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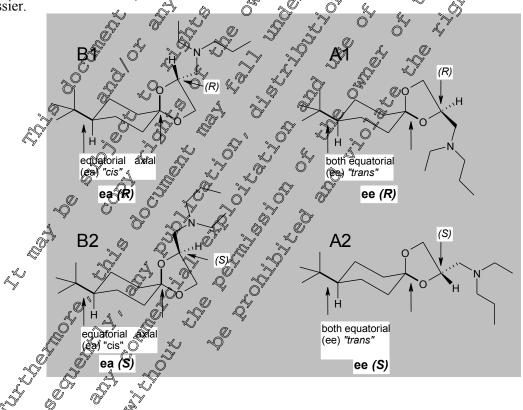


CA 8 ECOTOXICOLOGICAL STUDIES ON THE ACTIVE SUBSTANCE

Spiroxamine was included in Annex I to Council Directive 91/414/EEC in 1999 (Directive 1999/77/EC, Entry into Force on 1 September 1999). This Supplementary Dossier contains data which were not submitted at the time of the Annex I inclusion and first renewal of spiroxamine under Council Directive 91/414/EEC and which were therefore not evaluated during the first EU review and renewal. However, all studies submitted for the first approval and subsequent first renewal of spiroxamine have also been summarised according to current guidance and included in the dossier. Where studies meet relevant validity criteria, new robust study summaries have been provided in the appropriate dossier section. However, where studies do not meet relevant validity criteria and are not considered acceptable, less detailed summaries may have been provided alongside discussions of study-deficiencies. All relied upon study reports are submitted in Document K for this second reneval of approval dossier or in Document K for the previous Annex I inclusion and first renewal submissions.

All data which were already submitted by Bayer, AG (former Bayer GopScience) for the Annex I inclusion and first renewal under Council Directive 90/414/BEC are contained in the draft Be-Assessment Report (RAR) 2010 and its revised RAR 2017, and are included in the Baseline Dessier provided by Bayer AG.

Spiroxamine consists of four isomers (two diastereomers each with its corresponding two enantiomers which are in a 1:1 ratio) as shown in the schematic below. The isomer nomenclature presented in some historical documentation may differ with respect to the A/B and corresponding trans/eis notation as a result of a discrepancy in referencing, which is discussed in detail in position paper M-01468-01-1 (see CA 1.7/01). It is recommended that the stereo assignments depicted here, together with the A and B notation should be used exclusively going forward to ensure continuity of information throughout the dossier.



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



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CA 8.1 Effects on birds and other terrestrial vertebrates

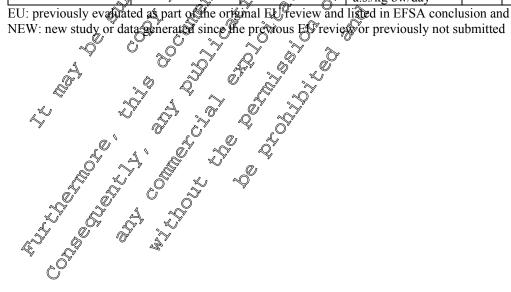
CA 8.1.1 **Effects on Birds**

The avian studies conducted with spiroxamine technical are summarised in the following table

Table CA 8.1.1/01	Summary of avian toxicity studies with spiroxamine	
1 abic CA 0.1.1/01	Summary of avian toxicity studies with spiroxamine	

1 abic CA 0.1.1/01	Summary of avia	ii toxicity studies w		
Organism	Test item	Test type	Endpoints	Reference 👋
Bobwhite quail (Colinus virginianus)	Spiroxamine	toxicity	LD ₅₀ 565 mg	EU <u>M-003095-05-1</u>
Canary (Serinus canarius)	Spiroxamine	Acute oral A toxicity		£U <u>\$1-008100-01-</u> £
Bobwhite quail (Colinus virginianus)	Spiroxamine	Short form dietary toxicity	LC 5000 mg a S kg diet LDD 3357 mg a.s./kg w/day)	HONSON -02-1
Mallard duck (Anas platyrhynchos)	Spiroxamine	Short-term	a.s./kg/uw/uay)	₩
Mallard duck (Anas platyrhynchos)	Spiroxonine	Short-term of dietary toxicity		£€ £€ 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2
Bobwhite quail (Colinus virginianus)	Spiroxamiline	Reproduênve 5	NOEC 293 mg S./kgaliet NOEL 2.02 mg a.s./kg bw/day NOAEC 38.6 mg a.s./kg dfet NOAEL 5.40 mg a.s./kg bw/day	EU <u>M-007470-03-1</u>
Mallard duck Afnas platyrhynchos)	Spirosamine	Reproductive test	NOEC 7808 mg a.s./kg dret NOFA 10.6 mg a.s./kg bw/day	EU <u>M-008186-01-1</u>

EU: previously evaluated as part of the original EU review and listed in EFSA conclusion and DAR





Data Point:	KCA 8.1.1.1/01
Report Author:	
Report Year:	1998 KWG 4168 (technical grade): A cute oral toxicity to holywhite guail
Report Title:	KWG 4168 (technical grade): Acute oral toxicity to bob white quail
Report No:	VB-028
Document No:	<u>M-008095-02-1</u>
Guideline(s) followed in	U.S. EPA E 71-1 (1982)
study:	<u> </u>
Deviations from current	None $\int_{\mathcal{A}} \int_{\mathcal{A}} \int_{$
test guideline:	$\Delta^{(0)} \qquad \Delta^{(0)} \qquad \Delta^{($
Previous evaluation:	yes, evaluated and accepter DAR (1997), RAR (2017) RAR (2017)
GLP/Officially	Yes, conducted under GLP/Officially recognised testing factifies
recognised testing	
facilities:	
Acceptability/Reliability:	Yes V V V V V

CA 8.1.1.1 Acute oral toxicity to birds

Executive Summary

Adult bobwhite quail (*Colinus virginianus*) were used to evaluate the acute oral toxicity of KWG 4168 over a period of 14 days.

Groups of ten birds, five per sex, were given a single oral dose of 125, 250, 500, 1000 oc 2000 mg a.s./kg body weight (b.w.) or an untreated control. Following exposure, all groups were held for a 14-day observation period.

No mortalities were observed on the control and 120 mg a S./kg bw. groups. One mortality occurred in the dose level of 250 mg a.s./kg b.w. five bours after dosing and one bird showed apathy for two days. At the 500 mg a.s./kg b.w. dose rate, three birds died within two days and four others showed signs of intoxication however recovered within four days. Of the ten birds dosed with 1000 mg a.s./kg b.w., all exhibited signs of toxicity, with nine dying within two days. Half of the birds of the highest dose group, 2000 mg a.s./kg b.w. died within one hour of application of the test substance, with the remaining birds dying in the following two days.

Marked ofinical signs of toxicity were apathy and loss of equilibrium. There were no visible sex-related differences in toxicity. Observations in post-modern examination of the birds dosed with 500, 1000 or 2000 mg a.s./kg by. showed not compound-related lesions.

Based on the results of this study, the LO₅₀ was determined to be 565 mg a.s./kg b.w. with 95% confidence intervals of 41300 773 mg a 5/kg b.w. The NOED and LOED were 125 and 250 mg a.s./kg b.w., respectively.

I. Materials and Methods A. Materials Test Material Lot/Batch #: 898144002 Purify: 958% Description: Clear liquid Stability of test Not reported compound: Reanalysis/Expiry 27 January 1994 date:



Density:	Not reported
Treatments	Le contraction de la contracti
Test rates:	125, 250, 500, 1000 or 2000 mg a s /kg body weight
	125, 250, 500, 1000 of 2000 ing a.s./kg body weight
Solvent/vehicle:	None of the second seco
Analysis of test	No (dose enclosed within capsule)
concentrations:	
Test organisms	
Species:	Bobwhite quail (Coling virginianus) aged 19 week
Source:	
Acclimatisation period:	14-day acclimation period & & &
period:	
Feeding:	Laying her ration available ad loitum except for an 18-hour fasting
Test design	Not reported 125, 250, 500, 1000 or 2000 mg a.s./kg body weight None No (dose enclosed within capsule) Bobwhite quail (<i>Colime virginianus</i>) aged 19 weeks 14-day acclimation available <i>ad (bitum</i> , except for an 48-hour fasting period pror to dosing Stainfess steel wife cage (18 x 23 x 10 cm)
Test vessel:	Stainless steel wire cages (18 x 3 x 16 cm)
Replication:	Trive female and five male bilds permeatment
No. of	Individually housed
animals/vessel: 📎	
Duration of tests	Dr4 days
Environmental test	14-day acclimation period Laying hep ration available <i>ad (bitum</i> , except for an 48-hour fasting) period pror to dosing Stainless steel wire cages (18 x 23 x 10 cm) Five female and five male binds permeatment Individually housed $20 \pm 2^{\circ}C$ $30^{\circ}-90\%$ Natural day length
Temperature:	$20 \pm 2C$ 4 5 6 6 6
Relative humidityz	
Photoperiod:	Alatural day length O
B. Study Design	
This study was conducted	to assess the active or toxicity of KWG 4168 on adult bobwhite quail

This study was conducted to assess the acute or toxicity of KWG 4168 on adult bobwhite quail (*Colinus virganianus*). Dose levels for this study were selected based upon the results of a previous range-finding test.

Five mate and five female birds were randomly assigned a treatment or control group, and housed individually in 18 x 23 x 13 cm stainless steel were cages. Cages followed a natural photoperiod and were maintained at $20 \pm 20^{\circ}$ with a relative hubidity of 30 to 90%.

After a 14-day acclimation period, food was withheld from all groups for approximately 18 hours prior to dose administration. The test substance was administered by oral application of one gelatine capsule per bird. Dose levels used in the study were 125, 250, 500, 1000 and 2000 mg a.s./kg body weight (b.w.) and an empty capsule-only control.

Observations for morality and toxicity were made continuously for the first hour, *ca.* hourly for the remainder of the first day and then daily on workdays for 14 days. Body weights were measured prior to test initiation (day -1), on study day 7 and at test termination. Feed consumption for each group was recorded on days 3, 7 and 14. At test termination, all surviving birds were sacrificed, and those in the 500, 1000 and 2000 mg a.s./kg b.w. dose groups were necropsied as well as all birds that died during the in-life phase of the study.



II. Results and Discussion

Validity criteria were not assessed as part of the study.

No mortalities were observed in the control and 125 mg a.s./kg b.w. groups.

Half of the birds of the highest dose (2000 mg a.s./kg b.w.) group died within one hour of application of the test substance, with the remaining birds dying in the following two days. Of the ten birds dysed with 1000 mg a.s./kg b.w., all exhibited signs of toxicity, with nine dying within two days

At the 500 mg a.s./kg b.w. dose rate, three birds died within two days and four others showed signs of intoxication however recovered within four days. One mortality occurred in the dose level of 250 mg a.s./kg b.w. five hours after dosing and one bird showed apathy for two days.

Marked clinical signs of toxicity were apathy and loss of equilibrium. There were no visible sex-related differences in toxicity. Observations in post-mortem examination of the birds dosed with \$00, 1000 or 2000 mg a.s./kg b.w. showed no compound-related lesions. Pale livers and pancreas were observed in some birds that had died during the study, however such findings were frequently observed in dead birds and not deemed to be treatment-related.

Table CA 8.1.1.1/01-1	Mortality and	signs of	f toxicity of	birds after	acute	oral expo	gure	to KWG 4	1068
-----------------------	---------------	----------	---------------	-------------	-------	-----------	------	-----------------	------

Dose	Mortality (%			Signs of oxic	ity (%) ¹ 5	
(mg a.s./kg b.w.)	Males	Females 0	Total	Males	Females	Total
Control	0	8 28	0	0,0,0,0,0	0	0
125	0 0					0
250		2000			400	20
500	de c			60 0	867	70
1000	80 0 ×	100 🖤 🔊	90 🔊 🐧	100	100	100
2000		100 60		100	100	100
		Ê	Y W	~		

Table (8.1.1.1/01-2 Body weights and body weight change of birds after acute oral exposure to KWG

Dose		Males			Females		
(mg a.s./kg h		Day -1 ~~~	Dav.7 %	Day 14	Day -1	Day 7	Day 14
Control	Mean SD	982.0 15.00	A84.8 Q 14.7 Z	186.0 75.3	173.0 8.6	174.4 12.3	175.2 <i>14.0</i>
125 4	Mean SD	180.6 26.2	1802 1800	179.6 <i>14.5</i>	182.8 19.5	186.0 23.7	191.0 26.2
250	Mean D		173.20 10.0	173.0 10.7	168.2 11.1	164.0 10.9	165.5 9.3
500	Mean	971.6 5 12.80	168.7 9.6	174.0 <i>13.1</i>	162.0 <i>18.7</i>	160.0 11.5	166.0 <i>13.4</i>
	Mean SD	.177.6 2.9	195.0 -	206.0	165.2 10.3	-	-
2000 کې	Mean SD	173.6 <i>10.1</i>	-	-	174.6 <i>8.5</i>	-	-



Dose (mg a.s./kg b.w.)	Mean daily feed consumption (g feed/bird/day)			Total daily fo (g feed/day)	on O S	
	Days 0-3	Days 3-7	Days 7-14	Days 0-3	Days 3-7	Days 2-14
Control	12.2	15.5	14.4	366	62	1010
125	16.8	19.1	16.4	503	(7765 🔊	9148 9 V
250	8.9	16.5	15.6	239	595	985 × 0
500	3.8	16.9	17.3	88 0	472 🖑	9857 0 C
1000	4.8	20.0	22.0	43 ^Q Q	° 80 2 4	154
2000	0.8	-		50 27		

T 11 CA 0 1 1 1/01 2	
1 able CA 8.1.1.1/01-3	Mean and total feed consumption during exposure and observation periods

III. Conclusion

G**A**168 Adult bobwhite quail (Colinus virginianus) were used to evaluate the acute of a toxicity of KW Å V over a period of 14 days. Ô

Groups of ten birds, five per sex, were given single oral dose of 125, 250, 500, 5000 of 2000 ang a.s./kg body weight (b.w.) or an untreated control. Following exposure, all groups were held, for a 14-day J. A. Ô observation period. 6

Based on the results of this study, the Lioso was determined to be 365 mg a.s./kgb.w. with 95% confidence intervals of 413 to 773 mg a.s. Ig b.w. The NOED and LOPD were 125 and 250 mg a.s./kg b.w., respectively. Ô

Assessment and conclusion by applicant

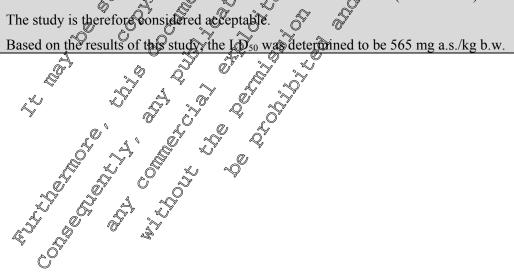
The study was considered to be consistent with the requirements of the current OECD 223 test guideline and is therefore considered acceptable.

 \bigcirc

The study was assessed against the current OECD test guideline OECD 223: Avian acute oral toxicity test", adopted 29 July 2016. toxicity test", adopted 29 July 2016.

Validity criteria according to OECD 223 were met:

Control portality to not exceed 10% of the end of the test (actual: 0.0%) C.S.





Data Point:	KCA 8.1.1.1/02
Report Author:	
Report Year:	1989
Report Title:	KWG 4168: Vogeltoxizitaet oral am Kanarienvogel (Serinus canarius), weblich, orientierend
Report No:	VK-335
Document No:	<u>M-008100-01-1</u>
Guideline(s) followed in study:	Not reported
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated, not accepted RAR (2010), RAR (2017) Due to poor test design and lacking reliable endpoints/the study cannot be used for risk assessment purposes. The acute oral 0.050 of spiroxamine in the canary (Serinus canarius) was estimated to be between 250 - 500 mg as/kg bw.
	Due to poor test design and lacking reliable endpoints the study cannot be used
	for risk assessment purposes. The acute oral D50 of spirox mine in the canary (Serinus canarius) was estimated to be between 250 - 500 mg as/ky bw. ^ ° No, not conducted under GLP/Otficially recognised testing facilities & @
GLP/Officially	No, not conducted under GLP/Officially recognised testing facilities
recognised testing facilities:	
Acceptability/Reliability:	Supportive Mily & S S S S S S

Executive Summary

test) on a non-standard bird species was conducted with A non-GLP screening study (acute oral toxicity female Serinus canarius. Ĉ

gavage dosed dietary concentrations of 25, 50, 100, Groups of five birds were exposed for seven days to 250, 500 and 1000 mg a.s./kg kw

The LD₅₀ was determined to be 250 500@mg

```
I.
     Materials and Methods
A.
     Materials
 Test Material
    Lot Batch #
    Purity:
    Description
                     Č.
    Stability of test
    compound:
                             reporte
    Readalysis/Expire
    date:
   Density:
                                ported
 Treatments
                         25, 50, 100, 250, 500 and 1000 mg a.s./kg bw
    Test rates:
                         Beionised water
    Solvent/vehicle:
     Analysic of test
                          Not reported
 Concentrations
 Test organisms
    Species:
                          Canary (Serinus canarius) - female
    Source:
                          Not reported
```



Acclimatisation period:	Not reported Q_{μ}°
periou	
Feeding:	Not reported
Test design	
Test vessel:	Not reported
Replication:	Not reported
No. of animals/vessel:	Not reported Not reported
Duration of test:	Not reported
Environmental test conditions	Not reported Not reported No
Mean temperature:	Not reported to the second sec
Relative humidity:	Not reported in the second sec
Photoperiod:	Not reported a star star star star star star star st
B. Study Design	
B. Study Design A non-GLP screening study	(acute oral toxicity test) on a non-standard bird species was conducted with
female Serinus canaries.	

Groups of five birds were exposed for seven days to gayage dosed dietary concentrations of 25, 50, 100, 250, 500 and 1000 mg a s./kg bw. Birds were starved for an hour prior to dosing dosing was conducted via a gavage.
II. Results and Discussion and the second starved for a formation of the second starved formation of the second starved for a formation of the second starved for a formation of the second starved formation of the second starv

Table CA 8.1. 1/02-1 Results summary	U
Applied amount of active	Remarks
Applied amount of active substance (mg a.s./kg by)	
	-
500 \$ 5/5 \$	-
	Vomit, cramps, lateral position
	Low cramps, apathy
	Certain cramps, apathy
	-

III. Conclusion

The LD₅₀ was determined to be 250-500 mg a.s./kg bw.

Assessment and conclusion by applicant:

This non-GLP screeping study on a non standard bird species was originally considered as non relevant. However, it supports the toxicity profile reported for Bobwhite quail and is therefore presented as supporting information only.

A control group does not appear to have been tested in this study therefore it is not possible to assess this against the OECD 223 test guideline criterion.

The LD₅₀ was determined to be 250 - 500 mg a.s./kg bw.



Data Point:	KCA 8.1.1.2/01
Report Author:	
Report Year:	1998
Report Title:	KWG 4168 (technical grade): 5-day dietary LC50 to bob white quail
Report No:	VB-025
Document No:	<u>M-008081-02-1</u>
Guideline(s) followed in	OECD 205 (1984)
study:	U.S. EPA E 71-2 (1982)
Deviations from current	None A O' A O O'
test guideline:	
Previous evaluation:	yes, evaluated and acceptor DAR (1997), RAR (2010), RAR (2017)
GLP/Officially	Yes, conducted under GLP/Officially recognised testing facilities
recognised testing	
facilities:	$A \mathcal{O} \mathcal{O} \mathcal{Q} A \mathcal{O} \mathcal{O} $
Acceptability/Reliability:	Yes $\sqrt{2}$ $\sqrt{2}$ $\sqrt{2}$ $\sqrt{2}$ $\sqrt{2}$ $\sqrt{2}$ $\sqrt{2}$ $\sqrt{2}$

CA 8.1.1.2	Short-term	dietarv	toxicity to	o birds
011 011111				

Executive Summary

Ø Young bobwhite quail (Colinus virginiants) were used to evaluate the dietary toxicity of KWG 4168 Î over a period of five days. L, C ð Ø1

Groups of ten birds were exposed for five days to dietary concentrations of 313, 626 1250, 2500 or 5000 mg a.s./kg feed or an untreated control. Following exposure, all proups were held for a three-day observation period on untreated feed.

Based on the results of this study the subacule dietary LC, of technical graded KWG 4168 to bobwhite quail was determined to be >5000 or a.s./kg feed requixalent to >357 or a.s./kg bw/day). The LOAEC and NOAEC were \$500 and 1250 mg a kg feed, respectively.

A decrease in body weight during the exposure period was noted in the group treated with 5000 mg a.s./kg diet, corresponding with a reduction in deed intake of the birds during the treatment phase, indicating the unpalatability of KWG 4268.

- Materials and Methods I.
- A. Materials

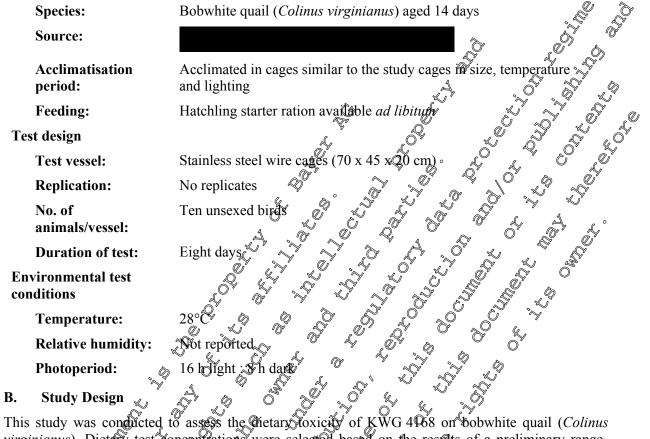
T

Test Material 🔊 🔺	Kew G 4168 2
Lot/Bate #: 2	
Purity:	
Description:	Clear liquid &
Stability of test	Stable under the conditions of this study (recoveries of 105 – 107%
compound:	after 24 hours)
Reanalysis/Expiry	911 August 1994
date:	
Density:	Bot reported
Treatments	S.
& Test rates:	313, 625, 1250, 2500 and 5000 mg a.s./kg
Solvent/vehicle:	40.0 g peanut oil
Analysis of test concentrations:	Yes, mean measured values of a.s. in diet $90.9 - 116\%$ of nominal



B.

Test organisms



virginianus). Dietary test concentrations were selected based on the results of a preliminary rangefinding study.

All birds were apparently healthy after arrival to the test facility, and were phenotypically similar to wild birds. At 14 days of age, ten birds of unknown sex weighing as to 41 g were randomly allocated to each of the treatment and control diets. Test vessels were stainless steel wire 70 x 45 x 20 cm cages which were maintained at a mean cage temperature of 28°C.

Test diets were prepared by mixing the bird ford, KWG 4168 and 40.0 g peanut oil in a glass beaker, then grinding the pre-max to homogeneity, and finally adding the required amount of feed to obtain the desired nominal concentrations. Nominal lest concentrations were 313, 625, 1250, 2500 and 5000 mg a.s./kg diet. Measured concentrations in the test diet ranged from 90.9 to 116% of nominal.

Birds were given relevant treated or control teed for five days, after which they received an untreated diet during a three-day observation period. Feed Consumption was recorded daily during the exposure period and at the end of the observation period. Food and fresh tap water were available ad libitum throughout the study.

Birds were weighed at test initiation, on day 5, and at test termination. Observations for mortality and clinical signs of intextication were recorded daily throughout the study. Necropsy examinations were conducted on all birds. Control birds and those receiving the 313, 625 or 1250 mg a.s./kg diet were not necropsied due to no abnormalities being observed.

Analytical method

Samples of feed were analysed using the validated analytical method VE-006, report reference M-00804 C-02-1 (see Doc MCA Section 4).



II. **Results and Discussion**

Table CA 8.1.1.2/01-1 Measured concentration of KWG 4168 in the animal feed

Validity criteria were not assessed in the study repo The measured concentrations of spiroxamine in the Table CA 8.1.1.2/01-1 Measured concentration of KV	test diets are summarised below.
Nominal dietary concentration (mg a.s./kg diet)	Measured concentration of nominal (86)
Control	- & 2 2 2
313	
625	
1250	
2500	98.4 Q Q Q A A
5000	90.9

Mortality of 10 and 20% occurred in the 2500 and 5000 mg w.s./kg thet test groups, respectively. It was unable to be judged whether these mortalities were due to toocity of to starvation because of the unpalatability of test diet, however clinical signs of toxicity were not observed

Post-mortem examinations of birds the survived or died during the study showed no gross lesions or macrosconic organ alterations macroscopic organ alterations.

Table CA 8.1.1.2/01-2 Cumulative mortality of birds after dietary exposure to K

				.0	÷		<u>).</u>		<u> </u>	
Nominal	Cumul	ative mo	rtality by	k day	°0 /	∀_¢Û	× Ø	, U	0 [′]	Total
dietary	0	1	ر م	3	4 🔊	5 🚿	6	. <i>¶</i> ?	è)8	(%)
concentration	°#	Q (-	~ \	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	>		
(mg a.s./kg	0	1			Ň	ŝ [,] u	° ≪			
diet)	Ž	<u>S</u>		-	\mathbb{Q}		K,	SN 1		
Control	00	0 2	. ×	~ 20		0	00.	0×	0	0.0
313		× 0	0	0	00	Ø		b0	0	0.0
625 Č		0 🞸	0	0		NO L	0	0	0	0.0
1250	0 0	0_{\odot}	Ø Ø	Ø 🕺	JŐ 🏷	0	0	0	0	0.0
2500	00	₩ _©	0	¥0 .~~	0 \$	1°	10	1	1	10.0
5000	0 ĸ	,0 🔊		10	1		02	2	2	20.0
3000				1 🥥		\sim		2	2	20.0

A decrease in body weight during the exposure period was observed in the 5000 mg a.s./kg diet group, which coincided with a reduction in the feed intake of the birds. This secondary effect on growth is therefore not assumed to be due to toxicity or a treatment-related adverse effect.

ŗ. 3-Body weights and borry weight change of birds after dietary exposure to KWG Table CA 8.1 4168 , Ôj Ŏ Ð.

Nominal [®] dietary	Bodý weight	(g) 🔍 🥫		Body weight	increase (g)	
dietary concentration (mg ^a .s./kg diet)	Day 0		Dav <mark>8</mark>	Days 0-5	Days 5-8	Days 0-8
Control	333.0	440	50.8	10.9	6.8	17.6
313	33.0	A3.4	51.3	8.3	8.0	16.3
625	\$33.4 ₽	39.4	46.5	6.1	7.0	13.1
1250	33.8	38.7	44.2	4.9	5.4	10.3
2500	34.9 5	36.7	41.0	1.8	4.4	6.2
5000 000	94.4 ×	32.6	38.0	-1.8	5.5	3.6

Ĉ



	periods				ذ 🛸
Nominal dietary concentration	Mean daily feed consumption (g feed/bird/day)			Mean a.s. intake	L'Y O'
(mg a.s./kg diet)	Days 1-5	Days 5-8	Days 0-8	mg a.s./bird/day	mg a.s./kg
Comtra 1	(1	()	(1		Dw/uay
Control	6.1	6.2	6.1	0.0	
313	6.0	6.9	6.3	1.9 🔊	48.4~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
625	4.8	5.5	5.1	3.0	8354 ~ 0
1250	4.4	5.0	4.6	5.5 Q	1652 .2 3 4
2500	3.8	5.7	4.4	9.4	262.7Q
5000	2.4	5.9	3.0		357(4 C

Table CA 8.1.1.2/01-4 Feed consumption and a.s. intake of KWG 4168 during exposure and observation

III. Conclusion

Young bobwhite quail (Colinus virginianus) were used to evaluate the distary toxicity of KWG 4168 over a period of five days. Ŧ

A decrease in body weight during the exposure period was noted in the group treated with 5000 mg a.s./kg diet, corresponding with a reduction in feed intake of the birds during the treatment phase, indicating the unpalatability of KW004168

Based on the results of this study the subacute dictary & 50 of rechnical graded KWG 4168 to bobwhite quail was determined to be >5000 mg &s./kg feed (eqpivalent to >357 mg as./kg.bw/day). The LOAEC and NOAEC were 2500 and \$250 mg a.s./kg/feed, respectively.

Assessment and conclusion by applicant:

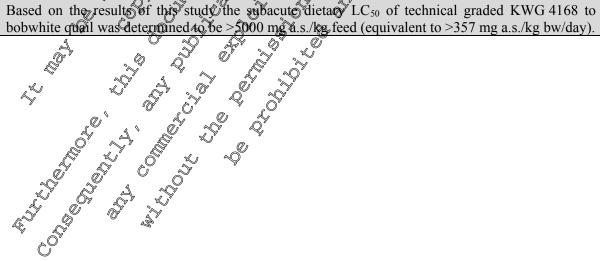
The study was conducted to the OFCD test guideline DECD 205; Avian Chetary toxicity test", adopted 4 April 1984 which is still the current version ...

Validity criteria according to the OEGD 205 (1984) Suideline were met: @

- Control mortanty to not exceed 10% at the end of the test (actual: 0.0%)
- Test Substance to temain at least 80% of nominal in the diet throughout the first five days of the test period (actual: 90.9 to 16%)
- The lowest treatment level should not resplit in any compound-related mortality or toxicity (actual: no toxicity at 313 mg a.s. kg diet) S K.

The study is therefore considered acceptable.

Based on the results of this study the subacuto dietary LC₅₀ of technical graded KWG 4168 to





Data Point:	KCA 8.1.1.2/02
Report Author:	
Report Year:	1998
Report Title:	KWG 4168 (technical grade): 5-day dietary LC50 to mallard duck
Report No:	VE-006
Document No:	<u>M-008047-02-1</u>
Guideline(s) followed in	OECD 205 (1984)
study:	U.S. EPA E 71-2 (1982) (now OCSPP 850.2200 (2012))
Deviations from current	Yes Methods: SANCO/3029/99 rev. No linearity data, no chromatograms, method oot described yes, evaluated and accepted DAR (1997), RAR (2010) RAR (2017)
test guideline:	Yes Methods: SANCO/3029/99 rev. No linearity data, no chromatograms, method oot described
	No linearity data, no chromatograms, method of described 🖉 🖉 🖉
Previous evaluation:	yes, evaluated and accepted
	DAR (1997), RAR (2010) RAR (2017)
GLP/Officially	Yes, conducted under GRP/Officially recognised testing facilities
recognised testing	
facilities:	
Acceptability/Reliability:	Yes A & Q Q O' Q' A

Executive Summary

Young mallard ducks (Anas platyrhytehos) were used tog Ğ 4168 ∰oxicit over a period of five days.

Groups of ten birds were exposed for five days to detary pricent ations of 1250, 2500 or 5000 mg a.s./kg feed or an unfreated control. Following exposure all groups were held for a three-day observation period on untreated feed.

The LOAEC was 5000 mg a.s./kg feed based on signs of intexication and mortality. The NOAEC was determined to be 2500 mg a.s. (kg feed) \cap 0

Based on the results of this study the subscute dietary DC 50 of technical graded KWG 4168 to mallard ducks was determined to be 5000 mg as /kg feed (equivalent to 874 mg a 4/kg bw/day).

I. Material and

- Materials A.
- **Test Material**

Purity:

Lot/Batch

Description: ear fiquid Stable under the Sonditions of this study (recoveries of 105 - 107% Stability of test compound:

Reanalysis **Adate:**

Density

Treatment

Testerates

303, 625, 1250, 2500 and 5000 mg a.s./kg feed

Solvent/Schicle: 60.0 g peanut oil

ot reported

Analysis of ŝ Yes, mean measured values of a.s. in diet 83.4 - 99.9% of nominal concentrations:

Test organisms

Species: Mallard ducks (Anas platyrhynchos) aged 7 days



Acclimated in cages similar to the study cages in size, temperature

Acclimatisation period:

and lighting

Feeding:

Test design

Test vessel:

Replication:

No. of animals/vessel:

Duration of test:

Environmental test conditions

Temperature:

Relative humidity:

Photoperiod:

B. **Study Design**

WC 4168 on malla the results of a p PE-foil coated stainless wire reages of $100 \times 70 \times 70 \text{ cm}^{+}$ No replicates Ten unsexed birds Eight days $27 - 33^{\circ}$ $40 - 60^{\circ}$ 16 h light \approx h datk This study was conducted to assess the dietary toxicity of KWG 4168 on mallard duck (Anas platyrhynchos). Dietary test concentrations were selected based on the results of a preliminary rangefinding study.

Hatchling starter ration available ad libitum

All birds were apparently health after arival to the test factity, and were phenotypically similar to wild birds. At six days of age, ten birds of unknown sex weighing 680 to 123.5 g were randomly allocated to each of the reatment and control diets. Yest vessels were PE-foil coated stainless steel wire 100 x 70 x 70 cm cages which were maintained at a mean cage emperature of 27 to 33°C.

Test diets were prepared by mixing the bird feed, KWG 4168 and 60.0 g peanut oil in a glass beaker, then grinding the pre-mix to know and findity adding the required amount of feed to obtain the desired nominal concentrations. Nominal jest concentrations were 313, 625, 1250, 2500 and 5000 mg a.s./kg. Measured concentrations in the test dicorange@ from 83.4 to 99.9% of nominal.

Birds were given relevent treated or control feed for five days, after which they received an untreated diet during a Oree-day observation period. Feed consumption was recorded daily during the exposure period and at the end of the observation period. Food and fresh tap water were available ad libitum throughout the study. Ø

Birds were weighed at test initiation, on day 5, and at test termination. Observations for mortality and clinical signs of intoxication were recorded daily throughout the study. Necropsy examinations were conducted on all birds of the 2500 and 5000 mg a.s./kg diet groups. Control birds and those receiving the 313, 625 of 1250 ing a.s. kg diet were not necropsied due to no abnormalities being observed.

Analytical method

Samples of feed were analysed using the validated analytical method VE-006, report reference M-008047-02-1 (see Doc MC& Section 4).

Results and Discussion

Validite criteria were not assessed in the study report.

The measured concentrations of spiroxamine in the test diets are summarised below.



Mean measured concentration of nominal (%)
- 25 8
86.4
99.9
93.8
86.0

Table CA 8.1.1.2/02-1 Mean measured concentration of KWG 4168 in the animal feed	Table CA 8.1.1.2/02-1	Mean measured concentration of KWG 4168 in the animal feed
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Mortalities and clinical signs of toxicity (slight apathy, tumbling) were observed for the birds exposed to 5000 mg a.s./kg feed from the fourth day of exposure onwards. Total mortality at the ord of the test was 0.0, 0.0, 0.0, 0.0, 0.0 and 50% in the control, 34,3, 625, 1250, 2500 and 5000 mg a.s./kg diet test groups, respectively. Thus, the LC₅₀ was considered to be 5000 mg a.s./kg diet.

Post-mortem examinations of surviving birds from the 2500 and 5000 mg #s./kg thet groups showed no gross lesions or macroscopic organ alternations, while the examination of those birds that died in the 5000 mg a.s./kg diet group showed pale discolouration of the pancreas, live, and kidneys as well as enlarged gall bladders.

Table CA 8.1.1.2/02-2	Cumulative mortal	lity of birds	after dietar	y exposure 1	to KWG 4168

			~	-0	/	<u>></u>				0
Nominal	Cumul	ative mo	reality by	y day	ð	Ñ	õ,	o ĉ) `}	Total
dietary	0	1 🕡	2 %	3 🔊	45	S I	% (C	7 🔊	8 🐇	(%)
concentration		, Ç			"0" "	v , Ô	\$. Ø	Ū.	0 [°]	
(mg a.s./kg		Ŵ	s s	or s		4		, Q	ra.	
diet)	•	ĝ (D' v		.ſ	- \	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~			
Control	0	0 4	0	0.5	. E		$\tilde{0}$ %	0	0	0.0
313	0 炎	0	Q	0	Č N		0 炎	0	0	0.0
625	00	0	Ø.	D0 S	0	0	00	0⊁	0	0.0
1250	D (0 %	~ 0	0	00	Ø		² 0	0	0.0
2500		0 🞸	0	0	Ú.		0	0	0	0.0
5000	0 0	0_{\odot}	Ø «	Ø ×	JŽ 🏷	5	5	5	5	50

During the five-day exposure period, a dose related reduction in feed intake was evident in groups receiving 625, 1250, 2500 and 5000 and a.s./kg diet. The reduced feed intake led to diminished body weight gain in all treated groups over the exposure period, a growth depression that could not be compensated in any of the treated groups during the following three-day observation period on untreated feed.

Table CA 8.1.52/02-3 Feed consumption and a.s. intake of KWG 4168 during exposure and observation

Nominal dietary concentration	Mean daily feed con	isumption	Mean a.s. intake, days 1-5			
(mg a.s./kg diet)	Days Ar S	Bays 68	mg a.s./bird/day	mg a.s./kg bw/day		
Control	48.80 0	°63.4 [°]	0.0	0.0		
313	50.0	603	15.6	84.0		
625	50.0 7 ~ 7 44.5 6 4 34.6 7 4 ~	57.5	27.8	163.7		
1250	¥34.6 🖉 🔬 👒	053.6	43.2	295.1		
2500 av av	1 22 1 ~	53.4	55.3	502.7		
<u>5000 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 </u>	15.1	51.3	75.5	871.1		



	416	8				Q° *	>
Nominal di	ietary	Body we	ight (g)		Body weight	change (%)	Ŷ,
concentrat	ion	Day 0	Day 5	Day 8	Days 0 – 5	Days 5 8	
(mg a.s./kg	diet)						
Control	Mean	84.87	200.25	277.90	+105.9	+38.8	
	SD	10.87	19.62	22.90	2	\$ ~ \$ B	
313	Mean	89.75	185.90	245.60	× 107.1 v	+32,1	
	SD	11.13	24.42	30.91			a
625	Mean	88.28	169.88	243.70 Q	+92.4	+33.5 %	s S
	SD	9.09	20.73	34.22 J	× ×)
1250	Mean	98.53	146.39	219.2Q	° +48.€	+49.	
	SD	11.53	18,10	22.Z7 °		b û	
2500	Mean	88.72	110.01	178.90	+24.0	\$\$58.1 \$	
	SD	12.81	49.98	23.56 L	× ¢	\sim	
5000	Mean	86.12	~86.64	Q 1/0,40	0 +0.6 °	+10 3. 6°	
	SD	10.94	18,89 ~	21.98			

Table CA 8.1.1.2/02-4 Body weights and body weight change of birds after dietary exposure to KWG 4168

III. Conclusion

Young mallard ducks (*Anas platyrhonchos*) were used to evaluate the dietary toxicity of KWG 4168 over a period of five days.

Groups of ten birds were exposed for five days to betary concentrations of 319, 625, 1250, 2500 or 5000 mg a.s./kg feed or an underted control. Following exposure all groups were held for a three-day observation period on untreated feed.

The LOAEC was 5000 mg a.s. As g feed based on signs of infoxication and mortality. The NOAEC was determined to be 2500 mg a.s. Kg feed.

Based on the results of this study he subacute dietary \widehat{PC}_{50} of technical graded KWG 4168 to mallard ducks was determined to \widehat{Pe} 5000 mg a.s./kg feed (equivalent to 874 mg a.s./kg bw/day).

Assessment and conclusion by applicant:

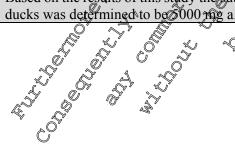
The study was conducted to the OEOD test guideline "OECD 205: Avian dietary toxicity test", adopted 4 April 1984 which is still the current version.

Validity criteria according to the OECD 05 (1984) guideling were met:

- Control mortality to not exceed 10% at the end of the test (actual: 0.0%)
- Test substance to remain a least 80% of nominal in the diet throughout the first five days of the lest period (actual: 83,4 to 99.9%)
- The lowest treatment to el should not result in any compound-related mortality or toxicity factual: no toxicity a0313 mg a.s./kg diet)

The study is therefore considered acceptable.

Based on the results of this stuff the subacute dietary LC_{50} of technical graded KWG 4168 to mallard ducks was determined to be 000 mg a.s./kg feed (equivalent to 871 mg a.s./kg bw/day).





Data Point:	KCA 8.1.1.2/03
Report Author:	Q° 🎓
Report Year:	1995
Report Title:	KWG 4168 (technical grade): 5-day dietary NOEC to mallard duck
Report No:	VE-007
Document No:	<u>M-008072-01-1</u>
Guideline(s) followed in	OECD 205 (1984)
study:	U.S. EPA E 71-2 (1982)
Deviations from current	None 🖉 🖉 🖉 🖉
test guideline:	
Previous evaluation:	No, submitted, not evaluated
	RAR (2010), RAR (2017)
	Since a NOEC value base on weight data could not be defined a follow-up
	study was conducted (report no. VE-000).
GLP/Officially	Yes, conducted under GLP/Officially recognised testing factities
recognised testing	
facilities:	
Acceptability/Reliability:	Yes A Y Y Y Y Y Y Y

Executive Summary

Young mallard ducks (Anas platyrbynchos) of KWG 4168 over were ušed to evaluate the a period of five days. M

Groups of ten birds were exposed for five days to dietary concentrations of 156 or 312 mg a.s./kg feed or an untreated control. Following exposure, all groups were held for a three-day observation period on untreated feed.

JEGN JEGN J J J J J J J J J J J J Based on the results of this story the 12 and 156 mg a.s./kg feed, respectively.

I. Materials and Methods

A. Materiak Test Material

Lot Batch #: **Purity:**

Stable under the couditions of this study (recoveries of 105 – 107% Description Stability of test, after 24 hours compound:

Reanalysis/Expiry date:

Density: Treatments

> 156 and 362 mg a.s./kg diet Test rates:

60.0 g peanut oil Solvent/vebicle

W&G 4168

8114002

porter

Yes, mean measured values of a.s. in diet 95.5 - 97.6% of nominal 'nf concentration

Test ørganisms

Aňalvsis

Species:

Mallard ducks (Anas platyrhynchos) aged 7 days



Acclimated in cages similar to the study cages in size, temperature

Acclimatisation period:

and lighting

Feeding:

Test design

Test vessel:

Replication:

No. of animals/vessel:

Duration of test:

Environmental test conditions

Mean temperature:

Relative humidity:

Photoperiod:

B. **Study Design**

totics of a preli-PE-foil coated stainless wire reages of $100 \times 70 \times 70 \text{ cm}^{+}$ No replicates Ten unsexed birds Eight days $29 - 31^{\circ}$ Not reported 16 h night 28 h datk This study was conducted to assess the dietary toxicity of KWG 4168 on mallard duck (Anas platyrhynchos). Dietary test concentrations were selected based on the results of a preliminary rangefinding study.

Hatchling starter ration available ad libitum

All birds were apparently health after arival to the test factity, and were phenotypically similar to wild birds. At six days of age, ten birds of unknown sex weighing 80 to 102 g were randomly allocated to each of the treatmend and control diets. Test vessels were PE-for coated stainless steel wire 100 x 70 x 70 cm cages which were maintained at a mean wage temperature of 29 to 31°C.

Test diets were prepared by mixing the bird feed, KWG 4168 and 60.0 g peanut oil in a glass beaker, then grinding the pre-mix to homogeneity, and findly adding the required amount of feed to obtain the desired nominal condentrations. Nominal test concentrations were 156 and 312 mg a.s./kg diet. Measured concentrations in the pest diet range of rom \$5.5 to \$7.6% of nominal.

Birds were given relevent treated or control feed for five days, after which they received an untreated diet during a Oree-day observation period. Feed consumption was recorded daily during the exposure period and at the end of the observation period. Food and fresh tap water were available ad libitum throughout the study.

Birds, were weighed at test initiation, on day 5 and at test termination. Observations for mortality and clinical signs of intoxication were recorded daily throughout the study. Necropsy examinations were conducted on all birds of the G12 mg a.s./kg diet groups. Control birds and those receiving 156 mg a.s./kg diet were not necropsed due to no abnormalities being observed.

Analytical method

Samples of feed were analysed using the validated analytical method VE-006, report reference M-008047-02-1 (see Doc MC& Section 4).

Results and Discussion

Validite criteria were not assessed in the study report.

The measured concentrations of spiroxamine in the test diets are summarised below.



Nominal dietary concentration (mg a.s./kg diet)	Measured concentration of nominal (%)	¢ ¢
Control	-	
156	97.6	
312	95.5	

Table CA 8.1.1.2/03-1	Measured concentration of KWG 4168 in the animal feed
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Mortalities and clinical signs of toxicity were not observed in any of the birds during the study. Post-op mortem examinations of surviving birds from the 312 mg a.s./kg diet showed no gross lessons of macroscopic organ alterations.

During the 5-day exposure period, a dose-related reduction in feed in ake and in body weight gath was noted in the treatment groups. The depression of body weight development was not deemed are diverse treatment-related effect, as the unpalatability of KWG 4168 reduces consumption of contaminated feed.

Table CA 8.1.1.2/03-2 Cumulative mortality of birds after dietary exposure to KW@4168

				0	, W		~0	R		1
Nominal	Cumula	ative moi	tality by	day 🔐	r C	, Q	0°		0	Total
dietary concentration (mg a.s./kg diet)	0	1		3 ,3 ,7 ,7 ,7 ,7 ,7 ,7 ,7 ,7 ,7 ,7 ,7 ,7 ,7						
Control	0	0 4				0.5	<u>A</u>			0.0
156	0	0	0 4	0	000		V V	0_0	0	0.0
312	0	0~~	0°%	Q	°¢ »	\$0 Ø	0	0 0	6 ×	0.0

During the five-day exposure period, a dose-related reduction in feed intake and in body weight gain was noted in the treatment groups.

