of the experiment and the remaining weight at sampling time allowed determination of the degree of degradation. Data were statistically analysed by Student-t-Test (two sided,  $\alpha = 0.05$ ). 

#### October 08, 2008 Dates of experimental work, March 24, 2003 -

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$\sim$	4 H	RFS			DISCOM	SSION	
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		Ô	$\bigcirc$		<u> </u>	×.,	
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Validity criteria:

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Validity Criter Document No 🤅	ia accordi	ng to QECI) Guidanc	e S	Obgrined
Document No c	10 (2000)	.4 &		Y`	
Degradation of s	straw after	6 months 2	60% in coi	ntogi	Yes
Coefficients of va	ariation \leq 4	40% for the	data gener	uted within	¹ O YOES
the first & month	ns for soil 1	itter degrada	tion of the	control	
- Ky	. U			<u> </u>	

Means of 6 plots Control Fluopscolide SC 480	% of Control
0-29 d* @ 5 5 5 5 5 5	
g straw degraded	
g straw degraded 0.77 0.78 0.78 0.78 0.78 0.78 0.78 0.78	100.7
0-92 d*	
g straw degraded	
% straw degraded 9 straw degra	94.5
0-184 d*	
g strave degraded 302 3.68	
% straw degraded \$ 93.03 91.92	98.9

*dag 0 was soon April 07, 2003 when litter bags had been buried



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The results of this study show that at no sampling time (29, 92 and 184 days after introduction of litterbags into the soil) a statistically significant difference in proportion of straw degradation could be observed between untreated control plots and the plots treated with Fluopicolide SC 480.

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

III. CONCLUSION

From the results of this study it can be concluded that residues of Fluopicolide SC long-term use (plateau concentration) have no influence on organic matter breakdown after 1 Ŕ months. Q,

Assessment and conclusion by applicant

The study is considered reliable. However, as linerbag@tudies@re not required anymore, this study is not further considered in the risk assessment.

Data Point:	
Report Author:	
Report Year:	
Report Title:	AE C638206-BAM (AEC653719): Effects on Soil litter degradation
Report No:	xC039658
Document No:	<u>M-257095-65-1</u> O S S S S
Guideline(s) followed	
study:	
Deviations from coverent	Method: none Study: Current Guideline: OECD Guidance Document No 56 (2006)
test guideline:	Study: Current Guideline: OECD Guidance Document No 56 (2006)
Or Ay	
Flevious evaluation.	yes, evaluated and accepted of
	in DAR (2005)
GLP/Officially	Yeoconducted under GLDOfficially recognised testing facilities
recognised testing	
facilities:	
Acceptability/Reliability	Yes of the former of the forme
	Yes on the string facilities

Executive Summary

The study investigated the influence of the test-term M-01 (AE C653711) on soil litter degradation. Therefore, six plots in the field winhohenseh 410 of Bayer Experimental Farm Höfchen Burscheid, Germany were treated at 4th of March at an application rate of 18.71 g test item/ha corresponding to 18.15 g p.m./ha/Six plots served as untreated control plots. All plots measured 9×9 m = 81 m². By careful harrowing the test item was incorporated into the upper 10 cm soil layer to achieve a plateau concentration of 121 µg as./kg soil dry weight in 0-10 cm soil depth. On the same day untreated summer barley, variety Scarlett, was sown onto all plots. On the 7th of April 2003, 40 litter bags (12 cm × 22 cm, mash size mm) filled with 4 g of dry straw each were buried per plot. Irrigation with 10 mm of water was performed on the 9th of April 2003. Soil samples were taken on the 25th of March 2003 one davafter application and incorporation of the plateau concentration. The application of the rate representing the plateau concentration of M-01 (AE C653711) resulted in soil residues of 9.73 µg fluopicolide/kg dry soil, which is 80.4% of the nominal amount of 12.1 µg/kg dry soil. The degradation of the straw was determined for the time periods of 0 - 29, 0 - 92 and 0 - 184 days by recording the weight of undegraded straw. The results of this study show that at no sampling time (29, 92 and 184

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days after introduction of litter-bags into the soil) a statistically significant difference in proportion of straw degradation could be observed between untreated control plots and the plots treated with M-01 (AE C653711). From the results of this study it can be concluded that residues of M-01 (AE C653711) in soil even after long-term use (plateau concentration) have no influence on organic matter break fown after 1, 3 and 6 months.