Table CA 8.1.1.2/03-3 Feed consumption and a.s. intake of KWG 4168 during exposure and observation

Nominal dietary	Mean daily feed con (g feed/bird/day) (bays 14.5		Mean a.s. intake, day	ys 1-5
(mg a.s./kg diet)	Days 165 🔊	Days 6-8	mg a.s./pird/day	mg a.s./kg bw/day
Control 🔊	55.5 💭 🖉	\$57.7° 0° 0	0.0 0	0.0
156	440 28	48.0	6.9	39.5
312	A5 x		JA/.8	81.4

Table CA 8.1.1.2/03-4 Body weights and body weight change of birds after dietary exposure to KWG

Nominal dieta		Body weight (g)ô Ô		Body weight	change (%)
concentration		Day 0	Day 5	Day 8	Days 0 – 5	Days 5 – 8
(mg a.s. kg di Control	<u>et) , 🖗 , 🤇 , 🤇 , 🤇 , 🤇 , 🤇 , 🤇 , 🤅 , 🤇 , 🤅 , 🤅</u>					
	Mean		201969	285.30	+94.7	+41.5
L.	SD V Q	~1393 J	~Z7.09	39.86		
156	Mean 🔗	Q100.09 🕺 🦼	Q [*] 73.72	248.40	+73.6	+43.0
	STD ,	15.88	23.63	27.27		
312	Mean SIX	10531	182.03	262.80	+72.9	+44.4
<u> </u>	SDC S	103931 14.52	25.74	29.99		

III. Conclusion

Young mallard ducks (*Anas platyrhynchos*) were used to evaluate the dietary NOEC of KWG 4168 over a period of five days.

Group of ten birds were exposed for five days to dietary concentrations of 156 or 312 mg a.s./kg feed or an untreated control. Following exposure, all groups were held for a three-day observation period on untreated feed.



Based on the results of this study the NOEC, NOAEC and LOEC were <156, >312 and 156 mg a.s./kg feed, respectively.

Assessment and conclusion by applicant:

The study was conducted to the OECD test guideline "OECD 205: Avian metary toxicity" test adopted 4 April 1984 which is still the current version.

Validity criteria according to the OECD 205 (1984) guideline were met:

- Control mortality to not exceed 10% at the end of the test (actual: 0.0%)
- Test substance to remain at least 80% of nominal in the diet Broughout the first for the test period (actual: 95.5 to 97.6%)
- The lowest treatment level should not result in any compound related mortality or toxicity (actual: no toxicity at 156 mg a.s./kg diet)

The study is therefore considered acceptable

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No LC<sub>50</sub> was reported but, due to the absence of mortality, the LC<sub>50</sub> is considered to be 312 \text{ mg}
a.s./kg feed (equivalent to >81.4 mg a.sc/kg bw/day).
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Data Point: KCA 8: R1.3/0 Report Author: 2004 Report Year: Report Title: Effects of a subchronic dietary exposure of KNCG 4168 techn, on bobwhite quail including effects on reproduction and health Report No: SXR REP 04 \bigcap M0007470-03-1 Document No: WECD Buideline 206 "Avian Guideline(s) followed in 1 Reproduction Test" from April 1984 and EPA Pesticide Assessment Guidelines / study: Subdivision/E, § 74-4 from July 1986 Deviations from current Yes Ô Methods: SAMCO/3029/99 rev. 4 test guideling: No correlation coefficient or equation of the line presented On three levels injected is singlizate for linearity wes, evaluated and accepted (Previous evaluation A ĎAR (2010), ROR (2010), ROR (2017) GLP/Officially Yes Sonducted under GLP Officially recognised testing facilities recognised testing ~0 facilities: Acceptability/Reliability:

CA 8.1.1.3 Sub-chronic and reproductive to sicity to birds

Executive Summary

This study was conducted to assess the effects of long-term dietary exposure of KWG 4168 on the health and reproductive performance of adult bobwhite quail (*Colinus virginianus*). Three test concentrations and a control were used, each of which cordained 20 pairs of one male and one female bobwhite quail. Nominal concentrations were 30, 77 and 200 mg a.s./kg diet.

After the 2¹-week dietars exposure period, no dose-dependent and thus treatment-related adverse effects on behaviour, arvival rate or body mass changes of adult bobwhite quail were observed.

No evaluation criteria concerning reproductive performance were found to be statistically significantly different to the control in any of the treatment groups. There was, however, a statistically significant difference in the mean body mass of the 14-day old surviving chicks.

The NOEC and LOEC for adult bobwhite quail were determined to be 204 and >204 mg a.s./kg diet, respectively, and for reproductive performance were 29.3 and 78.6 mg a.s./kg diet.



y the second sec **Materials and Methods** I. A. **Materials Test Material** KWG 4168 Lot/Batch #: 898114002 97.8% (27 July 1993), 97.5% (21 January 19 **Purity:** 1994) **Description:** Clear liquid Measured values after a 14-day storage period showed no inadmissible deviation (87 – 92% of initial) • Stability of test compound: **Reanalysis/Expiry** date: **Density:** Not reported al dort Treatments **Test rates:** Nomina /kg basal Measured: 293, 78% and 204 mg a.s None Solvent/vehicle: Ø, ses, measured concentrations of the s. in prepared Analysis of test concentrations: 402% o€nominal m **Test organisms** Bobwhite quail (Colonus virginianos) aged 25 weeks. Pen-reared and **Species:** pheaetypically indistinguishable from wild birds. Source: Acclimatisation After arrival to the test facility, alleast two weeks prior to the initiation of the Ost, birds were place on an 18 m² aviary for acclimation to climatic conditions Ŕ Feeding: Adults a full value Het for adult quail (Altromin 0721) ad libitum Matchlings: ed a commercial game bird feed (Altromin 0711) ad libitum Test design Test vessel: Ädułts: Staipless steel wire and sheet pens of 75 x 50 x 25 cm Hatchlings: Batteries of brooding pens of 55 x 33 x 18 cm Replication 20 pairs per group No.of Ône male and one female per pen animals/yesse Duration of test: â 21 weeks Environmental test conditions



Temperature:	Adults: $20 - 22^{\circ}C$
	$20 - 22^{\circ}C$ Hatchlings: First week: $35 - 38^{\circ}C$ Second week: $30 - 32^{\circ}C$
Relative humidity:	55 - 75%
Photoperiod:	Adults: First 8 weeks: 7 h light : 17 h dark with a 30 minute transition period
	From week 9: 17 h light 7 h dark with a 30 minute transition period
	Hatchlings:
	Outdoor light/dark Scie
Study Design	

B. Study Design

This study was conducted to assess the effects of long-term dietar@exposure of KWG 4068 on the health and reproductive performance of adult boowhite guail (Colinte virginitanus)

Test birds were pen-reared, 25-week old boowhite quail (*Solinus Virginanus*) that were healthy and phenotypically indistinguishable from wild birds. After arrival to the test facility, birds were placed in a large 18 m² aviary for acclimatisation.

Adult birds were housed indoor in stabiless steel wife and sheet pens of $75 \times 50 \times 25$ km, with a slight slope towards the feeding box. Pens were provided with prepared diets and tap water *ad libitum*, with any uneaten food being removed weekly. Flatchlings were housed in battery brooding pens of 55 x 33 x 18 cm.

Birds were randomly assigned to one of four treatment groups. Each group consisted of 20 pairs, with one male and one female birds per pen. To three of the groups were fed nominal concentrations of 30, 77 and 200 mg as kg thet, with the fourth group receiving untreated control diet. Mean measured concentrations of the diet were 29.3 78.6 and 204 mg a.s./kg diet corresponding to 98, 102 and 102% of nominal, respectively.

Fresh batches of diet were prepared in Aweelomterval's during the 21-week exposure period. Samples of the control and test diets were taken mmediately after preparation of four of the batches, and analysed using gas chromatography.

All adult birds were observed at each work day throughout the study for signs of toxicity or behavioural impacts. All birds were recropsied upon death or termination of the study. Body mass of adults was determined at study initiation, every second week until week 8 and after terminal sacrifice. Body mass was not measured during egglaying to prevent any potential adverse effects of handling on egg production. Feed consumption per pen was measured weekly throughout the study.

Eggs were collected daily throughout the course of the study, and stored for a maximum of seven days in a cooler. Mean storage temperature was maintained at $16 \pm 1^{\circ}$ C. After removal from the cooler, eggs were candled to identify any cracks, and such eggs were discarded. Non-cracked eggs were then placed into an incubator at 37.5 ± 0.5 °C, with a relative humidity during incubation of 50 to 65% and during hatch of 70 to 75%. In order to prevent adhesion of the embryo to the shell membrane, eggs were rotated every four hours. Eggs were candled again on day 11 of incubation to determine fertility and on day 18 for determination of embryo vability. On day 21 of incubation, eggs were placed into a hatcher, and all hatchlings, unfatched chicks and eggshells were removed 48 hours after emergence of the first chick.

To determine egg thickness, eggs were opened around the equator, washed out and left to dry with the membrane intact for at least 48 hours at room temperature. The thickness was then measured at four points around the waist using a calibrated micrometre.



Hatchlings were grouped according to parent treatment level and housed for 14 days in batteries of 55 x 33 x 18 cm, at 35 to 38°C. Birds were provided with untreated diet and water ad libitum. Grouped hatchling body mass was then recorded by parent treatment group at the end of the 14 days.

Analytical method

Samples of feed were analysed using the validated analytical method 00795, report reference $\frac{1000}{1000}$ 03-1 (see Doc MCA Section 4).

II. **Results and Discussion**

Validity criteria were not assessed in the study report.

The measured concentrations of spiroxamine in the test diets are summarised below Ø

Nominal	Measured con	centration by d	kot preparation	(mg a s/kg) (4
concentration	1 st	3 rd	5 th		Mean 🔿	Mean % of
(mg a.s./kg)	preparation	preparation	preparation	preparation		nominal
0	0	0	~Q~	le de la		
30	28.3	29.2	¢\$0.3 🖉 🕺	م 🕼 29.4%	29.3 0	₽98 ©
77	80.1	92.4 ⁰ *	71.1	70,70 ,5	78.6	1020
200	202	220	195	195	204	-102
C^{1} · · · · · · ·	. 1	m si	a a		<u>م م</u>	

Table CA 8.1.1.3/01-1 Measured dietary concentrations of KWG 4168 in control and test diet

Clinical observations and mortality

During the 21-day study period, a total loss of two of the 160 birds was recorded, one in the 29.3 mg a.s./kg diet group and one in the 204 mg a.s./kg diet group. No test substance-related symptoms were evident prior to death. Three birds in the highest treatment level, 204 mg a.s./kg diet showed short-term, reversible behavioural impacts following injuries on claws and egs. As similar injuries of feet were observed in all test groups and the control, it is assumed that such injuries are attributable to the cage environment rathers than to treatment effects.

Gross necrops

The gross pathological examination of adults at termination of the study showed in particular injuries on the next and heads of birds as a consequence of cage-mate interactions.

Birds from all treatment groups showed a reduced size of festicles/ovaries. Additionally, greenish discoloured testicles of some males were found throughout all concentration groups. There was, however, no correlation between these findings and the symptoms observed during the exposure period.

Other pathological findings such as enlarged spleen, foot lesions, enlarged kidneys among other findings did not show a clear dose response, and appeared to be distributed over all test groups at random. It is therefore assumed that the treatment did not adversely affect the body organs of the birds.

Adult body weights and feed consumption

Statistical analyses of body masy and feed consumption of adults revealed a significantly lower male body mass in the 29.3 mg a.s. As die $p \le 0.05$ treatment group at test initiation and at weeks three and five, however at test termination of statistically significant difference was recorded. No significant differences of female body mass were observed. Feed consumption was significantly reduced at weeks four, seven and eight. C

No significant differences in body mass were found at any time interval in the 78.6 mg a.s./kg diet treatment group. Ford consumption was significantly reduced at weeks four, eight and 11.

No statistically significant differences in male body mass were observed in the 204 mg a.s./kg diet treatment group to the controls, however female body weight at weeks three and five were significantly reduced. Statistically significant reductions in feed consumption were observed at weeks four, eight, 10, 15 and 20.



Mean measured	Sex	Mean bod	ly weight (g)				
concentration (mg a.s./kg		Week 1	Week 3	Week 5	Week 7	Week 9	Week 22
diet)					1		~~ <u>~</u> ~~
Control	Male	198	202	203	206	209	Ĩ♥217. [™]
	Female	193	199	202	204 🔊	207 📎	2479 4
29.3	Male	189*	194*	195* 196	199	202	240 0
	Female	187	193	196	200	204	\$250 🔊 🖉
78.6	Male	194	200	202	206	210 Q	216
	Female	190	196 🔟	199 🖌	203 <u>°</u>	206 3	240 _ @
204	Male	191	196 191*	197 👡	200 200 197 or	R205, Ö	B12 0
	Female	186	191*	193*	Ĵ97 @	202	€Ž33 ,^Ş

Table CA 8.1.1.3/01-2 Mean adult body weight after dietary exposure to KWG 4168

* Differences between the control were not significant $(p_{\phi}^{0.05})$

Table CA 8.1.1.3/01-3 Mean feed consumption during and after dietary exposure to KWG 4168

Mean measured	Feed c	onsump	۵Č		84 /	\sim	«O [″]		9 49 29 49 22	Ģ (
concentration (mg a.s./kg	1	2	3,0 ×	0	<i>"</i>	Ì Â		×8 5		10 مي پر	11
diet) Control	20.6	24:00	22.3	23.6	2109	20.6	24.3	22.4	0 24.5	[≫] 26.9	28.6
29.3	19.6	23.6	Q1.3	22.2*	Q1.3		19.8*	20,96	23.2	26.1	27.9
78.6	20.6	Ø3.5	Q2.2	22.3*	21.2	19.8	20,4	21.3*	23.2 23.8	26.3	26.5*
204	20.0	[≫] 23.3	21.4	22.0	21 16	2007	20.3	20.8*	23.1	25.4*	25.4
	12 🔊	13	14	15	16	×17	118 §	"19 »	20	21	-
Control	28,5	31.8	34 .8	® 6.8	37.4 🕺	¥39.3	37.2O	39.4√	38.9	39.4	-
29.3	\$6.9	3 0.8 s	\$5.1	36.2		39	35(5	3&A	37.0	41.4	-
78.6	27.4	30.0	33,1	36.0	36.7	389	36 .9	40 .7	39.8	43.1	-
204	28	29-2	368	39 .1		36.4	\$34.3	37.8	35.1*	39.3	_

* Significantly different from the control at p < 0.05 β \circ

Reproductive results

There were no statistically significant differences observed between the treatment groups and the controls in measured criteria of the reproductive performance (number of laid eggs, number of cracked eggs, eggshell thickness fertility, hat the rate, body mass of natchlings) over the 11-week reproductive period of the study.

Juvenile observations and mortality

Observations on the hearth and vitality of hatchlings during the 14-day post-hatching period showed no statistically significant differences between treatment groups and controls. At the end of the 14-day post-hatching period, mean body mass of hatchings was statistically significantly reduced in the 78.6 and 204 mg a.s./kg diet treatment groups when compared to the control group.

Table CA 8.103/01 Summary of mallard reproductive performance after dietary exposure to KWG

Parameter ,	Test concentration	(mg a.s./kg diet)		
	Centrol	29.3	78.6	204
Total eggs and / here	\$D.9	55.1	51.0	45.7
Total eggs set / hen	43.8	48.9	43.9	39.9
Eggshel thickness (mm)	0.21	0.20	0.21	0.20
No. of cracked eggs / hen (% of eggs laid)	8.9	5.0	8.0	5.7



Parameter	Test concentration (mg a.s./kg diet)						
	Control	29.3	78.6	204 _{@`} °			
No. of fertile eggs on	94.1	95.3	96.2	86.3			
Day 11 / hen (% of				N I			
eggs set)			Č.				
No. of viable embryos	90.2	92.8	94.3	85.0 4			
on Day 18 / hen (% of			~U				
eggs set)							
No. of hatchlings / hen	69.5	68.9	69.9	66.3 ~ ~			
(% of fertile eggs)		The second secon	<u> </u>				
No. of 14-day	27.2	30.0	26.80	21.2 ~ ~			
survivors / hen		"Q"					
No. of 14-day	62.2	61.4	61.0	53.90			
survivors / hen (%							
eggs set)		(k, 6° Š		$\mathcal{Y} \sim \mathcal{V}$			
Average body weight	-						
of hatchlings (g) (%	4			O' Q' A			
inhibition compared to	, K						
control)		N O S					
Average body weight	- Q	25,06		8.85			
of 14-day survivors (g)			L" 2 .5				
(% inhibition							
compared to control)				ŎĽ <u>ú</u> ľ			
* Significantly different	from the control at p	Q.05 ° L		, ^N			

III. Conclusion

During a 21-week dietary exposure to KWG \$168 technical at measured conceptrations of 29.3, 78.6 and 204 mg a.s./kg diet, no dose-dependent and this treatment related adverse effects on behaviour, survival rate or body mass changes of adult boby thite quail (*Colinus virginianus*) were observed.

No evaluation criteria concerning reproductive performance were found to be statistically significantly different to the control in any of the treatment groups. There was, however, a statistically significant difference in the mean body mass of the 14 day and surviving Dicks. \mathcal{Q}

The NOEC and LOEC for adult bobwhile quail were therefore determined to be 204 and >204 mg a.s./kg diet, respectively, and for reproductive performance were 29.3 and 78.6 mg a.s./kg diet.

Assessment and conclusion by applicant:

The study was conducted to the OECD test guideline "OECD 206: Avian reproduction test", adopted 4 April 1980 and preets the requirements second within. Due to there being only three test concentrations with a wave spacing factor between, the data are not considered suitable for reliable EC_{10} and EC_{20} calculation therefore this has not been conducted.

Validity criteria according to the OECD 206 (1994) guideline were met:

- Control mortality to not exceed 10% of the end of the test (actual: 0.0%)
- Average number of 14-day surviving chicks in the control to be at least 12 (actual: 27.2)
- The average shell thickness for eggs in the control group to be at least 0.19 mm (actual: 0,29 mm)

The study is therefore considered acceptable.

The NOEC determined in the study was 29.3 mg a.s./kg diet (equivalent to 2.02 mg a.s./kg bw/day) and has been based on the statistically significant effects on 14-day survivor body weight at 78.6 mg a.s./kg diet. This NOEC is considered to be very conservative because there was only a 3.8% reduction in body weights, relative to the control, at 78.6 mg a.s./kg diet. Whilst statistically significant, this reduction is not considered to be a true treatment related effect as the reduction is very minor and unlikely to cause an impact at the population level.



It may be a statistical anomaly instead of a substance related effect since over the weeks the body weights of 14 day survivors changed. They were statistically reduced in three of the weeks but in two of the weeks they were reduced without statistical significance. In one of the weeks the body weights were equal to the control, but in three of the weeks they were higher than the control. In the last two weeks for example the mean body weights of 14 day old survivors were 34.3 g and 33.7 g, at 78.8 me a.s./kg diet, while the control chicks weighed 32.3 g in that period. In contrast to these slight and partially contradictory effects, the results at the next highest test concentration (204 mg a.s./kg diet), were consistent and clear. The reduction of body weight of 14 days survivors compared to the control to 8.8 %. This also is not a dramatic decline but the average body weights were reduced over the whole exposure period (6 tunes statistically significance). These findings indicate that at this test concentration (204 mg a.s./kg diet) the effects have to be considered treatment related. It is therefore believed that the two EC is 204 mg a.s./kg diet (equivalent to 5.40 mg a.s./kg bw/day).

Further supporting data has been provided below (RCA \$1.1.3/03) in the form of historical control. data for 14-day old survivors to demonstrate that the mean body weight of 32.6 g achieved at 78.6 mg a.s./kg diet is well within the normal deviation of the historical controls

	KCA 8.1 43/02 7 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5
Data Point:	KCA 8.1 93/02 2 2 2 2 2 2 2 2
Report Author:	Č ×
Report Year:	
Report Title:	KWG 4168 Technical - A reproduction study with mallard (Anas platyrhynchos)
Report No:	
Document No: 2	<u>M-008186-019</u> <u>5</u>
Guideline(s) followed in	FIER 71_A (now @CSPD& 90 2300) (
study.	OBČD 206 (1984)
Deviations from current test guideline:	OPCD 206/(1984) Yes Methods: SANCO/3029/99 fev. 4 Accuracy it≤5 for some levels, precision not avaitable for 10 mg/kg level yes, evaluated and accepted RAR (2010), RAR (2017)
test guideline:	Methods: SANCO/3029/99 zev. 4 2 2
	Accuracy h 5 for some levels, precision not available for 10 mg/kg level
Previous evaluation:	yes, evaluated and accepted
(Co	RAR (2010), RAR (201/7)
GLP/Officially	Yes conducted under GLP/Officially recognised testing facilities
recognised testing	$\delta_{1}^{*} \sim \delta_{2}^{*} \sim \delta_{1}^{*} \sim \delta_{1}^{*} \sim \delta_{1}^{*}$
facilities:	
Acceptability/Reliability:	Yes by by O a
Executive Summary	$\frac{Y es}{\sqrt{2}} \frac{y}{\sqrt{2}} \frac{y}{\sqrt{2}} \frac{y}{\sqrt{2}} \frac{x}{\sqrt{2}} \frac{y}{\sqrt{2}} \frac{y}{\sqrt$

Mallard ducks (*Anas plat hynchos*) were exposed to KWG 4168 in the diet at mean measured concentrations of 28.4, 78.8 and 205 mg a.s. kg diet or a control for 20 weeks. Ducks were observed daily for mortality, abnormal behaviour and signs of toxicity. In addition, the effects of adult exposure on the number of eggs laid, normal development of eggs, embryo viability, percent hatchability, offspung survival and eggshell theckness were evaluated.

No mortalities, evert signs of boxicity or other treatment-related effects on body weight were observed at any of the concentrations tested. There were also no treatment-related effects upon feed consumption or reproductive performance at the 28.4 and 78.8 mg a.s./kg diet test concentrations.

At the 205 mg a 2/kg diet test concentration there was a treatment-related reduction in feed consumption and an increase in the number of hens displaying lesions of egg yolk peritonitis at terminal necropsy. Additionally, there were reductions in egg production, viable eggs and embryo survival that were reflected as reductions in the numbers of hatchlings and 14-day old survivors. There was also a treatment-related reduction in the body weight of hatchlings in the 205 mg a.s./kg diet treatment group.

Based upon the multiple effects seen at the highest test concentration, the NOEC and LOEC for this study were determined to be 78.8 and 205 mg a.s./kg diet, respectively.



I.	Materials and Metho	ods
A.	Materials	° S
Te	st Material	KWG 4168
	Lot/Batch #:	603-0152
	Purity:	100% (reported)
	Description:	Clear yellow liquid
	Stability of test compound:	KWG 4168 603-0152 100% (reported) Clear yellow liquid Analysis of diet samples collected from treders after being held at ambient temperatures for 7 days were 95, 104 and 92% of nominal for the 30, 77 and 200 mg/a.s./kg diet test concentrations Not available
	Reanalysis/Expiry date:	Not available
	Density:	Not available
Tr	eatments	
	Test rates:	ambient temperatures for / days were 95, 104 and 92% of nominal for the 30, 77 and 200 mga.s./kg diet test concentrations Not available Not available Nominab 0, 30, 77 and 200 mg a.s./kg diet Meanmeasured: 0, 28.4, 78.8 and 205 mg a.s./kg diet 100 mL acetone and 180 mL com oil Ses, mean measured concentration 95 – 103% of nominal
	Solvent/vehicle:	100 mL acetone and 180 mL com oil
	Analysis of test concentrations:	Ses, mean measured concentration 95 – 103% of nomical
Te	st organisms	
	Species:	Mallard ducks (Anas platyr) winch (3) age 23 weeks
	Sources a	\mathcal{R}_{n}
	Acclimatisation	Three week acclimation period
	period:	
	Feeding:	Feed formulated to test facility specifications
Te	st design 🖉 🎽	Feed formulated to test facility specifications
	Test vessel:	
		Sinyl-coated wire mesh batteries of 75 x 90 x 45 cm
		Hatchings V Viovil-coated wire mesh and stainless steel sheeting pens of 62 x 92 x
		2.5 cm ² 2 2
,	Replication:	Eight pairs per group
l'	No. of	Que male and one female per pen
	animals/yessel:	
	animals/yessel:	Acclimation: 3 weeks
		Pre-photostimulation: 8 weeks Pre-laying (with photostimulation): 1 week
		Egg laying: 10 weeks
Ľ,	Duration of fest:	Post-adult termination (incubation, hatching and 14-day offspring rearing period): 5 weeks
En	vironmental test	

conditions



Temperature:	Adults:
	21.7 °C (average) \bigcirc
	21.7 °C (average) Hatchlings: 38 °C until aged 5-7, then 29 °C
Relative humidity:	36% (average)
Photoperiod:	First eight weeks: 8 h light : 16 h dark at approx. 275 lux, From week nine: 17 h light of h dark at approx. 275 lux,

B. Study Design

This study was conducted in order to evaluate the offects of dietary exposure of KWG 416800 adult mallard ducks (*Anas platyrhynchos*) over a 20 week period. Test concentrations were based on expected environmental concentrations.

Test birds were pen-reared mallard ducks (*Anas flatyrhynchos*) that Gere apparently healthy and phenotypically indistinguishable from wild birds. All bird were from the same batch and were 23 weeks of age at test initiation.

All birds were given feed and water ad *librum* during acclimation and testing. The basal diet was formulated to contain at least 27% protein and 25 % tat, with no more than 5% fibre. Pulverised limestone was added to the adult diets at 5% w/w in order to provide calcium for eggshell formation.

Test diets were prepared by mixing relevant amounts of KWG 4168 with 50 mD acetone and 180 mL corn oil, and then adding half the required amount of basal diet. The mixing beaker was rinsed with a further 50 mL acetone, and the premix was then added to the remaining half of the basal ration and mixed. Nominal test concentrations were a control 30,77 and 200 mg a.s. Kg diet. Mean measured test concentrations were 0 28.4. 38.8 and 205 mg a sckg diet, corresponding to 35, 102 and 103% of nominal, respectively

Each test concentration contained eight pairs of one male and one female duck, housed indoors in vinylcoated wire mean batteries of 75 x 90 x 45 cm. These were maintained at an average temperature of 21.7 \pm 1.2 °C (SD), with an average relative humidity of $36 \pm 17\%$ (SD). The air handling system was designed to sent up to fifteen room volumes every hour. The photoperiod during acclimation and for the first eight weeks of the test was eight hours or less of light per 24 hours. From the beginning of week 9, the photoperiod was increased to 17 hours of light per day to induce egg laying. Illumination was provided by fluorescent lights at a mean of 2754ux.

Mallard ducks were observed daily for mortality, abnormal behaviour and signs of toxicity. Adult body weights were resourced at test initiation, on week 2, 4, 6,8 and at test termination. Feed consumption was measured weekly for each pen. Necropsies were performed on all adults surviving to test termination.

Weekly throughout the laying period, eggs were collected from alternate pens for measurements of eggshell thickness. Eggs were opened at the warst and the contents removed and rinsed before being allowed to air dry for at least one week. Measurements of the dried shell plus the membrane was determined by measuring five points around the waist of the egg using a micrometre.

Effects of adult exposure on the number of eggs laid, normal development of eggs, embryo viability, percent hat hability, offspring survival and eggshell thickness were evaluated.

Hatchlings were placed in vinyl-coated wire mesh and stainless steel sheeting pens of $62 \times 92 \times 25.5$ cm. These were maintained at approximately 38 °C from the time of hatching until the birds were five to seven days of age, after which the temperature was set to maintain an average temperature of approximately 29 °C.

Statistical analyses were conducted using Dunnett's method following arcsine square root transformation.



Analytical method

Samples of feed were analysed using the validated analytical method M-008186-01-1, report reference M-008186-01-1 (see Doc MCA Section 4).

II. **Results and Discussion**

Validity criteria were not assessed in the study report.

In measured of the second seco The measured concentrations of spiroxamine in the test diets are summarised below. Mean p test concentrations were 0, 28.4, 78.8 and 205 mg a.s./kg thet. Ň

Table CA 8.1.1.3/02-1 Measured concentration of KW& 4168 in the animal feed

Nominal dietary concentration	(mg a.s./kg diet)	Aean measur	red concentrat	tion (% of n	ominal) 🔗
Control	-	·	N W	ð .	4) ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~
30	× 8			Å.	× , ~
77	, M	02 Û		la L	à s.º
200		03		» 0	E O
Mortalities and clinical observ	ations				

Mortalities and clinical observations

of the concentrations tested. Some There were no mortalities or overt signs of poxicity observed at any were not deened to be treatmentincidental clinical observations and injuries were noted, however related. \bigcirc

All surviving adults were subjected to gross necropsy following test termination. All necropsy findings in the 28.4 and 78.8 mg_as/kg diet treatment groups were considered incidentak to treatment. While there were no treatment related findings among drakes in the 205 mg a.s./kg die freatment group, there may have been a treatment related increase in the mcidence of hens that exhibited lesions pathognomonic of egg yolk peritonitis.

Adult body weight

There were no apparent treatment delates effects Ъn adult body weight at any of the concentrations » O tested.

Mean	Şex 🞸	Mean body weigh	vt (g) 🖉 🎒		
measured 👸	6 1		<u> </u>		
concentration		Test initiation	Week 8-5	Test	Total change
(mg a.s./kg				termination	
diet) 👋			, O.		
Control	Male	SP180	1145	1204	24
Control	Female 4	1052	2049	1154	101
28.4	Male A	1184 0 %	1165	1197	12
N N	Female	14955 Q 🛇	1029	1130	74
78.8	Male "	9183 J	1196	1188	5
L.	Female 🖉	10 59 ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	1081	1156	97
205	Mate S	1183	1204	1211	28
	Małe V Pemalo	A052 ~ V	1076	1152	100

Mean adult body weight aften dietary exposure to KWG 4168 Table CA 8.1.1.3/02-2

Differences between the control and each treatment group were not significant (p>0.05) Only surviving birds were included in the calculations for each body weight interval

Adult Jeed consumption

There were no treatment-related effects on feed consumption in the 28.4 and 78.8 mg a.s./kg diet treatment groups. Slight and inconsistent significant reductions in feed consumption were occasionally observed, however were not considered to be treatment related.



At the 205 mg a.s./kg diet test concentration there were statistically significant treatment-related reductions in feed consumption during weeks 11 to 17. While not always significantly different, feed consumption for the 205 mg a.s./kg diet was consistently lower than that of the control group from week five until the end of the adult test period.

Mean measured	Feed consumption (g/bird/day) by week									Î) Î	
concentration (mg a.s./kg diet)	1	2	3	4	5	6		8		10 0 57 %	
Control	105	134	123	106	1 12	104 🖓	12 4 ,°	1:09	Q 14	0154 L	
28.4	106	143	126	109	108	103/	. 1 0 2	M5 \	104, 🖗	1490	
78.8	119	161*	141	131*	120	A95 🖌	132 <u> </u>	″ 118 [°] O	112	459	
205	119	147	126	108	Ø10 🗴	99 🖉	11.80	10	92	139	
	11	12	13	14	15	16.0	170	18	519	20 🔨	
Control	178	173	183 🔬	182 ~	1847	488	A76 é	190	187 🛇	193	
28.4	153	159	161	155*	b61	055**	163 🔊	169	175	436	
78.8	161	162	170	166	¥ 1 84	177 🔊	16	189	A 97	9 ₈₅	
205	141**	138**	A9**	148**	154	1A2@*	146*		163 🖉	163*	

				ρ <i>ω</i>
	Mean feed consumption		~~ V	a .
T 11 C 1 0 1 1 2/02 2		1 1 1 1 1		TATIO 11 (D)
	Nage tood consumption	during and attar dia	tory ovnosure to	K W/(- / 168%
A = A = A = A = A = A = A = A = A = A =				

* Significantly different from the control at p < 0.05** Significantly different from the control at $p \neq 0.01$

Reproductive results

There were no apparent treatment elated effects on reproductive performance at the 28.4 and 78.8 mg a.s./kg diet test concentrations

At the 205 mg a.s./kg thet test concentration there were slight reductions in a number of parameters that indicated effects on egg production, esg viability and embryo survival. The reductions were also reflected as reductions in the numbers of hatchlings and 14-day old survivors. While none of the differences from the control were statistically significant, they may nonetheless have been treatmentrelated.

Table CA 8.1.1.3/02-4 Summary of mallard reproductive performance after dietary exposure to KWG

^				
Parameter	Test concentration	(mga.s./kgdiet)	-	
Q.	Control ~	28.4	78.8	205
Total eggs laid	751 ° (783 0 0	767	642
Eggs cracked		5 8 8	7	15
Eggs set 🔊	634 2 4	698	684	546
	2605 Q	6 738 °∼γ	631	455
Live 3-week embryos	584 584	626	610	423
Hatchlings 💞	581 × ×	482	422	288
14-day survivors	1 991 (C)	494	411	285
Eggs laid/hen	47	Q49	48	40
Eggs laid/heroday1		0.61	0.60	0.50
14-day surveyors/hen	24 0	30	26	18

Differences between the control and each treatment group were not significant (p>0.05)

¹ Based on 80 days of egg production

There were no apparent treatment-related effects on eggshell thickness.



 \bigcirc

Mean shell thickness (±SD)	l l l l l l l l l l l l l l l l l l l
0.382 ± 0.023	, Å _ Å
0.385 ± 0.020	S 10
0.372 ± 0.025	
0.363 ± 0.020	
	$\begin{array}{c} 0.382 \pm 0.023 \\ \hline 0.385 \pm 0.020 \\ \hline 0.372 \pm 0.025 \end{array}$

Differences between the control and each treatment group were not significant ($p \ge 0.05$)

Offspring body weight

There were no apparent treatment-related effects on the body weights of hatchlings of 14-day old survivors at the 28.4 mg a.s./kg diet test concentration. While there were no treatment-related effects on the weight of hatchlings or 14-day old survivors at the 78.8 mg a.s./kg diet test concentration, there was a slight but statistically significant (p<0.05) decrease in the mean body weight of hatchlings. The difference between the mean of the control group (38 ± 2 g) and the mean of the 98.8 mg a.s./kg diet group (36 ± 2 g) was only about 5%, with both means well within historical control values for the parameter. This slight difference in mean hatchlings weight is therefore not considered to be biologically significant.

However, in the 205 mg a.s./kg diet test concentration there was a statisticall significant (>0.01), treatment-related reduction in hatching body weight. The difference between the test group and the control was greater than 10%, and the mean body weight (33° g) was outside the range of historical control values.

Table CA 8.1.1.3/02-6 Mean body weight othatchings and 14-day survivors after parental dietary exposure to KWG#168

<u> </u>		
Mean measured concentration	Maan body weight (g) (+SD)	
(mg a.s./kg diet)	Hatchlings S S	Aday survivors
Control		$289 \pm 20^{\circ}$
28.4		294 26
78.8	36*±2 ~ ~ ~	290/± 21
205	93**±9 0 0	@ 76 ± 32

Only surviving hatchlings were weighed

- Significantly different from the control at p < 0.05
- ** Significantly different from the control at p < 0.010

III. Conclusion

Mallard ducks (*Anas platymenchov*) were exposed to KWG 4168 at mean measured dietary concentrations of 0, 28.4, 78.8 or 205 mg a.s./kg diet for 20 weeks. There were no mortalities, overt signs of toxicity or other beatment-related effects on body weight at any of the concentrations tested. There were also no treatment-related effects upon feed consumption or reproductive performance at the 28.4 and 8.8 mg a.s./kg diet test concentrations.

At the 205 mg a.s./kg diet test concentration there was a treatment-related reduction in feed consumption and an increase in the number of hens displaying lesions of egg yolk peritonitis at terminal necropsy. Additionally, there were reductions in egg production, viable eggs and embryo survival that were reflected as eductions in the numbers of hatchlings and 14-day old survivors. There was also a treatment-related reduction in the body weight of hatchlings in the 205 mg a.s./kg diet treatment group.

Based upon the multiple effects seen at the highest test concentration, the NOEC and LOEC for this study were determined to be 78.8 and 205 mg a.s./kg diet, respectively.

Assessment and conclusion by applicant:

The study was conducted to the OECD test guideline "OECD 206: Avian reproduction test", adopted 4 April 1984 and meets the requirements set out within. Due to there being only three test



concentrations with a wide spacing factor between, the data are not considered suitable for reliable EC_{10} and EC_{20} calculation therefore this has not been conducted.

Validity criteria according to the OECD 206 (1984) guideline were met:

- Control mortality to not exceed 10% at the end of the test (actual: 0.0%)
- Average number of 14-day-old survivors per hen in the controls to be at least 14 (act 24)
- Average eggshell thickness in the control group to be at least 0,54 mm (actual

The study is therefore considered acceptable.

The NOEC determined from this study was 78.8 mgas./kg diet which is equivated a.s./kg bw/day based on a mean body mass of 1138 g/bird and a mean feed consumption 152.5 g/bird/d.

Data Point:	
Report Author:	
Report Year:	1998 A A A A A A
Report Title:	Results from the KWG 4168 northern bobwinte pilor reproduction study
Report No:	
Document No:	M-008 101-1
Guideline(s) followed in	
study:	
Deviations from current	None & C & A & A & A & A
test guideline:	No. pot previously submitted and a start of the second start of th
Previous evaluation:	
GLP/Officially	Yes conducted under GLOOfficially recognised testing facilities
GLP/Officially	
lacinues.	
Acceptability/Reliability	Supportive only 2 2 2 2

Executive Summary

The toxicit@of technical KWG \$168 was evoluated in a northern bobwhite pilot reproduction study using an & week exposure during egg aying. Nominal die ary levels were set at 0 (Control), 100, 447, and 1000 ppm a.i.. The results from this prior study were used in setting test levels for the definitive northern bobwhite and mallard reproduction studies.

Based on all parameters measured, then observed effect concentration (NOEC) for this study was <100 ppm and the lowest observe deffect concentration LOEC was 100 ppm.

W@ 4168

ot reported

lot reported

I. Materials and Method

A. Materials **Test** Material Lot/Batch Not reported Purity Not reported Description Stability of test compound:

Not reported Reamalysis/Expiry dâte:

Density: Not reported



Treatments

0, 100, 447 and 1000 ppm **Test rates:** Solvent/vehicle: Not reported Analysis of test None concentrations: 21 days - Upon arrival, the birds were housed as pairs. The photoperiod in the room was 17 hours light/7 hours dark per day to bring the birds into the reproductive state. **Test organisms Species:** Source: Acclimatisation period: Feeding: **Test design Test vessel:** Not reported **Replication:** Sot reported No. of animals/vessel: **Duration of test: Environmental tes** conditions Not reported Temperature: Not reported Relativedrumie 17 hour Hight and hours dark Photoperiod: A. B. Ő Study Designy The toxicity of technical KWG \$168 mas evaluated in a northern bobwhite pilot reproduction study

using an 8-week exposure during egg aying. Nominal dietary levels were set at 0 (Control), 100, 447, and 1000 ppm@a.i.. The results from this pilot study were used in setting test levels for the definitive northern bobwhite and mailard reproduction studies.

Adult northern bobwhites (approximately 22 weeks old) were obtained from Barrett's Quail Farm and were held for approximately 21 days prior to initiation of the study. Upon arrival, the birds were housed as pairs. The photoperiod in the room was 17 hours light/7 hours dark per day to bring the birds into the reproductive state. Prior to study initiation the Dirds were randomised into four test levels (control, 100, 447 and 1000 psm a.i, KWG 4168) of twelve pairs each. The appropriate feed was then presented to the birds for an eight-week exposure period. During this time adult body weight, feed consumption, survival, and reproductive success of ere monitored.

The monotored study end-points for the adult birds were body weight, feed consumption and survival, whereas, the coproduction or d-points were number of eggs laid per hen, number of cracked eggs per hen, number of fertile eggs, number of vible embryos, number of nominal hatchlings and hatchling body weights.

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II. **Results and Discussion**

There was a statistically significant reduction (p < 0.05) in female body weight in the 447 ppm treatment group at weeks 1, 2 and 3 and at study termination. This was not considered a treatment related reduction for three reasons: 1) the mean body weight of this group was 19 g lighter than controls at the indiation of the study and stayed approximately 30 g lighter than controls during the remainder; 2) the 4000 ppin group did not differ significantly from the control; and 3) there was no trend for body weight reduction. There were no trends or statistically significant effects noted in male body weights.

	Eight	Weeks	ŵ	Ć	S #		
Study	Nominal Dietary	Concentration ((ppm) 🕰	Q,		f h	
week	Control	100		447 🦴	Ű,	/ 1000 [″]	Ô Ô
Initial	276 ± 22	263 ± 21		•257 ± 20 2	y jø	281±34	
1	286 ± 15	271 ± 20		256 15		282 ± 28	y a
2	285 ± 14	276 ± 19		$255^{d} \pm 19^{0}$		282 ± 28	
3	293 ± 18	280 ± 22 🔬		$261^{d} \pm 16^{d}$	A C	288 ± 28	
Terminal	298 ± 17	288 ± 39 5		270 ^d 16		235 ± 27	
Change	21 ± 14	25 ± 39	L L	13 3/12		$@4 \pm 18$	0

Table CA 8.1.1.3/03-1 Body weight (g) for Female Northern Bobwhite Fer KWG 4168 in the Det for

dStatistically significant from control (Bunnett Fone-tabled test, $p \le 0.05$).

Table CA 8.1.1.3/03-2	D 1 1 (1)					. A		11.0		
Table CA 8.1.1.3/03-2	Body weight	(g) ¥ør	Mal@Nor	thern	Bolowhi	iteded	KW-G	4168 in 1	the Diet for E	ight
	Walte	\sim	~	"0"	d a	a S	a	<u> </u>	<u> </u>	0
	vv eeks 🖉 🔊	Ŷ	\approx		4	, V	(\mathcal{O})		0	

Study	Nominal Dietary C	oncentration (ppn)		, Q
week	Control 🔊		447	
Initial	263 ± 25 🔬 🦼	₹252 ±209 0° °	259 ± 21 (255 ± 16
1	269 ± 26	258¥21	260 ± 20 3	259 ± 16
2	270 ± 25 ~	208 ± 210	261 21	258 ± 18
3	277 ≭28 \	(262 ± 21 ~ ~ ~ ~ ~	266≇21 0 ~~~	260 ± 17
Terminal	2710 ± 36 ℃	269 ≇ 21 ,	272 ± 21	254 ± 41
Change	8 ¹ 27 5 4	17±8	95±10 0	-1.3 ± 37

Feed consumption was significantly reduced p < 0.05) in the A47 and 1000 ppm treatment groups compared to the control group (Table 3). This was considered ω be treatment related as there was a dose-response trend for reduced feed consimption over treatment groups.

Table CA 8.1.1.3/03-3 Gverall Mean Feed Consumption (g/bud/day) for Northern Bobwhite Fed KWG A168 in the Diet for Right Weeks

Nominal Dietary	Concentration (ppn	n) Q Q	Fean Feed Consumption	
Control			$\sqrt{2}2.8 \pm 1.3$	
100 🖉			21.8 ± 1.8	
447 🔬			$20.8^{d} \pm 1.6$	
1000/			$19.3^{d} \pm 1.5$	

dStatistically significant from control (Donnett' One-tailed test; $p \le 0.05$)

Reproductive totals how clear dose response effect. There was a 36%, 54%, and 82% reduction in number of beggs later compared to controls for the 100, 447, and 1000 ppm treatment groups, respectively. As expected, the trend is seen in the mean number of eggs laid per hen, the mean eggs set per hen, and the mean number of tratchings per hen, where all three treatment groups were significantly reduced compared to the control group. Fertility of the eggs was significantly reduced in the 1000 ppm treatment group. Only 46% of the 1000 ppm eggs set were fertile compared to 90% for the control group. Although only the 447 ppm treatment group had a significantly reduced percentage of hatchlings of live threeweek embryos, there appeared to be a trend for a treatment effect on all treatment levels for this parameter and for percentage of hatchlings of fertile eggs per hen.



Table CA 8.1.1.3/03-4 Reproductive Totals for Northern Bobwhite fed KWG 4168 in the Diet for Eight Weeks

Reproductive Parameter	Nominal Dietary Concentration (ppm)								
	Control	100	447	1000 5					
Eggs laid	540	345	251	95					
Eggs cracked	3	3	6 🔊	2					
Eggs defective	13	11	15 🔬	185 25 6					
Eggs set	524	331	230/ "						
Fertile eggs	469	312	æ03	2 ³⁹ 2 0					
3-Week viable embryos	465	<i>"</i> 309	R193	0 39 2 2					
Normal hatchlings	410	246	L 137						

Table CA 8.1.1.3/03-5 Reproductive Success for Northern Bobwhite fed &WG 4168 in the Diector Eight Weeks

VV CCR5	×.	Q N	N N		×Q
Reproductive Parameter	Nominal Dieta	y Concentrat	ion (ppm)	O L	4 00
	Control 🖉	100 - 2	447 _©	_≈	
Eggs laid per hen in 8 weeks (\bar{x})	45.0±14.8	$\sim 28.8^{d} \pm 38.3$	20.9 ^d ⊕ ^v 1	1.7≰ 7.9 [₫]	≟ 8.4,©
Eggs cracked of eggs laid per cage	\$\$ ± 1,0%	0.6 ± 11	◎ 2.& + 4.4		± 7.5
(%)				Č V	<u> </u>
Eggs defective of eggs laid per cage 炎	2.1 £02.9 >>	5.2±9.5	₹.9 ± 9.3	23.3	∲ 27.0
(%)	à à	$\delta \delta$		<u>Č</u> 'N	,
Eggs set per hen (\bar{x})	43.7 ± 10.3	27.6 27.4			± 7.7
Fertile eggs of eggs set per hep (%)	90.2 11.9	94.54 6.8 €	a (1)		$^{d} \pm 43.6$
Live three-week embryos of fertile	99 Q ± 1.4	99.2 ± 2.0 ×	293.8ª±	0.0 🍙 100.	0 ± 0.0
eggs per hen (%)	ØR.		~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~		
Normal hatchlings per hen (\bar{x})	34.2±43.1 🔊	20.46€ 14.4	$11.4^{d} \pm 1$	2.1 ^d	± 4.5
Normal hatchlings of fertile eggs per 🖉	88.3 ± 8.3	7 8 .7 ± 25 ∅°	67.1±3	9.3 73.6	± 35.6
hen (%) $\mathbb{Q}^{\mathbb{Y}}$		ð a	$\rho \sim$		
Normal hatchlings of live three-week	89.1 ± 8.1	Q79.3 £29.7 _	<i>≨</i> y 69, 9 ± 2	29.0 73.6	± 35.6
embryos per hen ()					

dStatistically significant from control (Dunnett's one-tailed test; $p \le 655$)

There was no trend or statistically significant effect for reduced body weight in hatchlings.

Table CA 8.1.1.3/03-ở Hatch Body Weight Pata for offspring of Northern Bobwhite fed KWG 4168 in the

Reproductive Parameter S Nominal Dietery Concentrat	tion (ppm)	
Canfrol 100	447	1000
Hatchling body weight		
Mean Thatch Weight (g) 3 6.2 6.2	5.8	6.2
Stargard deviation $\sqrt{2}$ $\sqrt{2}$ 0.6 $\sqrt{2}$ 0.6	0.5	0.6

III. Conclusion

Based on all parameters measured, the no observed effect concentration (NOEC) for this study was <100 ppm and the lowest observed effect concentration (LOEC) was 100 ppm.

Assessment and conclusion by applicant:

The study was not conducted to a specific test guideline but clearly followed the general principles of OECD 200. The study was conducted as a pilot study in light of the definitive mallard duck and boltwhite quail studies and was non-GLP. The results are deemed valid but the study has been submitted as supporting information only.



Data Point:	KCA 8.1.1.3/04	
Report Author:		~
Report Year:	2006	N N
Report Title:	Comment on study SXR/REP 04 (GLP-No.: E 298 0738-7 by	
	(1995): Effects of subchronic dietary exposure of KWG \$168 techn. on bobwhite	
	quail including effects on reproduction and health,	
Report No:	<u>M-279402-01-1</u>	
Document No:	<u>M-279402-01-1</u>	"C
Guideline(s) followed in study:	None	*)
Deviations from current test guideline:	None	
Previous evaluation:	yes, evaluated and accepted RAR (2010), RAR (2017)	
GLP/Officially		
recognised testing facilities:	not applicable	
Acceptability/Reliability:	Yes y y y y y y y	

Executive Summary

Comment on study SXR/REP 04 (GLR No.: E298 0738-7)

The statistical re-evaluation of the body weight of 14-day old survivors revealed a NOEC at 77 mg a.s./kg food for this parameter.

The reproductive NOEC, cited in the report, is based on this parameter. There were no further endpoints which showed significant differences to the control at that concentration. Therefore it is justified to define the NOEC at 77 mg a.s./kg bood (nominal) or 78 6 mg cs./kg food (measured).

I. Results and Discussion

Comments on the NGEC

The Test Results, cited on page 8 in the report, read: "Based on the results of this study, the NOEC for adult Bod white Quail is 204 ppm KWG 4168 technical as the highest test concentration. Accordingly, the LOEC is > 204 ppm.

The reproductive NOEC was 20.3 pprov. The reproductive OOEC was 78.6 ppm, based on body mass development of the hat hing during the 14 day post-hat probservation period."

The absolute differences of the mean body weights between the treatment groups and the control were rather small. The body weight amounted to 962% of the control at 77 ppm, and to 91.2% at 200 ppm. Due to this subtle distinction, it was decided to subject the respective data to a new statistical analysis to validate the results.

Comment on the statistical analysis of the endpoint of concern

The relevant data for the NGEC setting, the body mass data of 14-day old chicks, were displayed in Table 5 on page 37 of the oport:

Table CA 8.1.1.3404-1 Body mass of 14-day old chicks from bobwhite quail fed KWG 4168 (technical a.s.)

	Study	week n	umber								
Control	1	2	3	4	5	6	7	8	9	10	1 – 10
Mean body mass (g):	32.6	34.4	30.2	35.6	36.5	33.8	34.8	34.6	32.3	32.3	33.9
Standard deviation:	2.1	4.1	5.8	4.7	4.0	5.0	4.4	4.3	5.7	6.8	5.3
Total no. of survivors:	3	27	49	66	56	78	67	67	71	60	544



	Study	week n	umber								
29.3 ррт	1	2	3	4	5	6	7	8	9	10	1 - 10
Mean body mass (g):	32.1	30.9*	29.6	32.8*	33.4*	32.9	34.6	33.5	35.3	34.1	1 - 10 - 32
Standard deviation:		6.8	5.2	5.7	4.7	5.2	4.6	5.2	4.9	5.0	ĴА и
Total no. of survivors:	1	37	56	76	70	72	75	78 Ĉ	¢ 61	74 🤅	600
78.6 ppm	1	2	3	4	5	6	7	8 31.4*	9	10 🗇	1-40
Mean body mass (g):		32.6	31.5	34.1	30.1*	33.7	30.7*	3 1.4*	34.3	3Q:7	32,6*
Standard deviation:		4.9	4.0	3.8	6.2	3.6	4.6	Å ?4	4.3 🗞	<u>9</u> .9	¢.6 🔬
Total no. of survivors:	0	23	41	71	51 🗘	66	61 😽	80	60 🔬	83 🔊	\$536 \$
204 ppm	1	2	3	4	5 🕅	6	7	8	9	100″	2330 × 530 1 × 10 3029*
Mean body mass (g):		28.6*	29.7	30.3*	30,8*	30.4*	<i>3</i> 0.6*	31.3*	\$2.3	ð1.7	30 .9* (
Standard deviation:		7.8	3.9	4.6	s¥.0	4.4 🖌	0 ⁴ .3	₀5.1 _م	P4.0 °	3.9 (A.7 _@
Total no. of survivors:	0	22	31	54 .0	r 43	62	¥44 _?	64 Q	56	47	423

Reported are the mean body weights of all clicks per test group on a weakly basis (column 1, 2, 3 ... 10). The value of the column 1-10 is not the mean of all weekly data, but the mean body weight over all chicks per concentration, which were produced in this study (verified b) the original excel spreadsheet). This overall mean gives a good impression of the quarry of the chicks of the control and the treatment groups. But this way of condensing the data results in only one value per group. With such a data set, a statistical analysis is not possible.

It remained unclear, which data were subjected to statistical analysis. In principal 3 methods may be considered:

1. Statistic with the weekly means

The way the data are presented in the study suggests that this may have been the choice of the author. The number of replicates would be in our example -10. This procedure cannot be recommended since in many cases the reproductive success is seaker during the first weeks (including the control). This can lead to quite high standard deviation in all groups, which reduces the statistical power of the test, so that possible effects can easily be masked.

2. Statistic with the body weights of all single checks

The number of replicates would amount to some hundreds But since chicks of one cohort are reared together, the request of independent replicates is not obeyed. Another argument against this procedure is that results cannot be related to the exposure of the distinct pairs.

3. Statistic with mean body weight data per par

The most ofter used procedure is to calculate the mean body weight of all the 14 day old chicks, which were produced by each single pair. This method procedure is 18-20 replicates per group, which can be submitted to statistical analysis A problem of this procedure is the way how prematurely adult birds should be considered in particular if there is no indication of a treatment related mortality but more a housing artefact. It is a common practise to exclude them, to avoid an unreasonable increase of standard deviations and to eliminate statistical effects, caused by artefacts. But in general this method is the most appropriate and most accepted one. It is the way, US- EPA normally performs the statistic evaluation of bird reproduction studies, and the author of our study described this method as the one used. Unfortunately the underlying data sets are not displayed in the study.

Re-evaluation of the body mass of 14 day old survivor

Since it was not completely clear how the statistic was performed, it was decided to re-evaluate the data on a per par basis (method 3). We excluded one pair at 30 ppm and one pair at 200 ppm. In both cases one bird of these pairs died prematurely during the run of the study. At 200 ppm one pair produced no chick at all, therefore no analysable data exist for this pair and this endpoint (body weight of chicks).



Hence, the number of replicates amounted to 20 for the control, 19 for the 27 ppm group, 20 for the 70 ppm group and 18 for the 200 ppm group. The data were analysed on equal variance with the Bartlett's test. It revealed that homogeneity had to be rejected.

Then the Welch-t test for inhomogeneous variances with Bonferroni adjustment was performed. The program suggested a NOEC of 77.0 mg a.s./kg food (see below).

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Statistical Ev	(1.1.1) (1.1.1) (1.1.1) (1.1.1)	******			3	0X		> 0
ii				¥	đ	Q	Ű.	9 🖉
Relation of Mea	sured Re	esponse	on Conc	entration	n at 0,0 d		Nº Q	
				A	Q, ^y	e d	,0 , , , , , , , , , , , , , , , , , , ,	Õ.
ļ				O I		R Q	, 0	is a
Bartlett's Test F	Procedur	e on Var	riance Ho	mogene	ity 🔊 🧳	Y m	A X	J .S
			×	- Q			Q 7	
lomogeneity of varia	ance was te	sted (Alpha	a = 0.05); va	ar: wariance	df: deglees	officedom	Ber 🔗	A
variance; c: Bartlett o	correction; c	fm: deg. o	f freedom n	utiple test.	p(Chi ²), prob	ability of	·0	S V
Chi², if Ho: var1 = va			L.A	$\gamma \sim \gamma$	à L	, O	N.	No.
			V in	A N	× O	1		0
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(Chi ²) <= Alpha, bo	mogeneity h	voothesis	is rejected	s. Y	5° Q'	15		
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2Q2	Ű	\$ 8		S A		1		
Nelch-t test for	Innomo	geneous	Variance	es with E	Sonferroni	Adjustm	ent	
	9° 4	,C	~ ×	, &	2			
Aultiple sequentia	reiective co	moarisons	after Weich	of treatme	with "Cor	trol" by the		
test procedure, Sig							ple	
size; s²: varian@e; %l								
sample t; p(t): proba								
he differences are s								
neteros dascity. (T								licates
(i): 🕼 number of tre	atments)A		0, ×	Y and applied	a)		Guinentie	induced
n(i); k number of tre	S	2	Q ~~					
reatm. [mg a.s/k	Mean	S. P	√ √ df	%MDD	1	p(t)	Alpha(i)	Sign.
Control	433 224	10.85	Q^{ν}	,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,		P(4)	, (p)((d))	Jigin
	> 33,0	10,625	Ø 68	6,3	-0,15	0,880	0,050	
77 0	32 097		93				0,030	
2000	32, 9 97 30,050	0 10,625	41	8,0			0,023	
	00,000	10,020	4 1	0,0	-3	0,005	0,017	т
-: significant; -: n-q-signi	TICAN							

II. Conclusion

The statistical re-evaluation of the body weight of 14 day old survivors revealed a NOEC at 77 mg a.s./kg food for this parameter.



The reproductive NOEC, cited in the report, is based on this parameter. There were no further endpoints which showed significant differences to the control at that concentration. Therefore it is justified to define the NOEC at 77 mg a.s./kg food (nominal) or 78.6 mg a.s./kg food (measured).

Assessment and conclusion by applicant:

This non-GLP statistical re-analysis report has been submitted in order to justify the use of the NOAEC of 78.6 mg a.s./kg food (equivalent to 5.40 mg a.s./kg bw/day) derived in the botwhite quail reproduction study (M-007470-03-1).

The report is considered acceptable and has been used to help justify the use of a NOAEL 5.40 a.s./kg bw/day in the Tier I risk assessment.

Data Point:	KCA 8.1.1.3/05
Report Author:	
Report Year:	
Report Title:	Evaluation of historical control data on boowhite quail 12-d chick body weights
	to establish the NOAES in the study SOR/REP 04 with spiro amine
Report No:	$\underline{M-304591-0} (\underline{M} \times \underline{M} \times$
Document No:	M-304591 0Y-1 4 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7
Guideline(s) followed in	OECD 206 7 7 7 7 7 7 5 5
study:	
Deviations from current	None of the second seco
test guideline:	
Previous evaluation:	RAR (2010) RAR (2017)
2	
GLP/Officially	not applicable
recognised testing	
Acceptability/Reliability: (Yes Y L Y Y L A

Executive Summary

The effect of KWG 4168 (spirovamine) on the veproduction of Bolywhite Quail has been investigated by (1995) according to OECD guideline 206 (1984) and EPA FIFRA guideline 71-4 (study M-<u>M-007470-03-1</u>)

In this study, adult Bobwhite quail were exposed over 21 weeks to nominal dietary concentrations of 0 (control), 30, 77 and 200 ppm in the diet (equivalent to mean measured concentrations of 0, 29.3, 78.6 and 204 ppm in the diet).

Effects of exposure on the reproductive performance of the birds were evaluated based on the egg production over 11 weeks, hatching success and body weight of the chicks at hatch and 14-day posthatch.

The study author concluded that "The NOEC" for adult Bobwhite Quail was 204 ppm KWG 4168 technical a.s., the highest test concentration Accordingly, the LOEC is > 204 ppm. The reproductive LOEC was 78.6 ppm, based on body mass development of the hatching during the 14 day post-hatch observations period."

The mean 14-d chick body weight in this study at the different treatment levels, and relative differences to the control of presented in the table below.



Treatment level (ppm)	14-d chick bw (g)	Relative to control (%)	Difference	
0 (control)	33.9	100	-	P. Q.
29.3	33.2	97.8	2.2	
78.6	32.6	96.3	3.7	
204	30.9	91.1	۶ 8.9	

Table CA 8.1.1.3/05-1	Mean 14-d chick body weight in study SXR/REP 04
1 abic C/1 0.1.1.5/05-1	mean 14-d chick body weight in study 5200 KE1 04

The differences of the chick body weights between the treatment groups and the control were rather small (< 5% in the 78.6 ppm group), and the statistical significance of the differnce vatures according to the various methods used (e.g. 2006).

It is toxicological practise to consider differences $Sf \leq 5\%$ in sublethal parameter compared to control to be usually within natural variation and without toxiciolgoicabrelevances (NOAEL). S

I. **Results and Discussion**

 \bigcirc Historical control data for the endpoint of concern for the study of (1995) were obtained from 59 regulatory studies reported by various aboratories which met the above defined criteria.

These studies include 13 results of the Con-site historical control", Qe. the Bobwhite quait reproduction studies conducted to the same guide mes in the laboratory of

From any other laboratory, not more than a maximus of 10 studies was pecluded in this exercise, in order to avoid any potentially undue influence of a new more frequently encountered report sources in the archive of

An overview over some descriptive statistics for this pristories control is presented in the table below.

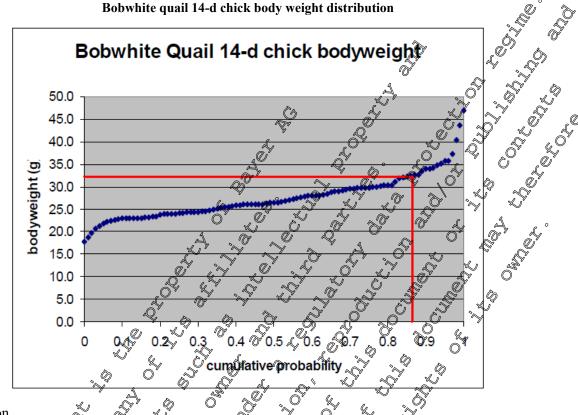
Table CA 8.1.1.3/05-2 Body mass of 14-dayold chicks from bobwhite quail fed KWG 4168 (technical a.s.) in diets for @21-week period L Ø

			<u> </u>	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~		
		14-d chick bo	dyweight (g)		K, V	
Lab code 🧳	# of stordies _ (🕽 Arithmetic 🐇	Standard	25 th	, 50 th	75th
		mean «	deviation	🛾 percentile 🛷	percentile	75 th percentile
BCS DE 🎻	13 🔬	287	7.9	22. 8	29.4	22.6
BCS US	10	Stó 7 🛇	6 .8	29.1	29.7	30.2
BLAL	4	30.9 6	2.0 × 4	29.9	31.3	32.3
EBA	4 3	265 1	1.	25.9	26.4	26.6
EPT	10 2			20	32.5	
	Ø7 .0 [%] (25.0 ° (2.5 0	@ 3.4	26.0	26.6
LPT 🔨					31.7	
NOTOX 🔔	4 ^{O*}	250 4		25.2	25.4	25.7
ProTox 🖉 🎽	5 . Q	3.4 V	A.6 🔪	24.3	25.0	25.8
Wildlife	10 ~~ 4	24.9~	2.2,~Q	23.0	24.0	26.0
			~			
Total	59 0	20.5	<u>5</u> Q'	24.1	26.5	29.8

As displayed in Fig. 1, the body weight of 32.6 g as reported from the 78.6 ppm treatment level in the is corresponding with the 87th percentile in the distribution of the historical control study of of 14-d chick body weights. The control group of this study represented the 90th percentile in the historical control data distribution.



Figure CA 8.1.1.3/05-1



Evaluation

The mean body weight of 14-d old chicks at the 786 ppor treatment level in the Bobwhite quail reproduction study by Schmuck (1995) was 32.6 g (377% dollerent from the concurrent control).

The mean body weight of 14-d old chicks of historical controls 327.5 g, with a standard deviation of \pm 5.1 g.

Thus, the 4-d body weight of 32.6 gobtained at 78 % ppm piroxamine is within the standard deviation of the historical controls.

Also the presented distribution of the historical control suggests that a body weight of 32.6 g is not to be considered biologically significantly reduced, since it is corresponding with the 87th percentile body weight of chiefs from (untreated) controls reported from 59 studies conducted according to the same guidelines.

Since the 94-d chick body weights at 98.6 ppm in the avian reproduction study with spiroxamine do not differ by more 3.7% of the concurrent control, and are well within the normal range of the historical control, this difference should not be considered as adverse, being within normal variability.

Therefore Bayer Crop Science propose that the 78.6 ppm level of the Bobwhite quail reproduction study (1995) should be considered as ecologically acceptable no (observed) adverse effect level (NOAEL) for the reproductive tisk assessment for spiroxamine.

II. Conclusion A

The historical control evaluation of the body weight of 14 day old survivors consisting of 59 comparable studies with Bobwhite quail suggest that the magnitude of effects (3.7%) observed at the 78.6 ppm level can be considered as being within the normal variation of untreated birds and as such not adverse.

There were no further observations with significant differences to the concurrent control at that concentration or below.



Therefore it is considered justified to define the NOAEC at 77 mg a.s./kg food (nominal) or 78.6 mg a.s./kg food (measured).

Assessment and conclusion by applicant:

This non-GLP historical control analysis report has been submitted in order to fustify the use of the NOAEC of 78.6 mg a.s./kg food (equivalent to 5.40 mg a.s./kg bw/day) derived in the bobwhite anall reproduction study (M-007470-03-1). The results demonstrate that the mean body weight of 30.6 g achieved at the 78.6 mg a.s./kg diet dose group was well within the normal deviation of the historical control data from 59 regulatory studies and is not therefore a biologically relevant reduction

The report is considered acceptable and has been used to help justify the use of a NOAEE of 5.40 a.s./kg bw/day in the Tier I risk assessment.

Relevant literature on birds

No relevant scientifically peer-reviewed open literature could be found on spiroxamine or its major metabolites, from an ecotoxicological perspective on birds? Details of the literature search undertaken can be found in the M-CA Section 9 of the current submission

CA 8.1.2 Effects on terrestrial vertebrates other than birds

A summary of essential data required for compliance with data point (A 8.12) is presented in the table below.

Organism	Test iten	Testaype	Endpoints		Reference
Rat	Spiroxamile	Acute oral		EU	<u>M-007791-01-1</u>
Mouse 39 39 39 39	Spirosomine &	Acute oral Soxicity Acute oral Acute or	LD_{50} 460 tog a@:/kg.bw?(male) LD_{50} 501 mg a.s./kg bw (female)	EU	<u>M-007804-01-1</u>
	Spiroxamiae	reneration	NOAEL (parental) 3/9 5.5 / 6.7 mg a.s./kg bw/day NOAEL (reproductive) 3/9 21.0 / 21.2 mg a.s./kg bw/day NOAEL (offspring) $3/9$ 6.5 / 6.7 mg a.s./kg bw/day	EU	<u>M-304231-01-1</u>

Table CA 8.1.2-1Summary of mammalian toxicity studies with spiroxamine

EU: previously valuated as part of the original EU review and listed in EFSA conclusion and DAR

Acute oral toxicity to mammals

Summaries of the available acute oral data used to derive endpoints for the wild mammal risk assessment are provided in Document M-CA Section 5.2.1 and Section 5.8.1.4.



CA 8.1.2.2 Long-term and reproduction toxicity to mammals

Summaries of the available reproductive data used to derive endpoints for the wild mammal ask assessment are provided in Document M-CA Section 5.2.1.

Relevant Literature on Terrestrial Vertebrates other than Birds

No relevant scientifically peer-reviewed open literature could be found on spiroxamine or its major metabolites, from an ecotoxicological perspective on wild mammals. Please refer to the A-CA Section 5 for details of the review of literature on mammals from a human toxicological perspective. Details of the literature search undertaken can be found in the M-CA Section 9 of the current submission.

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

According to the EFSA Guidance Document in Risk Assessment for Birds and Mammals (2009), for active substances with a Log $P_{ow} > 3$, an assessment of the risk of bioconcentration of the substance in the prev of birds and mammals (secondary poisoning) shall be provided in the document M-CP Section 10. Spiroxamine has a Log P_{ow} of 2.79 and 2.98 at pH 7 for diastomets A and B, respectively but at pH 9 these value are 4.88 and 5.08, respectively. Thus, the potential risk from bioconcentration needs to be addressed in the risk assessment.

The Log P_{ow} of spiroxamine-desethvl (M01) is 2,41, 197 and 3.64 a pH 4,7 and 9, respectively. The Log P_{ow} of spiroxamine-desproyel (M02) is 1.99, 1.41 and 20,44 at pH 4,7 and 9, respectively. The Log P_{ow} of spiroxamine-N-oxide (M03) is 2.82, 2.59 and 2.63 at pH 4,7 and 9, respectively. The Log P_{ow} of spiroxamine-carboxylic acid (M06) is 0.45, -0.25 and 0.10 at pH 4,7 and 9, respectively. Thus, an assessment of the potential risk from bioconcentration of spiroxamine-desethyl (M01) and spiroxamine-desproyel (M02) also needs to be addressed in the tisk assessment.

An earthworm bioconcentration study using spirovamine technical is available and has been summarised below.

Data Point: O	KCA 8.1.3/01
Report Author:	
Report Year:	
Report Title:	Study on the bioaccumulation of [1,3-dio olane-4-14C] spiroxamine on the
~``	earthworth Eiseola fetida, tested in artificial soil with 5 percent peat
Report No:	LRT-&G-B-Q[/11_0 0 0
Document No:	<u>M-491910-01-1</u> ~
Guideline(s) followed n	Proposal for a new Guiderine for OECD Guidelines for the Testing of Chemicals
study:	Bioaccumulation in teastrial Oligochaetes, Draft Document, November 2009
Deviations from current	Yes OEQD 317 (2010) pH value was not recorded for the separately produced artificial soil
test guidefine:	OEQD 317 (2010)
	pH on day 0 opsoil was 6.63, which is slightly outside of the specified range of
	6.0 ± 9.5 0
L . 1 ¹	Due to the equal composition of the artificial soil, the result of the TOC – content
	setermination for the first run was also used for the metabolism investigation part
	Sand the 2nd rup of the study.
	These deviations were not considered to have any influence on the study.
Previous evaluation:	No. not previously submitted
	Yes, conducted under GLP/Officially recognised testing facilities
recognisea testing	
facilities:	
Acceptability/Reliability:	Yes



Executive Summary

The purpose of this study was to determine the bioaccumulation of spiroxamine in earthworms, Eigenia fetida. Adult worms were exposed to soil, spiked with a mixture of ¹⁴C-labelled and non-labelled spiroxamine at a concentration of 5.09 mg spiroxamine / kg dry weight soil.

Test vessels without the addition of the test item served as controls. The test animals (one per test vessel, 3 replicates per treatment group) were exposed to the spiked soil for 21 days to assess the bioaccumulation kinetics of the test item in the test organisms (uptake phase). After the uptake phase the test animals were exposed to untreated control soil for a period of 21 days (elimination phase).

The calculated bioaccumulation factor (BAF) was 1.56. It can be concluded, that the bioaccumulation of spiroxamine in earthworms is considered to be low.

I. **Materials and Methods**

A. **Materials**





Number of organisms per vessel:	One Exposure: 21 days
Duration of test:	Exposure: 21 days
Environmental test conditions	
Temperature:	$20 \pm 2^{\circ}C$
pH:	
Water content:	Then, the soil was moistened with deconsed water to reach a water content of 58% of the maximum water holding capacity
Photoperiod:	16-hour light to 8-kour dargeness abotonessing and a light intensity of 429
B. Study Design	- 799 Lux

The purpose of this study was to determine the bioaccumulation of spiroxamine in earth corms, *Eisenia fetida*. Adult worms were exposed to soil spiked with a mixture of ⁴C-labelled and non-labelled spiroxamine at a concentration of 509 mg Spiroxamine / kg dry weight soil.

Test vessels without the addition of the test item server as controls of the test animals (one per test vessel, 3 replicates per treatment proup) were exposed to the spiked soft for 21 days to assess the bioaccumulation kinetics of the test item in the test organisms (uptake phase). After the uptake phase, the test animals were exposed to untreated compol soil for a period of 21 days (elimination phase).

In addition other parameters (*e.g.* mortality, weight, lipid content and total carbon content) were recorded for the eard worms during the study. Bioaccumulation factor (BAF) is based on the parent compound.

Three days prior to the tart of exposure, the set organisms were acclimated to the artificial soil and test temperature. The body wet weight of the test organisms at the start of the test ranged from 0.25 g to 0.44 g per worm? The worms were adult with a well developed clitellum and approximately 5 months old. The age of the worms from the synchronised culture differed by not more than 4 weeks.

The ¹⁴C - labelled test item was dissolved in actionitrile. The stock solution was prepared by weighing 0.0887 g of non – Dabelled test item into a 10 mL volumetric flask, adding 4.25 ml ¹⁴C - labelled test item solution (= 1.039 mg KMC 903204.25 MBq) and discriving this mixture in enough acetonitrile to achieve a total of 10 mL. All concentrations are based on the content of test item spiroxamine.

The stock solution was used to solden 470 g quartz sand. After evaporation of the solvent under a fume hood (overhight), the quartz sand was well nexed to ensure homogeneous distribution of test item.

160 g of the quartz sand / test item mixture was added to 1110.48 g (wet weight) of the artificial soil (pre-mixture). Subsequently, the remaining artificial soil (16.70 Kg wet weight) was divided by two and each of these portions was treated with equal amounts of the pre-mixture. Hence, the total wet weight amount of treated sou was 17.81 Kg (nominal 16 Kg dry weight). To ensure equal distribution of test item in the artificial soil, treatments were carried out by using a gyro wheel mixer (Elte 650, J. Engelsmann AG, Ludwigshafen, Germany), mixing terms: 15 min at 50 rpm. Subsequently both portions were thoroughly mixed together.

Approx. 2006 dwordry weight artificial soil) of the spiked artificial soil was filled into the test vessels to determine biological effects. This soil was equilibrated three days under test conditions. Approx. 500 g dws (by weight artificial soil) per vessel was used for metabolism investigations.

Three days prior to the start (2nd run) of the uptake/elimination test, required worms were held in untreated artificial soil for acclimatisation. At the start of the study, the worms were quickly washed



with water. Surplus water was absorbed on filter paper. Subsequently the worms were weighed and directly placed into the corresponding test vessels.

During the uptake phase, samples were taken at days 0, 1, 2, 4, 8, 10, 14, 17 and 21, during the elimination phase samples were taken at days 0.25, 1, 2, 4, 7, 10, 14, 17 and 21 after introduction of the worms into the soil. These measured values were used to determine the uptake and elimination of the test item in the worms, to calculate the uptake rate constant, the clearance rate constant and the steady-state bioaccumulation factor (BAF).

Samples for the uptake or elimination phase, respectively were incubated at a light intensity between 402 and 658 Lux (mean: 482 Lux, 16:8 light / dark frequency) and a temperature of $00 \pm 25^{\circ}$.

At the end of the incubation phase for metabolism investigations the worms were purged, washed weighed and frozen until the corresponding investigations. The worms remain in the substrate for 21 days.

Due to partly incomplete combustion of worfus within a first test, the obtained data were not suitable for further analysis. The corresponding data were archived with the raw data and were not suitable the report. Results of the metabolism investigation part of the first run were not affected by this technical error and therefore described in the report. The uptake and the elimination part of the study were repeated and the corresponding results were presented in the report.

II. Results and Discussion

The study was assessed against the validity criteria in the draft (2009) guideline DECD Guidelines for the Testing of Chemicals – Broaccumulation in terrestrial Oligochaetes

- Overall mortality in the control during the uptake and elimination phase 400 % (actual: 0 %)
- Overall mortality in the treatment group during the uptake and elimination phase $\leq 10\%$ (actual: 0%)
- Mean mass loss at the end of the uptake phase, compared to the initial fresh weight $\leq 20 \%$ (actual or none)
- Mean mass to ss at the end of the elimination phase, compared to the initial fresh weight $\leq 20\%$ (actual: none) \approx

The study was therefore, considered acceptable.

During the study earthworms and not show any cortality or signs of intoxication. The weight development was similar between earthworms exposed to proxamine and control soils.

Spiroxamine was mixed into the artificial soil theroughly and the concentration of spiroxamine in soil was confirmed by radiochemical analysis. The mean value measured during the uptake phase was 5.06 mg t.i. / kg dry weight soil, corresponding to 101.1 % of the nominal concentration.

During the elimination phase the mean value for the concentration of spiroxamine in the soil was lower than the natural background level radioactive radiation.

No test item was detected in the control soft (mean of the natural radioactive radiation of control soil from uptake of elimination phase, respectively: 0.32 Bq).

After an initial increase of the spiroxamme concentration during the first two sampling dates (day 1 and 2) of the uptake phase, the concentration in earthworms did not further increase over the testing period of 21 days (uptake phase). The average value measured in earthworms was 7.90 mg spiroxamine / kg dry, weight of worm in the treated group.

The calcolated bioaccumulation factor (BAF) was: 1.56.

The calculated kinetic bioaccumulation factor (BAFk) was: 1.68 g soil/kg of worm.



Based on the lipid content of the worms and the total organic carbon content (TOC) of the artificial soil, a biota soil accumulation factor (BSAF) was calculated: Q_{μ}°

BSAF: 0.72 kg TOC/kg lipid

Uptake of test item from the treated substrate: day 1 = 0.47 %, day 21 = 0.93 %

Remaining test item in worm after elimination: day 0.25 = 51.0 %, day 21 = 19.6 %

After extraction, the TRR (Total Radioactive Residue) in the earthworm sample was calculated from the sum of radioactivity in the extracts and remaining solids (PES – post extraction solids). The TBR amounted to 1.071 mg/kg. Approximately 80% of the TRR was extractable. The main part of the extracted residues in earthworms were parent compound (0.548 mg/kg, 51.1% of the TRR).

III. Conclusion

It can be concluded that the bioaccumulation of spiroxamine in earth vorms is considered to be how

The calculated bioaccumulation factor (BAF) was: 456.

Assessment and conclusion by applicant

The study has been assessed against the validity enteria of the current OECD 17 test guidaine – Bioaccumulation in terrestrial Oligo haetes

- At the end of the test, the overall mortality during what and elimination phase should not exceed 10% of the total number of the introduced worms factual. 0%)
- For *Eisenia fetida* the mean mass loss as measured at the end of the uptake and at the end of the elimination phase should not exceed 20% compared to the initial fresh weight (f.w.) at start of each phase (actual: none)

The validity criteria of the surrent lest guideline have been net therefore the study is considered acceptable.

The calculated broaccumulation factor (BAE) was; V.56.

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

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No additional studies on other terrestrial wertebrates are required in accordance with Commission Regulation (EU) No283/2013.

In the supporting publication by EFSA (2017)⁴ to review the biological relevance of the magnitude of effects observed in studies with amphibians and ceptiles, it is noted that fish-generated toxicity data seem to be appropriate to cover aquatic amphibians. For terrestrial organisms typically birds and mammals are shown to be more sensitive than amphibians and reptiles to a higher number of substances. Currently data do not allow for extrapolating between groups, however the frequency of cases in which amphibians or reptiles are more sensitive than birds or mammals is around 30%. In the absence of further available data with reptiles and amphibians of case is likely to be protective of the risk to amphibians and reptiles.

Relevant literature on other terrestrial yertebrate wildlife

No relevant scientifically peer reviewed open literature could be found on spiroxamine or its major metabolities, from an ecotoxicological perspective, on other terrestrial wildlife. Details of the literature search indertaken can be found in the M-CA Section 9 of the current submission.

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¹ EFSA supporting publication 2017; EN-1251. Biological relevance of the magnitude of effects (considering mortality, sub-lethal and reproductive effects) observed in studies with amphibians and reptiles in view of population level impacts on amphibians and reptiles.



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CA 8.1.5 **Endocrine disrupting properties**

A full assessment of the endocrine disrupting potential of spiroxamine, in accordance with the ECSA and ECHA Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009 (adopted 05 June 2018) and the OECD conceptual framework described in OECD TG 150 (2018), will be submitted. à

CA 8.2 Effects on aquatic organisms

The aquatic studies conducted with spiroxamine and its metabolites are summarised in the following table. The data include studies previously reviewed and included in the DAR and EFSA conclusion for spiroxamine as well as any previously unsubmitted or new studies which have also been conducted. Ä

Table CA 8.2-1 Su	immary of endpoir ganisms	nts for toxicity of	spiroxamine and m	etabolit	es to aquatic
Organism	Test item		Endpoints 8		Reference°
Fish	·			Ç	
Oncorhynchus mykiss (Rainbow trout)	Spiroxamine	Acute toxicity	90 hour IC ₅₀ 98,500 g a.s. A 7 (mm)	E	<u>16006246-01-1</u>
Lepomis macrochirus (Bluegill sunfish)	Spiroxamine	Acute toxicity 96 a (static)	96-bour LO ₈₀ δ) 30 μg a.s./L. (mm) Q	EUÔ	<u>M-006229-01-1</u>
Danio rerio (Zebra fish)	Spiroxamine	Acutetoxicity, 96 h(static)	96-hour LC. 2,410 μg a.s./L (mm)	EU Ø	<u>M-303809-02-1</u>
Oncorhynchus mykis (Rainbow trout)	Spiroxanine	toxicity (ELS) 93 d (flow 5 through).	NOEC <62.5 μg a.s. Φ (nom) (EC ₀) 14 μg as./L (nom)	, O EU	<u>M-006232-01-1</u>
		Statistical Re-analysis	EC ₁₀ 62.5 µg a.s E (nom)	NEW	<u>M-760407-01-1</u>
Oncorhynchus mykirs (Rainbow trout)	9 A	Chronic to Acity (ELS; radiolaborled), 96 d (tow through)	NOES 14.2 µg a.s. (mm)	EU	<u>M-006449-02-1</u>
		Statistical Re-analysis	CEC ₁₀ 91.5 μg a.s./L (mm) EC ₂₀ 195 μg a.s./L (mm)	NEW	<u>M-760405-01-1</u>
Oncorhynchus mykiss (Rahnbow trout)	Spiroxamine of	Chronic oxicity (ELS; sediment system; pulsed exposure) 56 d	NOEC 3 x 60 μg a.s./L (mm)	EU	<u>M-304369-01-1</u>
Danio Ferio	Spiroxamine	Chronic toxicity (FFLC) 230 d (flow through)	NOEC 2.6 μg a.s./L (nom)	EU	<u>M-304458-02-1</u>
		Statistical Re-analysis	EC ₁₀ 1.88 μg a.s./L (nom) EC ₂₀ 4.46 μg a.s./L (nom)	NEW	<u>M-760413-01-1</u>