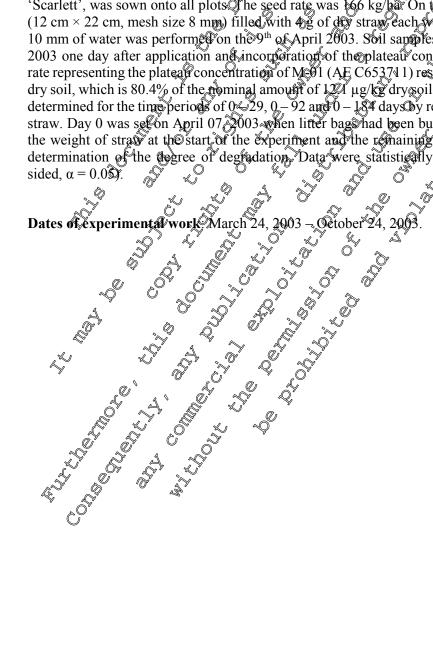
Objective:

10 on soil litter degradation. This study was designed to evaluate the influence of M-0 CAE C6537

I. MATERIAL AND METHOD

Test item: The study was conducted using M-01 (AE C653711) Batch No.: 8808018, development No.: 3000325964, purity: 97.0%.

Six plots in the field Amhohenseh 410 of Bayer Experimental Farth Höften Burscheid Gernany were treated at 24th of March at an application rate of 18.71 gyest item ha corresponding to 18.15 g p.m/ha. Six plots served as untreated control plots. All plots measured $9 \times 9m = 84 \text{ m}^2$. By careful hardwing the test item was incorporated into the opper 40 cm soil layer to achieve a plateat conceptration of 12.1 µg a.s./kg soil dry weight in 0-10 em soil depth. On the same day untreated summer barley, variety 'Scarlett', was sown onto all plots the seed rate was 166 kg/ba. On the 7th of April 2003, 40 litter bags (12 cm × 22 cm, mesh size 8 mm) filled with 4 g of dty straw each were boried per plot, Irrigation with 10 mm of water was performed on the 9th of April 2003. Soil samples were taken on the 25th of March, 2003 one day after application and incorporation of the plateau concentration. The application of the rate representing the platear concentration of MeOI (AE C653741) resulted in soil residues of 9.73 µg/kg dry soil, which is 80.4% of the minal amount of 1221 µg/kg dry soil. The degradation of the straw was determined for the time periods of $0 \le 29$, 0 - 92 and 0 - 184 days by recording the weight of undegraded straw. Day 0 was set on April 07 2003 when litter bags had been buried. Calculating the difference of the weight of straw at the start of the experiment and the remaining weight at sampling time allowed determination of the degree of degradation. Data were statistically analysed by Student-t-Test (two





Validity criteria:

alidity criteria:				
Validity Criteria according to OECD Guidance Document No 56 (2006)		Obtained		
Degradation of straw as	fter 6 months \geq 6	0% in control	Yes	
Coefficient of variation the first 6 months for se			Yes	
		(
Means of 6 plots	Control	M-01	% of Control	
		(AE C653741)	Ŷ, o	
0-29 d*		Q.		
g straw degraded	0.77	0.78		
% straw degraded	19.34	19258 0 0		
0-92 d*				
g straw degraded	2.17	2.06	95:0 ⁰ 2 ⁵ 0 ² 2 ⁵	
% straw degraded	54.14	51.43	95 N ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	
0-184 d*		S & S		
g straw degraded	3.92 %	387 4		
% straw degraded	°93.03 [°]	92.81 S	99.8 5	
lay 0 was set on April 07,	2003 when litter ba	gs had been buried		S OF
Ş		0, 5, 5		L'Y

The results of this study show that it no sampling time 29, 92 and 184 days after introduction of litterbags into the soit a statistically significant difference in proportion of straw degradation could be observed between untreated control plots and the plots treated with M-01 (AE C653711).

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

From the results of this stroy it can be concluded that residues of M-01 (AE C653711) in soil even after long-term the (plateau concentration) have no influence on organic matter breakdown after 1, 3 and 6 months.