Organism	Test item	Test type	Endpoints		Reference
organishi	i est item		EC_{10} (survival)		°
		Chronic	$23.3 \ \mu g \ a.s./L$		
		toxicity	(im)		
		(FFLC;	(IIII)	~	S O
Danio rerio		sediment	NOEC	<u></u>	<u>M-46799-03-0</u>
	Spiroxamine	system; pulsed	(biomarker	6 ^y	
(Zebra fish)	-	exposure)			
		56 d	VTG) 15.8 µg		
			a.s./L (im)		
		Statistical	EC ₁₀ not	NEW	M-729412-64+1
		Re-analysis	determinable	\$ I	
		«O"	Growth and	Ő	
			fertility not og	Ó I	
			affected at up to	× /	
		k, B°	and including	Ŭ,	× v
		o ^v . U [*] ;	(58.8 µg/a.s./L ~		¢ .4
			(mm) Ö	U U	
Pimephales promelas	Continue of the	Fish screening assay	No effects on		<u>M-304833407-1</u>
(Fathead minnow)	Spiroxamine	assay	endocrine ~	EU	<u>IVI-304833787-1</u>
			specific	Ĩ	R O
	,Ő¥	\$, \$, \$	biomarker 🔊		
	A l	or indiana series and a series	endpoints at up		ř. K
	~~ <u>0</u>	Â	wand including	<u> </u>	**Y
			18.9 12 a.s./L	\approx	%
			(mm)))) A.	O'
			BCF _{(whot} and 87		
Lepomis macrochirus	Q Q Q Snirotamine	A s.	DCI (wholesush) 07	de la companya de la	
(Dluggill gunfigh)	Spirexamine	BOST	CT ₅₀ 43 - 19	E	<u>M-006479-01-1</u>
	S' 41		hours	\sim	
Aquatic invertebrate				1	
Aquatic inverteorates.	Spiroxamike	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	48-hour EC50		
Daphnia magna	Spirovamika	Acute toxicity	6,100, ug a.s. 4	EU	M-006245-01-1
		@48 h (static) 📎	(inf)	EU	<u>IVI-00024J-01-1</u>
		A averative			
	Support of the second s	Acúte toxicity 48 h	A8-hour EC50		
Daphnia Daph	Spiroxamine		6,800 ag a.s./L	EU	M-006476-01-1
		(radiolabelled,	(mino)		
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~		v static) V	3		
Daphnia magna		Acouse toxicity	48-hour EC ₅₀		
Daphnia magna	Snitexamin	.48/h	[*] 3,000 μg a.s./L	EU	M-006523-01-1
Daphnia magna		Tradiolabelled;	(mm)	LU	<u></u>
~Q⁻ Ŭ	Spinoxamino	flow-through)	· · ·		
A	KWG9169	Acute toxicity	48-hour EC ₅₀		
Daphnia 🕅 🕺 🖉	$\Lambda W Q + 1007 $	Static)	>100,000 µg/L	EU	<u>M-006702-01-1</u>
		(Static)	(nom)		
	$\mathcal{A}$ $\mathcal{A}$	Chromic			
N G		toocity	NOEC 100 µg	<b>FU</b>	M 00(401 01 1
a, `	L D	2 d (static-	a.s./L (nom)	EU	<u>M-006401-01-1</u>
~ ^ _ \	Q° XS	Fenewal)			
Daphnia ma@a 🚿	Spiroxamine		EC ₁₀ 120 µg		
	)* <u>*</u> ~ ?	Statistical	a.s./L (nom)		
	Ň	Re-analysis	$EC_{20} 200 \ \mu g$	NEW	<u>M-761546-01-1</u>
	~~~~	Re-analysis			
	<u> </u>	Character	a.s./L (nom)		
NO O I	· *	Chronic			
		toxicity	110501		
	а. [.]		NOEC 34 ug	D7	11.00/2222.01.1
Daphniomagna	Spiroxamine	21 d	NOEC 34 μg a.s./L (mm)	EU	<u>M-006555-01-1</u>
Daphnia magna	Spiroxamine	21 d (radiolabeled; flow-through)	NOEC 34 µg a.s./L (mm)	EU	<u>M-006555-01-1</u>



Organism	Test item	Test type	Endpoints		Reference
UI Samon		<u> </u>	Endpoints EC_{10} 32 µg a.s./L		0
		Statistical	(mm)*		
		Re-analysis	EC ₂₀ 68 μg a.s./L	NEW	<u>M-760409-61 </u>
		ite ulurysis	(mm)*		
		Chronic		Q.	
		toxicity	1	D'	
		21 d	NOEC 47 µg 🔬	EU	MO06466 201-1
		(radiolabeled;	a.s./L (mm) 🔬 🎽	LU	
Daphnia magna	Spiroxamine	static-renewal			
Duphnia magna	Sphoxalline	Static-Tellewalk	EC10 39 00 a.s./L	.0	
		Statistical	(mm)		Q A K
		Re-analysis	EC.	NEW	<u>M-7615¢4-01-1</u> ©
		Re-allarysis	EC_{20} 65 μ g acs. ² /L (mm)	Qʻ, (
Sediment-dwelling organis					
Sediment-dweining organis			EC15 S		
			(development	0	A A .º
	1		$(4000) \sim 5.600 \mu g$	Ç	
	Ĺ	Chronic A	$1 \text{ a.s./L} (\text{porn}) \sim 3,300 \text{ µg}$	ľ 🔬	
	<u> </u>	toxicity \sim	a s./L (born)		
	Ő¥	28 d (static;	NQEC	ÆŬ	@ <u>1-006549-01-1</u>
Chironomus riparius	Spiroxamine	radiolabelled)	(emergence)		
	~~ ~		(0.000 mm 2 s)	Ŭ	°~/
		o Ş	\$500 µg a.s./L	~	&
			(nom)		0″
		Stati@tical	EC ₁₀ /EC anot	p Ø	
\$		Statierical Resanalysis	determinable	NEW	<u>M-760403-01-1</u>
. //		Resaliarysis	EC10 4,120 µg	<u>Š</u>	
	a s			\sim	
Į D ^v		Chronic 2	(form)	1	
I umbriculus variaditus .	Spirovamine V	toxicity \$		NEW	M-688127-01-1
Lumoriculus variesalus	Spiroxamine	28 d (static)		INL: W	<u>IVI-000127-01-1</u>
	0 0 %		110155 10,700		
	V A		sediment (mm)		
Amphibia					
			No indication of		
			endocrine		
\$9 [′]	4 8 %		activity on the		
8 <u>1</u>			thyroid axis		
	S C		concluded A		
			statistically		
×	D AN CA		significant		
Xenopus Wevis	Spiroxamin	XXETA.	increase in	NEW	M-762327-01-1
			flouresence was	1,1,1,1,1	
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	A nº Ó		observed at the		
Ϋ́ ····	V X Q	A Start	1.6  mg/L		
<i>@</i> , ^{\$}		<u> </u>	treatment but this		
~~ ^^ `	Q' N	₽ [°]	concentration		
			was above the		
	p" ~		MTC		
Lumbriculus varies Amphibia Amphibia Xenopus tevis Algae Scendesmus straspicator	<u> </u>	1		1	<u> </u>
N R A	S.		ErC50 12 119		
Scenedesmus	×		a.s./L (mm)		
subspicatos	Spiroxamine	72 h (static)	$E_bC_{50} 32 \mu g$	EU	<u>M-006228-01-1</u>
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~			a.s./L (mm)		
	1	1)	1	<u> </u>



Organism	Test item	Test type	Endpoints		Reference
8		✓ <u>1</u>	Е _r C ₁₀ 1.56 µg		
			a.s./L (mm)		
			E _r C ₂₀ 3.51 µg		
			a.s./L (mm)	×	
			ErC ₅₀ 11.9 µg	Â,	
		Statistical	a.s./L (mm)	ð"	
		Re-analysis	$E_y C_{10} 0.84 \ \mu g$	NEW	<u>M-361401-01-1</u>
		A contraction of the second se	a.s./L (mm)		
			$E_{v}C_{20}$ 1.44 gg	Ő	
		¥.	a.s./L (mm)	. Õ	
			$E_v C_{50}$ 3.28 µg	Ŵ	Q Ô ^y N
		A	a.s./L (mm) \mathcal{O}°	A	
			ErC 19.43 4 4 g	Ŷ,	
		~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	$L_r C_{30}$ 19.59 $\mu g$ as $L_r (nom)$		
		120 h (static)		EU	<u>M-006518-01-1</u>
			$E_bC_{50}$ 5,42 µg	O.	A A C
	Spiroxamtue	120 h (static)	E ₁₀ 9.20 ug	<b>X</b>	
	L.		1.2010 9.2001g	E CONTRACTOR	
	Į į		$E_rC_{20}(0.9 \text{ ugs}^{-1})$	<u> </u>	
Selenastrum	Å.	KY A N	a.s/D(nom)		
capricornutum	Spiroxamtne	$\mathcal{F}' \stackrel{*}{\sim} \mathcal{F}' \stackrel{*}{\sim}$	$E_x G_{50} 15.2 $ $Que C$		
capricornatian	Q' &	Stafetical	L	Õ	°∼y ĭ
		Re-analysis	$E_{\rm v}C_{10}$ $E_{\rm c}C_{10}$ $E_{\rm c}C_{10}$	NW	M-761402-01-1
			a.s.A. (nom)		0
			$E_yC_{20}$ 4.70 µg		
\$			$E_yC_{20}$ 4.40 µg	s s	
2	× A		Даг.s./L (попп) ДЕ _v C ₅₆ 7.99 µg	S	
$\sim$			a.s. $\mathbb{Q}$ (nom)	$\sim$	
<u> </u>		96 h (static) ()	$E_{2} = 811 \mu \sigma$	l'	
		~ <u>~</u>	$F_{0}C_{50} > 8.14 \ \mu g$		
a S			$E_bC_s$ 5.5 µg		
		06 h (Stric)	$L_b C_{20} 0.5 \ \mu g \otimes$	EU	M-006533-01-1
			a.s $(im)$ EC ₅₀ (cell	EU	<u>IVI-000333-01-1</u>
		S, O	EC ₅₀ (cell) density 5.7 $\mu$ g a.s./ $D$ (im)		
Å Ö			aciistiv 93.7 μg		
Ky "O			a.s./Q(IIII)		
Ň,	4 × .0		E ₄ C ₁₀ 4.93 μg a.s./L (im)		
Selenastrum	Spir		a.s./L (im) $P_rC_{20}$ 10.5 µg a.s./L (im)		
capricornutum	Spinesramme	N Q A	$= L_{\rm r} C_{20}  10.3  \mu g$		
	Ď 🔊 🔺		$E_rC_{50} > 8.14 \ \mu g$		
~~ ~ ~ ~ ~		Statistical	$E_rC_{50} > 8.14 \ \mu g$ a.s./L (im)		
A .		De analysia	a.s./L (IIII) E C., 1 20	NEW	<u>M-761427-01-1</u>
	Q V	analysis	E _y C ₁₀ 1.29 μg a.s./L (im)		
		, <u> </u>	$E_yC_{20}$ 2.18 µg		
		Š	$L_y C_{20}$ 2.18 µg a.s./L (im)		
″ _ ~ ~ ~	× ,0′ ,, ×		a.5./L (IIII) E C 5 00~		
ĴÛ , \		Č, ^y	$E_y C_{50} 5.90 \ \mu g$		
		¥	a.s./L (im) E.C. $< 0.52$ ug		
			$E_r C_{10} < 9.53 \ \mu g$		
			a.s./L (nom) $E C = 11.4 \text{ mm}$		
			E _r C ₂₀ 11.4 μg	EU	M-273962-01-1
Desmodernus	Spyoxamine	72 h (static)		EU	IVI-2/JJ02-01-1
Desmodermus D subspicatius C	Spyoxamine	72 h (static)	a.s./L (nom)	EU	<u>IVI-273902-01-1</u>
Selenastrum capricornutum	Sproxamine	72 h (static)	a.s./L (nom) $E_rC_{50}$ 175 µg a.s./L (nom)	EU	<u>IVI-273902-01-1</u>



<u>Test item</u> Spiroxamine	Test type         Statistical         Re-analysis         96 h (static)	Endpoints $E_yC_{10}$ not determinable $E_yC_{20}$ not determinable $E_yC_{50}$ 10.5 µg a.s./L (nom) $E_rC_{50}$ 6.3 µg a.s./L (im) $E_rC_{10}$ not	NEW S	<u>M-761456501-1</u>
Spiroxamine	Re-analysis 96 h (static)	determinable $E_yC_{20}$ not determinable $E_yC_{50}$ 10.5 µg a.s./L (nom) $E_rC_{50}$ 6.3 µg a.s./L (im)		
Spiroxamine	Re-analysis 96 h (static)	$\begin{array}{c} E_yC_{20} \text{ not} \\ \text{determinable} \\ E_yC_{50} 10.5 \ \mu\text{g} \\ \text{a.s.}/L \ (\text{nom}) \\ \hline E_rC_{50} \ 6.3 \ \mu\text{g} \\ \text{a.s.}/L \ (\text{im}) \end{array}$		
Spiroxamine	Re-analysis 96 h (static)	determinable $E_yC_{50}$ 10.5 µg <u>a.s./L (nom)</u> $E_rC_{50}$ 6.3 µg <u>a.s./L (im)</u>		
Spiroxamine	96 h (static)	$ \begin{array}{c} E_y C_{50} \ 10.5 \ \mu g \\ a.s./L \ (nom) \end{array} \\ \hline E_r C_{50} \ 6.3 \ \mu g \\ a.s./L \ (im) \end{array} $	EU	
Spiroxamine	`´``````````	$\begin{array}{c} a.s./L \text{ (nom)} \\ \hline E_r C_{50} \text{ 6.3 } \mu g \\ a.s./L \text{ (im)} \end{array}$	EU	
Spiroxamine	`´``````````	E _r C ₅₀ 6.3 μg	EU	
Spiroxamine	`´``````````	, a.s./L (im)	EU	
Spiroxamine	T T		1	<u>M-761414-01-1</u>
Spiroxamine				
Spiroxamine	a la	determinable	,Ŵ	
Spiroxamine		$E_r C_{20}$ not	Ž	Q Ô K
Spiroxamine	A	datarmanahlan [°]	L	
Spiroxamine	or "	determinable $\hat{c}$	Ŷ,	Ď a ay
-	Statistical 0	E ₁ C ₃₆ 6.33 44g ass/L (iso)		
	Statistical °	ass./L (int)	NEW	<u>M-761414-01-1</u>
	Reanalysus		NEW	of the so
4	1. ° ^ ©	deter fillindele -	$\sim$	
×	Statistical Reanalyses	$E_{20}$ not	×	<u>M-761414-01-1</u>
Ő,		determinable 🔊	, Q	
Q,		E _y C _{50x} ],29 μg	Ű	
O×		a.s./10(im) 5		Ç Q
Â ^Y .		$E_{6}^{\infty}$ (cell $^{\circ}$		<u>M-006537-01-1</u>
Spiroxamine	96 b (static)	Consity) >990 pg	ЕЦ	<u>M-006537-01-1</u>
		a.s./Lenm)	ð	8
	See all and a second	$E C^{(1)}_{11} 1 05 $		
	96 h (static) 🔗		FEU 🔊	<u>M-006542-01-1</u>
	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~			
	S S (DE C. & 8 36 µg	65	
à v		$2 \times \mathcal{P}(mm)$	\sim	
	Statistical	TeC 0.44	EII	M-280532-01-1
	Re-analysio	$10020 9.44 \mu g$	EU	<u>IVI-200332-01-1</u>
4 6				
Spiroxamine (Ø KY S	$L_{\rm r} \sim 1.7 \mu S$		
\checkmark	<u> </u>			
19 A	S. O	<u>72-hour</u>		
		$\Psi_{y}C_{10}$ 6,83 µg		
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	StatistiQi 🔍	a.ş./Q(mm)		
i ^y 🖉 d	Re-apalysis	E ₄ C ₂₀ 7.60 μg	NEW	<u>M-761458-01-1</u>
		a.s./L (mm)		
S a		¥E _y C ₅₀ 9.32 μg		
N. C	<u>ૻૼ૾૾ૼ૽ૼૻ૽ૼ૽ૼૺૻ૽ૼ૽ૼ૽ૻૼૺ</u>	a.s./L (mm)		
jõ 🗸 🏹		ErC ₁₀ not		
) ₂₀ <u>4</u>	B .U	determinable		
ð ú		$E_r C_{20} 42.9 \ \mu g/L$	EU	M 200222 01 1
	as n (static)	(nom)	EU	<u>M-288232-01-1</u>
A Nº O		E _r C ₅₀ 737 це/L		
₩WGA2168- ®	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	(nom)		
desethvl (MØ1)	- V	E.C. not		
	Q,	determinable		
	Statistical	E C., not		
<u>)</u> ~ ~ ~	De enel-reie	LyC20 IIOt	NEW	<u>M-761465-01-1</u>
a í	Re-analysis			
$\sim$		E _y C ₅₀ 30.6 μg/L		
K) ^Y		(nom)		
Y				
Y				
	Spiroxamine	Spiroxamine 96 F(statio) 96 F(statio) 96 h (statio) 96 h (statio) 97 h (statio) 98 h (statio) 98 h (statio) 98 h (statio) 99 h (statio) 90 h (	Spiroxamine 96 P(static) a.s./L(im) 96 P(static) a.s./L(im) 96 P(static) a.s./L(imin) 96 P(static) a.s./L(imin) 96 h(static) a.s./L(imin) 572-hour FrC188.36 µg a.s./L (imin) 672-hour FrC209.44 µg 6.s./L (imin) 6.s./L (imin) 6.s./L (imin) 6.s./L (imin) 6.s./L (imin) 7.2.hour 8.s./L (imin) 8.s./L (imin)	Spiroxamine 96 h (static) a.s./ $L$ (map) 96 h (static) a.s./ $L$ (map) 96 h (static) a.s./ $L$ (map) 96 h (static) a.s./ $L$ (map) $E_{rC}$ (s.36 µg a.s./ $L$ (map) $E_{rC}$ (s.3737 µg/ $L$ (nom) $E_{rC}$ (s.3737 µg/ $L$ (nom) $E_{rC}$ (s.30.6 µg/ $L$ NEW



Organism	Test item	Test type	Endpoints		Reference
~		~	E _r C ₁₀ 20.3 μg/L		<u></u> °
			(im)		
			E _r C ₂₀ 55.7 μg/L		
			(im)		
	KWG 4168-		$E_rC_{50}$ 383 µg/L		
Pseudokirchneriella		72 h (statis)		NEW	<u>M-680695-194-2</u>
subcapitata	despropyl	72 h (static)	(im)	NEW	<u>IVI-080095-09-2</u>
1	(M02)		$E_yC_{10}$ n.d.		
		Ĉ	E _y C ₂₀ 14.8 μg/L		
		- The second sec	(im)		
			EyC50 425 ag/L		
			(im) 🖑	õ	
		A	ErC10 058 µggL°	Ň	
				Qʻ	Ď ^v č Č ^v <u>M²2⁄88235⁵ • 01-1</u>
		· · · · · · · · · · · · · · · · · · ·	$E_{20} 2,500 \mu g/L^{0}$	EU	
		72 [°] (static)	E ₂₀ 2,500 μg/L ⁰	EU	M-288235-01-1
		0.0	K(nom) E _r C ₅₀ 31,700	- Or	L A o
		5 ~ 0	LrC 1,700		
Desmodesmus	KWG 4168-N- 🛒			Y ^V	
subspicatus	oxide (M03)		E, C ₁₀ 248 µg/E		
			Mnomk ~	Ĩ	Q O
	Ű.	Statistical	EvC20526, ugyL	S.	
	A l	Re-analysis	(nom)	<b>MNEW</b>	<u>M-761467-01-1</u>
	~~ ~		10 - 2835 11040	Ŭ_Û	°~~
		o S	nom ₂		<b>%</b> ,
				U	0 ^w
		or in the the	ErCto >3,200		с -
			μg/L (nong)	EG	
		72 (station	$E_rC_{20} > 3,200$	E	M-309818-01-1
S. 1			Φμg/L%(nom),		<u>IVI-307616-01-1</u>
			$E_{r}C_{S}^{O} > 3.200$	× *	
Desmodesmus	KWG 4968-		$\mu g / L (nom)$	1	
Desmodesmus subspicatus	KWG 4468- acid (A466)	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	State of the second sec		
Ö a	l Sía		datarmunahla		
<u></u>		Statistical 📎		NEW	M 7(14(0 01 1
O' A'		Re-analysis	$E_{y} = S_{30}^{30}$ considered	INEW	<u>M-761469-01-1</u>
Ô	2 A		to be $> 5,200$		
			ug/L/(nom)		
Aquatic plants	<u> </u>		<u>, 0'</u>		
			14 day EC50		
, Alexandre and Alexandre a			(frond counts)		
Č A			910 µg a.s./L		
	S C	MA d A &	(mm)	EU	M-006497-01-1
	\$ . N ~	(static)	14-day E Cro	20	
, ¥		Q D	2.650  mg o g/I		
			2,050 µg a.s./L		
	Û.		(mm)		
		\$~,~\$`	trond number		
Lemma gibba 🔬 🖉	<b>Sp</b> iroxa@nne @		7-day $E_rC_{10}$		
۲. Marine ( Marine )	\$ 67 [~] ~	O ^Y	2,060 µg a.s./L		
<i>(</i> ), [\]	L L L	1 A	(mm)		
К A [^]	Q" N	Statistical	7-day ErC20		
Q" ~~~		Re-analysis	3 110 110 25 /	EU	<u>M-303421-01-1</u>
	$\delta^* \sim \delta^{}$	ive-anarysis	$\int \frac{3,110 \ \mu g}{(mm)} a.3./L$		
67 A.S. (	Ĩ, Ĩ, Î				
			$/-day E_r C_{50}$		
N 68 A	1 AV		6,780 μg a.s./L		
<u> </u>			(mm)		
4 4	Le la				
Ča Č					
Ű					
Desmodesmus subspicatus					



Organism	Test item	Test type	Endpoints		Reference
			frond number		Reference
			14-day ErC ₁₀		
			1,260 µg a.s./L		
			(mm)	ð	
			14-day $E_rC_{20}$	Ğ.	
			1.820 ug a.s./L	Ø.	
			(mm) A		
		<i>i</i>	14-day ErCsof		
			$3170\mu g a QL$	Č	
		Ŵ	(mm)	, Ŵ	
		a.		Ž	Q Ô N
		A	7-day & C10 220	L,	
		Statistical Recanalysis °	$/ \operatorname{uny} \operatorname{Lyc}_{10} = 0$	Ŷ,	
		De analysis o	$\pi P$ $\mu g$ $\alpha$ s./L. (18411) $\pi P$ $\mu g$ $\alpha$ s./L. (18411)	ŇEW	<u>M-760417-00-1</u>
		Realiarysis	$\mathcal{A}_{\text{ug}}$ as $\mathcal{A}_{\text{ug}}$	Č.	× ×
			Ang a.s. A (min)	1 Contraction of the second se	L A co
		1. 0° 0	$7 - u_{40} = y_{50} = 0$	$\gg$	
	, K		$5,020 \ \mu g a s./L$		
	Ő			<i>S</i>	
	Q				
	L. I		14-020 EyC 0-360		Q
	Q'		μg a.s./L (gam) C		
		a s	$O_{20}$ -day $E_{3}C_{20}$	~Õ~	()
			ug a.s. (mm)	0°	Ň
	<i>К</i> ′ <u>к</u> ,		14-day E _y C 56	ð,	$\begin{array}{c} \begin{array}{c} & & & & \\ & & & & \\ & & & & \\ & & & & $
	\$ O \$		1,990 μg.a.s./L 🗞		
<u> </u>	Y A Q		(mm) 🔍 🏸	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	
×.			D14-day EC _{50 (frond}	, Ó	
¹ C ³			number 2,760 µg		
	L . 6	14 d∼ _ ⊃	ag,/L (mm)	FU	M-006540-01-1
Š,		(static)	94-day FC 50	LU	<u>M 000340 01 1</u>
	× &		(biomagy 9,380 ug		
ð s	O O X		a.s (mm)		
	× Ø A		frond number		
		O	$\mathcal{O}$ -day $EC_{10}$		
je g			3,510µg a.s./L		
			(mana)		
\$9 °			$7-day E_rC_{20}$		
A 3			04,130 μg a.s./L		
	Ĵ C		(mm)		
Lemna gibba	Spiroxamine		7-day $E_r C_{50}$		
	d and a		5,600 µg a.s./L		
	N OF		(mm)		
	Q,	Statistical	<b>`</b> ,	EU	M-303443-01-1
	AN	Ke-anarysis	dry weight		
ky ₩	Ş ^ı xy Q	Ś	14-day ErC ₁₀		
~ \	۲ _۵ ۵ _۵ , ۲		4 760 ug a s /I		
		Q, [¥]	(mm)		
OY LA	Š V "	¥	$14$ -day F $C_{22}$		
	$\sim$ $\sim$		$7.960 \mu \sigma 20$		
	Í DÍ Í		(mm)		
			(1111)		
	1 V		14-uay $E_r C_{50}$		
Lemna gibba Control of the state of the stat	$\sim$		$21,200 \ \mu g \ a.s./L$		







CA 8.2.1	Acute toxicity to	o fish
011 01211	reduce conficiely co	, 11011

Data Point:	KCA 8.2.1/01
Report Author:	
Report Year:	1994
Report Title:	KWG 4168 techn Acute toxicity (96h) to rainbow trouvin a static test
Report No:	DOM 93062
Document No:	<u>M-006243-01-1</u>
Guideline(s) followed in	M-006243-01-1         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         X         <
study:	
Deviations from current	
test guideline:	OECD 203 (2019) Conditions during the acclimation period were not reported (however \$% of the
	Conditions during the acclimation period were not reported (however 40% of fish
	died). A dissolved oxygen concentration <60% was observed on one individual
	measurement in the bwest test concentration, however later readings were above
Previous evaluation:	yes, evaluated and accepted A A A A
	DAR (1997), KAR (2010), RAK (2017)
GLP/Officially	Yes, conducted under GLP/Officiality recognised testing facilities
recognised testing	
facilities:	
Acceptability/Reliability:	Yes Yes Y Y Y Y Y Y Y Y Y Y Y Y Y Y Y Y

#### **Executive Summary**

The acute toxicity of KWC4168 to rainbow trout (*Oncorhynchus myKss*) was determined in a static 96hour test. Seven test concentrations were assessed along with a control and solvent control. Each treatment group contained 20 fish.

At test termination, mortalities of 0, 0, 0, 0, 0, 0, 45 and 100% were observed in the control, solvent control and 0.78, 0.48, 3.03, 4.33, 8.80, 18.0 and 26.2 mg as /L test concentrations. Sub-lethal signs of intoxication were observed from the 8.80 mg a.s./L test concentration onwards, and included lying on back/side, tumbling paring swimming and fish staying mainly on the bottom of the tank.

The resulting 96-hour LC₄₀ was determined to be 18,5 mg a C/L, with 95% confidence intervals of 8.80 to 26.2 mg a.s./L. The DEC and NOEC were 8.86 and 433 mg a.s./L, respectively.

```
I.
          Materials and Method
  A. Materials
Test Material
    Lot/Batch #:
    Putrity:
  Description:
                            ColourlessInquid
    Stability of tes
                                  shown by percent ranges for measured analytical values
    compound:
                              Januar 1994
    Reanal
    dat
                             ot reported
    Děns
Treatment
    Test rates:
                           Nominal: 1.0, 1.78, 3.16, 5.62, 10.0, 17.8 and 31.6 mg a.s./L
                           Mean measured: 0.78, 1.48, 3.13, 4.33, 8.80, 18.0 and 26.2 mg/L
    Solvent/vehicle:
                           100 µL acetone/L test water
```



Analysis of test concentrations:	Yes, mean measured concentrations $78 - 101\%$ of nominal
Test organisms	
Species:	Rainbow trout (Oncorhynchus mykiss)
•	
Source:	
Acclimatisation period:	48 hours Not fed for 48-hours proor to or during the test None
Feeding:	Not fed for 48-hours prover to or during the test
Treatment for	None
disease:	
Test design	
Test vessel:	Glass aquaria of 32 x 36 x 38 cm containing 40 E test solution
Test medium:	Reconstituted water
<b>Replication:</b>	No representation of the second secon
No. of	
animals/vessel:	
Duration of test:	96 hours of a a a a a
Environmental test	
conditions 🔬	
Temperature:	Not fed for 48-hours proof to or during the test None Glass aquaria of 32 x 36 x 38 cm containing 40 £ test solution Reconstituted water No repficates 20 fish 96 hours 11° 6 65 – 10 3 mgA (approx. 58.93 – 93@8% of saturation) 6.9 – 9.4 16 fi light 8 h dark
Dissolved oxygen	65 – 10.3 mg/L (approx. 58.95 – 93@8% of saturation)
рН: 🔗 👸 🗶	$\bigcirc 6.9 - \bigcirc 4$
Photoperiod: B. Study Design	16 h light 8 h dark
	OT AT OT AT OT
B. Study Design	
This study was anducted	in Order to accord the Quite Privicity of KWG 4168 to rainbow trout

This study was conducted in order to assess the acute oxicity of KWG 4168 to rainbow trout (*Oncorhynchus mykiss* on a 96 hour static test. Test concentrations were determined based on the results of a preliminary range-finding test?

The mean body weight of fish at the beginning of the test was  $2.3 \pm 0.5$  g (±SD), and the mean length was  $6.2 \pm 0.4$  cm (±SD). Loading was 1.15 fish/bytest medium.

Test vessels vere glass aquaria of  $32 \times 36 \times 38$  cm containing 40 L test solution. To each vessel were added 20 fish, which were observed after four hours and then daily for mortalities and symptoms of intoxication.

Dissoftwed or gen and pH values were determined daily in each aquarium, and the water temperature of the control aquarium was recorded hourly.

Analytical determinations of the active substance were made in the test medium at test initiation and termination.



#### Analytical method

Samples of water were analysed using the validated analytical method 00252 M001, report reference <u>M-008490-02-2</u> (see Doc MCA Section 4).

#### II. Results and Discussion

Validity criteria according to the OECD 203 guideline were met:

• Control mortality to not exceed 10% at the end of the test (actual 50.0 and 0.0% for the control and solvent control, respectively)

One of the criteria was not met:

• Dissolved oxygen concentration to be  $\geq 60\%$  of the air saturation value (abtual: 58.93 to 93.38%)

A dissolved oxygen concentration <60% was observed in only one individual measurement in the forwest test concentration, and later readings were above 60%?

Table CA 8.2.1/01-1 N	leasured concentrations	of KWG-4168	in test	medium	ć
-----------------------	-------------------------	-------------	---------	--------	---

Measured	Measured 0	Mean measured	Mean measured
concentration $\mathcal{Q}$	concentration	test concentration	test concentration
(% of nominal) at	6% of nominal sat	(% of nominal)	(ngg a.s./L)
test initiation 🖉 🍡	test termination ¹		o v
- 0, 4	- 8 4		) - _{(4.}
- ~~~~		- 0 [×] & 0	- 0
86 🔌 📞		78 ~	<b>Q</b> 78
80 S O S	86	83 2 2 2 2	JI.48
97 7 4	1005	99, 🗸 🏹	[*] 3.13
	80 0 ~	76 & 27	4.33
		88 O A	8.80
98 8 20 20	103 ,	101 L	18.0
28 4 6		83 ^b	26.2
	concentration (% of nominal) at test initiation - - - - - - - - - - - - - - - - - - -	concentration       concentration         (% of nominal) at       test initiation         test initiation       -         -       -         -       -         -       -         -       -         -       -         -       -         -       -         -       -         -       -         -       -         -       -         -       -         -       -         -       -         -       -         -       -         -       -         -       -         -       -         -       -         -       -         -       -         -       -         -       -         -       -         -       -         -       -         -       -         -       -         -       -         -       -         -       -         -       -         -       -      <	concentration       concentration       test concentration         (% of nominal) at test initiation       test concentration       % of nominal) at test concentration         -       -       -       -         -       -       -       -         -       -       -       -         -       -       -       -         -       -       -       -         -       -       -       -         -       -       -       -         -       -       -       -         86       -       -       -         97       -       -       -         100       -       -       -         27       -       -       -         97       -       -       -         100       -       -       -         287       -       -       -         98       -       -       -       -         103       -       101 4       -         28       -       -       -       -

¹ Mean of two replicates , corrected for recoveries 🖉

^a Measured concertoration at day 2

^b Mean-calculated concentration between day 0 and day 2

The results have been presented based on the mean measured concentrations.

At test termination, mortalities of 0, 0, 0, 0, 0, 0, 0, 45 and 100% were observed in the control, solvent control and 0.78, 0.48, 3-13, 4.25, 8.80, 18.0 and 26.2 mg a //L test concentrations. Sub-lethal signs of intoxication were observed from the 8.80 mg a.s. b test concentration onwards, and included irregular swimming behaviour lying on back/side tumbling during swimming and fish staying mainly on the bottom or furface of the tank. In the 3.45 mg as /L test concentration at 96 hours, 10% of fish showed slight turbbling during swimping, however in the next higher test level all 20 fish were without symptoms. This observation is the fore pot conspiered to be treatment-related.

 Table CA 8.2.1/01-2
 Mortalities and symptoms of intoxication of rainbow trout during exposure to

 KWG 4/68
 Q

Mean measured	Number of mo	Number of mort arties / number with symptoms of intoxication ¹					
concentration (mg a A)	(4)h 🔊	24 h	48 h	72 h	96 h		
Control &		0 / 0	0 / 0	0 / 0	0 / 0		
Solvent control	0,70	0 / 0	0 / 0	0 / 0	0 / 0		
0.38	0 0	0 / 0	0 / 0	0 / 0	0 / 0		
1.48 p°	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0		
3.13	0 / 0	0 / 0	0 / 0	0 / 0	0 / 2		
4.33	0 / 20	0 / 0	0 / 0	0 / 0	0 / 0		
8.80	0 / 20	0 / 20	0 / 20	0 / 20	0 / 20		



Mean measured	Number of	Number of mortalities / number with symptoms of intoxication ¹					
concentration (mg a.s./L)	4 h	24 h	48 h	72 h	96 h 🖉 °		
18.0	2 / 20	9 / 20	9 / 20	9 / 20	9 / 20		
26.2	5 / 20	19 / 20	20 / 20	- 🏷	- 02 &		
LC ₅₀ (mg a.s./L)	-	18.5ª	18.5 ^b	18.5 ^b	18.54		
(95% CI)		(15.6 – 20.5)	(8.8 – 26.2)	(8.8 – 26.2)	$(8_{2} - 26_{2})^{2}$		

20 fish were introduced per aquarium

Dead fish were added to the sum of fish with symptoms

- Calculated using binominal probability
- b Calculated using probit method

#### III. Conclusion

The acute toxicity of KWG 4168 to rainbow trout (Oncorhynchus myliss) was determined in a static 96hour test. Seven test concentrations were assessed along with a control and solvent control. Each treatment group contained 20 fish.

At test termination, mortalities of 0, 0, 0, 0, 0, 0, 0, 45 and 100% were observed in the control, solvent control and 0.78, 1.48, 3.13, 4.33, 8.80 48.0 and 26,2 mg a s/L test concentrations. Sub-lethal signs of intoxication were observed from the 8.80 mg a.s./E test concentration opwards and it cluded lying on back/side, tumbling during swimming and fish staying mainly of the forttom of the tank.

The resulting 96-hour LC₅₀ was determined to be 18 5 mg a structure and 18 5 mg a structure and 18 8.80 to 26.2 mg a.s./L. The LOEC and NOEC were 8.80 and 4.33 mg @s./L, gespectively.

#### Assessment and conclusion by applicant:

The study was conducted to the OEGD test guideline 203, the most up-to-date version of which is the "Fish acute toxicity test", adopted 48 June 2019

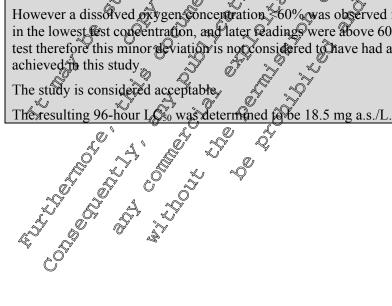
Validity criteria according to the most up-to-date QECD 203 guideline (2019) were met:

Control mortality to not exceed 10% at the end of the test (actual: 0.0 and 0.0% for the control and solvent control, respectivel

One of the criteria was not me

• Dissolved of generation to be  $\geq 60\%$  of the air saturation value (actual: 58.93 to 93.38%) 🚿

However a dissolved oxygen concentration \$60% was observed in only one individual measurement in the lowest test concentration, and later rading were above 60%. Control mortality was 0% in this test therefore this minor reviation is nonconsidered to have had any detrimental impact on the results





-		
Data Point:	KCA 8.2.1/02	
Report Author:	<u> </u>	8
Report Year:	1994	1
Report Title:	KWG 4168 techn Acute toxicity (96 h) to bluegill in a static test	
Report No:	DOM 93063	
Document No:	<u>M-006229-01-1</u>	
Guideline(s) followed in	OECD 203 (1992)	
study:		
Deviations from current	None None	Ø
test guideline:		×.
Previous evaluation:	yes, evaluated and accepted DAR (1997), RAR (2010), RAR (2017)	
	DAR (1997), RAR (2010) <b>A</b> AR (2017) <b>C</b>	
GLP/Officially	Yes, conducted under Gb Officially recognise Desting facilities	
recognised testing	Yes, conducted under Gb Officially recognise desting facilities	
facilities:		
Acceptability/Reliability:	Yes A C C C C A A A A	

#### **Executive Summary**

The acute toxicity of KWG 4168 to bloegill sunfish (*Lepoints macrochinits*) was determined in a static 96-hour test. Seven test concentrations were assessed, along with a control and solvent control. Each treatment group contained 20 bluegill sunfish.

At test termination, mortalities (#0, 0, 0, 0, 0, 0, 80, 400 and 400% were observed in the control, solvent control and 0.34, 1.30, 2.50, 4.22, 9.40, 15 4 and 26.2 mg a.s./L test concentrations. Sub-lethal signs of intoxication were observed from the 4.22 mg a c/L test concentration onwards, and included lying on back/side, tumbling during swimming and fish staying mainty on the bottom or surface of the tank.

The resulting 96-hour C₅₀ was determined to be 743 mg a.s./L with 95% confidence intervals of 5.91 to 8.37 mg a.s./L. The LOFC and SOEC were 4.22 and 2.50 mg a.s./L, respectively.

```
Chand Change
                                                     Materials and Methods
  I.
  Α.
      Materials
Test Material
    Lot/Batch #:
    Purity:
                                            y percent anges for measured analytical values
   Description
                              lourless 1
    Stability@f test
                                 ₫S
    compound:
    Reamalysis/Expire
    date:
  ∕∽Ďensity:
                                 porte
Treatment
                           Nominal: 20, 1.78, 3.16, 5.62, 10.0, 17.8 and 31.6 mg/L
    Test
                           Mean measured: 0.34, 1.30, 2.50, 4.22, 9.40, 15.4 and 26.2 mg a.s./L
                         𝟹00 µL acetone/L test water
    Solvent/wehicle
                           Yes, mean measured concentrations 34 – 94% of nominal
    concentrations:
Test organisms
   Species:
                           Bluegill sunfish (Lepomis macrochirus)
```



Source:	o
Acclimatisation period:	14 days
Feeding:	Fish were not fed for 48 h before or during the story
Treatment for disease:	Not for 70 days prior to the start of the study $40 \text{ L}$ to t solution $32 \times 36 \times 38 \text{ cm}$ containing $40 \text{ L}$ to t solution $32 \times 36 \times 38 \text{ cm}$ containing $40 \text{ L}$ to t solution $32 \times 36 \times 38 \text{ cm}$ containing $40 \text{ L}$ to t solution $32 \times 36 \times 38 \text{ cm}$ containing $40 \text{ L}$ to t solution $32 \times 36 \times 38 \text{ cm}$ containing $40 \text{ L}$ to t solution $32 \times 36 \times 38 \text{ cm}$ containing $40 \text{ L}$ to t solution $32 \times 36 \times 38 \text{ cm}$ containing $40 \text{ L}$ to t solution $32 \times 36 \times 38 \text{ cm}$ containing $40 \text{ L}$ to t solution $32 \times 36 \times 38 \text{ cm}$ containing $40 \text{ L}$ to t solution $32 \times 36 \times 38 \text{ cm}$ containing $40 \text{ L}$ to t solution $32 \times 36 \times 38 \text{ cm}$ containing $40 \text{ L}$ to t solution $32 \times 36 \times 38 \text{ cm}$ containing $40 \text{ L}$ to t solution $32 \times 36 \times 38 \text{ cm}$ containing $40 \text{ L}$ to t solution $32 \times 36 \times 38 \text{ cm}$ containing $40 \text{ L}$ to t solution $32 \times 36 \times 38 \text{ cm}$ containing $32 \times 36 \times 38 \text{ cm}$ cm}
Test design	
Test vessel:	Glass aquaria of 32 x 36 x 38 cm containing 40 L tot solution
Test medium:	Reconstituted water $2^{2}$
<b>Replication:</b>	Glass aquaria of 32 x 36 x 38 cm containing 40 L test solution
No. of animals/vessel:	14 days Fish were not fed for 48 h before or during the study Not for 70 days prior to the start of the study Glass aquaria of 32 x 36 x 38 cm confarining 40 L test solution Reconstituted water No replicates 20 fish 96 hours 19 – 2020 8.6 – 9.3 mg/L (approx. 92)67 – 102.25% of saturation) 7.0 – 7.8 – 6 h light : 8 h dark
<b>Duration of test:</b>	
Environmental test conditions	
Temperature:	
Dissolved oxygen:	8.6 -9.3 mg (approx. 9267 – 102.25% of saturation)
pH:	8.6 - 9.3 mg2 (approx. 92)67 - 102.25% of saturation) 7.0 - 7.8 76 h light : 8 h dark
Photoperiod: 💭	A6 h kight : 8 h dark
B. Study Design	7.0 - 7.8 7.0
This study was conducted i	n order to assess the active toxicity of IEWG 4168 to bluegill sunfish (Lepomis
<i>macrochirus</i> ) in a 96-hou preliminary range-finding	r static test. Test concentrations were determined based on the results of a test.
The mean body weight of	fisher the beginning of the test was $30 \pm 0.5 \mathrm{g} (\pm \mathrm{SD})$ and the mean length
was $3.8 \pm 0.6$ cm ( $\pm$ OD). L	fish at the beginning of the test was $10 \pm 0.5$ g (±SD), and the mean length bading was 0.5 g fisher test medium.
Nominal test concentration	ns were 1.0, 1.78, 7.16, 5.62, 100, 17.8 and 31.6 mg a.s./L. Mean measured b, 0, 75, 94, 87 and 83% of nominal, corresponding to 0.34, 1.30, 2.50, 4.22,
	uaria of $32 \times 36$ x $38$ cm containing 40 L test solution. To each vessel were e observed after four hours and then daily for mortalities and symptoms of

Dissolved oxygen and pH values were determined daily in each aquarium, and the water temperature of the control aquarium, was received hourly.

Analytical determination of the active substance were made in the test medium at test initiation and termination.

#### Analytical method

Samples of water were analysed using the validated analytical method 00252 M001, report reference M-0084@-02-2 (see Doc MCA Section 4).

#### II. Results and Discussion

Validity criteria according to the OECD 203 guideline were met:

Ľ



- Control mortality to not exceed 10% at the end of the test (actual: 0.0 and 0.0% for the control and solvent control, respectively)
- Dissolved oxygen concentration to be ≥60% of the air saturation value (actual: 92,67 to 102.25%)

Mean measured values ranged from 34 to 94% of nominal. The low recovery seen in the lowest test concentration (1.00 mg a.s./L) had no impact on test results as the subsequent two higher test verses showed no adverse effects on the fish. The results have been presented based on the mean measured concentrations.

Nominal test	Measured	Measured	Mean measured Moan measured
concentration	concentration	concentration	stest concentration test concentration
(mg a.s./L)	(% of nominal) at	(% of nominal) at ^	(% of nominal) (mg a.s./L)
	test initiation ¹	test termination ¹	
Control	0	A	
Solvent control	0		
1.00	29	38, ~	¥ 34 0 [°] 4 [°] 6.34 [°] 0.34 [°]
1.78	69	75	73 C & 1.30
3.16	87	nt in w	79 8 2 250 V
5.62	67 👋 õ		§75 0 0 Q4.22 ×
10.0	90 0 5	98 0 2 0	94 Q 9.40
17.8	62		870, 0 15.4
31.6	83		83 26.2
1 Maan of true no	nlipping for real		

 Table CA 8.2.1/02-1
 Measured concentrations of KWG 4168 in test/medium

¹ Mean of two replicates corrected for recoveries

Table CA 8.2.1/02-2 Mortalities and symptoms of intostication of bluegill sunfish during exposure to KWG 4168

Mean measured			ber witth sympton	ms of intoxication ¹	
concentration		29 h 🖑	\$48 h	72 h	96 h
(mg a.s./L)					
Control			» 0.40°	0 / 0	0 / 0
Solvent contro			0000	0 / 0	0 / 0
0.34	0,00		0 / 0	0 / 0	0 / 0
1.30	070	0×/0, 0		0 / 0	0 / 0
2.50	∞ 90 / 0 Q	0/00	0 / 0	0 / 0	0 / 0
4.22		$\rightarrow 0$	0 / 20	0 / 20	0 / 20
9.40	× 0\$20 ×	Q 20 S	0 / 20	4 / 20	16 / 20
15.4		10/20	16 / 20	19 / 0	20 / 20
26.2	× 20 / 20	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	-	-	-
$LC_{50}$ (mg a.s. $\mathbb{Q}^{2}$ )	- <u>~</u>	5.4ª	13.2 ^a	11.1 ^b	7.13 ^b
LC ₅₀ (mg a.s.O.) (95% CI)		<b>A</b> ¶9.40−26.2)	(9.40 – 15.4)	(9.93 – 12.5)	(5.91 - 8.37)

¹ 20 from were introduced per aquarium

Dead hish were added to the sum of fish with symptoms

Salculated using binominal probability

^b Calculated using probit method



#### III. Conclusion

The acute toxicity of KWG 4168 to bluegill sunfish (*Lepomis macrochirus*) was determined in a satic 96-hour test. Seven test concentrations were assessed, along with a control and solvent control Each treatment group contained 20 bluegill sunfish.

At test termination, mortalities of 0, 0, 0, 0, 0, 0, 0, 80, 100 and 100% were observed in the control, solvent control and 0.34, 1.30, 2.50, 4.22, 9.40, 15.4 and 26.2 mg a.s./L test concentrations. Sub-tethal sons of intoxication were observed from the 4.22 mg a.s./L test concentration onwards, and included bying or back/side, tumbling during swimming and fish staying mainly on the bottom or surface of the tank.

The resulting 96-hour LC₅₀ was determined to be 7.13 mg a.s./L, with 95% confidence intervals 95.9 k to 8.37 mg a.s./L. The LOEC and NOEC were 4.22 and 2.50 mg a 7/L, respectively.

#### Assessment and conclusion by applicant:

The study was conducted to the OECD test guideline 203, the most up-to date version of which is the "Fish acute toxicity test", adopted 18 June 2019.

Validity criteria according to the OECD 203 (2019) guideline were met:

- Control mortality to not exceed 10% at the end of the test (actual 0.0 and 0.0% for the control and solvent control, respectively)
- Dissolved oxygen concentration to be  $\geq 60\%$  of the air saturation value (actual: 92.67 to 102.25%)

The study is therefore considered acceptable.

The resulting 96-hour LC was determined to be 7.13 mg a st/L.

×.	
Data Point:	KCA 8201/03 @ 5 2 0 4
Data Point:	
Report Year:	
Report Title: 🖉 🔗	Aqute toxicity of spiroxamine to gebra fish (Danjo rerio) over 96 hours
Report No:	BAY-033/4-11 2 2 2
Document No:	<u>M-309809-00-1</u>
Guideline(s) followed in	QEGD 203, 1992, Direction 92/69/ EEC, Part C.1
study:	$\lambda^{\gamma} \stackrel{\sim}{\sim} \stackrel{\circ}{\circ} \frac{\gamma}{\sqrt{\gamma}} \stackrel{\sim}{\ll} \stackrel{\circ}{\sim} \circ$
Deviations from current	Yes $0^{\circ}$ $0^{\circ}$ $0^{\circ}$ Methods: SANCO/5029/99 rev. 4 $0^{\circ}$ $0^{\circ}$ $0^{\circ}$
test guideline:	Methods: SANCO/3029/99 rev. 4
	Aecuracy G=1 O' O' O'
	Accuracy of a similar length and
	Ecotox cology OECO 203 (2019)
	The age of the test lish is not reported, however they are of a similar length and
	weight $\sim$ $\sim$
	Freedings chedule is not reported, nor if any mortalities were observed during the
	acclamation period O
Drawiewa ewaladiewa 1	The Fight intensity of not reported
Previous evaluation:	yes, evaluated and accepted RAR (2010), RAR (2017)
GLP/Officially	Yes, Sonducted under GLP/Officially recognised testing facilities
recognised testing facilities:	
	¥ui Wes
Acceptability Reliability	/1 55

## Executive Summary

The acute toxicity of KWG 4168 to zebra fish (*Danio rerio*) was determined in a static 96-hour test. Six test concentrations were assessed, along with a control. Each treatment group contained ten fish.



Mortality of 0, 0, 0, 0, 30, 100 and 100% were observed in the control and 0.278, 0.589, 1.22, 2.22, 4.27 and 9.27 mg a.s./L test concentrations.

Sub-lethal effects of spiroxamine exposure could be observed in the 1.22 mg a.s./L test concentration and above. Such signs included uncoordinated swimming, lateral positioning, dorsal positioning, tail-heavy swimming and dark colouring.

The 96-hour LC₅₀ was determined to be 2.41 mg a.s./L. The NOEC and LQEC were determined to be 0.589 and 1.22 mg a.s./L, respectively.

I. Materials and I	Methods
A. Materials	
Test Material	KWG 4168 (spiroxamine)
Lot/Batch #:	EDTH004650
Purity:	97.0% w/w
<b>Description:</b>	Light brown dil of a start of a s
Stability of test compound:	Methods       Methods         KWG 4168 (spiroxamine)       Methods         EDTH004650       Methods         97.0% w/w       Methods         Light brown Ail       Methods         Not reported       Methods         Methods       Methods         02 Abgust 2009       Methods         No information       Methods         Methods       Methods         Methods       Methods         Methods       Methods         02 Abgust 2009       Methods         Methods
Reanalysis/Expiry date:	02 August 2009
Density:	No information
Treatments	No information (1997) Nominal: (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997) (1997)
Test rates: Solvent/vehicle: Analysis of test	Oceonnean. 0.248, 0.389, 1.2292.22, 42/ and 9.2/ mg a.s./L
Solvent/vehicle:	Note is a start w
Solvent/vehicle:	Yes, geometric mean measured concentrations 85 – 98 % of nominal Debra fish ( <i>Danio refio</i> )
Test organisms	Debra fish (Danio repio)
Species:	Debra fish (Danio repio)
Test organisms Species: Source:	
Acclimatisation	Fish were reared in water of the same quality as used in the test for at least three months until the start of exposure
Feeding:	NOLARI UUUM9/IDRA/EN
Treatment for	None reported
disease:	
Test design	
Test kessel;	12 L glass aquaria containing approx. 10 L test solution
Test mediam:	Furified drinking water
Replication:	No replicates
No off	Ten fish
animals/vessel:	
<b>Duration of test:</b>	96 hours



Contraction of the second seco

# Environmental test conditions

Temperature:	$22.6 - 23.2^{\circ}C$
Dissolved oxygen:	90-100% saturation
pH:	7.7 - 8.6
Photoperiod:	12 h light : 12 h dark

#### B. Study Design

This study was conducted to assess the effects of 96-hour exposure of 'zebra fish (Danio Ferio) to spiroxamine in a static test. Test concentrations were selected based on the results of a non-GLP ratge-finding test.

Fish were of an in-house culture, and had been reared in water of the same quality as used in the test for at least three months prior to exposure. At test inflation fish were weighed and measured, and rareed from 2.2 to 2.9 cm (mean 2.5 cm) and 0.40 to 0.52 g (mean 0.95 g).

Test vessels were 12 L glass aquaria containing approximately 10 L test solution. Test medium was drinking water purified by filtration, with activated charceal, passage brough a limestone column and oxygen aeration to saturation. Aquaria were held at 22 6 to 23 2 C under a 10 hour tight to 22 hour dark photoperiod.

Six test concentrations were assessed along with a control. A stock solution was prepared by mixing 500 mg spiroxamine with 1 L dilution water, which was then further diluted to obtain required test concentrations. Nominal test concentrations were 6.31, 663, 1.25, 2.59, 5.06 and 10.0 mg a.s./L. Analyses of fresh and aged exposure media revealed concentrations of 56 to 123% of nominal values, thus endpoints were 0.278, 0.589, 1.22, 2.22, 4.27 and 9.27 mg a.s./L, corresponding to 89, 94, 98, 89, 85 and 93% of nominal respectively.

The temperature, ph and exygen concentration of the water were measured in each vessel directly at test initiation and then daily for the remainder of the test. Fish were measured for length and weight at the beginning of the study and observed daily for mortalities and any abnormalities in appearance and behaviour.

LC_x values were determined by probit analysis using the programme ToxRat Standard v3.3.0.

#### Analytical method

Samples of water were analysed using the validated analytical method <u>M-303809-02-1</u>, report reference <u>M-303809-02-1</u> (see Doc MCA Section 4).

II. 🧖 Results and Discussion

Validity criteria according to the OECD 203 gordeline were met:

- Control mortality to got exceed 10% at the end of the test (actual: 0.0%)
- Discolved oxygen concentration to be  $\geq 60\%$  of the air saturation value (actual: 90 to 100%)

Table CA 8.2.1/93-1	Measured	concentrations of spiroxamine in test medium
---------------------	----------	----------------------------------------------

Nominal test concentration (mg a.s.D.)	(mg/E)	% of nominal	48 h (mg/L)	% of nominal	96 h (mg/L)	% of nominal	Geometric measured concentra mg/L	
0.31	0.340	109	0.300	96	0.210	67	0.278	89



Nominal test concentration (mg a.s./L)	0 h (mg/L)	% of nominal	48 h (mg/L)	% of nominal	96 h (mg/L)	% of nominal	Geometric measured concentra mg/L	0	
0.63	0.770	123	0.530	85	0.500	80	0.589	.04 ,	
1.25	1.48	118	1.49	119	0.830	66 0	1.22	98	
2.50	2.93	117	2.65	106	1.41	56 A	2.22	89	Ì
5.00	6.07	121	3.55	71 🔊	3.62	72	4.27	85	
10.0	11.2	112	10.2	102 💎	6.96	Øð	9.20	<b>9</b> 3 Ø	
LOQ Limit of	quantificatio	on: 0.003 mg	z/L		Ô	,¥			Ő

The results have been presented based on the mean measured concentrations.

Mortality of 0, 0, 0, 0, 30, 100 and 100% were observed in the control and 0.278, 0.589, 1.22, 2.22, 4.27and 9.27 mg a.s./L test concentrations.

Sub-lethal effects of spiroxamine exposure could be observed in the 1.22 mg a.S./L test concentration and above. Such signs included uncoordinated swimming, lateral positioning, dorsal positioning failheavy swimming and dark colouring.

Table CA 8.2.1/03-2	Cumulative morta	lity of zebra fisl	Auring expos	urê to spirêx	amine
		#// P * %	· · · · · · · · · · · · · · · · · · ·		

					N N
Geometric mean	Cumulativezno	ortality (%)	S L		¢.
measured concentration	3.h ~	24 h 🔗	48 h	72 h 🏹 🕺	<b>∦96 h</b>
(mg a.s./L)					
Control	0 × v	Ó Á O		0 ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	0
0.278	0,00				0
0.589		65 20	Ó ^V & .	J B	0
1.22		0 2 2	>0 0° %		0
2.22		0 ~ ~	10	30	30
4.27	0 🔆 🗸	160, 50	100	<b>L19</b> 0	100
9.27			100 × ×	<u>7</u> 100	100
10 fish ware added not non	Aration O (		Y A		

10 fish were added per concentration O

The 96-hour  $LC_{50}$  was determined by probit analysis using the programme ToxRat to be 2.41 mg a.s./L. The NORC and LOEC were determined to be 0.589 and 1.22 mg a.s./L, respectively.

Endpoint Q S S M mg a SL
NOEC 0 0 0 0 0 0 0.589
$LC_{10}$
LC ₅₀ & 2.41
(95% confidence limits) $A$ $\gamma$ $\gamma$ (1.97 – 2.95)

Table CA 8.2.1/03-3 Endpoints after 96-Pour exposure of zebra fish to spiroxamine

## III. Conclusion

The acute toxicity of KWG 4968 to zebra fish (*Danio rerio*) was determined in a static 96-hour test. Six test concentrations, were assessed, along with a control. Each treatment group contained ten fish.

Mortality of 0, 0, 0, 30, 100 and 100% were observed in the control and 0.278, 0.589, 1.22, 2.22, 4.27 and 9.20 mg as //L test concentrations.

Sub tethal offects of spiroxamine exposure could be observed in the 1.22 mg a.s./L test concentration and above. Such signs included uncoordinated swimming, lateral positioning, dorsal positioning, tail-heavy swimming and dark colouring.