Assessment and conclusion by applicant

The study is considered rehable. However, as litterbag studies are not required anymore, this study is not further considered in the risk assessment.

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CA 8.8	Effects on biological methods for sewage treatment
011 010	

KCA 8.8/01
Activated Sludge, Respiration Inhibition Test of AE C638206 Technical
B003666
<u>M-240681-01-1</u>
OECD 209 (1993)
Current Guideline: OECD 209 (2010) The test was conducted as a range-finder only with less reploates per
The test was conducted as a range-finder only with less replicates per concentrations but more concentrations of the test substance. Nitritication respiration was not checked in the test. However, there was no need to
respiration was not checked in the test. However there was no need to
differenciate heterotrophic and nitrification respiration in this est since there was
no significant inhibition of total respiration. The duration of the test was 30
minutes, whereas the new guideline recommends adjuration of 3 bours. The
suspended solids concentration was 3.8 g/L instead of 15 g/L in the new version
of the guideline. These deviations are not considered to invalidate the conclusion
of the test since the validity criteria are all met. The combination of the higher concentration of a crivitated studge and shorter test ouration produced a control
concentration of activated Sudge and shorter test ouration produced a control
oxygen up take high enough to reveal an inhibition by the test item if there were
any. A g g g g g g g g g g g g g g g g g g
yes, evaluated and accepted of the second se
in DAR (2005)
Xes, conducted under QUP/Officially recognised testing facilities
Yes a strong the second s
KCA\$8.8/02 ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~
2018
Statement - Certificate of analysis for fluopicatide study on activated sludge
respiration mibition (Heine 2001, M-240681-01-1)
M-634699-01-1 ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~
$\frac{M-634699-013}{2}$
Not applicable y y
No pet prevenue and the
No, pet prevously admitted
And ann Marshle Q - X
My634699-01-1
No off previously submitted and applycable Very Very Very Very Very Very Very Very



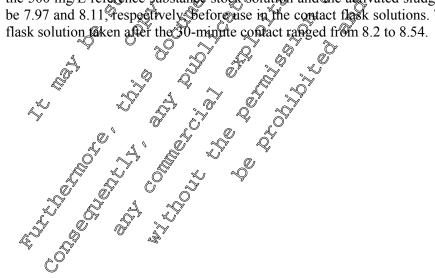
The objective of this study was to evaluate fluopicolide technical for its potential inhibitory effects on microbial respiration in an activated sludge suspension. The respiration rate of an activated sludge inoculum in a synthetic sewage suspension, after aerating for 30 minutes in the presence of fluopicolide technical, was compared to the respiration rate of an activated sludge inoculum in a synthetic sewage suspension to which no test substance was added. A range-finding test was conducted at fluopicolide technical concentrations of 1.4, 3.3, 6.4, 12.0, and 25.4 mg a.s./L in order to provide a range of treatments. A reference substance 3,5-dichlorophenol, a known microbial inhibitor was tested to verify normal sensitivity of the microbial population. The two control flasks containing an activated slugge and synthetic feed mixture with no fluopicolide technical exhibited respiration rates within 19% of each other. Exposure of the activated sludge and synthetic feed mixture to the reference substance resulted in percent inhibition values of 22.8, 57.2, and 85.9% for the 3.2, 10, and 32 mg/L treatments, respectivel The corresponding EC₅₀ is 8.1 mg/L. The respiration rate of the abiomy control, 120^7 mg/Q₂ / L/3 h indicated that oxygen uptake by fluopicolide technical was 10% of the mean respiration rate of the controls, 78.4 mg O₂ / L × h. Inhibition of the respiration rate was 4.7, 0, 3, 31, 5, 11.2, and 13.2% for the 1.4, 3.3, 6.4, 12.0, 25.4 mg a.s./L treatments, respectively. Because respiration rate reduction was < 50% in all fluopicolide technical treatments, an EC 50 value could not be calculated and was estimated to be > 25.4 mg/L, the highest concentration rested. A definitive rest was not conducted since the inhibition at the highest concentration dested was < 50%.