The 96-hour  $LC_{50}$  was determined to be 2.41 mg a.s./L. The NOEC and LOEC were determined to be 0.589 and 1.22 mg a.s./L, respectively.



#### Assessment and conclusion by applicant:

The study was conducted to the OECD test guideline 203, the most up-to-date version of which is the "Fish acute toxicity test", adopted 18 June 2019.

Validity criteria according to the up-to-date OECD 203 (2019) guideling were met:

- Control mortality to not exceed 10% at the end of the test (actual: 0.0%)
- Dissolved oxygen concentration to be ≥60% of the air saturation value (actual: 90 to 100%)

The study is therefore considered acceptable.

The 96-hour LC₅₀ was determined to be 2.41 mg a.s./

## CA 8.2.2 Long-term and chronic toxieity to fish

For procedural reasons studies listed in the Table 8.2.2.1 below are included in the current dosser as available data or information previously submitted but not necessarily evaluated. However, these ports have been fully superseded by newer studies. Consequently, no summaries of the reports have been included in the dossier.

#### Table CA 8.2.2-1: Studies previously submitted and not relied upon for the risk assessment

Data	Document	Date Title 🔨 🚕	"0" ~~ ₍ )~	B . O
Point	No.		Å Ø Å	
KCA	M-468005-			essmen for fist based on a refined-
8.2.2/01	01-1 🔬	exposure Fish	ull Life Cycle Qudy &	

Data Point:	KCA 8,2.2.1/01
Report Author:	
Report Vear:	
Report Title:	KWG 4168 techEarly life stage toxicity to rainbow trout (Oncorhynchus
	mykig under flow-through conditions
Report No:	DON 94016 × C
Document No	<u>MC006232-01-1</u> O , O , O
Guideline(s) followed in	9ECD240 (1292) 0 0
study:	
Deviations from current	Yes y
test guideline:	QECD 210 (2015)
	Temperature deviated from the recommended $10 \pm 1.5^{\circ}$ C, with a max. daily
	temperature of 12°C Prequently observed. The max. temperature on day 22 was
	14% ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
	Sean total length of the control fish at test termination was not reported. The
	mean standar dength of the pooled controls was 34.2 mm.
	Fry were first fed 14 days post-hatch rather than 19 days post-hatch, and were
	orly fed once daily on weekends and holidays.
Previous evaluation:	Wes, evaluated and accepted
	DAR (1997), RAR (2010), RAR (2017)
GEP/Officially	Yes, conducted under GLP/Officially recognised testing facilities
recognised testing	
facilities:	
Acceptability/Reliability:	Yes
· · · · ·	

## CA 8.2.2.1 Fish early life stage toxicity test



### **Executive Summary**

The chronic toxicity of KWG 4168 to rainbow trout (*Oncorhynchus mykiss*) was determined in a flowthrough early-life-stage toxicity test. The effects on 35 eggs and 15 fry were observed over a total of 93 days. Nominal test concentrations of 62.5, 125, 250, 500, 1000 and 2000  $\mu$ g a.s.  $\mu$  were tested

Survival at post-hatch Day 34 and Day 63 resulted in a NOEC and LOEC of  $\frac{1}{2000}$  and 2000  $\frac{1}{410}$  g a.s./L

Egg hatchability resulted in a NOEC at 2000  $\mu$ g a.s./L and the LOEC at > 2000  $\mu$ g a.s. threshold could not be determined.

Time to hatch resulted in a NOEC at 2000  $\mu$ g a.s.  $\beta$  and the LOEC at > 2000  $\mu$ g a.s.  $\beta$ . Apeffect threshold could not be determined.

Time to 94 percent swim-up resulted in a NOEC at 500  $\mu$ g a.s. and the LOEC at 1000  $\mu$ g a.s. b. The effect threshold (EC₀) was calculated to be 70%  $\mu$ g a.s. L.

Growth, expressed as dry weight, resulted in a NOFC at  $< 62.5 \mu$  gas.s./L and the LOEC at  $62.5 \mu$  g a.s./L. The effect threshold (EC₀), based on a concentration-response model (threar regression model), is  $4^{4} \mu$  g a.s./L.

Growth, expressed as length, on post thatch bay 34 resulted in a NOEC at 62.5  $\mu$ g a.s./L and the LOEC at 62.5  $\mu$ g a.s./L. The effect threshold (EC₀), based on a concentration response model (linear regression model), is 29  $\mu$ g a.s./L.

Growth, expressed as length, on post-hatch Day 63 resulted in a NOEC at  $\leq 62.5 \ \mu g$  a.s./L and the LOEC at  $\leq 62.5 \ \mu g$  a.s./L. The effect threshold (EC), based on a concentration response model (linear regression model), is 26  $\mu g$  a.s./L.

The effect threshold for KWGA168 was based on the most sensitive endpoint (growth, expressed as dry weight), and determined to be 14 kg a.s. 4

weight), and determinated to be	
I. Materials and M	lethods why a second se
A. Materials	KWG 4168
Test Material 10° ~	KWG 4168
Lot Batch #: Purity:	KWG 4168 \$98114602 97.8%
	97.8% OF LY & LY
Description	Colourless liquid
Descriptions Stability@f test compound:	Coourless liquid Certified unto 27 Jaouary 1994
compound:	
Reamalysis/Expire	2 ⁹ Janu@y 1924 、 ^人
date:	× ~ <u>A</u> , Q
Density:	Not reported Not reported Nominal: © .5, 125, 250, 500, 1000 and 2000 μg a.s./L Mean measured: 61.1, 126.8, 220.5, 452.8, 995.6 and 1874.5 μg a.s./L
Treatments	Nominal: <b>@</b> .5, 125, 250, 500, 1000 and 2000 μg a.s./L
Test rates:	Nominal: @.5, 125, 250, 500, 1000 and 2000 µg a.s./L
	Mean measured: 61.1, 126.8, 220.5, 452.8, 995.6 and 1874.5 µg a.s./L
Solvent/xehicle1	Acetone
Analysis of test	Yes, mean measured values 88 – 101% of nominal
👋 concentrations:	
Test organisms	
Species:	Rainbow trout (Oncorhynchus mykiss)



Source:	
Acclimatisation period:	Not applicable
Feeding:	Not applicable Fry were fed with live bring Shrimp ( <i>Artenna salina</i> ) natelli and ground trout/salmon starter <i>ad libitum</i> starting on day d4 postilated Food was added to aquaria twice daily during the week, and once daily at weekends/holdays, with eadl aquarium receiving an approximately equal quantity of food None Egg hatching: 14 x 9 cm with a water depth of 17 c cm (volume 2.2 L) Growth phase: 18 x 22 on with a water depth of 19 cm (volume 7.5 L) Reconstituted water No feplicates Egg hatching: 35 eggs per ressel Growth phase: 19 alevin per vessel 9 - dA*C (mean: 40.3 - 44.9°C) 9 - dA*C (mean: 40.3 - 44.9°C) 9 - 10% of saturation 7.1 - 7.5 16h light' 8 h dark at 455 lux
Treatment for disease:	None & & S & S & S & S & S & S & S & S & S
Test design	
Test vessel:	Egg hatching:
	Growth phase: 18 x 22 cm with a water depth of 19 cm (volume 7.5L)
Test medium:	Keconstituted Water
Replication:	No replicates
No. of animals/vessel	Egg hatching: O G G G G G G G G G G G G G G G G G G
No. of animals/vessel	Growth phase:
Duration of test:	$\gamma$ 93 days $\gamma$ $q$ $\gamma$ $\gamma$ $\gamma$ $\gamma$ $\gamma$
Environmental test conditions Temperature Dissolved oxygen	
Temperature	$7^{9} - 4^{\circ}C$ (mean: $40.3 - 44.9^{\circ}C$ )
Dissolved oxygens	95 – 105% of saturation
pH: v v	97.1 - 7.5 ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~
Photoperiod:	16 h light: 8 h dark at 435 lux
B. Study Design	7.1 - 7.5 16 h light. 8 h dark at 435 lux G 4108 to rambow frout ( <i>Oncorhynchus mykiss</i> ) was determined in a flow-
The chronic toxicity of K	G 4108 to rannow frout (Oncorhynchus mykiss) was determined in a flow-

The chronic toxicity of KWG 4168 to ranbow frout (*Oncorhynchus mykiss*) was determined in a flowthrough early-life-stage toxicity test ever 93 days. Test concentrations were chosen based on historical toxicity results

Nominal test concentrations of 62.5, 123, 250, 500, 1000 and 2000 µg a.s./L were tested. Prior to the initiation of the oudy, test solutions had been flowing through the test system for nine days.

Unfertilised ggs and milt from three female and three male adult brood fish. Eggs were fertilised in dilution water at  $19 \pm 1$  with the aid of gentle stirring, after which they were rinsed to clarity and randomly distributed to incubation cups.

Eggs were incubated in 1.8 mm diameter cups constructed with perforated stainless steel plate on the bottom. These incubation cups were suspended in each replicate test chamber. The glass aquaria used



for egg hatching measured approximately  $14 \times 9$  cm with a water depth of 17.5 cm, yielding an approximate chamber volume of 2.2 L. The test solution was changed approximately 41 times per hour.

Test chambers were held under a 16 hour light to eight hour dark photoperiod at 435 lux. Developing mbryos were shielded from excess UV light exposure by leaving lights off until completion of batch.

To each of the incubation cups were added 35 impartially selected for filised eggs. Each test concentration had four replicates. Four replicates of 50 eggs each were placed in additional exposure chambers for a viability determination. All incubation cups were observed daily, except weekends, for egg mortality, determined by a distinct change in colouration. Any deadeggs found were discarded

The number of eggs hatched in each incubation cup was recorded dail@except weekends until three days post-hatch. The post-hatch period began after 95% of living eggs of the control had hatched, on study day 30. Alevin were impartially thinned to 15 individuals per replicate of day 50 ost-hatch. Observation of behaviour and mortality were made daily except weekends

Glass aquaria used as growth chambers for the post diatch phase measured approximately 18 x 22 cm with a water depth of 19 cm, yielding an approximate chamber colume of 7.5 L. The dest solution was changed approximately 12 times per hour.

Fry were fed with live brine shrimp (*Aplemidesalina*) nauplit and ground fout/satarion starter *adlibitum* starting on day 14 post-hatch. Food was added to aquariativice daily during the week and once daily at weekends/holidays, with each aquarium receiving an approximately equal quantity of food

Growth was determined by measurement of the standard length on day 34 post-hatch using slide photography. At termination of the biological phase on day 62 post-hatch, after a total of 93 days, surviving fish were sacrificed and the standard lengths and wet weights determined. Once these measurements were completed, fish were dried at 60°C for 52 hours, and individual dry weights were determined.

Temperature was preasured working darly in the control and a data logger documented hourly temperatures from a centrally-located test chamber. Dissolved oxygen and pH were measured in one replicate of the control, solvent control, and 62.5, 500 and 2000 ug a.s./L test concentrations approximately weekly.

### Analytical method

Samples of water were analysed using the validated analytical method 00252 M001, report reference  $\underline{M-008490-02-2}$  (see Doc MCA Section  $\Phi$ ).

### II. Results and Discussion

Validity criters according to the most recent OECD 210 guideline (2013) were met:

- Dissolved oxygen concentration to be  $\geq 60\%$  of the air saturation value throughout the test (sectual: 95 to 109%) a
- Overall survival of pertilised egg(*i.e.* hatching success) and post-hatch survival in the controls should both be 25% for rainbow troot (100 and 90.5% achieved, respectively, in the pooled control 3%

One of the coveria was not met:

• Writer temperature to not differ by more than  $\pm 1.5^{\circ}$ C between test chambers or between test chambers or between test days at any time during the test. The temperature was only measured in one test chamber, with one deviation of  $\pm 2^{\circ}$ C to preceding and successive days recorded on study day 22 The mean temperature on day 22 was 11.7 °C  $\pm 0.9$  (SD).

Mean measured values ranged from 88 to 101% of nominal. At days 42 and 49 in the 2000  $\mu$ g a.s./L test group, measured concentrations of 43258 and <0.1  $\mu$ g a.s./L, respectively, were found. These two values were most likely outliers resulting from sample handling errors, and as a result were excluded from further statistical analysis. On several sample days, KWG 4168 was detected in control and solvent



control samples at amounts in the same range as in the blank samples, due to cross-contamination during the analytical phase. 2°

Nominal test concentration (µg a.s./L)	Mean measured concentration (µg a.s./L)	Standard (μg a.s./L	deviation )	Mean measured Concentration	
Control	-	- 🔊	"W"	- ,*> *	
Solvent control	-	- 🖓	Ű	- 8 2	
62.5	61.1	13.0 *	R.	97.8 0 5	
125	126.8	22.3	4	1007 8	
250	220.5	\$9.2	Q Q°	882 4	
500	452.8	<i>R</i> 82.8	$\sim$ $0^{\circ}$	90.6 \ ^{O'} \$	Ű
1000	995.6	375:3		99.6	J.
2000	1874.5 C	677.2 🔊	27 × 67	93	٩
Results corrected for	recoveries		ja or		

Table CA 8.2.2.1/01-1	Measured concentrations of KWG 4168 in test medium
	measured concentrations of it to a froo in test measure

The results have been based on nominal test concentrations.

Percent egg hatchability was not significantly affected by the treatment at any concentration when compared to the pooled controls. Mean fry survival on day 64 (post-hetch day 34) was 93, 95, 98, 88, 97, 93, 87 and 82%, with survival in the highest test concentration, 2000 up a.s./Ic being significantly reduced compared to the pooled controls. On day 93 post-batch day 63, nean frequencies was 87, 94, 98, 88, 93, 92, 87 and 73%, with fry survivation the 2000 µg a.s./Lagain significantly reduced compared to the pooled controls. m

Table CA 8.2.2.1/01-2 Mean egg hatchability and frx survive with exposure to KWG 4168

Nominal test	Mean egg hatch (%)	Mean post-hatcholay	Mean post-hatch day
concentration &		34 survival (%)	63 survival (%)
Nominal test concentration (µg a.s./L)			
Control O	100 ~ ~ ~	93	87
Solvent control	J100 × × ×	95% O	94
62.5		98 a, ~~	98
125	HOO & A	88 5 2	88
250		97 %	93
500	100 0 0	Sey A	92
	1000 2 2	87 🐎	87
2000	100 20 24	82*	73*

Results are means of four replicates ~

* Statistically significantly different from the pooled controls ( $\alpha = 0.05$ )

Ŵ

¹Post-hatch day 3

Egg hatching began of study day 28 and continued until study day 33. Percent hatch on study day 33 was 190% for all test levels, and there was no statistically significant different in percent hatchability at any treatment level. As 295% of the eggs in the control had hatched on day 30, this was taken as day 0 of the post-hater period.

Time to hatch of Ginbow trout fry with exposure to KWG 4168 Table CA § 2.1/04-3

Nominal test concentration	Percent hatch (%)								
(µg a.s./L) Control	Pay 27	Day 28	Day 29	Day 30	Day 31	Day 32	Day 33		
Control	0	2	74	96	99	100	100		
Solventcontrol	0	9	87	99	100	100	100		
62.5	0	13	73	97	100	100	100		
125	0	16	73	100	100	100	100		



)0 )0

8

4

4

Nominal test concentration	Percent hatch (%)							
(µg a.s./L)	Day 27	Day 28	Day 29	Day 30	Day 31	Day 32	Day 33	
250	0	15	77	96	99	100	1000	
500	0	25	68	97	98	P00	100 0	
1000	0	24	69	98	99	<b>@</b> 100	100	
2000	0	17	64	98	100 🔬	100	<u>100</u>	

Results are means of four replicates

Newly hatched fry began swimming up from the bottom of test chambers on study day up was achieved on day 49 in the pooled controls, during which time there was a statistically difference in the 1000 and 2000 mg a.s./L test concentrations.

					-p 01 1	Ŵ.	Ô	5 j	5	×,	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	) O	°~	,	Ĵ.
Nominal test conc.	Perc	ent sw	vim up	by stu	dy day	y ())	N.		, ,	چ ج	ĵ,	Ø	Å.	A.	2
(μg a.s./L)	46	47	48	49	50 x	51	52	~53	54 [°]	55	56	7 57 K	58	<b>(\$</b> 9	<b>G</b>
Control	0	57	80	93	80/	659	73, ®	83 👡	×90 _	<b>9</b> 5	<u>\$9</u> 2	<b>90</b> ×	95	95	95
Solvent control	0	67	88	95	<b>69</b> 7	90 D	20 >>	25	97	98	98	<b>9</b> 8	<b>98</b>	<u>98</u>	98
62.5	0	64	57	88	850	90 Ø	95	95	695	<u>J</u>	20	97Ô	100	100	10
125	0	18	36	<b>Ø</b> 81	76	710	69	66 0	79 🔬	⊉79	9ľ	98	1Q0	100	10
250	0	48	34	78	73	\$5	52 ّ	53	85 Ø	~63 🖉	73 🔊	44	9 <u>1</u>	91	91
500	0	32	77	93	86	68 (	40	<b>®</b> 5	21	14	61	33 🖉	55	53	51
1000	0	2	<b>ب</b> ية	41*	5Ø	52	42 🔊	42	24	×22	20	12	7	5	5

Ø Table CA 8.2.2.1/01-4 Percent swim-up of rainbow trout fry with exposure to KWG 4168

2000 0 Results are means of four replicates

0

Statistically significant difference the pooled control (2-0.05)

Fry growth expressed as standard length on post-hatch day 34 was significantly reduced at all test concentrations when compared to the provided controls. A concentration-response model (linear regression) demonstrated an effect threshold (ECR) at 29 µg a.S.L.

8

Fry growth expressed as standard length was also significantly reduced on post-hatch day 63 in all test concentrations compared to the pooled controls. A concentration-response model (linear regression) demonstrated an effect threshold ( $EC_0$ ) a Q6  $\mu$ g a.s./L  $\leq$ 

Fry growth expressed as dry weight was significantly reduced on post-hatch day 63 in all test concentrations compared to the pooled controls of concentration-response model (linear regression) demonstrated an effect threshold  $\langle EC_0 \rangle$  at 14 µg/a.s./L A.

			N.	rout with exposure to KWG 4168
Table C A (0 ) 1 /01 5	Kalloon La orth	and water of	want and the second	nout with announce to VWC 1169
1 able CAND.2.2.1/01-3		and weight of	ranadow u	rout with exposure to K w G 4100
				I I I I I I I I I I I I I I I I I I I

		<u>~~</u>		
Nominal test	Post hatch day 34	Post-hatch day 63		
concentration	leingth (mm)			
(μg a.s./L)	<i>(</i> )	Length (mm)	Wet weight (mg)	Dry weight (mg)
Control		34.5	552	91.9
Solvent control 📣	3699 🔬 🖓	33.9	491	86.8
Pooled control	Q0.5 X	34.2	522	89.4
62.5 5 5 4	28.4	31.9*	-	77.3*
125 2 2 2	26.6*	29.4*	-	71.3*
125 A Q Q	24 ⁷ .7*	26.5*	-	69.5*
125 5 250 5 500 5	21.9*	23.2*	-	59.1*
1000 C	20.4*	21.9*	-	55.5*
2000	19.1*	21.2*	-	50.8*

Results are means of four replicates



Nominal test concentration	Post-hatch day 34 length (mm)	Post-hatch day 63		0			
(µg a.s./L)	iongen (inni)	Length (mm)	Wet weight (mg)	Dry weight (mg			
* Statistically significant different to the needed control (g=0.05)							

Statistically significant different to the pooled control ( $\alpha$ =0.05)

During the post-hatch period, the following morphological and behavioural effects were observed: darkened colouration, swollen belly, oversized yolk sac, exophthalmia, kyphosis, scoliosis, fish lying, on side or back, loss of equilibrium and vertical orientation. The lack of a dose-response coupled with the level of random mortality observed in the controls, indicated no compound-related effects in the case of swollen belly, oversized yolk sac, exophthalmia, kyphosis, scoliosic and loss of equilibrium.

In the case of darkened colouration, there was a dose-response relationship observed between post-hatch day 33 and the end of the study in the 250, 500, 1000 and 2000 µg a.s./Lorest concentrations. In the case of vertical orientation there was a dose-response between post-hatch day 33 and the end of the study in the 500, 1000 and 2000 µg a.s./L test concentrations. In the case of fish lying on side or back there was a transient dose-response between post-hatch day 18 and post-batch day 22 at all test levels. This symptom was observed at the end of the study only in the 500, 1000 and 2000 µg as./L dest concentrations.

168 are summarised in the table The endpoints resulting from exposure of rainbow trout below:

Endpoint	NOÈC & S &		Effect threshold (EC ₀ )
Survival at post-hatch	NGEC & Star		1414
day 34			. 65
Survival at post-hatch	1000 5		~~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~
day 63			×
day 63			>2000
	2000 %	√2000 ° ,/> %/	>2000
Time to 94% Sym-up		1000	707
Growth, expressed as dry	[*] ≪62.5 ↔	625 2 2	14
weight 🛰			
Growth Standard length	<65	62.5	29
on post-hatch day 34			
Growth (standard length)	×62.5 ×	623	26
on post-hatch day 3	×62.5 5 2 2 7 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5	, or	

Table CA 8.2.2.1/01-6 Endpoints (µg as /L) after exposure to

#### Conclusion III.

Based on the statistical analysis of survival, esg hatchability, time to hatch, time to swim-up and growth (expressed as weight and length), the NOEC and LOECs were determined. In addition, effect thresholds (EC₀) were estimated with a good curve fitting for growth parameters based on a concentration-response model (linear regression model).

Survival at post-hatch Day 3@ and Day 63 resulted in a NOEC and LOEC of 1000 and 2000 µg a.s./L, respectively,  $\hat{\mu}$  both days. The effect threshold (EC₀) was calculated to be 1414 µg a.s./L.

Egg hatchability resulted in a DOEC at 2000 µg a.s./L and the LOEC at >2000 µg a.s./L. An effect threshold could not be determined.

Time to hatch resulted in a NOEC at 2000  $\mu$ g a.s./L and the LOEC at > 2000  $\mu$ g a.s./L. An effect threshold could not be determined.

Time to 94 percent swim-up resulted in a NOEC at 500 µg a.s./L and the LOEC at 1000 µg a.s./L. The effect threshold (EC₀) was calculated to be 707  $\mu$ g a.s./L.



Growth, expressed as dry weight, resulted in a NOEC at  $< 62.5 \ \mu g a.s./L$  and the LOEC at  $62.5 \ \mu g a.s./L$ . The effect threshold (EC₀), based on a concentration-response model (linear regression model), is 14 \ \mathcal{\mu} g a.s./L.

Growth, expressed as length, on post-hatch Day 34 resulted in a NOEC at  $< 62.5 \ \mu g a.s./L$  and the LOEC at 62.5  $\mu g a.s./L$ . The effect threshold (EC₀), based on a concentration-response model (linear tegression model), is 29  $\mu g a.s./L$ .

Growth, expressed as length, on post-hatch Day 63 resulted in a NOEC at  $62.5 \ \mu g$  a.s./b and the LOEC at 62.5  $\mu g$  a.s./L. The effect threshold (EC₀), based on a concentration-response model threat regression model), is 26  $\mu g$  a.s./L.

The effect threshold for KWG 4168 was based on the most sensitive endpoint (growth, expressed as drewight), and determined to be 14  $\mu$ g a.s./L.

### Assessment and conclusion by applicant:

The study was conducted to the OECD test guideline 210 (1992), the most up-to-date version of which o is the "Fish, early-life stage toxicity test", adopted 26 July 2013.

Validity criteria according to the most recent OECD 210 guideline 2013) were set:

- Dissolved oxygen concentration to be 20% of the air saturation value throughout the test (actual: 95 to 109%)
- Overall survival of fortilised eggs (*i.e.* hatching success) and post-hatch survival in the controls should both  $e \ge 75\%$  for rainbow trout (100 and 90.5% achieved, respectively, in the pooled controls)

One of the criteria was not met

• Water temperature to not differ by more than  $\pm 1.5^{\circ}$  between test chambers or between successive days at any time during the test. The temperature was only measured in one test chamber with one deviation of  $\pm 2^{\circ}$  to preceding and successive days recorded on study day 22. The mean temperature on day 22 was  $\pm 1.7^{\circ}$  C  $\pm 0.9^{\circ}$  SD).

The water temperature deviation was transient and only slight, and thought to have no impact on the integrity of the study. The study is therefore considered acceptable

Statistically significant effects were determined at the lowest concentration tested which was 62.5  $\mu$ g a.s./L. As a result on EC was calculated and determined to be 14  $\mu$ g a.s./L. Whilst the study is considered to be valid the EC endpoint is not conventionally used in risk assessment and should therefore be treated with caution.

The data have been subjected to statistical recovaluation and the results have been presented in the following study summary.

The NOEC was determined to be  $\leq 62.5 \,\mu\text{g}$  a.s.  $\Phi$ . The EC₀ for KWG 4168 was based on the most sensitive endpoint (growth) expressed of dry veight), and determined to be 14  $\mu$ g a.s./L. A more conservative chronic fish endpoint is available from a fish full life cycle study and has been used in the risk assessment.

The data have been subjected to statistical re-evaluation and the results have been presented in the following study summary.

A B B A A



Data Point:	KCA 8.2.2.1/04
Report Author:	
Report Year:	2020
Report Title:	Calculation of EC10 and EC20 values for Oncorhynchus mykiss with
	spiroxamine TG in an early life stage study
Report No:	0471836-ECO1
Document No:	<u>M-760407-01-1</u>
Guideline(s) followed in	none
study:	
Deviations from current	None V Q Q Q Z
test guideline:	
Previous evaluation:	No, not previously submitted
GLP/Officially	No, not conducted under GEP/Officially recognised testing facilities
recognised testing	
facilities:	
Acceptability/Reliability:	Yes O V V V V A

### **Executive Summary**

The report M-006232-01-1 on the effects of Spiroxamine TG in the rainbow Fout Oncorrynchus mykiss) early life stage study did not provide estimates of C10 or EC20 values Therefore, these values have been calculated in accordance with the Annex to Com. Reg 283/2013. Effect Concentrations with a 10% and 20% effect on length when compared to the pooles controls were re-calculated. As effects were less than 50% an EC₅₀ could not be determined. As effects were >10%, no  $C_{10}$  calculations were conducted for dry weight. Atthough ECx values and 95% confidence intervals were calculated for % swim up, as the EC₁₀ is way above the test rate that showed an effect abound 10% the results are deemed unfit for use within a risk assessment. As effects of 5% and 9% were seen in the controls, which were not corrected for in the test item concentration surgival, actual effects would therefore be minimal and so reliable ECx calculations could not be determined. As there were no effects on hatchability and time to hatch, with a lack of dose response, no ECx values could be calculated.

The resulting EG10, and EC20 values for length at 34 days post-batch (dph) of 67.87 (95%CL: 23.52 -123.02) and 288.97 (95% CLP. 173.78 - 408.69) ag a.s. P, respectively, are considered reliable as the criteria for coodness of fit were fact. The resulting EC₀ and EC₂₀ values for length at 63 dph of 58.21 (95%CL: 27.16 - 93.81) and 327.46 (95%CL: 232.84 - 459.19) µg a.s./L, respectively, meet the goodness of fit criteria and are therefore considered reliable. However, from a visual interpretation of the data an EC10 062.5 for a sould still be considered more appropriate for use in the risk 

Effect concentrations with 10 and 20% from the test item treatment when compared to the pooled controls were calculated but due to lack of effects and the non-existence of a dose response these could not be determined for some parameters. IC10 and EC20 values were determined for length and % swim up. For the determination of the  $GC_{10}$  and  $EC_2$  values for length at 34 and 63 dph a Probit function using linear maximum likelihood regression and lotear weighted regression, respectively was used along with 95% ECx condidence limits For the determination of the EC₁₀ and EC₂₀ values for % swim up at 49 dph a Weibull function using linear maximum likelihood regression was used along with 95% ECx confiden@limits

#### **Results and Discussion** II

A more detailed explanation is given for regression analysis endpoints for dry weight, total length and % swim Ap. These details can be found below. For the parameters hatchability and time to hatch, no statistical calculations were possible to perform as no effects were observed.

Total length at 34 days post hatch (dph)



Regarding the calculation of  $EC_{10}$  and  $EC_{20}$  values for total length at 34 dph, the criteria for goodness of fit were met as the P(Chi²) value was 1.00, showing no significant deviation between fit and data, and a statistically significant concentration/response was found (p(F) < 0.001) for this parameter.

The resulting  $EC_{10}$  and  $EC_{20}$  values and the respective confidence intervals are represented following table below.

# Table CA 8.2.2.1/04-1 Results of the Probit analysis (max. likelihood regression) with total ongth at 34 dph: Selected effective concentrations (ECx) of the test item and their 95% confidence limits (according to Fielder's theorem)

Parameter EC10 Total length EC26 EC26 (95 % confidence interval) (95 % confidence interval)		4			y N
(05.%  confidered interval) $(05.%  confidered interval)$		Joint To	tal length	, ^e	
	Parameter	(05 % confidence interval)	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	of EC26 confidence int	erval)
		<u>με α</u>		[μg@a.s./L]>γ	″ ≪J ^v
Effect on total length at 34 $67.87 (2052-123402)$	Effect on total length at 34	67.87 (2 D52-12 3 02)	2 <b>8</b> 8.	.97 \$73.78-408	.69
$\frac{dph}{dph} = \frac{1}{\sqrt{2}} \frac{1}{\sqrt$	dph	<u> </u>		~ O [×]	

The resulting  $EC_{10}$  and  $EC_{20}$  values of 67.87 (95% CL 23.52 123.62) and 288.97 (95% CL: 178.78 – 408.69) µg a.s./L, respectively, meet the goodness of fit criteria and therefore the estimated  $EC_{0}$  value is considered reliable for use in the risk assessment.

### Total length at 63 days post hatch (aph) @

Regarding the calculation of  $RC_{10}$  and  $EC_{20}$  values for total length at 63 dph, the criteria for goodness of fit were met as the P(Chi²) values vas 1.00, showing no significant deviation between fit and data, and a statistically significant concentration/response was found (p(F) < 0.001) for this parameter.

The resulting  $EC_{10}$  and  $EC_{20}$  values and the respective confidence intervals are represented in the following table.

Table CA 8.2.2.1/0422 Results of the Probit analysis (hinear weighter) regression) with total length at 63 Oph: Selected effective concentrations (EC ) of the test item and their 95%confidence limits (according to Fielder's theorem)

			Fotalde	ngth
	A 1945 0/ Q	EC 10 O	val)	EC ₂₀
		μg, as./L]	val) ~	(95 % confidence interval) [μg a.s./L]
Effect on total length a 63	58.21	(27.16 - <b>9</b> 3.81)	5	327.40 (232.84 - 459.19)

The resulting  $EC_{10}$  and  $EC_{20}$  values of \$8.21 (95% Cf. 27.16 – 93.81) and 327.40 (95% CL: 232.84 – 459.19) µG a.s./L, respectively meet the goodness of fit criteria and therefore the estimated  $EC_{10}$  value is considered reliable. However, from a visual interpretation of the data an  $EC_{10} > 62.5 \mu g$  a.s./L should still be considered more appropriate for ose in the risk assessment.

### <u>% swim up</u>

Regarding the calculation  $(EC_{10} \text{ and } EC_{00} \text{ values for } \% \text{ swim up at 49 dph, the criteria for goodness of fit were met as the P(Chio) value was 1.00, showing no significant deviation between fit and data, and a statistically significant concentration/response was found (p(F) < 0.001) for this parameter.$ 

The resulting  $EC_{10}$  and  $EC_{20}$  values and the respective confidence intervals are represented in the following table and figure below.



# Table CA 8.2.2.1/04-3Results of the Weibull analysis with % swim up at 49 dph: Selected effective<br/>concentrations (ECx) of the test item and their 95%-confidence limits (by<br/>bootstrapping (1000 resamplings); bias-corrected)

	% sw	vim up
Parameter	<b>EC</b> ₁₀	EC20 2
	(95 % confidence interval)	(95 % confidence interval)
	[µg a.s./L]	
Effect on % swim up at 49	321.75 (176.14 - 519.90)	502.71 (327.4k 7703.62)
dph		

The resulting  $EC_{10}$  and  $EC_{20}$  values of 321.75 (95% CF. 176.14 – 549.90) and 505 71 (95% CL: 327.4 16 – 703.62) µg a.s./L, respectively, meet the goodness of fit criteria and therefore the estimated  $EC_{10}$  value is considered reliable. However, as the endpoint way above the test rate that showed an effect around 10% the results are deemed unfit for use within a risk assessment.

A summary of the obtained endpoints is presented in the following table

# Table CA 8.2.2.1/04-4 Overall endpoints of the statistical re-calculation of the *Oncorpynchus mykis* study with spiroxamine

Parameter	The second se
rarameter	🖓 💦 EG 10 (95% confidence jutervals) 🖉 🥎
Survival 34 dph	
Survival 63 dph	$\gamma$ $\gamma$ $\gamma$ $\gamma$ $\gamma$ $\gamma$ $n.d. \gamma \gamma \gamma \gamma \gamma \gamma \gamma \gamma \gamma \gamma$
Dry weight	
Length 34 dph	
Length 63 dph	A
% swim up	a And A Ar
Hatchability	$p^{\gamma}$ , $q^{\gamma}$ , $q^{\gamma}$ , $q^{\gamma}$ , $q^{\gamma}$ , $q^{\gamma}$ , $q^{\gamma}$
Time to hatch	
2	

Due to the lack of a concentration dose response when compared to the controls, the calculation of  $EC_{10}$  and  $EC_{20}$  values for batchability and time to hatch was not possible and therefore no  $EC_{10}$  or  $EC_{20}$  values were determined. Due to effects at all the tester rates being more than 10% below the controls values, and as recommended by OECD (OECD 54), the  $EC_{70}$  could not be calculated for dry weight. Due to a mortality of 5 and 9% in the pooled controls at 34 and 63 dph/respectively, which were not used to correct test item superior,  $\frac{1}{2}C_{10}$  values could not be reliably calculated for survival as the real effects would be even smaller than those presented.

### III. Conclusion

The resulting EC₁₀, and EC₂₀ values for length at 34 den of 67.87 (95%CL: 23.52 – 123.02) and 288.97 (95%CL: 27.78 - 408.69) µga.s./L, respectively, are considered reliable as the criteria for goodness of fit were met. The resulting EC₁₀ and EC₂₀ values for length at 63 dph of 58.21 (95%CL: 27.16 – 93.81) and 327.40 (95%CL: 232.84 – 459.19) are a.s./C, respectively, meet the goodness of fit criteria and are therefore considered reliable. However, from a visual interpretation of the data an EC₁₀ >62.5 µg a.s./L should still be considered more appropriate for use in the risk assessment.

### Assessment and conclusion by applicant:

The statistical @e-evaluation of the data has determined EC₁₀ and EC₂₀ values for some of the parameters but marks of these values, although reliable according to the statistical software, were considered unsuitable for use in the risk assessment. Expert judgement has therefore been used for some of these parameters. The most critical endpoint determined remains to be a NOEC of <62.5 µg a.s./L based on effects on dry weight. However, due to the limited effects seen at this test concentration there is argument to use expert judgement to set the NOEC at 62.5 µg a.s./L.



An EC₀ of 14  $\mu$ g a.s./L was determined in the original study report and has been taken as the most critical endpoint for this study in order to remain consistent with the original study report.

The values determined in the re-evaluation work are considered to be fully valid.

Data Point:	KCA 8.2.2.1/02
Report Author:	
Report Year:	
Report Title:	2004 14C-KWG 4168 - Early life stage toxicity to randow trout (Ore or hypertus mykiss) under flow-through conditions (supplemental raw data)
Report No:	DOM 95017 A Q & A A O Q
Document No:	$\underline{M}_{-006449-02-1} \qquad \qquad$
Guideline(s) followed in study:	OECD Guideline 210: "Fish, Early-life®tage, Toxicity/Fest"
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted DAR (1997) RAR (2010), RAR (2017)
GLP/Officially	Yes, conducted under GLROfficially recognised esting facilities
recognised testing	
facilities:	
Acceptability/Reliability:	Yes y y y y y

### **Executive Summary**

A flow-through early-life stage toxicity lest was conducted to determine the toxicity of ¹⁴C-KWG 4168 to the early-life stages of the rambow rout (*Oncorronchus mykiss*).

Six test concentrations (3,75, 7.5, 15, 36, 60 and 120 µg as L) were investigated, each with four replicates of initially 100 fertilised eggs. The effects of exposure on different life stages of the fish, including early life stages, juvenile growth, reproduction and early life stages of the filial generation were assessed onder continuous exposure.

Based on the statistical analysis of survival, egg hatchability dime to hatch, time to swim-up, and growth (expressed as weight and length), the no-observed-effect-concentrations (NOECs) and the lowest-observed-effect-concentrations (LQECs) were determined as follows. All test levels listed are based on mean measured concentrations (365, 7:4), 142, 28.9, 61.8 and  $119 \mu g$  a.s./L) of the test substance:

Survival at day 71 (poophatch day 36) resulted in a NOEC at  $\geq$ 119 µg a.s./L and the LOEC at >119 µg a.s./L

Survival at 96 (post-hatch day 61) resulted into NOEC at  $\geq$ 119 µg a.s./L and the LOEC at >119 µg a.s./L

Egg hatchability at day 38 resulted in a NOEC at  $\geq$ 119 µg a.s./L and the LOEC at >119 µg a.s./L.

Time to hatch at day 38 resulted in a NOEC  $g \ge 119 \ \mu g$  a.s./L and the LOEC at >119  $\mu g$  a.s./L.

Time to swim up at day 55 resulted in a NOEC at  $\geq$ 119 µg a.s./L and the LOEC at >119 µg a.s./L.

Growth at day 71 (post-hach day 36), expressed as standard length, resulted in a NOEC at 28.9 µg a.s./L and the LOEC at 61.8 µg a.s./D.

Growth at day 96 (port-hatch day 61), expressed as standard length, resulted in a NOEC at 14.2 µg a.s./L and the LQEC at 28.9 µg a.s./L.

Growth at day 96 (post-hatch day 61), expressed as dry weight, resulted in a NOEC at 28.9 µg a.s./L and the LOEC at 61.8 µg a.s./L.



The lowest effect threshold (geometric mean of NOEC and LOEC) for ¹⁴C-KWG 4168 was based on the most sensitive endpoint, the fry growth, expressed as standard length, on study day 96 (post hatch day 61). So, the lowest effect threshold in this study was calculated to be 20.3 µg test substance/L

- I. **Materials and Methods**
- A. Materials

st in the second Non-radiolabelled Radiolabelled **Test Material** KWG 4168 technical **THS 442** Lot/Batch #: 898114002 Not reported 96.7% **Purity: Description:** Colourless liquid Not reported **19**94 Stability of test Certified until 25 Januar compound: **Reanalysis/Expiry** 25 January 19 porte date: **Density:** Not report Treatments 60 and 320 **Test rates:** Solvent/vehicle: ceton Ø es on days Analysis of test and 96 (mean measured value concentrations: 📞 03% of nominal) **Test organisms Species:** Rainbow trout (Oncorhynchus mykiss Source: Not applicable Acclimatisation period: ve byne sharp (Artemia salina) and trout/salmon starter pellets Feeding obtained from certified disease free hatchery Treatment for disease: Test design 18 ¢m x 2⊈cm with a water depth of 19 cm = 7.5 L volume Test vesset Reconstituted water Test medium: Replication Four replicates per control and test group Μα' of animals/vessel Duration of test: 96 days **Environmental test** conditions



Temperature:	9.1 – 10.7°C (mean: 9.8°C)
Dissolved oxygen:	93 – 100% of saturation
pH:	7.0 - 7.6
Photoperiod:	16 h light : 8 h dark at 435 lux

### **Study Design**

100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - 100 - A flow-through early-life stage toxicity test was conducted to determine the toxicity to the early-life stages of the rainbow trout (Oncorhynchus mykiss).

Nominal test concentrations of 3.75, 7.5, 15, 30, 60 and 120 µg a.s. were tested. Prior to the mitiation of the study, test solutions had been flowing through the test system.

Eggs transported in plastic bags, were transferred to addry stamless steel bowl and the milt was mixed with the eggs (with an autoclaved goose feather). Milt, Gransported in a separate plastic bag, was examinated under the microscope. There were a negative findings Dilution water (10 & 1°C) as added until the eggs were covered.

A total amount of 135 L/h dilution water was used in this study. This dilution water was split into 9 different water streams of 15 L/h, controlled by flow meters. A diluter system with two programmed Microlab Hamilton MT 4-fold syringe pumps was used for the intermitten introduction of 1.5 mL/h (volumes of 25 µL each 60 seconds) of stock solutions with different concentrations of C-KWG 4168. Stock solutions of ¹⁴C-KWG #168 in acetore were added to each of these streams of dilution water (resulting in a concentration of 100 µL accord por litre of dilution water), except for the dilution water control group and the fertilisation control group. The solvent control group was also prepared with acetone. The maximum concentration of acetone in the test system, was 100 µLD.

All stock solutions were continuously agilated during the test by magnetic stirrers. Flow splitting cells divided the water streams after the introduction of stock solutions and after passing mixing chambers into four aliquotoper test concentration before being delivered to replicate test chambers.

The accuracy of the st solution splits was checked prior to the test institution and after test termination for both the egg hatching chamber system (for embryos from fertilisation to hatching) and the growth chamber system (for alevins). The accuracy of splits was within 10% of nominal. The diluter system and syringe pump function were checked and documented working daily.

Eggs were incubated in incubation cups, constructed from 8 cm diameter teflon pipes with stainless steel plates perforated (hole diameter. 1.8 mm) on the bottom. These incubation cups were suspended in each replicate test Mamber? To facilitate circulation of water and keep eggs clean, incubation cups were oscillated vertically with a lifting movement of approximately 4 cm in the test chamber by means of a rocker and apparatus driven by a low from electric motor.

The glass aquaria used for egg hatching measure approximately 14 cm x 9 cm with a water depth of 17.5 cm, yielding an approximate chamber volume of 2.2 liters and resulting in approximately 41 changes of test solution per da? The glass aquaria used as growth chambers for the post hatch phase measured approximately 18 on x 22 cm will a water depth of 19 cm, yielding an approximate chamber volume of 7. Oliters and resulting in approximate 12 changes of test solution per day. All glass aquaria used as growth chamber for the post hatch phase were covered with stainless steel screens to prevent escaping of alevons.

The number of eggc hatched in each incubation cup was recorded until 3 days post-hatch. The posthat the period began after \$5% of all living eggs in the control had hatched (study day 35, post hatch day 0). Aleying were impartially thinned to 15 individuals per replicate on study day 38 (post-hatch day 3). This was accomplished by transferring 15 impartially selected fry from each replicate and by releasing in the corresponding replicate aquarium on the same day. Observations of abnormal behaviour, normal



swim-up behaviour, abnormal physical changes, and mortality were recorded daily by visually inspecting each growth chamber. Dead fry were removed and discarded.

Feeding began on day 47 (post-hatch day 12). Fry were fed *ad libitum*, taking care that each aduaria received an equal quantity of food. Food was added to the aquaria twice daily, except on weekends/holidays when food was added once daily. Live brine shrimp (*Artenna salina*) nauplii were fed to the fry until one day before study termination. In addition, the fry were also fed from post-batch day 14 with ground trout/salmon starter until one day before study termination.

Growth was determined by measurement of standard length (mm) on study day 71 (post hatch day  $\delta$ ) using slide photography. The water depth in each test chamber was lowered to approximately 2 cm in order to take the photo. A millimeter scale and tank identification plate with study number, test concentration and replicate number were positioned on the bottom of the chamber. The developed photographic slides were projected parallax-free on a wall and standard length of each fish was measured using the photographed metric scale as the standard.  $\delta$ 

At the termination of the biological phase (post hatch day 61) after a total of 96 days the surviving fish were sacrificed. The standard length (mm) was determined and recorded for each individual fish. Standard lengths were determined by measuring from the tip of the snoat to the tip of the caudal pediancle using a millimeter scale. Wet weight of control and solvent control fish was recorded for evaluation of test system loading. Fish were blotted on paper towels to remove excess more than placed into labelled open pans and placed in a 60% drying over for 60 hours. The thy weights of the individual fish were measured to  $\pm 1$  mg using an analytical balance.

### II. Results and Discussion

Validity criteria were not assessed as part of the study report?

The mean measured concentrations of ¹⁴CrKWG 4168 during the test over 3.65, 7.41, 14.2, 28.9, 61.8, and 119  $\mu$ g a.s./L. These mean values ranged from 94 103 of nominal during the total test period and for all test levels. All reported results are related to the mean measured concentrations of test substance. The stability of ¹⁴CrKWG 4168 in the prime stock solution, used for preparing of all 5 series of stock solutions with acetone, was >99% during the tridy. The stability of ¹⁴CrKWG 4168 in the fifth series of stock solutions with acetone was >98.8%. The stability of ¹⁴CrKWG 4168 in water samples from the aquaria had an overall mean of 95% during the tridy. Thus, C-KWG 4168 was stable under test conditions.

Nominal test concentration	Mean neasured concentration	Mean N	SP.	Measured (X) concentration in	Measured (X) concentration
concentration (μg a.s./ΙΟ 3 75	(µg@ps./L)		$\sim$	%	in μg/L
3.75 ⁽¹⁾	5,226 5.3120 5.226	5.2 <b>5</b> 8 ~ ~ ~	0.038	98	3.66
7.5	5.091 J		0.126	98	7.34
		3.065	0.313	94	14.11
$\begin{array}{c} 15 \\ \begin{array}{c} & & \\ & \\ \end{array} \\ 30 \\ \begin{array}{c} 5 \\ \end{array} \\ \end{array} \\ \begin{array}{c} & \\ \end{array} \\ \end{array} \\ \begin{array}{c} & \\ \end{array} \\ \begin{array}{c} & \\ \end{array} \\ \end{array} \\ \end{array} \\ \begin{array}{c} & \\ \end{array} \\ \end{array} \\ \end{array} \\ \begin{array}{c} & \\ \end{array} \\$	Ø.167 4.989 4.952	5.036	0.094	94	28.07
120	5.243 5.072 5.213	5.176	0.075	96	115.39

Table CA 8.2.2.1/02-1 analytical measurements of 4C-KWC 4168 in test water



Nominal test concentration (µg a.s./L)	Mean measured concentration (μg a.s./L)	Mean	SD	Measured (X) concentration in %	Measured (X) concentration • in μg/L
Lab recovery	564.665	538.301	12.119	-	- X
spikes (before	529.227			ð	
test series)	535.91				
Lab recovery	537.329			"O"	
spikes (after test	531.566				
series)	561.107			L L	

Fry survival was analysed on study day 71 (post-hatch day 36). On post batch day 36 (by survival ranged from 95 percent to 100 percent: Control (95%), Solvent Control (98%), 3.65  $\mu$ g/c (100%), 7.40  $\mu$ g/l (100%), 14.2  $\mu$ g/L (100%), 28.9  $\mu$ g/L (100%), 61 &  $\mu$ g/L (97%), and 119  $\mu$ g/L (98%). There was no statistical difference in percent hatchability in any test treatment compared to the pooled control data.

Fry survival was again analysed on study day % (post hatch day 61, study termination). On post-hatch day 61, fry survival ranged from 95 percent to 100 percent: Control (95%). Solvent Control (98%), 3,65  $\mu$ g/L (100%), 7.41  $\mu$ g/L (100%), 14.2  $\mu$ g/L (98%), 28.9  $\mu$ g/L (100%), 61.8  $\mu$ g/L (97%), and 119  $\mu$ g/L (98%). There was no statistical difference in percent hatchability in any test treatment compared to the pooled control data.

Percent egg hatchability was evaluated on study day 38 (post hatch by 3). Hatch data, corrected for viability, ranged from 93 percent to 100 percent. Control (98 %), Solvent Control (97 %),  $3.65 \mu g/L$  (98 %), 7.41  $\mu g/L$  (93 %), 14.2  $\mu g/L$  (93 %), 28.9  $\mu g/L$  (96 %) 61.8  $\mu g/L$  (100 %) and 149  $\mu g/L$  (95 %). There was no statistical difference in percent hatchability in any test treatment compared to the pooled control data.

Table CA 8.2.2.1/02-2	Mean	eggahat	chabilit	v and	fry sur	vivat wit	h exposu	reto	14CKWG 4168	;
	. // .	<i>70,1</i>	ČA ·	$^{\prime}$ O		. 0	×~• .		. 05	

Mean	Post-hatch day 3 (study day 38) Survival (%)	Post-hatch day 61
measured	a by a a gurvival (%)	(study termination)
test	Mean egg hatch     Mean post-hatch day     Survival (%)       (%) ¹ 34 survival (%)     34 survival (%)       89 ¹⁰ 984     95	Survival (%)
concentration ₍	(%) ¹ 34 survival (%)	
(µg a.s./L) 🚿		
	89 0 2 98 2 2 2 2	95
Solvent	$\circ \circ \times \times \circ \circ$	98
Control Solvent Solvent		
3.65	91 7 4 98 7 4 100	100
7.41	84 93× 0 100	100
14.2	<b>§</b> 4 A § <b>§</b> 3 . A 100	98
28.9		100
61.8 ~ 9		97
119 🕰	86 95 95 95 98	98

Fry growth, expressed as standard length, was measured on study day 71 (post-hatch day 36). Analysis of data showed a significant difference from the pooled controls in the 61.8, and 119  $\mu$ g/L test levels. Fry growth, expressed as standard length, was again measured on study day 96 (post-hatch day 61; study termination). Analysis of data showed a significant difference from the pooled controls in the 28.9, 61.8, and 119  $\mu$ g/L test levels.

Fry growth, expressed and dry weight, was measured on study day 96 (post-hatch day 61; study termination). Analysis of data showed a significant difference from the pooled controls in the 61.8, and 119  $\mu$  get test weight.

The biomass loading factor for the study was determined using the wet weights of the control and solvent control fish at study termination. The mean wet weight was 346 mg/fish in the pooled controls. The biomass loading factor based upon the 7.5 litre volume of a single growth chambers was 692 mg fish per litre. The biomass loading factor based upon a flow of 90 litres per day (12 changes of test solution



per day) through each single test chamber was 58 mg fish per litre and day. These loads were well within the requirements to ensure adequate dissolved oxygen levels and to avoid crowding of fish.

## Table CA 8.2.2.1/02-3 Mean standard length, wet and dry weight of Rainbow trout (Oncorhynchus mykiss) exposed to 14C-KWG 4168 in an early life-stage to the stage to th

Mean	Post-hatch day 36	Post-hatch day 61	Ø.	
measured	mean length	Mean length (mm)	Mean wet weight	Mean dry weight
test concentration (μg a.s./L)	(mm)		(mg) (mg)	Mean dry weight (mg) y y y y y y y y y y y y y y y y y y y
Control	26.4	30.9	331	
Solvent control	25.9	31.6	361 Q Q	60.3
Pooled control	26.1	31.2	346 27 0	8 . S
3.65	26.6	31.5		\$55.0
7.41	27.0	31.7	<u>, p- , or or (</u>	56 2 × ~ ~ ~ ~
14.2	27.4	31.6	- <u>~~</u>	57.2 & Ø
28.9	26.3	29.97		30.7 × 2
61.8	25.0*	29.0* & &		50.0 °C
119	22.2*	Q7.3* × ×	9-20-5 5	

*denotes statistically significant difference (Williams test) from pooled controls

Time to hatch was evaluated for all test levels. Egg hatching began on study day 3 and continued until day 38. The mean percent hatch on study day 38 ranged from 98 percent to 100 percent Control (98%), Solvent Control (100%), 3.65 ug/L (100%), 7.44 ug/L (100%), 14.2 ug/L (100%), 28.9 ug/L (100%), 61.8 ug/L (100%), and 119 ug/L (100%) There was no statistical difference in time to hatch in any test treatment compared to the pooled control data.

Table CA 8.2.2.1/02 Mean time of haten for Rainbow prout (Oncorhynchus mykiss) eggs during the ¹⁴C-KWG early life-stage toxicity study

				A	
Mean 🔬	Percent hatehed ¹⁾ stu	dy da 🖉 📈 🏠		<i>.</i>	
measured 🚬 🔍	33 1 4 34	35. J	360 36	37	38
test 🔍 🧟			O T		
test concentration (μg a.s.L)	Ŭ S &				
(µg a.s.)L)					
Control		× ⁰ 96 ≪ × ≪ 930	98	98	98
Solvent	\$ A L	× 930°	900	100	100
control					
3.65	0 ℃ ↓2 `~	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	100	100	100
7.41		Q 969 Q	99	100	100
14.2 28.9 2	0	1 . W	99	100	100
28.9			99	100	100
61.8	0 5 5 6	<u>رُوْمَ</u> 92 مُ	99	100	100
1197		98	97	100	100
1				1 07 1 100	

¹ Percent hatch [# of alevin]/4 of alevin + # of eggs on Day 38, post-hatch day 3] * 100

Newly hatched fry began swimming up from the bottom of the test chambers on study day 50 (posthatch day 15). Swim-up was observed for a 7 day period between study day 50 and 57. A >95% swimup was achieved on day 55 in the pooled controls (control: 96 percent; solvent control: 95 percent). For all other treatment levels on day 55 a swim-up between 91 and 100 percent was reached. There was no statistical difference in time to swim up in any test treatment compared to the pooled control data.



Table CA 8.2.2.1/02-5	Mean percent swim-up of newly hatched rainbow trout (Oncorhynchus mykiss)	fry
	during the 14C-KWG 4168 early-life stage toxicity study	<i>a</i> .°

Mean	Percent hatched ¹⁾ study day							
measured test concentration (µg a.s./L)	50	51	52	53	54	55	56	5767 0 4
Control	3	5	20	20	56	96 🔊	100 %	97 97 4
Solvent control	3	15	32	42	<b>G9</b> 2	95 ⁴	98	
3.65	0	15	30	35	73	98	28 Q	98 8
7.41	3	20	45	40 🕰	87 🖧	2 100 ∘	J00 5	100
14.2	3	5	23	350	68 🔊	100	¶100 O	\$00 O
28.9	2	12	33	43		98 0	98	98 5
61.8	3	8	17	×32 v	78	100 🖉	400	100
119	0	7	17	27	69 0	100 × 91 >	900	100 <u>(</u> °

Egg viability was checked 12 days after tertilisation with the 200 addition eggs (50 per incubation cup) that were placed in separate egg incubation cups at test initiation. The viability determination (fertilisation success) in the four replicates tanged from 8606 94 percent with a mean (689.5, percent.

Table CA 8.2.2.1/02-6 Rainbow trout (Oncorhynchus mickiss) embryo sabilit during the 146-KWG 4168 early life stage toxicity study

			<u> </u>
Total eggs in egg cup	Number of viable ggs	Number of monviable	<b>Percent</b> viability
		eggs	
50	147 J 2 2	34 5. 4	×94 ~~
50		$\mathcal{F} \sim \mathcal{F} $	86
50		7 1 0	86
50	46 0 29	400 20 1. (	_{7,92}
Mean:			89.5

### III. Conclusion

Based on the statistical analysis of survival, egg hatchability time to ratch, time to swim-up, and growth (expressed as weight and length), the no-observed-effect-concentrations (NOECs) and the lowest-observed-effect-concentrations (LQECs) were determined as follows. All test levels listed are based on mean measured concentrations (3,65, 7,4), 14, 28.9 (1.8 and 119  $\mu$ g a.s./L) of the test substance:

Survival at day 71 (post hatch day 36) resulted in a NOEC at  $\geq$ 119 µg a.s./L and the LOEC at >119 µg a.s./L

Survival at 96 (post-hatch day  $\Re$ ) resulted in a NOEC at  $\geq$ 119 µg a.s./L and the LOEC at >119 µg a.s./L and the LOEC at >119 µg

Egg hatchability at day 38 tosulted in a NOEC at  $\geq$ 119 µg a.s./L and the LOEC at >119 µg a.s./L.

Time to hatch at day 38 resulted in a  $MOEC_{1} = 119 \ \mu g \ a.s./L$  and the LOEC at >119  $\mu g \ a.s./L$ .

Time to swim up at day 55 coulted in a NOEC at  $\geq$ 119 µg a.s./L and the LOEC at >119 µg a.s./L.

Growth at day 71 (bost-hatch day 36), expressed as standard length, resulted in a NOEC at 28.9 µg a.s./L and the LOEC at 01.8 µg a.s./D.

Growth at day 96 (post-hatch day 61), expressed as standard length, resulted in a NOEC at 14.2 µg a.s./L and the LOEC at 28.9 µg a.s./L.

Growth at day 96 (post-hatch day 61), expressed as dry weight, resulted in a NOEC at 28.9  $\mu$ g a.s./L and the LOEC at 61.8  $\mu$ g a.s./L.



The lowest effect threshold (geometric mean of NOEC and LOEC) for ¹⁴C-KWG 4168 was based on the most sensitive endpoint, the fry growth, expressed as standard length, on study day 96 (post hatch day 61). So, the lowest effect threshold in this study was calculated to be 20.3 μg test substance/Is Assessment and conclusion by applicant: The study was conducted to the OECD test guideline 210 (1992), the most up ad-date version of is the "Fish, early-life stage toxicity test", adopted 26 July 2013. Validity criteria according to the most recent OECD 210 guideline (2016) were met Dissolved oxygen concentration to be  $\geq 60\%$  of the air saturation value throughout the (actual: 95 to 98%) Overall survival of fertilised eggs and post-hatch success in the controls to be≥75% (95 98%, in the control ad solvent control, respectively) Water temperature to not differ by spore than  $\pm 1.5^{\circ}$ C between dest chambers or between successive days at any time during the test, The temperature range for the duration of the test was 1.6°C, daily temperatures were not reported, however, it can be assumed that the criteria was met based on the temperature range for the entire test period. The validity criteria according to the current test guideline have been met therefore this study is considered to be valid. 61), expressed as standard length resulted in the lowest NOEC at Growth at day 96 (post-hatch day S. 14.2 µg a.s./L. statistical re-saluation and the results have been presented in the The data have been subjected following study summary Data Point: Report Author: Report Year: 🕻 2020 Calculation of EC10 and EC20 values for Oper or hypothese mykiss with 14C Report Title spire amine OG in an early life stage study 04Ø≸836-ÉÈO3 Report No: Document No: M-760488-0 None Guideline(s) followed in study: Deviations from current Nône test guideline? Previous evaluation: No, not previously submitted GLP/Officially YeO conducted under GLR Officially recognised testing facilities recognised testing facilities: Accéptability/Reliability Executive Summary

The report  $\sqrt{1-006449-020}$  on the effects of ¹⁴C Spiroxamine TG in the rainbow trout (*Oncorhynchus mykiss*) early life stage study fid not provide estimates of EC₁₀ or EC₂₀ values. Therefore, these values have been calculated in accordance with the Annex to Com. Reg. 283/2013. Effect Concentrations with a 10% and 20% effect on length when compared to the pooled controls were re-calculated. As there were no effects (<10%) on survival, hatchability, % swim up and time to hatch, with a lack of dose response ECx values were estimated to be >119.00 µg a.s./L. Although ECx values and 95% confidence intervals were large, spanning more than 2 concentrations and the dose response curve is not covered by the data, it was not possible to determine reliable ECx values.



The resulting EC₁₀, and EC₂₀ values for length at 36dph were 96.73 (95%CL: 91.75 – 100.84) and 138.15 (95%CL: 131.86 – 146.92)  $\mu$ g a.s./L, respectively. The resulting EC₁₀, and EC₂₀ values for length at 61dph were 91.46 (95%CL: 72.83 – 114.65) and 195.28 (95%CL: 146.45 – 369.35)  $\mu$ g 48./L, respectively. Although the EC₁₀ values are considered reliable as the criteria for goodness of  $\mu$  were met, as effects were <20%, the EC₂₀ cannot be considered reliable as it was calculated. Cased on extrapolation.

### I. Methods

The statistical evaluation was performed with statistical software ToxRaf Standard v3

Effect concentrations with 10 and 20% from the test item treatment when compared to the pooled controls were calculated but due to lack of effects and the non-existence of a dosc response these could not be determined for some parameters.  $EC_{10}$  and  $EC_{20}$  values could only be determined for dry weight at 61 dph and length at 36 and 61 dph. For the dry weight at 61 dph a Probit function using linear maximum likelihood regression was used along with 95% ECx confidence limits to calculate  $EC_{10}$  and  $EC_{20}$  values. For the length at 36 and 61 dph, a Pogit function using linear maximum likelihood regression was used along with 95% ECx confidence limits to calculate  $EC_{10}$  and  $EC_{20}$  values.

### II. Results and Discussion 🔏

A complete list of the obtained endpoints is presented below. A more detailed explanation is given for regression analysis endpoints for total length and dry weight. These details can be found below. For the parameters survival, hatchability, the to hatch and % swim up no statistical calculations were possible as no effects were observed.

### Dry weight at 61 days post hatch (dph)

Regarding the calculation of EC₁₀ and E $\hat{b}_{20}$  values for dry weight at  $\hat{b}$  dph the criteria for goodness of fit were met as the P(Chi²) value was 1.00, showing to significant deviation between fit and data, and a statistically significant concentration response was found (p(F) = 0.019) for this parameter.

The resulting EC  $\infty$  and EC  $_{20}$  values and the respective confidence intervals are represented in the following table.

Table CA 8.2.2.1/05-17 Results of the Probit analysis (max. likelihood regression) with dry weight at 61 dph: Selected effective concentrations (BCx) of the test item and their 95%confidence limits (according to Fieller's theorem)

	A C X & DAY	veight
Parameter	5 (95% contidence interval)	EC20 (95 % confidence interval)
(Š ^v		[µg a.s./L]
Effect on dry weight at 6	3394 (4.89 - 61 99)	115.92 (63.47 – 979.11)

The resulting  $EC_{10}$  and  $EC_{20}$  values of 33.94 (95% CL: 4.84 – 61.93) and 115.92 (95% CL: 63.47 – 979.14) µg a.s./L, respectively, meet the goodness of fit criteria. However, as the  $EC_x$  confidence intervals span more than 2 concentrations and the dose response curve is not covered by the data (as effects were between 1.0% and 20.3%), the  $EC_{10}$  value is not considered reliable for use within a risk assessment.

### Total length at 36 dph

Regarding the calculation of  $C_{10}$  and  $EC_{20}$  values for total length at 36 dph, the criteria for goodness of fit were met as the P(Chi⁺) value was 1.00, showing no significant deviation between fit and data, and a statistically significant concentration/response was found (p(F) < 0.001) for this parameter.

The resulting  $EC_{10}$  and  $EC_{20}$  values and the respective confidence intervals are represented in the following table and figure below.



# Table CA 8.2.2.1/05-2 Results of the Logit analysis with total length at 36 dph: Selected effective concentrations (ECx) of the test item and their 95%-confidence limits (according to Fieller's theorem)

	r s theorem)	
	Total length	
Parameter	EC ₁₀ (95 % confidence interval)	EC20 (95 % confidence interval)
	[µg a.s./L]	
Effect on total length at 36	96.73 (91.75-100.84)	138.65 (131.86-146,92) ~ 2
dph	96.73 (91.75-100.84)	

The resulting  $EC_{10}$  and  $EC_{20}$  values of 96.73 (95% CL 91.75 – 100.84) and 138.15 (95% CL 161.86 4) 146.92) µg a.s./L, respectively, meet the goodness of fit criteria and therefore the estimated  $EC_{10}$  value is considered reliable for use in the risk assessmed.

### Total length at 61 dph

Regarding the calculation of  $EC_{10}$  and  $EC_{20}$  values for total length at 61 dph, the criteria for goodness of fit were met as the P(Chi²) value was 1,00, showing no significant deviation between fit and date and a statistically significant concentration/response was found (p(F) = 0,003) for this parameter.

The resulting  $EC_{10}$  and  $EC_{20}$  values and the respective confidence intervals are represented in the following table below.

Table CA 8.2.2.1/05-3 Results of the Logit analysis (max. likelihood regression) with total ength at 61 dph: Selected effective concentrations (ECA) of the test item and their 95%-confidence limits (according to Fieller's theorem)

×.	Fotal leagth S S S S
Parameter	EC10 (95% confidence intervat) (95% confidence interval)
	$  \mathbf{kg} \mathbf{a}.\mathbf{s}.\mathbf{f}   \sim \mathbf{f} = \mathbf$
Effect on total length at 61	Q91.46 (\$\vert 2.83 \vert 4.65) \$\vert 4.65 \vert 2 \vert 4.65 \ve
dph 🖓 🖓	

The resulting  $EC_{10}$  and  $EC_{20}$  values of 91.46 (95% CL: 72.89–11465) and 195.28 (95% CL: 146.45–369.35) bg a.s./L, respectively meet the goodness of fit enteria and therefore the estimated  $EC_{10}$  value is considered reliable.

A summary of the obtained endpoints is presented in the following table.

 Table CA 8.2.01/05-4 Overall endpoints of the statistical re-calculation of the Oncorhynchus mykiss study with spire and points of the statistical re-calculation of the Oncorhynchus mykiss study

Parameter	γ – ζ Endpoint (μg a.s./L)
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	EC 10 (95% confidence intervals)
Survival 36 dph	$\sqrt{2}^{\prime}$ $\sqrt{2}^{\prime}$ $\sqrt{2}^{\prime}$ $\sqrt{2}^{\prime}$ >119.00
Survival 61 dph	<i>[™] [™] [™]</i>
Dry weight 61 dph	n.d.
Length 34 dp	96.73 (91.75-100.84)
Length 61 Cph	
% swim up	>119.00
Hatchability 2	>119.00
Time to hat the	>119.00

The calculation of EC_{10} and EC_{20} values for dry weight at 61 dph did not generate reliable values, therefore no EC_{10} and EC_{20} values are presented. Due to the lack of effects above 10% when compared to the controls, the calculation of EC_{10} and EC_{20} values for hatchability, % swim up, hatchability and



time to hatch was not possible. The EC₁₀ and EC₂₀ values for hatchability, % swim up, hatchability and time to hatch are therefore estimated to be above the highest tested rate of 119.00 µg a.s./L.

III. Conclusion

The resulting EC₁₀, and EC₂₀ values for length at 36 dph were 96.73 (95% CL 91.75 – 100.84) and 138.15 (95% CL: 131.86 – 146.92) μ g a.s./L, respectively. The resulting EC₁₀, and EC₂₀ values for length at 61 dph were 91.46 (95% CL: 72.83 – 114.65) and 195.28 (95% CL: 146.45 – 369.35) μ g a.s./L, respectively. Although the EC₁₀ values are considered reliable as the criteria for goodness of far were met, as effects were <20%, the EC₂₀ cannot be considered reliable as it was calculated, based on extrapolation. The calculation of EC₁₀ and EC₂₀ values for determined. The EC₁₀ and EC₂₀ values for hatchability γ swim up, hatchability and time to hatch were estimated to be above the highest tested rate of 119.00 μ g a.s./L.

Assessment and conclusion by applicant:

The statistical re-evaluation of the data has determined EC_{10} and EC_{20} values for some of the parameters assessed but the EC_{20} values are largely based on extrapolation and are therefore not considered suitable for use in the risk assessment.

The lowest EC_{10} value of 33.9 µg a ΔL was determined for dry weight but has been considered to be unsuitable for risk assessment due to the wide confidence intervals has not been considered suitable for use in the risk assessment.

The NOEC of 14.2 μ g as \mathcal{K} from the original study report shall remain the critical endpoint determined for this study

The values determined in the re-evaluation work are considered to be fully valid

Data Point:	(KCA 80)2.1/05 ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~
Report Author:	
Report Year:	2008 0 4 2 6 2
Report Title:	Effects of spiroxamine technical on selected early life stages of rainbow trout
	(Oncorhynchus mykies) in a static water/sedonent system
Report No.	EBOWX000
Document No:	My 1043,69-01-1 0 2
Guideline(s) followed in	VIFRA Guideline 72-4 OPPT Guideline 850.1400 (draft); OECD Guideline 210
study:	
Deviations from current	Yes Arethods SANSO/3029/99 rex. 4
test guideline	Methods: \$8AN&@/3023/39 rex. 4
D' D	Some fortification levels have no precision
	The calibration curve only has 4 data points
	Ecotoxicology: DECD 210 (2013) The test follows a non-standard exposure regime
	The test follows a non-standard exposure regime
	There were only two replicates per concentration rather than the minimum four
	specified
Previous evaluation.	Ses, evaluated and accepted
	RAR (2010), RAR (2017)
GLP/Officially	Yes conducted under GLP/Officially recognised testing facilities
recognised testing	
facilities:	
Acceptability/Reliability:	Yes

Executive Summary

This study was designed to evaluate the chronic toxicity on the most sensitive early life stages of rainbow trout (*Oncorhynchus mykiss*) (swim-up phase, growth phase) exposed to spiroxamine under



consideration of more realistic exposure conditions. This was achieved by using a static water/sediment system that used three pulsed application times. For this purpose fish were exposed in a static water/artificial sediment system using three pulsed application times to a control, solvent control and initial nominal pulse concentrations of 60.0, 180, 540, 1620 and 4860 µg a.s./L.

Each test concentration had two replicates of 20 eyed eggs, which were thinged to 15 alevin after the hatching phase. The test duration was 56 days.

Sub-lethal effects were observed in the two highest test concentrations, \$620 and 4860 ug as survival was significantly reduced compared to the controls in the 4860 Jg a.s./L test concentration

At test termination, fish length and weight were significantly reduced on the 180, 540 and 1520 µg a.s test concentrations. No measurements were made in the 4680 og a.s./L test concentration due mortality.

The overall chronic NOEC and LOEC for the most sensitive carly life stage of ranbow trout (Oncorhynchus mykiss) exposed to spiroxamue in Estatic water artificial sediment system were 60.0 and 180 µg a.s./L, based on the effects observed on fish length and weight

in system we. I. **Materials and Methods** A. Materials Spiroramine technica **Test Material** Lot/Batch #: TH004650 espiry date **Purity: Description:** Light brown Stability of test 25 Stable compound: Reanalysis/E date: t reported **Density** Treatments Test rates: 1620 and 4860 Solvent/vehicle acetone Ses, measured test concentrations 69 – 126% of initial nominal in the Analysis of test 126% of initial nominal information of -126% of initial nominal information of 0.0, 180 and $540 \ \mu g$ a.s./L concentrations, and 122 - 126%concentrations: 408% in the nominally 320 and 4860 µg a.s./L concentrations Test organisms **Species**: Rainbow trout (Queorhynchus mykiss) Source: Acclimatisation ggs acclingated from 0.5°C to 9.9°C over approx. 4 hours period: Frine shrimp (Artemia salina) starting on study day 22. Preatment for None reported disease: Test design



Test vessel:	34.3 x 21.6 x 30.5 cm 22-L glass aquaria with a 3 cm sediment layer and a 21.3 cm deep water column containing 15.8 L test medium Q°
Test medium:	Soft blended water (spring water blended with RO water)
Replication:	Two replicates
No. of animals/vessel:	20 eyed eggs at experimental start, thinned to 15 alevin after hatching phase 56 days
Duration of test:	56 days $\sqrt{2}$ $\sqrt{2}$ $\sqrt{2}$ $\sqrt{2}$ $\sqrt{2}$
Environmental test conditions	56 days $\sqrt{2}$ $\sqrt{2}$ $\sqrt{2}$ $\sqrt{2}$ $\sqrt{2}$ $\sqrt{2}$ $\sqrt{2}$ $\sqrt{2}$
Temperature:	10.7 – 11.5°C
Dissolved oxygen:	$10.7 - 11.5 ^{\circ}\text{C}$ 9.4 - 11.0 mg/L 85 - 100% saturation 7.6 - 8.0
pH:	
Photoperiod:	16 h light 58 h dark with 30 minute transition period at 627 to 860 fux (mean 707 lux) Developing onbryos larvae were shielded from light esposure until
	one week post hateh.
B. Study Design	
This study was designed to	valuate the choonic texicity on the most sensitive carly life stages of rainbow

This study was designed to evaluate the choonic texicity on the most sensitive early life stages of rainbow trout (*Oncorhynchus mykiss*) (swim-up phase, growth phase) exposed to spiroxamine under consideration of more realistic exposure conditions

Due to the fast dissepation of spiroxamine from the water phase and ELS study under flow-through conditions would be unrealistic, therefore a special test design was followed.

For this purpose fish were exposed in a static water/astificial sediment system using three pulsed application times to control, solvent control and initial nominal pulse concentrations of 60, 180, 540, 1620 and 4860 μ g a.s./L

Test sediment was prepared based on OECD 210 (draft) and constituted 4% finely ground air-dried sphagnum peat moss, 76% white duartz 08iO₂ and and 20% kaolinite clay. Calcium carbonate was added to the mixture to obtain a pH value of the 6.5 to 8.6. Approximately 2.5 kg dry sediment was added to each test vessel to give an approximate sediment layer of 3 cm. Test vessels were filled to a depth of 24.3 cm, a water volume of 15.8 C. Formulated sediment was conditioned for eight days under test conditions prior to use in the study of the sediment was conditioned for eight days under

Serially divided stock solutions were added to the squaria in 10-day intervals on study days 22, 32 and 42.

Test solutions were prepared by adding 1.58 m of the relevant stock solution into the upper water phase of the corresponding aquaria, and gently stirring. Test solutions had a solvent load of 0.1 mL/L. Solvent controls were prepared by adding 4.58 mL to the upper water phase, and the control was prepared by gently stirring.

On study day 0 20 eggs were impartially placed in each egg cup and transferred to test vessels. Eggs were observed daily for mortality and hatching, with any non-viable eggs removed.

On post-hatch day 3, alexin were impartially thinned to 15 per replicate, and were observed daily for hatchability, abnormal behaviour, physical changes, swim-up behaviour and mortality. Growth expressed as standard length and dry weight was measured at test termination on day 56.



Fish were fed brine shrimp (*Artemia sakina*) starting on study day 22 at least once daily on weekends and holidays and at least two times daily on weekdays until approximately 24 hours prior to study termination.

Analytical method

Samples of water were analysed using the validated analytical method $\underline{M-304369-01-1}$, report reference $\underline{M-304369-01-1}$ (see Doc MCA Section 4).

II. Results and Discussion

Validity criteria according to the most up-to-date OECD 210 guideline (2013) were and

- Dissolved oxygen concentrations to be greater than 60% of the air saturation value throughout the test (actual: 85 to 100%)
- Water temperature to be 10 ± 1.5 °C (actual: 10.7 to 13.5 °C
- Overall hatching success in the controls to be at least 75% factual 95 to 100% across all groups)
- Overall post-hatch survival in the controls to be at least 75% (actual 97 and 97%)

Initial measured concentrations on application days ranged from 69 to 126% G nominal for the test concentrations 60, 180 and 540 µg a.s./L. In the two highest test concentrations, 1620 and 4860 µg a.s./L, unexpectedly high spiroxardine concentrations of 122 and 408% of nominal were found.

It appeared that spiroxamine bound quickly to the sospended solids in water and was extracted out of the suspended solids together with spiroxamine from the water. As a result, spiroxamine was found higher than the nominal concentrations. This way not noted at the lower treatment levels. Stock solution results of all test levels ranged from 87 to 105% of nominal values and were therefore within acceptable ranges. As expected from the physic ochemical properties, spiroxamine, disappeared rapidly from the water body of the test system. The calculated D450 was 3.5 days. The toxicity values were calculated based on initial nominal concentrations.

Study day 👸	Nominal concentration	(µg(L) ~ ~ ~	N.	
	60	2 540 ~ ~	/1620	4860
22	94		103	97
32	96 92	. Õ [×] ∡ [×100 & ⊥ [×]	91	105
42	95 5 4 02	<u>7</u> 7 90 0 ~	98	87

Table CA 8	.2. 2 .003	-1 M	easursed	concent	rations	ofspir	oxâmin	e in sto	ck solution
	(O) ^p	A V	, U	V	× v	Con 1		õ	a.

Study day	Nominal co	Nominal concentration (µg)							
(DU)	Control 🔊	Solvent @	60	4180	540	1620	4860		
		control	\mathcal{A}	1					
22 🖉	<loq td="" y<=""><td>Z\$LOQ ⊘″</td><td>60.9</td><td>151</td><td>370</td><td>2662</td><td>11613</td></loq>	Z\$LOQ ⊘″	60.9	151	370	2662	11613		
- S	<loq a<="" td=""><td>×LOQ ×</td><td>39.4</td><td>146</td><td>438</td><td>3320</td><td>10310</td></loq>	×LOQ ×	39 .4	146	438	3320	10310		
32	<body> <body> <body></body></body></body>	<lqq q<="" td=""><td>67.6</td><td>159</td><td>486</td><td>1982</td><td>7769</td></lqq>	67.6	159	486	1982	7769		
	SLOQ 1	∠¢ÓQ _√ y	66.2∜	151	560	2473	8173		
42	LOQ /	ČLOQ	<i>5</i> 9⁄.4	191	600	3785	12822		
S	<lqq td="" ~<=""><td>CLOQ I</td><td>64.5</td><td>176</td><td>681</td><td>4689</td><td>19831</td></lqq>	CLOQ I	64.5	176	681	4689	19831		
Mean % of	-2 1		105	90.2	96.8	194.6	241.8		
nominar	& A	XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX							
Ranse %		>	99 - 113	81 - 106	69 - 126	$122 - 290^{a}$	$160 - 408^{a}$		
of nominal	43	8							

Table CA 8.2.20/03-2 Measured concentrations of piroxamine in test medium

Calculations were made using unrounded data

- ¹ Calculated using rounded data
- ^a In the two highest test levels unexpectedly high spiroxamine concentrations were found

The results have been presented based on nominal test concentrations.



Time to hatch was recorded for all test levels from study day 7 until study day 13 (post hatch day 3). The post-hatch period began after 90% of all living eggs in the controls had hatched. Percent hatch was not statistically analysed as all test levels were untreated at the time of hatch. Hatching success ranged from 95 to 100%.

From study day 14 onwards observations of fish were made with reservations due to the turbidity of the water caused by the fish stirring up the sediment. In the highest test concentration, 4860 up a.s./L fish were on the bottom, and showed a loss of equilibrium and dark colouration.

Time to swim up could not be quantified due to the high turbidity of the water.

Fry survival on day 56 in the control, solvent control and 60, 186 540, 1620 and 4860 μ g as L test concentrations was 97, 97, 97, 100, 97, 93 and 0% respectively. A statistically significant reduction in fry survival compared to the control was found in the 4860 μ g as L test concentration.

		- Al
Initial nominal conce	ntration (µg a.s./L) 🖉 🏹 Fry Survival (%) 🐴 🔿	Ş
Control		,
Solvent control		
60	L & Y 97 V L & L L	
180		
540		
1620		
4860		
* 0::6		

Table CA 8.2.2.1/03-3 Fry survival of rainbow trout after 56 days exposure to spiroxamine

* Significantly different (p≤0.05) from the controls

At test termination (study day 56) fish were sacrified and measured for standard length and dry weight. No measurements were made in the highest test concentration, 4860 μ g a.s./L, as all fish had died by test termination. The mean lengths of surviving fish ranged from 25.2 to 38.1 mm. Length was significantly reduced from the pooled controls at test concentrations 180,540 and 1620 μ g a.s./L. The mean dry weights of fish ranged from 49.6 to 114.8 mg. Dry weight was significantly reduced from the pooled controls at test concentrations 180,540 and 1620 μ g a.s./L. The mean dry weights of fish ranged from 149.6 to 114.8 mg. Dry weight was significantly reduced from the pooled controls at test concentrations 180, 540 and 1620 μ g a.s./L.

Table CAS.2.2.1/03-4 Affects on length and weight of rainbow troot after 56 days exposure to

Initial nominal concentration	Standard length (mm)	Dry weight (mg)
Control	38.1 0 0 0	113.6
Solvent control	37.50 0	114.0
60	366 2	114.8
180	31.9* &	92.1*
540 1620	28.4* >	90.7*
1620	25.20 ~~	49.6*
4860		_ ^a

^a All fish had died prior to tot termination Q

Significately different (180.05) from the controls

III. Constasion

This study was designed to evaluate the chronic toxicity on the most sensitive early life stages of rainbow trout *(Oncortiynchus' mykiss)* (swim-up phase, growth phase) exposed to spiroxamine under consideration of more realistic exposure conditions. This was achieved by using a static water/sediment system that used three pulsed application times. For this purpose fish were exposed in a static water/artificial sediment system using three pulsed application times to a control, solvent control and initial nominal pulse concentrations of 60, 180, 540, 1620 and 4860 µg a.s./L.



Each test concentration had two replicates of 20 eyed eggs, which were thinned to 15 alevin after the hatching phase. The test duration was 56 days.

Sub-lethal effects were observed in the two highest test concentrations, 1620 and 4860 µg a.s. Fry survival was significantly reduced compared to the controls in the 4860 µg a.s./Lest concentration.

At test termination, fish length and weight were significantly reduced in the 180, 540 and 1620 µg a.S./L test concentrations. No measurements were made in the 4680 µg a.s./L test concentration one tog mortality.

The overall chronic NOEC and LOEC for the most sensitive early life stage of raidbow that (Oncorhynchus mykiss) exposed to spiroxamine in a static water/artificial sediment system were of an 180 µg a.s./L, based on the effects observed on fish length and weight.

Assessment and conclusion by applicant:

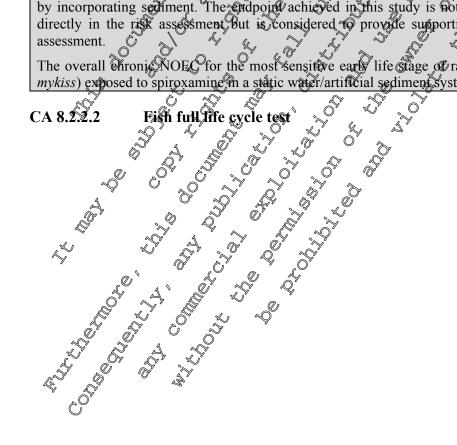
The study was conducted to the OECD test guideline 210, the most up-to date version of which is the of the second se "Fish, early-life stage toxicity test", adopted 26 July 2013

Validity criteria according to the most up to-date OECD 210 guideline (2005) were met:

- Dissolved oxygen concentrations to be greater than 60% of the air saturation value throughout the test (actual: 85 to 100%) 2
- Water temperature to be $D^{\circ} \pm 1.5^{\circ}$ C (actual: 10.7 to $D^{\circ}.5^{\circ}$
- (actual: 95 to 100% across all Overall hatching success in the controls to be at teast 75% groups)
- Overall post-hatch survival in the control's to be at least 75% Tactual 97 and 97%)

The study is considered acceptable on the basis that the validity criteria for OECD 210 were achieved. It is noted that this study used a non-standard tes design by adopting a pulsed exposure regime and by incorporating sediment. The endpoint achieved in this study is not considered suitable for use directly in the risk assessment but is considered to provide supporting information for the risk

The overall thronic NOEC for the most sensitive early life stage of rainbow trout (Oncorhynchus mykiss) exposed to spiroxamine in a static water/artificial sediment system was 60 µg a.s./L.





Data Point:	KCA 8.2.2.2/01
Report Author:	
Report Year:	2009
Report Title:	Zebra fish (Danio rerio), life cycle test, flow through conditions
Report No:	47758001
Document No:	<u>M-304458-02-1</u>
Guideline(s) followed in study:	-OECD Guideline for Testing of Chemicals, 210 Fish, Early Life Stage Toxistiv Test, 1992 -OECD Guideline for Testing of Chemicals, 215 Fish, Jovenia Growth Test, 2000 -OECD "Drat Proposal for and W Guideline Pish Too- generation Test", 2002EPA-FIFRA § 72-5/SEP-EPA-540/926-137 Standard Evaluation Procedure: Fish Life-Cycle Toxigny Tests"(OPPTS 850.000), 1986 - Nagel, R. (1998): Der vollständige Life Cycle Test (Complete Life Cycle Cest, CLC Test) mit dem Zebrabarbling (Danio rerio, Formals Brachydanio rerio), Entwuf. UBA-Texte 58/98
Deviations from current test guideline:	Yes Methods: SANCO/3029/99 rev. 4 Accuracy n=4 Ecotoxicology (OECD 210 (2013) and OECD 215 (2090) It was not reported if flow rates were checked to not vary by 10% Water was not held at 26 ± 1.5°C unstead at 23.7 27.8°C Juvenile fish were not were held to determine detection of a minimum variation of significant growth rate Frequency of feeding was nor reported Dilution water had exceedances in iron, copper and zinc concentrations Amended by additional endocrine test parameters and endpoints Mean measure concentrations ranged in week 4 between 31 2/17 %
Previous evaluation:	yes, evaluated and accepted RAR (2010), RAR (2017) The overall NOLC for the FFLC test is the EC 10 for the survival observed in the F1-ELS of 2.0 µg as L and should becompared to the PECSW max.
GLP/Officially C recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities

Executive Summary

This was a fish full life cycle study conducted to examine the potential for long-term adverse effects of spiroxamine exposure to populations of zebratish (*Danio refio*).

Five test concentrations were investigated, each with four replicates of initially 100 fertilised eggs. The effects of exposure on different life stages of the zebrafish, including early life stages, juvenile growth, reproduction and early life stages of the filial generation were assessed under continuous exposure.

At the early life stage ho direct effects on hatching could be found, however survival and growth had a NOBC of 6.4 μ g a.s./L.

At the juvenile growth stage, the NOPC values for survival, length and 'pseudo' specific growth rate were 16, 6.4 and 6.4 and 6.4 and as de, respectively.

At the adult stage, the NOEC values for eggs/female/day, cumulative egg number, fertilisation rate, survival and sex atio were 16016, 16, 40 and 6.4 μ g a.s./L, respectively. NOEC values for male length, female length male values for male and female weight were 6.4, 16, 40 and 16 μ g a.s./L, respectively. Biomarker NOEC values for male and female vitellogenin content were 40 and 2.6 μ g a.s./L, respectively, and for histology were 16 and 6.4 μ g a.s./L in males and females, respectively.

In the farly life stage of the filial generation, population NOEC values for hatching and survival were 16 and 2.6 μ g a.s./L, and growth NOEC values for length and weight were both 16 μ g a.s./L.



Based on the most sensitive endpoint, survival of the F_1 generation, the overall NOEC was found to be 2.6 µg a.s./L.

Materials and Methods I. the suse h A. Materials Spiroxamine **Test Material** Lot/Batch #: AE 1344293-01-01 97% **Purity: Description:** Light brown oil Stability of test Stable at pH 7 and 9 and 25°C with half compound: **Reanalysis/Expiry** 02 August 2009 date: **Density:** No informatio Treatments roxami and **Test rates:** 40 Solvent/vehicle: nomi Analysis of test nal concentrations: **Test organisms** Debratish (Danio recto). Fertilised eggs four cells used for the study **Species:** were collected from a glass spacening tray placed at the bottom of the adult holding ressels. Source: Acelimatisation period: 0 vae were fed daily ad libitum from day 6 onwards with finely Feeding: ground breeding food (TetraMin Baby). From day 9 onwards brine shripp napplii (Appenia Calina) were added ad libitum, and from day 6 onwards ground TetraMin flake food were added ad libitum. Treatment for ر disease: **Test design** 28 x 28 cm 28-L aquaria containing approx. 25 L solution Test vessel Purified drinking water Test medium: Replication: our replicates 100 fertilised eggs per replicate, later reduced to 50 fish and then 30 animäls/vessel: **Duration of test:** 56 days



Environmental test conditions

Temperature:

n:	24.0 - 27.8°C (four measurements rewith a minimum recorded of 23.7°C) $68 - 102%$	corded temperatures	s <24.0°C 5 5 5 5
	7.4 - 8.6	A A	
	12 h light : 12 h darkness 🖑	Í Š	
n			

Dissolved oxygen:

pH:

Photoperiod:

B. Study Design

This was a fish full life cycle study conducted to examine the potential for long term averse effects of spiroxamine exposure to populations of zebrafish (Danio rerig). The effects on different the stages of the zebrafish, including early life stages, juvenile growth, reproduction and early the stages of the filial generation were assessed under continuous exposure

Test vessels were glass 28-litre aquaria of 40 x 28 x 28 cm containing approximately 25 litres test solution. Test solution was prepared in mixing charaber by the addition of relevant appoints of stock solution to purified drinking water, with a daily water exchange rate of approximately five volumes. The flow-through system was served by yest solutions at least 24 hours prior to the additions of the eggs.

The study was conducted using nominal concentrations of 2,0, 6.4, 16, 40 and 100 µg spiroxamine/L and a control, each with four replicates under flow-through conditions. Exposure was started with 100 fertilised eggs per vessel and replicate. Lave were fed the first ad hitum from day 6 onwards with finely ground breeding food (TetraMin Baby). From day 9 onwards brine shrimp nauplii (Artemia salina) were added ad libitum, and from day 6 onwards ground CetraMon flake food were added ad libitum.

After 28 days, the fish number was randomly reduced to 50 per replicate for investigation of juvenile growth. Fish were photographed on days 28 and 56 to determine survival and growth.

After 56 days, fish numbers were reduced to 30 per keplicate for the investigation of reproduction. After the last reduction to \$0 individuals, glass spawning trays were introduced and monitored daily for spawned eggs. The time until first findings of eggs was recorded. Egg production per female per day and fertilisation rate was measured.

All fish were measured for length and weight. A blood sample was taken and the biomarker vitellogenin was measured for a subsample of five males and five females per replicate, if possible. In all replicates of the control and 2.6 µg a.s./Lesst concentration all male fish were measured for vitellogenin content.

To confirm the macroscopic Sex determination, all gonads were histologically inspected, with a detailed examination performed of five make and five female fish per replicate, if possible. Blood samples of the same fish were analysed for viterlogen.

To start the F_1 generation, 100 fertilised eggs per test vessel were placed in stainless steel fry chambers. Larvae were fed daily addibiting with finely ground breeding food (TetraMin Baby). From day 9 onwards brine shrimp nauplii (*Artemia, saling*) were added *ad libitum*, and from day 16 onwards ground TetraMin flake food were added addibitum? After 35 days, F1 fish were sacrificed and measured for length and weight. ~C

Chemical analysis of the test solutions was performed weekly in all test concentrations and replicates. Sample Swere analysed by HPLC-MS/MS, with a LOQ of 0.8 μ g/L. The temperature, pH and oxygen conceptration of the water was measured in each aquarium was measured directly before adding the fish and afterwards twice weekly.

Data were analysed for statistical differences as compared with the untreated control by performing ANOVA followed by Williams' or Dunnett's test or respective non-parametric approaches. All statistical tests and probit analysis were conducted using the software ToxRat Professional 2.09.



Analytical method

Samples of water were analysed using the validated analytical method M-304458-02-1, report reference M-304458-02-1 (see Doc MCA Section 4).

II. **Results and Discussion**

Validity criteria according to the study report were achieved:

- Total survival in the control replicates was >70%
- Hatching rate in the control replicates to be $\geq 70\%$ (actual: 91) •
- Dissolved oxygen concentration to be $\geq 60\%$ all test vessels throughout 102%)
- Water temperatures were kept within 26 •

The overall mean measured concentrations, determined for each replicate vessel were between 92 and 101% of the nominal concentrations. Thus, the evaluation of the effect concentrations was based on nominal concentrations of the test item.

The single test media concentration granger between 31 and 167% of nominal values. Due to a malfunction of the dosing system, the analytical results for week 4 showed reduced recovery fates of 31 to 82% over all treatment groups. Hweek 20, a pump malfunction resolted in low test item concentration in two replicates of the 16 µg @s./L test concentration. A decrease in sock solution and test vessel concentrations could be observed in week 20, 24 and 30, resulting in recoveries of 46 @ 77%. All other measurements were between 68 and 167%

Table CA 8.2.2.2/01-1 Measured concentrations of speroxandre during the test &

	\sim			J ing		~~ ~	
	Nominat co	ncentration (µ	g a@y./L) 🔊	, L	0 0	\$°	
	Control	<u>م</u> 2.6 م	6,4		, A	40	100
Mean meas	ured test con	centration ±SD				Ş	
μg a.s./L		2.6 ± 0	() () () () () () () () () () () () () (2 🔬 14.9	6 0.1	38.8 ± 1.4	96.8 ± 3.2
%	Ģ Å	≪ 99 ± 2.3	¥95,±22.9	9 🖉 93 €		97 ± 3.5	97 ± 3.2
SD Sta	Adard deviatio	n ation Ø.8 µg/s	<u> </u>	NO DI	. 7		
LOO Am	nit of quantific	ation 0.8 ugs			Š		

 F_0 generation, early life phase

A slight delay and reduction in hatching could be observed in the 6.4, 16, 40 and 100 µg a.s./L and above compared to the control This thought to be due of the suppression on microbiological growth of the egg surface by the active substance resulting in an increased stability of the egg shell. No negative impact on related endpoints was observed, therefore the finding is regarded as an indirect effect and is not considered to be relevant for the texicity assessment.