QI. MATERIAL AND METHODS:

Test item: Fluopicolide technical, Batch code: Ar C698206 001090005, Batch No: 2050190/PP241024/2; purity 97.1% w/w.

The activated sludge was exposed to fluopicolide technical a different concentrations (1.4, 3.3, 6.4, 12.0 and 25.4 mg a.s./L) is a range-finding test. Two control flasks containing an activated sludge and synthetic feed mixture with no substance added were also tested. An abiotic control was dosed with fluopicolide at 25.2 mg a s/L during the test and was used to measure chemical oxygen uptake. No inoculum was added to this blask. A reference substance 3,5 dichlorophenol, a known microbial inhibitor, was tested to verify normal sensitivity of the microbial population. Reference substance concentrations of 3.2, 10 and 32 mg/L were tested. The respiration rate of each mixture was determined after a 30 minute contact period with permanent aeration.

The temperature of the environmental chamber ranged from 19.2 to 19.8°C during the test. The pH of the 500 mg/L reference substance stock solution and the activated sludge inoculum were measured to be 7.97 and 8.11, respectively, before use in the contact flask solutions. The pH values for the contact flask solution taken after the 30-minute contact ranged from 8.2 to 8.54.





Validity Criteria

The test was performed according to the OECD guideline 209 published in 1984, the corresponding validity criteria are met. The validity criteria of the updated guideline (2010) are the following:

Validity criteria (OECD 209, 2010)	Required	Observed S
Oxygen uptake in blank controls	$\geq 20 \text{ mg O}_2 / \text{g suspended}$	$178.4 \text{ mg O}_2/16\text{h}$ at 38 g suspended solid/L so 20.6 mg O ₂ /g suspended solid/L 1000
Coefficient of variation of oxygen	á Ő ^v	
uptake in the blank control replicates	∠ € 30%	Within a 15% range
at the end of the definitive test		
EC ₅₀ for 3.5-DCP for total respiration	within 2 to 25 mg/L	& mg/L ~~~~

Analytical Findings:

The test item and reference compound concentrations were based on nominal concentrations. bo analytical methods they 'n,

Biological Findings:

Percent inhibition values for the test substance treatments in the range-finding test ranged from 0.3 to 31.5% and showed no dependence through the range of concentrations tested (1.5 @ 25 mg a.s./L). Based on these results, an EC₅₀ value for thopicalide could not be determined and addefinitive test was not performed. The respiration rate of the abjotic coptrol, $12.7 \text{ mg/O}_2 / 12^{\times} h$, indicated that oxygen uptake by fluopicolide was 16% of the mean respiration rate of the controls, 78.4 mg $O_2 / L \times h$. The EC₅₀ for 3.5-DCR is 8.1 mg/L

Since fluopicolide sid not produce significant inhibition of oxygen respiration in the range-finding test, no definitive testowas performed. Ô

Respiration rates and percent inhibition values for thiopic dide and 3,5-Dichlorophenol

Treatment [mg a.s./L]	Respiration rate	% Inhibition
	$[\mathbf{mgO}_2 / \mathbf{L} \otimes \mathbf{h}]$	
Control 5 5 4	× 80.9 ×	-
Control 2	\$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$	-
Control, mean	§ 78.4	-
1.4 mg a.s./Dest item	66×8	14.7
3.3 mg a L test item	ح _ ∞78.1	0.3
6.4 mg a.s./L test item	<u>م</u> م 53.7	31.5
12/0 mg a.s./L test item	69.6	11.2
25.4 mg a.s./L test item	68.0	13.2
25.2 mg a.s. D test item (abionic control)	12.7	-
3.2 mg/L terence item	60.5	22.8
10 mg Freference item	33.6	57.2
32 mg/L reference mem	11.0	85.9
<u> </u>		

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III. CONCLUSION:

Under the conditions of the test, the EC_{50} of fluopicolide is clearly in excess of 25.4 mg a.s./L, the highest tested concentration due to solubility limit.

the solution of the solution o Assessment and conclusion by applicant: ð This test was conducted as a range-finder with concentrations up to the solution with concentrations up to the solution of the latest OECD suideline are mot. The result of the solution of the latest OECD suideline are mot. A DE LA DE L criteria of the latest OECD guideline are met. The results show that fluopicolide does not