Survival of the hatched larvae as well as the length of the larval fish on day 28 post fertilisation was significantly reduced in the 16, 40 and 100 µg a.s./L test concentrations.

Parameter	Nominal concentration (ug a.s./L)						
	Contra	2.6	6.4	16	40	100	
Hatching, 🔿	26.8∉ 16.8	16.4 ± 11.2	13.1 ± 7.2	13.9 ± 7.2	$10.6 \pm 4.8*$	$6.3 \pm 6.4*$	
day 4(%)							
Hatching, & day 5 (%)	9P.1 ± 55	90.5 ± 4.4	81.7 ± 5.8*	$73.6 \pm 12.3*$	$80.6 \pm 10.2*$	$75.6 \pm 6.4*$	
	1						
Survivors,	92.4 ± 6.2	93.9 ± 3.0	93.4 ± 7.1	88.5 ± 3.8	$81.0 \pm 8.0*$	81.7 ± 2.5*	
day 14 (%)							
Survivors,	86.0 ± 4.8	88.5 ± 5.2	80.1 ± 10.1	$69.4 \pm 14.4*$	$24.5 \pm 13.5*$	$7.6 \pm 6.8*$	
day 21 (%)							

Table CA 8.2.2.2/01-2 Effect of spirexamine exposure on the early life phase of the Fo generation



Parameter Nominal concentration (µg a.s./L)						
	Control	2.6	6.4	16	40	100 °.
Survivors,	79.3 ± 3.4	83.1 ± 6.3	77.6 ± 7.8	65.0 ± 12.2*	$14.2 \pm 4.4*$	2.0 ± 2.4
day 28 (%)						2.0 ± 2.4
Length,	0.84 ± 0.04	0.80 ± 0.02	0.78 ± 0.03	$0.76 \pm 0.01*$	0.75 0.09*	0.74 0.02*
day 28 (cm)					S.	

Results are means of four replicates \pm standard deviation

* Significantly reduced compared to the control (William's test, p < 0.05)

F_0 generation, juvenile growth phase

Survival of the juvenile fish on day 56 was significantly reduced \hat{On} the 40 and 100 \hat{Of} a.s. \hat{O} test \hat{O} concentrations.

Fish length on day 56 and 'pseudo' specific growth rate, based on the length measurements on day 2^{2} and 56, were significantly reduced in the 16 and 40 µg a.s./L test concentrations.

Table CA 8.2.2.2/01-3	Effects of spiroxamine	exposure on t	he juveQile	growth phas	se of the Fo go	meration
-----------------------	------------------------	---------------	-------------	-------------	-----------------	----------

Parameter	Nominal concen	tration (µg a.s./L)			
	Control	2.6	6.4 1		40 0
Survivors, day 56 (%)	98.5 ± 1.9	98.5 ± 1.9	98.051.6	91,0±10,0 0 0 5	\$25.9* \$
Length, day 56 (cm)	2.26 ± 0.05	₹2.25¢ 0.03 ¢			1.7 3 †
Pseudo specific growth rate	3.583 ± 0.40	3.722 = 0.05	3.720 ± 0.14		2.864*

Results are means of four replicates \pm standard deviation \swarrow

* Significantly reduced compared to the control (William's test, p < 0.05)

[†] Significantly reduced compared to the control (Domnett's fest, p < 0.05)

F_0 generation, adult phases f_0 generation, f_0 generation

Reproduction No dose-dependent effects of spiroxamine exposure on reproduction could be detected. Due to the low number of remaining fish at the highest test concentration, the 40 µg a.s./L level was excluded from the statistical evaluation. However, the fertilisation rate as well as the cumulative number of fertilised eggs was significantly reduced at the lowest test concentration.

Since no clear dose response could be detected, the NOEC with regard to reproduction was determined to be 16 μ g a.s./L, the highest level tested doring this phase.

Table CA 8.2.2.2/01-4 Effects of spiroxamine exposure on the reproduction of the adult phase of the F_0 generation $\mathcal{O}^{\mathcal{O}}$

Parameter	Nominal concentrati	on (ug a.s./Q)		
	Control	2.6	6.4	16
Time to first	104±9° °°	¥24 <u>≠</u> Q0	114 ± 14	101 ± 6
spawning (days				
Eggs/female/day	£±0.6 € 45	6 ± 4.6	4 ± 1.3	8 ± 1.7
Cumulative	≰ 3 723 ≠\$84	$O_{0}^{\dagger}065 \pm 1334^{\dagger}$	5331 ± 2439	6710 ± 1.0
number of		<i>v</i>		
fertilised eggs	.1 ~~			
Fertilisation rate	8077¥5,5≪J	$75 \pm 9.8*$	82 ± 1.0	82 ± 1.0
Fertilisation rate	10 AN			

Results are means of four replicates ± standard deviation

- * Significantly reduced compared to the control (William's test, p < 0.05)
- [†] Significantly reduced compared to the control (Dunnett's test, p < 0.05)

Survival and growth



No exposure-related effects on survival of adult fish could be observed in the study. Length and weight of fish was found to be increased for female fish in the 40 μ g a.s./L test concentration. A significant decrease was found in male fish at the 16 and 40 μ g a.s./L test concentration. Male weight was not affected.

Table CA 8.2.2.2/01-5 Effects of spiroxamine exposure on the survival and growth of the adult phase the F₀ generation

	8			A	
Parameter	Nominal concen	tration (µg a.s./L)	Ĉa	Ľ.	
	Control	2.6	6.4	16 ″	
Survival at	87 ± 9.4	90 ± 17.8	98 ± 5.0	§78 ± 16.2	80,57 ,67
termination (%)			, O A	í "Õ	
Length,	3.7 ± 0.07	3.7 ± 0.08	3.7 ± 0.03	3. 6 5±°0.035℃	\$3.6* K
males (cm)			\sim	Û × v	
Length,	3.6 ± 0.06	3.7 ± 0.07 «	3.6°± 0.05	3.6 ± 0.08	3.9
females (cm)		O V	o to to		C A
Weight,	0.484 ± 0.03	0.490 ± 0.03	0.478 ± 0.02	0.466 ± 0.02	0.493
males (g)				A	
Weight,	0.493 ± 0.03	0.546/± 0.03	0,528±0,04	0.521 ≠ 0.03	0-263†
females (g)			K X K		<u> </u>
D 1	0.0 1.				

Results are means of four replicates \pm standard deviation

- * Significantly reduced compared to the control (William's test, p=0.05)
- [†] Significantly reduced compared to the control Dunnet s test p < 0.03

Sex ratio

Regarding sex ratio, a shift powards an increased number of males could be detected in the 16 and 40 μ g a.s./L test concentrations. It must, however, be noted that the number of males per group was quite small in the control and low test concentrations.

In the 16 μ g a.s./L test concentration the number of males significantly increased, however values remained within the historical range. In the 40 μ g a.s./L test concentration, the percentage of males was over 80%. The resulting NOEC for sexual development was determined to be 6.4 μ g a.s./L.

Vitellogenin

A decrease of vitellogen concentrations in female blood plasma could be observed in the 6.4, 16 and 40 μ g a.s./L test concentrations compared to the control. Vitellogenin concentrations in male blood plasma were not significantly affected.

Table CA 8.2.2.2/01-6 Effects of spiroramine exposure on the sex ratio and vitellogenin concentration of the adult phase of the Fo generation

Parameter ,	Nominal concentration (µg 42s./L)				
Sex rạtiõ	Control Q	2.6	6.4	16	40
Sex ratio					
Mates (%)	33.4±€3″ ∾	20.6 4.4	28.9 ± 2.8	$56.7 \pm 6.3*$	91.7*
Females (%)	66.6±6.5 °	$79.4 \pm 6.5^{\circ}$	71.1 ± 2.8	$43.3 \pm 6.3*$	8.3*
Vitellogenin 🦼					
Males (ng/µg)	Q T 54 ± 2005	[≈] ∕0.348 ₂ ≠ 0.28	0.139 ± 0.01	0.131 ± 0.02	0.095
Females (ng µg)	×198.8 58.0 ×	162Q ± 51.8	$90.0 \pm 13.9^*$	$114.1 \pm 4.3*$	77.7*

Results are means of four teplicates ± standard deviation

* Significantly reduced compared to the control (William's test, p < 0.05)

Historathology

No specific findings indicative of substance-related toxic effects could be seen in the test fish. Histopathological investigation of the gonads and gonadal ducts revealed in females a higher grade of inflammatory changes in the ovary and oviduct in the 16 μ g a.s./L test concentration.



Concomitantly, the incidence of egg debris in the oviduct was slightly elevated. In a few fish, the body cavity also showed inflammation/granulomas. A relation to the treatment could not be fully excluded, although inflammatory changes were seen at a higher test rate in only one of the replicates.

*F*¹ generation, early life stage phase

Hatching rate of the F1 generation was greater than 80% in the control and treatment groups. Survival of fish was significantly reduced at test concentrations 6.4 µg a.s./L and above, resulting in an F generation survival NOEC of 2.6 µg a.s./L. Ô

Parameter	Nominal concentration	on (µg a.s./L) 🔬	Â ^y	
	Control	2.6		16 2
Hatching,	91.2 ± 3.0	93.2 ± 8.7		$8^{2} 8 \pm 9^{4}$
day 4 (%)		k o°	S & K	
Hatching,	92.2 ± 2.3	90.4±Q.4	90.9 ± 2.4	90.2∉0.8
day 5 (%)		A. O.		O' Q' A
Hatching,	92.2 ± 2.3	90.4 ± 0.4 ×	909 ± 2,4 0	90.2 ± 0.8
day 6 (%)		Ø in a	A O XY	
Survivors,	82.2 ± 9.7	\$2.6# 4 .4	78.1 8.5 0	71.0 8.1*
day 14 (%)	L. L.	oʻ 'n "	78.1 \$8.5 0	
Survivors,	79.7 ± 7.4	70,8±58		58.3 ± 14.8*
day 21 (%)				р _«
Survivors,	77.0 ± 6.3	√66.3 ± 6.7	59.5 3 .6†	50.90 ± 18.3†
day 28 (%)	¥		4 <u>4</u> 9	(à)
Survivors,	75.0 ± 6.0	63.1 ± 5,0	55.3 ± 3.5	≪∯1.1 ± 17.4 [†]
day 35 (%)				
Length,	1.01 0.03	1.05 ± 0.005	1.02 0.02	0.94 ± 0.10
day 35 (cm)				
Weight,	0.013 ± 0.001	0014 ± 0.001 ~ 0	@.013 ≠0.001 @	0.011 ± 0.003
day 35 (g)			<u>pří "Sví – </u>	
D		and and an interior		

Table CA 8.2.2.2/01-7	Effects of spiroxamine exposure	on the early	life phase of th	e Foreneration
	r r r r r r r r r r r r r r r r r r r	r · · · · ·		

Results are means of four replicates ± standard deviation

sults are means of four replicates \pm standard deviation Significantly reduced compared to the control (William's test, p=0.05) Significantly reduced compared to the control (William's test, p=0.05)

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A sumptory of the relevant NOEC and LOEC endpoints is presented below.

% Ô Table CA 8.2.2.2/01 8 Summary of endpoints after exposure of zebrafish to spiroxamine

Population	Encroint S		NOEC	LOEC
			(µg a.s./L)	(µg a.s./L)
F ₀ generation,	Population 🔍	Platching Survey al Length	2.6 ^a	6.4
early life stage		Survey al	6.4	16
L.	Growth	Length	6.4	16
F ₀ generation,	Population	Survival A	16	40
juverile growth	Population Growth	Length	6.4	16
01			6.4	16
F ₀ generation	Population	Eggs/female/day	16	>16
adult		Cumulative egg number	16	>16
ý d		Fertilisation rate	16	>16
		Survival	40	>40
		Sex ratio	6.4	16
	G rowth	Male length	6.4	16
	L.	Female length	16	40
F ₀ generation for adult		Male weight	40	>40
\checkmark		Female weight	16	40
	Vitellogenin	Males	40	>40
		Females	2.6	6.4

A



Population	Endpoint		NOEC	LOEC
			(µg a.s./L)	(μg a.s./L) •
	Histology	Males: no change	16	40
		Females: inflammatory changes in the ovaries, egg debris	6.4	40 16
F ₁ generation,	Population	Hatching	16	>16 4
early life stage	ropulation	Survival	2.6	6.4
	Growth	Length	16	~96 BY *
		Weight 🖏	16	× 16 × ~
^a Endpoint not	relevant as base	d on indirect effects	~	

III. Conclusion

This was a fish full life cycle study conducted to examine the potential for long term adverse effect spiroxamine exposure to populations of zebrafish (Danio reright

Five test concentrations were investigated, each with your replicates of initially 100 fertilised eggs. The effects of exposure on different life stages of the zebrafisk including early life stages Quvenile growth, reproduction and early life stages of the filial generation were assessed under continuous exposure

At the early life stage, no direct effects on hatching could be found how ever surgeval and growth had a NOEC of 6.4 μ g a.s./L. m

At the juvenile growth stage, the NOE@value@for survival@ength and 'poeudo' @pecific growth rate were 16, 6.4 and 6.4 µg a.s./L, respectively.

At the adult stage, the NOEC values for eggs/female/day, cumulative/egg_mamber, fertilisation rate, survival and sex ratio were 16, 16, 16, 46 and 64 µg as./L, respectively. NOEC values for male length, female length, male weight and female weight were 624, 16, 40 and 16 µg a.s./L, respectively. Biomarker NOEC values for male and formale vitellogenin content were 40 and 2.6 µg a. S.L., respectively, and for histology were 16 and 6.4 µg a.s. In makes and remales, respectively.

In the early life stage of the filtal generation, population NOEC volues for hatching and survival were 16 and 2.6 µg as./L, and growth NOEC values for length and worght were both 16 µg a.s./L.

Based on the most sensitive endpoint, survival of the b generation, the overall NOEC was found to be 2.6 μg a.s.%Σ

Assessment and conclusion by applicant:

The study was conducted to the OECD test gridelines 210, the most up-to-date version of which is the "Fish, early-life stage to scity test", adopted 26 July 2013 and 215 "Fish, juvenile growth test", adopted 21 January 2000 There is no formal DECD test guideline for a fish full-life cycle test therefore the study validity has been based on the OECD 210 guidelines. The OECD 215 test guideline is no longer used and therefore the validity criteria of this guideline have not been assessed here.

The study report listed it's own validity criteria (see above) and these were considered to have been achieved. However, validity griteria according to the most up-to-date OECD 210 (2013) test guideline have also been assessed and the following criteria were met:

- Hatchingstrate in the control replicates to be $\geq 70\%$ (actual: 91.1%)
- Post-hatch survival in the control replicates to be \geq 75% (actual: 79.3%)
- Dissolved oxygen concentration to be $\geq 60\%$ in all test vessels throughout the test (actual: 68) 102%)

One of the criteria were not met:

Water temperature to be at $26 \pm 1.5^{\circ}$ C during the test (actual: $23.7 - 27.8^{\circ}$ C). Four measurements recorded temperatures <24.0°C, with a minimum recorded of 23.7°C.



L.

The deviations noted above are not considered to have adversely affected the integrity of the test therefore the study is considered to be acceptable.

Based on the most sensitive endpoint, survival of the F_1 generation, the overall NOEC was found to be 2.6 µg a.s./L.

The data have been subjected to statistical re-evaluation and the results have been presented in the following study summary.

Ĉa

Data Point:	KCA 8.2.2.2/04
Report Author:	
Report Year:	
Report Title:	Calculation of EC10 and EC20 values for Danio rerio with spiroxamino TG inca
	full life cycle test & & & & & & & & & & & & & & & & & & &
Report No:	0471836-ECO23 0 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2
Document No:	<u>M-760413-01-1</u> A & Q Q A & Q Q A
Guideline(s) followed in	None y y y y y y y y
study:	
Deviations from current test guideline:	None V V V V V V V V V V V V V V V V V V V
Previous evaluation:	No, not previously submitted of the company of the company submitted of the company of the company submitted of the compa
GLP/Officially	No not conducted under GLP/Officially recognized testing facilities
recognised testing facilities:	$\begin{array}{cccccccccccccccccccccccccccccccccccc$
Acceptability/Reliability:	Yes a way of

Executive Summary

The report M-30458-0201 on the effects of Spiroxanine 1G in the zebrafish (*Danio rerio*) full life cycle test did not provide estimates of EC₁₀ or EC₂₀. Therefore, these values have been calculated in accordance with the Annex to Com. Reg. 283/2015. Effect concentrations with a 10 or 20% effect on F_0 length at 28 dpf and 56 dpf, F_0 pseudo specific prowth ate, F_0 sex ratio based on females, F_1 post-hatch survival at 28 and 35 dpf and FL survival at 35 dpf (EC_{10,20}) were re-calculated.

I. 🔊 Methods

The statistical evaluation was performed with statistical software ToxRat Professional v3.3.0.

F0 generation (early lifestages

The calculation of an EC₁₀ value for F_0 length after 28 days was performed with a Probit function using linear maximum likelihood regression and with the confidence limits determined by Normal approximation. The determination of reliable EC₁₀ and EC₂₀ values for F_0 hatching after 4 and 5 days was not possible due to the lack of a dose response or effects with a magnitude of 10% among the tested treatment rates. The determination of reliable EC₁₀ and EC₂₀ values for F_0 survival after 14, 21 and 28 days was not possible due to a poor goodness of fit in the data and the data scattering around the dose response.

F0 generation (juvenile growth stage)

The calculation of EC_{10} and EO_{20} values for F_0 length after 56 days and pseudo specific growth rate after 56 days post fortilisations (dpf) was performed with a Probit function using linear maximum likelihood regression and with the confidence limits determined by Fieller's theorem. Due to a lack of data for survival a 56 dpf within the study report, EC_{10} and EC_{20} calculations could not be performed.

F₀ generation (adult life phase)



The calculation of EC_{10} and EC_{20} values for F_0 sex ratio based on females was performed with a Probit function using linear maximum likelihood regression and with the confidence limits determined by Fieller's theorem.

The determination of reliable EC_{10} and EC_{20} values for F_0 egg number per female per day, cumulative egg number, fertilisation rate, vitellogenin concentration in male and female plasma and sex ratio based on males, was not possible due to the lack of a dose response. Due to the lack of effects above 10% when compared to the control on F_0 length at termination in males and females, and weight at termination in males and females, and weight at termination in males and females, the EC_{10} and EC_{20} values are estimated to be >40 kg a.s./L.

Due to a lack of data for survival at termination and time to spawn within the study report EC_{20} calculations could not be performed.

F1 generation (early life stage)

The determination of reliable EC_{10} and EC_{20} values for F_1 survival at 14 and 21 drf was not possible due to the lack of a dose response. The EC_{10} and EC_2 values for F post-harch survival at 28 and 35 dpf were performed with a Probit function using linear maximum likelihood regression and with the confidence limits determined by Fieller's theorem. The EC_{10} and EC_5 values for F survival (based on number of eggs introduced) at 35 dpf were performed with a logit function using linear maximum likelihood regression and with the confidence limits determined by Fieller's theorem.

Due to the lack of effects above 10% when compared to the control of F_1 hatching day 4 and 5, length at 35 dpf and weight at 35 dpf the EC_{14} and EC_{20} values are estimated to $bc > 16_{+}Q$ a.s./L.

II. Results and Discussion

An explanation is given for regression analysis endpoints and endpoints where the statistical software could not produce a reliable results and where expert adgment was needed. These details can be found below.

Fogeneration (earl dife stage)

Length at 28 days post fertilisation (dpf)

Regarding the calculation of the ECⁿ value for length at 28 dpf the criteria for goodness of fit were met as the P(Chi²) value was 1.000, showing no significant reviation between fit and data, and a statistically significant concentration response was found (p(F) $\gtrsim 0.000$) for this parameter.

The resulting EC_{10} value and the respective confidence intervals are represented in the following table.

Table CA 8.2.2.2/04-1 Results of the Probit analysis (max. likelihood regression) with length at 28 dpf: Selected effective concentrations (E63) of the test item and their 95%-confidence limits (by Normal approximation).

	Le Revenue de la companya	ngth
Parameter	\sim \simeq	EC20
	Q5 % confidence interval)	(95 % confidence interval)
<u> </u>	Q [μg x(\$./L]	[µg a.s./L]
Effect on length at 28 dp	$^{\circ}$ 42,896 (24.536 - 74.994)	n.d.

n.d.: not determinable

The resulting EC_1 value of 42.896 (95% CL: 24.536 – 74.994) µg a.s./L, meet the goodness of fit criteria and therefore the calculated EG_{10} value is considered reliable.

Fogeneration (juvenile growth stage)

Length at B days post fertilisation (dpf)

Regarding the calculation of EC_{10} and EC_{20} values for length at 56 dpf, the criteria for goodness of fit were met as the P(Chi²) value was 0.999, showing no significant deviation between fit and data, and a statistically significant concentration/response was found (p(F) = 0.015) for this parameter.



The resulting EC_{10} and EC_{20} values and the respective confidence intervals are represented in the following table.

Table CA 8.2.2.2/04-2 Results of the Probit analysis (max. likelihood regression) with length at 56 dpf: Selected effective concentrations (ECx) of the test item and their 95%-confidence limits (by Fieller's theorem)

		8	
	Le	ngth 🕰	
Parameter	EC10	EC20	
Parameter	(95 % confidence interxal)	(95 % confidence	interval) 0° 0
	[µg a.s./L]	[μg, &.š./L	
Effect on length at 56 dpf	14.915 (6.341 – 21, 39)	32.974 (23,982 –	\$.492)

The resulting EC₁₀ value of 14.915 (95%CL: 6.341 \approx 21.039) and 32.974 (95%CL: 23.982 – 51.492) tg a.s./L, meets the goodness of fit criteria and therefore the estimated EC₁₀ value is considered reliable.

Pseudo specific growth rate at 56 days post featilisation (dph

Regarding the calculation of EC_{10} and EC_{20} values for pseudo specific growth rate at 56 dpf she criteria for goodness of fit were met as the P(Cht²) value was 1,000, showing no significant deviation between fit and data, and a statistically significant concentration/response was found ($\rho(F) = 0.003$) for this parameter.

The resulting EC_{10} and EC_{20} values and the respective confidence intervals are represented in the following table.

 Table CA 8.2.2.2/04-3
 Results of the Proble analysis (max@likelihood regression) with escudo specific growth rate at 56 opf: Selected effective concentrations (EC, of the test item and their 95% confidence limits (b) Fieller's theorem)

~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	
	🖉 🔬 🖉 Pseudo specific growth rate
	$EC_{20}$
Parameter 🔊 🔿	(95 % confidence interval) (95 % confidence interval)
	μg a.s./L] μg a.s./L]
Effect on pseudo specific 🔬	24.665 $(21.482)$ $27.163$ $(37.154 - 43.187)$
growth gate at 56 dpf	
growing are at 50 upr	

The resulting EC₁₀ value of 26665 (95%CL 21.482 – 27733) and 39.833 (95%CL: 37.154 – 43.187)  $\mu$ g a.s./L, meets the goodness of fit criteria and therefore the estimated EC₁₀ value is considered reliable.

#### Fogeneration (agait life phase

Sex ratio (based on females)

Regarding the calculation of EC and EQ₂₀ values for sex ratio (based on females) at 56 dpf, the criteria for goodness of fit were met as the  $P(Ohi^2)$  value was 0.999, showing no significant deviation between fit and data, and a statistically significant concentration/response was found (p(F) = 0.003) for this parameter.

The resulting EC to and EC 200 values and the respective confidence intervals are represented in the following table. A  $\mathcal{L}$ 

 Table CA \$2.2.2/974
 Reputs of the Probit analysis (max. likelihood regression) with sex ratio (based on females at 56 dpf: Selected effective concentrations (ECx) of the test item and their \$95%; confidence limits (by Fieller's theorem)

	Sex ratio (bas	ed on females)
Parameter	EC ₁₀ (95 % confidence interval) [µg a.s./L]	EC20 (95 % confidence interval) [μg a.s./L]
Effect on sex ratio (based on females) at 56 dpf	9.767 (8.043-11.038)	12.084 (10.598-13.187)



The resulting EC₁₀ value of 9.767 (95%CL: 8.043 – 11.038) and 12.084 (95%CL: 10.598 – 13.187)  $\mu$ g a.s./L, meets the goodness of fit criteria and therefore the estimated EC₁₀ value is considered reliable.^o

#### **<u>F</u>₁ generation (early life stage)**

Post-hatch survival at 28 days post fertilisation (dpf)

Regarding the calculation of  $EC_{10}$  and  $EC_{20}$  values for post-hatch survival at 28 dpf, the criterity for goodness of fit were met as the P(Chi²) value was 0.739, showing no significant deviation between fit and data, and a statistically significant concentration/response was found (p(F) = 0.032) for this parameter.

The resulting  $EC_{10}$  and  $EC_{20}$  values and the respective confidence intervals are represented in the following table below.

#### Table CA 8.2.2.2/04-5 Results of the Probit analysis (max: likelipood regression) with pseudo specific growth rate at 56 dpf: Selected effective concentrations (EC) of the test item and their 95%-confidence limits (by Fieller's theorem)

Donomotor	$\mathcal{A}$ $\mathcal{A}$ $\mathcal{A}$ Post-fratch survival $\mathcal{O}$ $\mathcal{A}$ $\mathcal{A}$
	$\mathcal{L}$
Parameter	(95% confidence interval) (95% confidence interval)
Effect on post-hatch	$^{\circ}$ 1.945 (1.038 - 2.7 $^{\circ}$ ) $^{\circ}$ $^{\circ}$ $^{\circ}$ 5368 (4.645 - 7.269)
survival at 28 dpf	

The resulting EC₁₀ value of 1.915 (05%CL F.038 (2.742) and 5.868 (95%CL A.545 – 7.269) µg a.s./L, meets the goodness of fit oriteria however as 4 falls below the lowest dose of 2.6 µg a.s./L and in accordance with the OECD Series on Testing and Assessment Number 54, the estimated EC₁₀ value should not be considered reliable.

#### Post-hatch survival at 35 days por fertilisation (dpf)

Regarding the calculation of  $EC_{10}$  and  $EC_{20}$  values for post-hater survival at 28 dpf, the criteria for goodness of figures met as the P(Chi²) value was 0.880 showing no significant deviation between fit and data, and a statistically significant concentration response was found (p(F) = 0.010) for this parameter.

The restricting  $EC_{10}$  and  $EC_{20}$  values and the respective confidence intervals are represented in the following table.

#### Table CA 8.2.2.2/04-6 Results of the Probit analysis (max. likelihood regression) with post-hatch survival at 35 dpf: Selected effective concentrations (EC_x) of the test item and their 95%confidence mits (by Fieller's theorem)

	D Post-hatc	h survival
Parameter 9		EC20 (95 % confidence interval) [µg a.s./L]
Effect on post-hatch survival 35 dpt	2.263 0 ^{1.} 619-2.868)	4.828 (3.998-5.625)

The resulting EC, value of 2.263 (95% CL: 1.619 – 2.868) and 4.828 (95% CL: 3.998 – 5.625)  $\mu$ g a.s./L, meets the goodbess of fit criteria however as it falls below the lowest dose of 2.6  $\mu$ g a.s./L and in accordance with the OECD Series on Testing and Assessment Number 54, the estimated EC₁₀ value should not be considered reliable.

#### Survival at 35 days post fertilisation (dpf)

In line with previous EU reviews of the data, survival based on eggs introduced has also been calculated at 35 dpf.



Regarding the calculation of  $EC_{10}$  and  $EC_{20}$  values for survival at 35 dpf, the criteria for goodness of fit were met as the P(Chi²) value was 0.905, showing no significant deviation between fit and data, and a statistically significant concentration/response was found (p(F) = 0.008) for this parameter.

The resulting  $EC_{10}$  and  $EC_{20}$  values and the respective confidence intervals are represented in following table.

# Table CA 8.2.2.2/04-7 Results of the Probit analysis (max. likelihood regression) with post-Outch survival at 35 dpf: Selected effective concentrations (ECx) of the test item and their 95%-confidence limits (by Fieller's theorem)

		Q		6
	⁴	Surviyar		¢,
Parameter	EC10	Q [°] , °	L EC20 C	<i>"</i> ©″
I al alletel	(95 % confidence miterval)	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	6 confidence interval)	Y
	[µg a.s./L] 。		- [μg _S a,s./L] 🗸 👋	
Effect on survival at 35 dpf	1.878 (1,271-2.469)		456 (\$ 610-5.243)	

The resulting EC₁₀ value of 1.878 (95%CL; 1.271  $\pm$  2.465 and 4.456 (95%CL; 3.610  $\pm$  2.43 and a.s/L, meets the goodness of fit criteria however as it falls below the lowest does of 2.6 µg a.s./L and in accordance with the OECD Series on festing and Assessment Number 54, the estimated EC value should not be considered reliable.

A summary of the obtained endpoints is presented in the following table.

# Table CA 8.2.2.2/04-8 Overall endpoints of the statistical re-calculation of the Danio reriestudy with spirox amine

	Endpoint (µg		
Period	Development	Parameter & C	EC ₄₀ (95% confidence intervals)
	stage 🔊	Parameter & O O &	EC(1)(95/8COandence intervals)
	Early li	Hatching d4 S	n.d.
	stage	Hatching d4 2 2 2 4	Ay.d.
	Development stage Early life stage	A.s./L) Parameter Hatching d4 Hatching d5 Sufwival at 14 dpt 28 dpf Lerenth at 28 dpf	n.d.#
		Sufficiency of the second sec	n.d.# R.g.#
,		28 dpf	
\$~		Length at 25 dpf 0 0	@42.896 (24.536-74.994)
	Juvenile	Servival & 56 dpf	×_*
~ ¥	growth 🔊	Vength at 56 dow	14.915 (6.341-21.039)
	Juvenile growth	Pseudo specific growth rate	24.665 (21.482-27.133)
	Aduto A		-*
F0	a sõy	Egg nuntber per temale per day	n.d.
generation	~°°°°	Cumulative egg number FertOsation arte Survival C	n.d.
2	Ő	Fertosation arte 6	n.d.
			-*
	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	Sex ratio males $\sqrt{2}$	n.d.
A.	w L	Sex ratio females	9.767 (8.043-11.038)
¥	, O	Length males O	>40
	Ĩ,	Length females	>40
		Length males Length females Weight males	>40
, de		Weight females	n.d.
Ű		Vitellogenin males	n.a.
, S	à 1	Artellogenin females	n.a.
, Á	Parly life 🕺 🐧	UHatching d4	>16
L'S' Q	stage 0	Hatching d5	>16
F1 O ⁴ generation	Parly life	⁺ PH survival 14 dpf	n.d.
generation		PH survival 21 dpf	n.d.
Seneration		PH survival 28 dpf	1.915 (1.038-2.742)**
		PH survival 35 dpf	2.263 (1.619-2.868)**
		⁺ Survival 35 dpf	1.878 (1.271-2.465)**



	Endpoint (µg	a.s./L)		
Period	Development stage	Parameter	EC10 (95% confid	lence intervals
		Length	≥16	N a
		Weight	≥16	

Due to the lack of a concentration dose response or when the test rate with 10% when compared to the controls are below the tested range of concentrations, the calculation of EC_{10} and EC_{20} values for the parameters marked in the above table as "n.d." was not possible and therefore no EC_{10} or EC_{20} values were determined. Ω 's data as not available within the study report, statistical analyses of parameters marked with * was not possible.

Where the calculated EC₁₀ values were below the lowest dose, as per the OEQD series on testing and assessment number 54 (Current approaches in the statistical analysis of ecotoxicity data: A guidance to application these values are not considered reliable enough and are marked with a **.

*Post-hatch survival was used to calculate the ECx values for the M generation resulting in survival being independent of hatch. However, as the number of eggs hatched at day 5 was less than the number surviving on day 14 (eggs assumed to have continued to hatch after the measured time point of day Thowever raw data not available to confirm), this was not possible for the F_0 generation and therefore number of eggs introduced was used to determine survival at 14 dpf, 21 dpf and 28 dpf. Survival was therefore, not independent of hatch for this stage.

The determination of reliable EC10 and EC values for parameters marked with a was not possible due a poor goodness of fit in the data and the data scattering around the dose response.

As vitellogenin concentration is a nonapical endpoint, this parameter was not deemed televant for further analysis and therefore ECx values were not determined for both mate and female fish.

III. Conclusion

Effect concentrations with a 10 of 20% effect on F_0 length at 28 dof and 56 dpf, F_0 pseudo specific growth rate, F0 sex ratio based on females, F1 post-hatch sorvival at 28 and 35 opf and F1 survival at 35dpf (EC_{10,20}) were re-calculated.

The lowest determined EC₁₀ value was 1.878 µg a.s./L for survival of the F₁ generation but it is noted that this is an extrapolated value and should be used with cathion.

Assessment and conclusion by applicant

The statistical re-evaluation of the data has determined EC_{10} and EC_{20} values for many of the parameters assessed. It must be noted that EC₁₀ values for survival are extrapolated values below the concentration range lested and therefore should be treated with caution.

In the RAR (2010), RAR (2017) an EG10 was determined by the RMS and used in preference to the NOEC in the original study report.

The lowest BC10 value of 688 µg a.s./L based on overall survival, is lower than the overall NOEC of 2.6 µg a.s./L and therefore has been taken as the capitcal endpoint determined for this study. It must be noted that the EC to value of 1.88 dig a structure lowest test concentration and therefore



D + D + +	
Data Point:	KCA 8.2.2.2/02
Report Author:	
Report Year:	2009
Report Title:	Statement concerning questions related to the fish full life cycle test listed to the draft re-assessment report on Spiroxamine
Report No:	<u>M-347595-01-1</u>
Document No:	<u>M-347595-01-1</u>
Guideline(s) followed in	None
study:	
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	Yes Yes Y

In the study <u>M-304458-02-1</u> [BVL Doc. 10 1798135; Zeora fish, life over test, flow through conditions.] the following aspect needs further classification.

During the critical phase for sexoal development of zebra fish, in the fourth week, the mean test concentrations dropped down to 71 % of nominal at the NOFC-level (4.5 for L inclead of 6.4 μ g/L). At the LOEC-level the measured concentration was 6.1 μ g as/L (38 % of nominal). At the lowest test concentration the measured value was 1.6 μ g as/L compared to 2.6 μ g as/L nominal (71.2 %). The decrease of the concentrations was due to malfunctions of the pumps. Probably, the concentrations were measured a second time for control purposes in the 40 week. This information may be helpful to define the time period of low exposure. Such data are not shown in the study report, probably they are available from the raw data and can be provided.

Since effects from exposure to endocrine discuptors may result from relatively short exposure periods of critical developmental stages, it can not be excluded that the effects on sexual development found in this study are underestimated, if related to the nominal concentrations.

During the evaluation of the Fish Full Life cycle report questions concerning the exposure situation especially at week 4 acose. The respective questions were discussed with the study director (

) and the following

answer can be given: The stock solutions for the flow through device were prepared freshly each day. Thus, the weekly

chemical analysis is only a representative one Additionally, the consumption of the stock solution was checked at least daily, assuring that malfunctions of the dosing pumps, if occurring, could be detected within 24 hours. On working days, the correct function of pumps was checked twice daily, therefore it can be stated that a severe malfunction would have been detected within 15 hours.

The problems observed within week of the experiment were caused by two different incidents.

1) Until week the test item concentrations in the aquaria were representatively measured to be between 75 and 102% of the normal concentrations. The first measurement in week 4 (Feb 27, day 1 of week 4) revealed a drop of concentrations down to values ranging between 18 and 81 %. To overcome the low exposure situation, the amount of test item weighed in was increased from that time point onwards. Due to daily preparation of the stock solution and the at least 5-fold water exchange rate of the flow through device, it can be assumed that the measured concentrations in week 4 (first measurement) represent the lowest values of the development of test concentrations between the weeks 3 and 4 and a recovery of test concentrations took place immediately, as confirmed for most of the replicates by the second measurement of week 4 (Feb 29).



2) Independently from this, malfunctions of the dosing pumps were observed on Friday of week 4 (Feb 29). Two replicates each of test level 1 (replicate 1 and 2) and test level 4 (replicate 3 and 4) were affected. The pumps were immediately repaired and samples were taken for analytical measurements (as second sampling point during week 4). No further analytical samples were taken at the following day (Saturday) due to the weekend situation. The visual control of the stock solution consumption from Friday onwards was in the expected range again. The results of the accompanying chemical analysis for week 5 (80-123 % recovery) demonstrate the correct function of the flow through device. Due to the at least 5 fold exchange of the water volume per day it can be assumed that the aimed concentration were achieved rapidly again after repairing of the flow through device on Friday.

The first analytical measurement of week 4 (Feb 27) was on day 21 after test initiation and day 56 post hatch. The pump malfunction occurred on Feb 29, which is day 23 after test initiation (post fertilisation) and day 18 post hatch. Therefore, this low exposure event took place during week 3 post hatch and was terminated at the beginning of week 4. The results for week 5 pepresenting week 4 post hatch) and the following weeks are within the acceptable range (mean values abaye 80% of neurinal). Based on the available literature concerning gonad development of zebrafish, the time period of low exposure is just before the early beginning of the critical phase of gonad development of zebrafish. This is confirmed by the homogeneity of the study results of the different replicates: the two replicates not negatively influenced by pump malfunctions did not show different effects compared to the ones affected by the pump malfunctions. In conclusion, there is clear evidence that the existing problems of the flow through system revealed on Feb 27 and 29 did not negatively affect the general outcome of the study. Therefore the use of the nominal concentrations which was based on mean measured values ranging between 92 and 101 % of the nominal values seems reasonable.

In order to be conservative, a worst case assumption for the calculation of the exposure concentrations in week 4 in the study report by applying a weighted approach (see Table 24). The lower values measured at the first sampling were weighted 6 told. The second measurement in week 4, revealing by far higher recovery values than the first measurement in most cases (except the 4 replicates negatively impacted by pump malfunctions), was weighted only one fold.

The evaluator of the Pish Full Life cycle used the LC_{10} instead of NOEC for the endpoint F_1 survival (day 35). Thickeems to be questionable. The Fish Full Life (Scle study was performed using a test design appropriate to generate a NOEC ("NOEC-approach"). Five concentrations tested in four replicates each were investigated. This is in line with existing guidelines for Fish Full Life Cycle Tests and other chronic fish studies (e.g. ELS –tests according OECD 210) and represents the state of the art.

The use of EC_{10} values is a possibility to create risk essessment relevant results for a chronic study in cases where a NOEC determination is not possible. In the presented study, a NOEC confirmed by statistical analysis was achieved. The evaluator relevant at this endpoint by calculating an LC_{10} and additionally excluded the replicate with the lowest survival rate. It was stated that the respective replicate was not fulfilling the validity orteria according to QECD TG 210. However, the validity criteria are defined for the mean value rater than for the single replicates. Especially for the second generation early life stages of a firsh Full Life Cycle fest the validity criteria of the OECD 210 should be discussed anyway. Nevertheless BCS evaluated the data for the endpoint under discussion again excluding the respective replicate as well. This evaluation were carried out. The originally presented NOEC of 2.6 $\mu g/L$ ranged an all cases within the 95% confidence limits of the LC₁₀ for the endpoint F1 survival (day 35).

On page 212 of the spirovarine Draft Re-assessment Report (yellow draft) it is stated that:

"Since it can not be excluded that also short term exposure of females may impair the egg quality permanently and hence the fish population, the use of PEC_{twa} values for the risk assessment is not appropriate and the PEC max values have to be preferred, as discussed in the guidance document on aquatic Ecotoxicology". In the Aquatic guidance document the following can be found on page 27:



"The use of PEC_{twa} may not be appropriate for use with endocrine disrupting compounds since these effects may result from relatively short periods of exposure at critical developmental periods." \mathcal{Q}_{p}°

Zebrafish is known to be a sensitive fish species concerning sex reversal. The critical developmental period for this is lasting for about 3 to 4 weeks. Thus, it should be possible to use a PEC_{twa} of days, especially when the single peaks originating from exposure modelling are not higher than the NQEC generated in a Fish full life Cycle study covering the most sensitive developmental period. The advatic guidance document states that it may not be appropriate to use the PEC_{twa}, therefore the detrivitive decision in each case should be depending on the available data package. In the case of zebrafish and Spiroxamine the possibility to use the PEC_{twa}, should be discussed on the basis of the respective exposure patterns.

Assessment and conclusion by applicant:

The supporting statement summarised above has been submitted here for completeness and to present the arguments regarding the concerns over the analytical measurements in week of the test. On this basis it is considered to be acceptable.

Some of the other issues discussed here (such as use of PEC twa values) are no longer considered relevant for the current submission. Please refer to Document M-CD Section 10 for the discussion on the selection of toxicity endpoints and the exposure values for the risk assessment.

	KCA8.2.2.203 7 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2
Data Point:	KCA8.2.2.2.03 07 57 07 57 50 6
Report Author:	
Report Year:	$ _{\mathcal{A}}^{014} \cap^{v} \stackrel{\sim}{\rightarrow} 0$
Report Title:	Zebrafisch (Danio rego), full life cycle test under static conditions in a water
× 1	sedifipent system - Test item SpiroQumine
Report No:	$\underline{M0467979403-1}_{0}, \qquad \qquad$
Document No:	M-46789-03-07 ~ ~ Q
Guideline(s) followed in	Special study, considers to QPCD 210; US PA QCSPP 850.1500
study:	
Deviations from current	None of the of t
test guideline:	None y g g g g
Previous evaluation:	yes evaluated and accepted
L Ž ^{, v}	\mathbf{RAOR} (2017) \mathbf{N} \mathbf{N} \mathbf{O}
GLP/Officially	Yes, conducted onder GDP/Officially recognised testing facilities
recognised testing	
facilities:	
Acceptability/Reliability.	yes evaluated and accepted RAB (2017) Yes, conducted ander GDP/Officially recognised testing facilities

Executive Summary

The 56-day chronic toxicity of spiroxamine to different life stages of the zebrafish (*Danio rerio*) was studied under static renewal conditions. Three proups consisting of different life stages of zebrafish (fertilised eggs, juveniles and mature spawning adults) were concurrently exposed to four spiroxamine concentrations under static condition in 260 L subdivided glass aquaria with a water column of 48 cm depth and a 3 m layer of artificial sediment. The nominal initial spiroxamine concentrations were 12, 24, 48 and 192 µga/s./L. Sediment was included in the test vessels to simulate a realistic dissipation of the test substance mimicking exposure and effects following two applications at a 14-day interval.

à

Organisms were exposed to mean initial measured test concentrations of 15.8, 30.4, 63.9 and 255 μ g a.s./b

The survey al of F_1 fish larvae was interpreted as the most sensitive population relevant endpoint. The corresponding effect concentration (EC₁₀) was calculated to be 23.3 µg spiroxamine/L.



Beside larval survival, the sex ratio after juvenile exposure as well as the growth of adult females showed similar sensitivity (NOEC: 30.4 µg spiroxamine/L). A decrease of the concentration of the biomarker vitellogenin could be observed at \geq 30.4 µg a.s./L thereby giving a NOEC for this parameter of 155 µg a.s./L. The histopathological evaluation of the fish gonads revealed no substance related effect. . ~

- **Materials and Methods** I.
- Materials A

Test Material	Spiroxamine
Lot/Batch #:	AE 1344293-01-05 (origin Fatch: EDTH 08883)
Purity:	98.2%
Description:	Light yellow liquid at a set of a set o
Stability of test compound:	Spiroxamine AE 1344293-01-05 (origin batch: EDTH608883) 98.2% Light yellow liquid Not reported 05 July 2014 Not reported Initial nominal: 12, 24, 48 and 192 µg a.s./L Initial measured: 15.8, 30.4, 63.9 and 255 ag a.s./L Noto Xes, mean measured initial concentrations 127 – 133% of nominal
Reanalysis/Expiry date:	05 July 2014
Density:	Not reported and the second se
Treatments	
Test rates:	Intrial nominal: 12, 24, 48 and 192 ug a.s./Loo (nitial measured: 15, 8, 30.4, 63.9 and 255 ag a.s./L
Solvent/vehicle: 🦃	None of the second seco
Analysis of test	x s, mean measured initial concentrations 127 – 133% of nominal
Test organisms	
Species:	Zebrafish (Dario rerip)
Source:	
period:	Fish were raised in the test facility, and holding water was of the same quality as that used in the test
Feeding:	Fed daily ad abitum uitable diet depending on fish age
Treatment for discuse:	Spiroxamine AE 1344293-01-05 (origin batch: EDTH608883) 98.2% Light yellow liquid Not reported 05 July 2014 Not reported Intrial nominal: 12, 24, 48 and 192 up a.s./L Initial measured: 15.8, 30.4, 63.9 and 255 ag a.s./L Norio Nets, mean measured bittial concentrations 127 – 133% of nominal Zebraffsh (<i>Davio revo</i>)
Test design	
a contraction of the second se	colump and 3 cm deep layer of artificial sediment
Test medium:	Purified draking water according to OECD 215
Reptrcation.	Bur control replicates, three per test concentration
No. of S	A: 50 fertilised eggs
Animals/vesser:	B: 30 four-week old juveniles C: 30 adult fish
Duration of test:	56 days



Environmental test conditions

Temperature: $24.0 - 26.9^{\circ}C$ **Dissolved oxygen:** 82 - 125% saturation pH: 5.6 - 8.9**Photoperiod:** 12 h light : 12 h dark

B. Study Design

Vifferent life and a op This study was conducted in order to assess the effects of spiroxamine exposure on different life stage of the zebrafish, Danio rerio, including early life stages, juvenile growth, reproduction and early fre stages of the filial generation. Test concentrations were selected based on available data on the chronic toxicity of spiroxamine to fish.

Three groups consisting of different life stages of zebratish (Crtilised eggs, juveroles, and mathre spawning adults) were concurrently exposed to four spiroxamme concentrations under static conditions in 260 L subdivided glass aquaria with a water column of 48 cm depth and a 3 cm layer of artificial sediment. The nominal initial spiroxamine concentrations were 12, 24, 48 and 92 µg/spiroxamine/L. Three aquaria replicates were used per test concentration, and four untreated aquaria served as controls.

Sediment was included in the test vessels to simulate a realistic dissipation of the test substance, mimicking exposure and effects following two applications at a 16 day interval. Dissipation in artificial sediment still represents a worst case exposure as the shows lower biological activity compared to natural systems.

Fertilised eggs were collected from a fealthy parental stock and the time from spawning to exposure in the test vessels did nov exceed two hours.

Test medium was frinking water purified according to OECI215, the filtration with activated charcoal, passage through a limestone column and agration while oxygen seturation. The sediment was prepared according to OECD 209, and was composed by dry weight as 4 to 5% peat, 20% kaolin clay (kaolinite content >30%) and ⁴⁵ to 76% quartz sand. The final organic carbon content was determined to be 1.2%.

Application solutions of the test substance were prepared by weighing adequate amounts and dissolving it in dilution water. Vessels were treated trace with a 14-day interval by replacing 10 L test media with 10 L application solution, with 3333 ml being introduced into each of the compartments with different fish life stages.

The in-life phase began with the simultaneous application of the test item to 50 fertilised eggs, 30 4week old juveniles and 30 adult ish. Each group was carefully segregated in separate compartments within each test aquaria. When the fish from tertilised eggs reached the age of 28 days (juveniles) their numbers were reduced to 30.

Measurements of mortality and abnorma behaviour were made daily.

At 28 and 56 days of age, fish were digitally photographed. Survival rates and fish lengths as well as the pseudo-specific growth rate (based on the length measurements) were determined by evaluating photograph using electronically supported counting and analysis. In the pre-adult life stage, *i.e.* day 56 and later spawning trags were introduced and monitored daily for spawned eggs. The time until first findings of eggs was recorded. The spawning success was monitored for at least 20 days beginning from when which sufficient egg numbers (at least 15 eggs per replicate vessel) and a fertilisation of ≥80% was achieved on three successive days. Egg production per female per day and fertilisation rate was measure diaily. After termination of the parental generation, a blood plasma sample was taken from each fish and the concentration of vitellogenin was measured in blood plasma of five males and five females. Finally, histopathology of the fish gonads was conducted for five males and five females. Primary evaluation criteria were increased proportion of spermatogonia, presence of testis-ova,



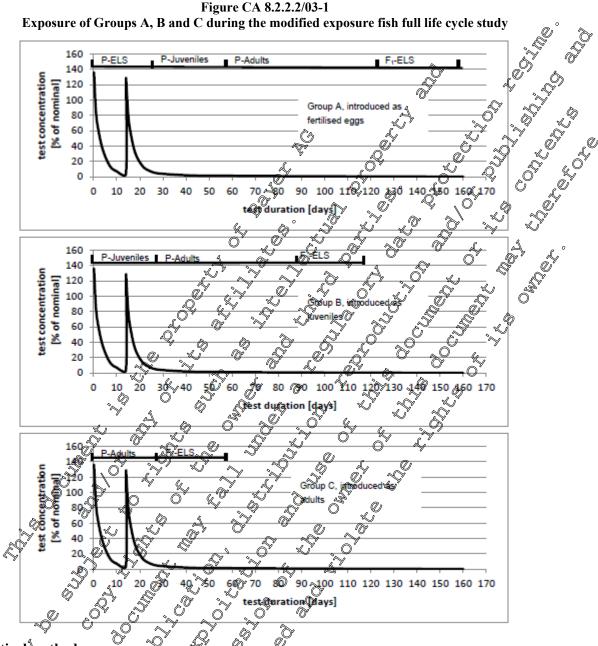
increased testicular degeneration and leydig cell hyperplasia/hypertrophy for males, and increased oocyte atresia, perifollicular hyperplasia/hypertrophy, decreased yolk formation and change in gonadal staging for females. Secondary evaluation criteria were decreased proportion of spermatogonia, increased proteinaceous fluid, asynchronous gonad development, altered proportions of spermatocytes and spermatids, altered gonadal staging and granulomatomous inflammation in males, and interstinal fibrosis, egg debris in oviduct, granulomatomous inflammation and altered mimber of post-ovulatory follicles in females.

For the filial generation (F_1) of each group, survival rates during the early life stage period (up to 28 days of age) were observed. After finishing the early life stage period, the fish were measured for total length. Furthermore, the group dry weight was measured and the single dry weight per fish was calculated.

Measurements of water temperature were made continuously in all test vessels. The oxygen concentration and pH of the water was measured in each vessel chrectly before adding the fish and afterwards at least twice weekly. Nitrate, nitrite and among the measured of ce per week

, n marken des) and ge three exposure the three the three three the three th atterwards at least twice weekly. Nitrate, nitrifie and announding weige measured once per week. The different life stages tested were marked as group A (fish introduced as spring adults). The sigure presented below summarises the three xposing groups tested. The different life stages tested were marked as abup A (fish introduced as fortilised eggs embrus),





Analyticalmethod

Samples of water were analysed using the validated analytical method <u>M-467979-03-1</u>, report reference <u>M-467979-03-1</u> (see Doc MICA Section 4).

II. Results and Discussion

Validity criteria according to the study report were met:

- Survival cate in the controls was greater than 70%
- Dissolved oxygen concentration to be >60% saturation throughout the test (actual: 82 to 125%) • Water temperature was within $25 \pm 2^{\circ}$ C throughout the study

1st application (day 0)

The measured concentrations of the application solutions corresponded to 104 to 137% of nominal. One hour after treatment, the measurements of the water samples revealed concentrations between 118 and



143% of nominal (mean 134%). After 4 hours, the results were between 117 and 151% of nominal (mean 136%). After 1 day the analysed values were between 63 and 104% of nominal (mean 86.0%).

The dissipation characteristic was similar in all test aquaria. On day 2 of the study, between $36 \operatorname{and} 80\%$ of nominal were found in the water column (mean value of 62.1%). On day 4, 7 and 100 mean concentrations of 35.1, 15.2 and 8.3% of nominal, respectively, were measured. On day 14, before the second application, the mean concentration was measured to be 4.8% of nominal.

2nd application (day 14)

The measured concentrations of the application solutions corresponded to 107 to 144% of nominal. hour after treatment, the measurements of the water samples revealed concentrations between 148 and 140% of nominal (mean 128%). After 4 hours, the results were between 114 and 131% of nominal (mean 121%). After 1 day the analysed values were between 68 and 92% of nominal mean 80.1%

The dissipation characteristic was similar in all aquaria. On day 2 after the 2rd approximition, between 43 and 69% of nominal were found in the water Column (mean value of 57,6%). On day 4, 7 and 10 after the 2nd application, mean concentrations of 313, 14.6 and 21% of nominal, respectively, were measured. On day 14 after the 2nd application, the mean concentration was determined to be 5,4% of nominal. For the following sampling dates on day 28, 42, 70 and at test end, only low amounts or amounts below the LOQ of 0.6 µg spiroxantine were detected.

10 The mean measured initial concentrations were calculated to be 158, 304, 63.9 and 255 µg a.s./L. Results have been presented based on these mean measured initial concentrations

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Nominal concentration (µg	Replicate	Measure	d eonce	NDration S	×		measuro tration	ed	
concentration (µg a.s./L)		🔊 h after	1 st	1 brafter	2 nd	By rep	licate	Total	
, S		applicati	on	applicati	øn 🚕				
		µg/L	~%	μg/L	%& ^{>}	μg/L	%	μg/L	%
Control	(A O O)	SLOQ*	- 2	<lqq*< td=""><td>TQ.</td><td>-</td><td>-</td><td>-</td><td>-</td></lqq*<>	TQ.	-	-	-	-
	B	<lqq*< td=""><td>) -</td><td><lŏq*< td=""><td></td><td></td><td></td><td></td><td></td></lŏq*<></td></lqq*<>) -	<lŏq*< td=""><td></td><td></td><td></td><td></td><td></td></lŏq*<>					
		° <loq*< td=""><td>ē.</td><td>LOQ*</td><td>r</td><td></td><td></td><td></td><td></td></loq*<>	ē.	LOQ*	r				
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12.0	DA <u>v</u> v	ji7.2 🖉	143	16,2	135	16.7	139	15.8	131
12.0	B S	a 14	120″	16.8	140	15.7	131		
		14.2		5.7	131	15.0	125		
24.0		231	ົ້າ30 ໃ	20.2	118	29.7	124	30.4	127
	B O O	30.7 S	128	29.6	123	30.2	126		
A		30.7	JØ1	32.1	134	31.3	130		
48.0		68.6	⇒43	59.0	123	63.8	133	63.9	133
	¢₿ <u>₹</u> ′∽	68.8 🔊	143	61.1	127	64.9	135		
	C C C	66.6	139	59.0	123	62.8	131		
192		261 ^O	134	251	131	256	133	255	133
	B OF AC	203	142	248	129	260	136		
Ú ^Y A		_@ 263	137	233	121	248	129		
* I init of Santifalor	(15) (15) (10) (10)	沂							

ñ \bigcirc Table CA 8.2.2.2/03-1 Mean measured initial concentrations of spiroxamine (maximum peak) during the

* Limit of grantification (BOQ) 🔬 0.6 pg/L

Survivaland

The survivabol the S₁ fish larvae was found to be the most sensitive population relevant endpoint after peak exposure to spiroximine with statistically significant effects observed at test concentrations of 30.4 μ g/ \mathbb{O} and higher in all test groups.



For the F₁-generation of group C, a statistically significant difference compared to the control was detected for survival at \geq 30.4 µg spiroxamine/L (NOEC: 15.8 µg/L). An EC₁₀ of 23.3 µg spiroxamine/L (based on mean measured initial concentrations) was calculated.

The survival of parental fish larvae (group A) was affected at the same concentration level as the F_1 fish larvae of groups A and B (NOEC: 30.4 µg/L). In contrast, survival of juvenile and adult fish was not affected in any group.

Growth retardation could be observed at all parental life stages at 63.9 μ g/L and higher. No impact of growth could be observed for the filial fish. The parental barvae (Group A) and juverile fish (Group A) and juverile fish (Group A) and B) showed an impact on growth in terms of reduced lengths (NOFC: 63.9 μ g spiroxamme/L). The adult females of group B showed significant decrease of weight at the two highest test concendations (NOEC: 30.4 μ g spiroxamme/L). Group C fish were not affected.

Reproduction

Fecundity, represented by the egg number peofemal@and day, was not affected after peak exposure to the test item in any of the tested concentrations. There was also no impact on the testilisation fate (NOEC: $\geq 255 \mu g$ spiroxamine/L).

Sexual development, biomarkers and histopathology of gonads

A significant shift in sex ratio towards an forceased number of male fish could be observed in group B (fish exposed as juveniles) at test concentrations ≥ 63.9 ug/L (NOEC 30.4 g spirovamine/L). Also for group A (fish exposed as fertilised eggs) an effect on sex ratio could be detected An increased number of males was found at the highest test level (NOEC: 63.9 µg spirovamine/L).

Beside the apical endpoints, the biometker viellogenin (VTG) was analysed. This parameter was analysed to provide additional information on physiological processes in the exposed fish and to assist the interpretation of the effects observed for the population relevant endpoints.

No effect on VTG concentrations is blood plasma of both female and male fish could be detected in the parental fish groups exposed as adults and juveniles (group B and C). However, for the parental group A (fish exposed as fertilised eggs) a significant decrease of TG concentrations was detected at $\geq 30.4 \ \mu g \ spiroxamine L$.

The decrease of VTG anount was quite pronounced, but a dear dose response relationship was missing. As a further parameter, which encourages the interpretation of apical effects, a histopathological analysis of fish gonads was performed. The histopathological evaluation of the adult animals of all groups (A, B and C) revealed to substance related effects on fish gonads. There were single findings of egg debris, fibrosis, atresia in females and testis ova in males but there were not treatment related.

			-	-				
Endpotent 🗞	Mean measure	Mean measured initial concentration (µg a.s./L)						
	Control 🔊	45.8	30.4	63.9	255			
Number of replicates		$\mathbb{Q}_3 \mathbb{S}^3$	3	3	3			
Number of eggs	200	150	150	150	150			
introduced $$		"Q"						
Survival, day 21 pf (%)	90 ± 4.3	8 9.3 ± 3.1	90.0 ± 2.0	$75.3 \pm 4.6*$	$46.0 \pm 15.9*$			
Survival, day 28 pt (%)		89.3 ± 3.1	90.0 ± 2.0	$74.7\pm4.2^\dagger$	$41.3\pm16.8^\dagger$			
Length day 28 (%)	1.27 0.04	1.31 ± 0.05	1.29 ± 0.02	1.30 ± 0.07	$0.89\pm0.09*$			

Table CA 82.2.2/03-2 Group A 9-generation: Barly life stage: hatch, survival and growth, day 28 pf

Mean values & standard deviation are presented

pf Post feetilisation

Statistically significantly different to the control, p < 0.05, Williams test, one-sided smaller

Strustically significantly different to the control, p < 0.05, Welch t- test, one-sided smaller



Table CA 8 2 2 2/03_3	Group A, P-generation: Ju	ivenile stage: hatch	survival and growth	n dav 56 nf
1 abic CA 0.2.2.2/03-3	Group A, I -generation. Jt	ivenne stage. naten,	sui vivai anu growu	i, uay so pi

Endpoint	Mean measure	Mean measured initial concentration (µg a.s./L)						
-	Control	15.8	30.4	63.9	255			
Number of replicates	4	3	3	3	3 5 0			
Number of eggs introduced	121	90	90	89				
Survival between day 28 and 56 pf (%)	99.2 ± 1.6	98.9 ± 1.9	98.9 ± 1.9					
Length, day 56 pf (cm)	2.76 ± 0.09	2.81 ± 0.03	2.84 ± 0.05	2.73 ± 0.07	2.48 ≇0.06			
Pseudo specific growth rate (based on length)	2.779 ± 0.16	2.735 ± 0.14	2.842 ± 0.11	2.667 ± 0.23	3,707 ± 0,32 [#]			
Mean values \pm standard deviation are presented pf Post fertilisation * Statistically significantly different to the control, $p<0.05$, Williams test, one-aded smaller								
* Statistically significantly different to the control, p (0.05, Williams test, one sided greater Table CA 8.2.2.2/03-4 Group A, P-generation: Reproduction								

Table CA 8.2.2.2/03-4 Group A, P-generation: Reproduction

Endpoint	Mean measured initial concentration (µg a L)
	Control 2 15,8 30,4 63.9 255
Number of replicates	$ \begin{vmatrix} 4 & \downarrow \\ \downarrow$
Time to regular	71 ± 1
spawning (days)	
Egg number per day	6 ± 3 6 ± 3 6 ± 9 742 65 ± 2 711 ± 1
and female	
Fertilisation rate (%)	97.4 ± 0.9 95.7 ± 1 97.8 ± 0.5 98.0 ± 0.2 97.0 ± 0.7
36 1 1 1	

Mean values ± standard deviation are presented

Table CA 8.2.2.2/03-5 Group A, Perneration: Termination - survival, growth, sex ratio and biomarker

O

Endpoint	Meanmeasure	d initial concept	ration (µg a.s./L)		
	Control 🖉	15,8	30.4	×63.9	255
Number of repretes	4,0 U	34° 60° 4		3	3
Survival (%)	99.2 ± 12	100 ≠ 0%0		96.6 ± 3.3	100 ± 0.0
Length mates (cm)	3.6 ± 0 1	3.0 ± 0.1	3 09≠ 0.1° ≫	3.5 ± 0.1	3.6 ± 0.1
Weight males (g)	0.4100±0.019	0.448 ± 0.042	0.414 ≠ 0.045	0.390 ± 0.009	0.439 ± 0.025
Length females (cm)	3.05 ± 0.1		⊿3.5 ±200	3.4 ± 0.0	3.5 ± 0.2
	40.424 ±Ø.017√	0.435 @ 0.009	0.420 ± 0.012	0.430 ± 0.023	0.485 ± 0.105
Sex ratio (% females)	60.4 8.0 0	682 ± 9.8	$54,2 \pm 8.8$	63.7 ± 1.0	$23.3 \pm 16.2*$
Sex ratio (% nales)	39.6± 8.0	303 ± 9	45.8 ± 8.8	36.3 ± 1.0	$76.7 \pm 16.2^{\#}$
Vitellogenin males	1991 ± 001	0.01 ±0.00 C	0.02 ± 0.01	0.08 ± 0.12	0.20 ± 0.34
$(ng/\mu g)$					
Vitellogenin females	309. Q± 142.1	27 3 ± 40 5	143.3 ±	$126.8 \pm 33.4*$	$165.5 \pm 3.3*$
(ng/µg)	A		101.2*		

Mean values ± standard deviation are presented

Statistically significantly different to the control, p < 0.05, Williams test, one-sided smaller

Statistically significantly different to the control, p<0.05, Williams test, one-sided greater # S *a*,

Table CA 8.2.2.2/03-6	Group A: E1-generation: Early life stage – hatch, survival and growth, day 28 pf

Endpoint &	Mean measure	ured initial concentration (µg a.s./L)					
	Control	15.8	30.4	63.9	255		
Number of replicates		3	3	3	3		
Number of eggs	202	152	152	153	151		
introduced							
Survival, day 21 pf (%)	85.2 ± 9.0	75.0 ± 1.7	75.7 ± 8.9	$63.4 \pm 12.5*$	$61.5 \pm 12.3^*$		
Survival, day 28 pf (%)	82.7 ± 6.4	72.4 ± 2.1	75.1 ± 7.8	$62.1 \pm 11.4*$	$60.2 \pm 13.4^*$		
Length, day 28 pf (cm)	0.99 ± 0.04	0.94 ± 0.06	0.99 ± 0.01	1.03 ± 0.06	1.07 ± 0.09		



Endpoint	Mean measur	Mean measured initial concentration (µg a.s./L)				
	Control	15.8	30.4	63.9	255 °	
Group dry weight, day 28 pf (mg)	70.0 ± 4.2	50.7 ± 12.5	48.1 ± 7.0	59.7 ± 20.2	61.9 ± 19	
Single dry weight, day 28 pf (mg)	1.7 ± 0.2	1.4 ± 0.3	1.3 ± 0.1	1.9 ± 0.4		

Mean values \pm standard deviation are presented

				~~ .	
Table CA 8 2 2 2/03_7	Group R P-generation	Juvenile growth	survival and growth	_ d/a& 56 z	n fin
1 abic C/1 0.2.2.2/05-7	Group B, P-generation:	ouvenne grømen.	Sur viva and growth	, u ay 50 j	ry/

 Mean values ± standard 6 * Statistically signific 	cantly different to	the control, $p < 0.1$	05, Williams test, one-sided smaller growth: survival and growth, day 56 pt tration (μg a.s./L) 30.4 4.53.9 4.255
Table CA 8.2.2.2/03-7GEndpoint	roup B, P-gener	ation: Juvenile g	growth: survival and growth, day 56 pto the survival and growth, day 56 pto the survival and the survival an
•	Control	15.8	30.4 2 53.9 255 2
Number of replicates	4	3	$3 \sim 03 \sim 03 \sim 0$
Number of eggs introduced	120	91	
Survival between day	99.2 ± 1.7	96,8±5.6	95.6 ± 1.8 08.9 ± 1.9 198.9 £ 1.9 £°
28 and 56 pf (%)			
Length, day 56 pf (cm)	2.49 ± 0.10	(2.30 ± 0.10)	$2.25 = 0.09 * 2.39 \pm 0.05 * 2.00 \pm 0.54 *$
Pseudo specific growth	4.793 ± 0.22	4.540 ± 0.05	$4.54/5 \pm 0.47$ 4.746 ± 0.31 $3.900 \pm 0.27*$
rate (based on length)	,Ô ^v		$4.34/5 \pm 0.4\% = 4.746 \pm 0.31$ (3.900 ± 0.27*
Mean values \pm standard of	leviation are pres	ented	

pf Post fertilisation

Post fertilisation Statistically significantly different to the control, *p* 0.05, William Gest, one-side Gmaller

Table CA 8.2.2.2/03-8 Group B, Pgeneration: Reproduction

Endpoint	Mean-measure	d initial concent	ration (µg a.s./L)		
, K	Control 🔬	15.8	39.4	63.9	255
Number of replicates	4	L S		3	3
Time to regular spawning (days)	68±1°~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	Ĵ71 ±↓	73	69@⊭ 1	71 ± 0
spawning (days)	Š Š G.	N' L'			
Egg number per day	14 0 3	k5⊈2 ,≪″	∂5±3	13 ± 3	17 ± 4
and female	w n				
Fertilisation rate (%)	94.1 ±2,3 🏾 🏯	96.2 O.5	94. @ ± 0.6 °	95.2 ± 0.3	96.3 ± 1.6
Mean values ± standard	eviation are prese	ented.			

"" **V**au

Table CA 8.2.2.2/03-9	Group B	, Regeneration:	Termination	ı – sürvival, g	rowth, sex ratio and biomarker
é.Y	, 1				,

Endpoint 2	Diapoint (µg u.s. D)								
	Control 👡		30.4	63.9	255				
Number of replicates	4° \sim	ð íð íð	* 3	3	3				
Survival (%)	97.5±0.7	98.9 91.9	96.4 ± 6.2	100 ± 0.0	93.3 ± 6.7				
Length males (cm)	3.7 ±Q.04	3.7 0.08	3.7 ± 0.09	3.6 ± 0.09	$3.5 \pm 0.06*$				
Weight males (g)	0.426 ± 0.03	0.430 ± 0.03	0.425 ± 0.03	0.396 ± 0.03	0.408 ± 0.02				
Lerigth females (cm)		Q3.5 ± Q05	3.5 ± 0.05	$3.4 \pm 0.04*$	3.4 ± 0.15				
Weight females (g)	0.458 £0.03	0.418±0.01	0.425 ± 0.04	$0.383\pm0.01\texttt{*}$	$0.398 \pm 0.05*$				
Sex ratio (% females) 🔬 🔌	53.5 10.4	$41Q' \pm 8.3$	45.1 ± 10.8	$37.4 \pm 2.3*$	$19.2 \pm 8.1*$				
Sex ratio (% @ales)	465 ± 10.4	5.7 ± 8.3	54.9 ± 10.8	$62.6 \pm 2.3^{\#}$	$80.8 \pm 8.1^{\#}$				
Vitellogenmmales	0.02 ± €.01 ~ ≪	0.02 ± 0.00	0.02 ± 0.01	0.01 ± 0.00	0.01 ± 0.00				
$(ng/\mu g)$									
Vitelløgenin females	221 ± 67.0	180.7 ± 47.5	213.0 ± 6.3	171.0 ± 81.3	281.7 ± 35.0				
(ng/ng)	\sim								

Mean values \pm standard deviation are presented

Storstically significantly different to the control, p < 0.05, Williams test, one-sided smaller Statistically significantly different to the control, p < 0.05, Williams test, one-sided greater

#



Endpoint	Mean measure	ed initial concent	ration (µg a.s./L))	QÛ ^
-	Control	15.8	30.4	63.9	255
Number of replicates	4	3	3	3	3 5 0
Number of eggs introduced	200	150	152	153	
Survival, day 21 pf (%)	87.5 ± 1.0	80.7 ± 7.0	84.9 ± 6.0	77 <u>4</u> ± 5.2*	75.7 ± 2,5 %
Survival, day 28 pf (%)	86.5 ± 1.9	80.7 ± 7.0	84.2 ± 7.0	74.5 ± 3.8*	₩75.7 ±2.7*
Length, day 28 pf (cm)	0.99 ± 0.04	0.94 ± 0.06	99 ± 0.01	0.03 ± 0.06	1.07¥0.09
Group dry weight, day	63.3 ± 22.6	53.3 ± 10.9	50.6 ± 15.1	51.7 ± 20.0 0	$ \frac{1.07}{689} \pm 8.9^{\circ} $
28 pf (mg)			L.		
Single dry weight, day	1.5 ± 0.5	1.3 ± 0.2	1.2 ± 0.4 $\%$	1.4 ± 0.6	1.8 ± 0.3
28 pf (mg)				\$n° ~~ .0	
Mean values \pm standard of	deviation are pres	ented 🖉 🔊	· 0 /		
* Statistically signific	antly different to	the control, $p \leq 0$.)5, Williams fest,	one-sided smalle	r a
fable CA 8 2 2 2/03-11 G	roun C D gonor	otia Denadua		one sided smalle	

Table CA 8.2.2.2/03-10 Group B, F1-generation: Early life stage – hatch, survival and growth, day 28 pf

Table CA 8.2.2.2/03-11 Group C, P-generation: Reproduction

Mean values ± standard deviation are presented * Statistically significantly different to the control, p (105, Williams test, one sided smaller Table CA 8.2.2.2/03-11 Group C, P-generation: Reproduction							
Endpoint	Mean measured initial concentration (µg a SL)						
	Control 2 15x8 2 304 63.9 2 255						
Number of replicates							
Egg number per day and female	$11 \pm 2 \qquad \qquad$						
Fertilisation rate (%)	$95.1 \pm 2.2 \qquad 94.0 \pm 36 \qquad 95.6 \pm 0.8 \qquad 96.0 \neq 0.9 \qquad 96.5 \pm 0.5$						

Mean values ± standard deviation are presented Table CA 8.2.2.2/03-12 Group C, P-generation: Termination – survival, growth, sex ratio and biomarker

Endpoint	Mean measure	d initial concept	ration (µg a k/L)		
	Control 🐇	15.8	30.4	×63.9	255
Number of represes	4_0_0 98.4 ± 109	× 6		3	3
Survival (%)	98.4 ± 109	§95.6 🛫 🦻 🥱		95.6 ± 5.1	93.2 ± 6.9
Length makes (cm)	4.0 ± 0,09	⁹ 4.0 ± 0.04	4.4 年 0.06	4.1 ± 0.05	4.1 ± 0.10
Weight males (g)	0.499 ± 0.03	$0.5.17 \pm 0.01$	6.520 ± 0.02	0.539 ± 0.01	0.514 ± 0.02
Length females (cm)	4.0 ± 0.08	$\hat{\Phi}$ 1 ± 0,04 «	4.0 ± 0.05	4.0 ± 0.01	4.1 ± 0.05
Weight females (g)	₄0.623 ±00.06 √	0.659° 0.10 °	0.569 ± 0.03	0.608 ± 0.02	0.639 ± 0.05
Sex ratio (% females)	49.7 £15.4	37 A ± 6.5	389 ± 6.2	38.2 ± 9.4	41.2 ± 8.1
Sex ratio (% notes) Sex ratio	50 S ± 15 4 °	$606 \pm 6.0^{\circ}$	©1.1 ± 6.2	61.8 ± 9.4	58.8 ± 8.1
Vitellogenin males	40.92 ± 0.07		$= 0.04 \pm 0.03$	0.03 ± 0.02	0.03 ± 0.01
$(ng/\mu g)$					
Vitellogenin females	333 D± 65.9	2450 ± 23.7	343.7 ± 96.8	217.5 ± 15.9	311.4 ± 96.3
(ng/µg) ~~~~	1 ×				

Mean values ± standard de Galion are presented

Table CA 8.2.2,2403	3-13 Gr	oup C, F	1-generation:	Early	life stage –	- hatch, sur	vival and	growth, day	28 pf
Š	à	s í		•	0	,		0 / V	1

Endpoint &	Mean measure	ean measured initial concentration (μg a.s./L)						
	Control	15.8	30.4	63.9	255			
Number of replicates	4	3	3	3	3			
Number of eggs	4 0	152	151	151	152			
introduced of								
Survival day 21 pf (%)	92.9 ± 4.9	88.8 ± 4.6	82.8 ± 3.9	$45.6 \pm 9.7*$	$5.2 \pm 9.1*$			
Surviva, day 28 pf (%)	92.9 ± 4.9	88.8 ± 4.6	$82.8 \pm 3.9*$	$37.8 \pm 0.4*$	$5.2 \pm 9.1*$			
Length, day 28 pf (cm)	1.18 ± 0.08	1.17 ± 0.06	1.16 ± 0.08	1.23 ± 0.17	1.31 ¹			
Group dry weight, day	147 ± 30.7	139 ± 28.6	123 ± 32.2	$75.0 \pm 34.6*$	40.0* ¹			
28 pf (mg)								



Endpoint	Mean measu	Mean measured initial concentration (µg a.s./L)					
	Control	15.8	30.4	63.9	255		
Single dry weight, day	3.2 ± 0.9	3.1 ± 0.5	2.9 ± 0.7	3.9 ± 1.8	5.0 ¹	<u></u>	
28 pf (mg)						S.	
	1				(<u>h</u>	

Mean values \pm standard deviation are presented

Mean values \pm standard down * Statistically significantly different to the control, $p \sim \infty$ ¹ Surviving fish larvae could only be observed at replicate B at 255 µg/L. Thus, only one replicate \cdots A summary of the resulting endpoints, based on mean measured concentrations, is presented in the table below

Group A (introd	uced as	Group B (introd	nced as	Group Controd	weed as adults
fertilised eggs)		juveniles)		South Structure	
Life phase,	NOEC (µg/L)	Life phase, 🏷	NOEC (pg/L)	Life phase, 🖉	NÔÆC (µg/L)
Parameter		Parameter		Parameter	e q
Parental, early lij	fe stage	Parental juvenil	f growld 🔍	Reproduction	
Hatching	_1	Survival	≥253 °°	Bgg number/ L	$ \begin{array}{c} \begin{array}{c} \begin{array}{c} \begin{array}{c} \begin{array}{c} \end{array} \\ \end{array} $
Survival	30.4	Length 2	\$63.9	Fettilisation	\$255 \$
Length	63.9	Reproduction		Œilial,œarly li fe s	tage 🦘
Juvenile growth		Time to regular spawning	255 JU D	Surveval 4	€C ₁₀ : 23.3
Survival	≥255	Egg number/	≥2555° ×	Cength Weight	≥255
Length, growth	63.9	Fertilisation gate	@255 ° (v)	Termination par	ental adults
Reproduction		Filial early lifes	tage	Survival	≥255
Time to regular spawning	\$255 ° *	⁹ Survival	tager 30.4 & A 2 ~ ~ ~ ~ ~	Sex ratio	≥255
Egg number/		Dength, weight	≥2587 3° S ⁷	Length, weight	≥255
Fertilisation rate	≥255 € 5	Termination par	ental adults	Biomarker VTG	≥255
Filial, early life s		Survive 🖓 🔊	≥2,55 ≧%	-	-
Survival	20 .4 × S	Sausana X	36.4		
		Length, male	_≥255 ℃		
Termination, pare		length, female	≥255°		
Survival 🔊	≥265 ~	Weightymale >>	<u>≥255</u> ⊂		
Sex ratio	63.9 🔍 🔍	Weight, female	30 .4		
Length, werght	≥255¢ Q	Bi@markety	≪≱255		
Biontarker VTC					

Table CA 8.2.2.2/0-14	Summary of endpoints for	each
-----------------------	--------------------------	------

Due to the presence of sediment in the fry chambers, it was not possible to monitor hatching success. The statistical evaluation of length at day 50 revealed a significant difference to the control at $\geq 15.8 \ \mu g$ spiroxamme/L. However, the calculated difference of the single treatment levels were found to be <10% compared to control at 5.8, 30.4 and 63.9 $\mu g/L$, with 7.3%, 9.3% and 3.9% effect, respectively. Since variables the inclusion of length at the single treatment levels were found to be <10% compared to control at 5.8, 30.4 and 63.9 $\mu g/L$, with 7.3%, 9.3% and 3.9% effect, respectively. Since variables the inclusion of length at the single treatment levels were found to be <10% compared to control at 5.8, 30.4 and 63.9 $\mu g/L$, with 7.3% of the single treatment levels were found to be <10% compared to control at 5.8, 30.4 and 63.9 $\mu g/L$, with 7.3% of the single treatment levels were found to be <10% compared to control at 5.8, 30.4 and 63.9 $\mu g/L$, with 7.3% of the single treatment levels were found to be <10% compared to control at 5.8, 30.4 and 63.9 $\mu g/L$, with 7.3% of the single treatment levels were found to be <10% compared to control at 5.8 $\mu g/L$. 2 variation between the single eplicates was very low, the statistical test was able to detect a significant difference aready for a small decrease in length. Moreover, no clear dose response relationship of the fish Ength could be bserved within the tested concentration range. At 63.9 µg/L, only a minor deviation from the control was found (3.9%).

3 A slight but significant decrease of male lengths could be observed at 255 µg/L. However, this effect was less than 5%, compared to the control males, and thus can be considered to be not biologically relevant.



Group A (introduced as		Group B (intro	luced as	Group C (introduced as adults)		
fertilised eggs)		juveniles)			<i>•</i>	
Life phase,	NOEC (µg/L)	Life phase,	NOEC (µg/L)	Life phase,	NOEC (µg/s)	
Parameter		Parameter		Parameter	N G	

In the filial generation of the parental fish, introduced as adults, the decrease of survival was >50%(related to the number of introduced eggs) compared to control and the effects showed a clear dose-related response. An EC_{10} was therefore able to be calculated.

III. Conclusion

The survival of F₁ fish larvae was interpreted as the most sensitive population relevant endpoint.

The corresponding effect concentration (EC₁₀) was calculated to be \Im 3.3 µg a.s./I

Beside larval survival, the sex ratio after juvenile exposure as well as the growth of adult females showed similar sensitivity (NOEC: 30.4 µg a.s./L). A decrease of the concentration of the biomarker vitellogenin could be observed at \geq 30.4 µg a.s./L thereby giving a NOEC for this parameter of 15.8 µg a.s./L. The histopathological evaluation of the fish gonads revealed no substance-related effect

Assessment and conclusion by applicant

Validity criteria according to the study report were onet

- Survival rate in the controls was greater than 70%
- Dissolved oxygen concentration to be >60% saturation throughout the fest (actual: 82 to 125%) 0
- Water temperature was within 25 2°C throughout the study

The study report listed it's own validity criteria (see above) and these were considered to have been achieved. However, validity conteria according to the most op-to-date QECD 210 (2013) test guideline have also been assessed anothe following criteria were met: O

- Hatching ate in the control replicates was 200%
- Post-hatch survival in the control replicates was 295%
- Dissolved oxygen Oncentration to be 30% in all test vessels, throughout the test (actual: 82 125%)

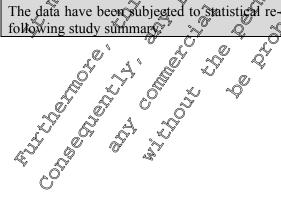
One of the criteria wefe not thet but this was a very minor deviation:

Water temperature to be at 26,±0.5°C during the test (actual: 24.0 – 26.9°C).

The study is therefore considered acceptable. It is acknowledged that this was a non-standard test design but the study was considered to have been successfully conducted in accordance with its aims. The use of this study in a defined risk assessment has been discussed in Document M-CP Section 10.

The lowest NOEC determined in the study was 15.8 µg a.s./L.

The data have been subjected to statistical re-evaluation and the results have been presented in the





Data Point:	KCA 8.2.2.2/05
Report Author:	
Report Year:	2020
Report Title:	Calculation of EC10 and EC20 values for Danio rerio with spiroxamine Tom a full life cycle test
Report No:	0471836-ECO18
Document No:	<u>M-760412-01-1</u>
Guideline(s) followed in study:	None
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted
GLP/Officially	No, not conducted under GLP/Officially recognised tearing facilities
recognised testing facilities:	
Acceptability/Reliability:	Yes the second s

Executive Summary

The report <u>M-467979-03-1</u> on the effects of spiroxamine TG in the zebratish (*Danio reso*) fulfilife cycle test did not provide estimates of EC_{10} or EC_{20} . Therefore, these values have been calculated in accordance with the Annex to Com. Beg. 283/2013. Due to the lack of effects above 10% when compared to the control, the EC₁₀ and EC₂₀ values for Group A Fa survival at 56 days post fertilization (dpf), length at 56 dpf, fertility, survival at termination, length at 28 dpf, Group B Fotength at 28 dpf, survival at 56 dpf, survival at females), F₁ length at 28 dpf, Group B Fotength at 28 dpf, survival at 56 dpf, survival at females), F₁ length at 28 dpf, Group C F₀ egg number per day and female), F₁ length at 28 dpf, Group C F₀ egg number per day and female), F₁ length at 28 dpf, Group C F₀ egg number per day and female), F₁ length at 28 dpf, Group C F₀ egg number per day and female), F₁ length at 28 dpf, Group C F₀ egg number per day and female), F₁ length at 28 dpf, Group C F₀ egg number per day and female), termination (males), weight at termination (males), F₁ length at 28 dpf, Group C F₀ egg number per day and female), termination (males), weight at termination (males), the remination (males), weight at termination (males), F₁ length at 28 dpf). Group C F₀ egg number per day and female, fertility, survival at termination (males), weight at termination (males), the remination (males), weight at termination (males), the remination (males), weight at termination (males), the remination (males), weight at termination (males), the remaining parameters, no reliable EC₁₀ or EC₂₀ values were possible to calculate.

I. Methods

The statistical evaluation was performed with Statistical software ToxRat Professional v3.3.0.

Group

The determination of reliable EC $_{0}$ and EC $_{20}$ values for F_{0} length after 28 days, pseudo specific growth rate, eggs per female per day, sex ratio and F survival at day 21 and day 28 and F₁ weight at day 28 was not possible as the obtained results do not to with the raw data due to the lack of a significant monotone dose response. Due to the lack of effects above 10% when compared to the control on F₀ survival after 56 days, factulity, survival at test remination, fongth after 28 days, the EC₁₀ and EC₂₀ values are estimated to be $\geq 255 \ \mu g \ a.s./L$. The determination of reliable EC₁₀ and EC₂₀ values for F₀ survival after 21 and 28 days was not possible due to a poor goodness of fit in the data.

Group B

Due to the lack of effects above 10% when compared to the control on F_0 length after 28 days, survival at 56 days, eggs per female per day, fertility, survival at termination, length of males and females at termination, weight of males at termination and F_1 total length at 28d, the EC₁₀ and EC₂₀ values are estimated to be 255 pg a.s. L. The determination of reliable EC₁₀ and EC₂₀ values for F_0 total length at 56 days, pseudo specific prowth rate, sex ratio based on male, weight of females at termination, F_1 survival after 21 and 28 days and weight at 28 days was not possible due to the lack of a significant monotone dose response. Due to the lack of effects below 10% on F_0 sex ratio based on female on all treatments, no reliable EC₁₀ value could be calculated inside the test concentration range. Therefore, no EC₁₀ is presented for F_0 sex ratio based on female.



Group C

Due to the lack of effects above 10% when compared to the control on F_0 eggs per female per ay, fertility, survival at termination, male length at termination, male and female weight at termination, F_1 weight at 28dpf, EC₁₀ and EC₂₀ values are estimated to be >255 µg a.s./L.

The determination of reliable EC_{10} and EC_{20} values for F0 sex ratio based on forhales and males, ferfule length at termination and F_1 total length at 28dpf was not possible due to the lack of a significant monotone dose response.

The determination of reliable EC_{10} and EC_{20} values for F survival after 21 and 28 days was not possible due to a poor goodness of fit in the data and the data scattering around the dose response \mathcal{A}

Q

II. Results

Due to the lack of effects above 10% when compared to the control, the EC_{10} and EC_{0} values for Group A F₀ survival at 56 dpf, length at 56 dpf, fertility, survival at termination, length at termination (males and females), b length at 28 dpf, Group B F₀ length at 28 dpf, survival at 56 dpf, egg number per day and female, fertility, survival at termination (males and females), weight at termination (males), F₁ fength at 28 dpf, Group C Fe egg number per day and female, fertility, survival at termination (males), weight at termination (males), weight at termination (males), F₁ fength at 28 dpf, Group C Fe egg number per day and female, fertility, survival at termination (males), weight at termination (males), F₁ fength at 28 dpf, Group C Fe egg number per day and female, fertility, survival at termination (males), weight at termination (males), F₁ fength at 28 dpf, Group C Fe egg number per day and female) and Female, fertility, survival at termination (males and female) are supported at termination (males), weight at termination (males and female) are supported at termination (males). For the remaining parameters, no reliable EC_{10} or EC_{20} values were possible to calculate.

A summary of the obtained endpoints is presented in the following able.

Table CA 8.2.2.2/05-1	Overall endpoin	ts of the	e stælisti	calme-	calcúla	tion of	the De	rerio _s study wi	th
	spirfxamin		Ű.			4 g	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	~~~``	

Spirexamme	
Parameter	Cindpoint (μg as./L) ECig(95% confidence intervals)
Group A A O S	
Parental larvae (Fp)	
Survival at 21 dry	al.d.*
Survival at 28 apr	n.d. Q
Length at 28 dpf	ng y O'
Survival at 56 dpf	
	>255 ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
	n.d &
Egg number per day and female	n. O 🏷
Egg number per day and female	255 2 2
Survival at terterination	>255
Sex ratio (males)	n.do O
Sex ratio (granales)	A.
Length ar termination (males)	\$255 ×
Length at termination (remailes)	>255
Weight at termination (males)	>2\$5
Waight at termination (familia)	\$255
Vitellogenin in males 1 0 0	Rn.a.
Vitalloganin in familiad	n.a.
F1 generation Survival at 21dpt Survival at 28dpf	
Survival at 21dpt	n.d.
Survival at 2860 f	n.d.
Length at 28 dpf	>255
Weight at 38 dpf	n.d.
Group B	
Parental larvae (F0)	
Length at 28 dpf	>255
Survival at 56 dpf	>255



Parameter	Endpoint (µg a.s./L)
r ar anneter	EC10 (95% confidence intervals)
Length at 56dpf	n.d.
Pseudo specific growth rate	n.d.
Egg number per day and female	>255
Fertility (fertilisation rate)	>255
Survival at termination	>255
Sex ratio (males)	n.d.
Sex ratio (females)	n.d. O A A A
Length at termination (males)	>255 🐨 🖉
Length at termination (females)	>255 4 0 5 6
Weight at termination (males)	
Weight at termination (females)	n.d. Q' Q Q Q
Vitellogenin in males	n.a. ' o 'O' 'Y Or N Y
Vitellogenin in females	
F1 generation	
Survival at 21 dpf	
Survival at 28 dpf	$n_{\rm e}$
Length at 28 dpf	§285 (· · · · · · · · · · · · · · · · · ·
Weight at 28 dpf	And The t
Group C	
Egg number per day and female	>255 8 2 2 8 4
Fertility (fertilisation rate)	≥255 <u> </u>
Survival at termination Sex ratio (males)	
Sex ratio (males)	
Sex ratio (females)	@d. ~~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~
Length at termination (males)	>255 5 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2
Length at termination (females)	n.d. 255 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2
Weight at termination (mates)	2255 2 2 2 2
Weight at termination (comales)	
Vitellogenin iomales V	n.a. Ø Ø Ø
Vitellogenin in females	ng 0 a
Survivação 21 upi	h.d.* O' * ` O
Survival at 28 dpf	n.d.* 0 *
Length at 28 dpf 3 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4	n. O h
Weight at 28 dpf	

Due to the lack of a concentration deserves when compared to the control, the calculation of EC_{10} and EC_{20} values for the parameters macked in the above tabless "n.d was not possible and therefore no EC10 or EC20 values were determined. The determination of reliable $\mathbb{B}C_{10}$ and $\mathbb{E}C_{20}$ values for parameters marked with a * was not possible fue to a poor groodness of fit in the data and the data scattering around the dose response. ** Due to variability within the data set and the observation of effects in all concentrations, there is uncertainty in the NOEC determined by the statistical software, Tox Rat Professional.

As vitellogenin concentration is non-apical entroint, this parameter was not deemed relevant for further analysis and therefore \mathcal{K}_x values were not determined for both male and female fish. **III.Conclusion** $\mathcal{K}_y = \mathcal{K}_y$

III. Conclusion 3° 4° 3° Due to the lack of effects above 10% when compared to the control, the EC₁₀ and EC₂₀ values for Group A F₀ survival at 56 dpf, length at 56 dpf, fertility, survival at termination, length at termination (males and temales), weight at termination (males and females), F1 length at 28 dpf, Group B F0 length at 28 dpf, survival at 56 dpf, egg number per day and female, fertility, survival at termination, length at termination (males and females), weight at termination (males), F_1 length at 28 dpf, Group C F_0 egg number per day and female, fertility, survival at termination, length at termination (males), weight at



termination (males and females) and F₁ weigh at 28 dpf, were estimated to be > 255 μ g a.s./L. For the remaining parameters, no reliable EC_{10} or EC_{20} values were possible to calculate.

Assessment and conclusion by applicant:

The statistical re-evaluation of the data did not allow for the determination of reliable EC_{10} and ECvalues for many of the parameters assessed. This was largely due to the absence of an adequate dose response or due to poor fit of the data. 5⁵⁷ G

The lowest endpoint determined remains the NOEC of (15.8 µg a.s./L based on effects on VTG female fish therefore this continues to be the critical endpoint determined for this study. The values determined in the re-evaluation work are considered to be fully valid

Bioconcentration in fish [&] CA 8.2.2.3

Data Point:	KCA 8 2 2 3/01
Report Author:	KCA 8.2.2.3/01
Report Year:	
Report Title:	KWG 4168 Bioconcentration in boregill-sunfish O
Report No:	BF-011 & & V V V V V V
Document No:	M-006499-01- M & & & & & & & & & & & & & & & & & &
Guideline(s) followed in	HAMELINK,
study:	Aquatic Toxicology and Hazard Evaluation, ASTON publication STP 634, 149 -
Deviations from current	None 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6
test guideline:	
Previous evaluation:	yes valuated and accepted Y O S Y
Previous evaluation:	DĂR (1997), RAR (2010), RAR (2017)
GLP/Officially	Yes, conducted under GLP (Officially ecognised testing facilities
recognised testing	
GLP/Officially recognised testing facilities:	
Acceptability/Reliability:	Yes A A A A A A A A A A A A A A A A A A A
ý)	

Spiroxamine has a Log Pow of 2.79 and 2.98 at pH 7 for diastomers A and B, respectively but at pH 9 these value are 4.88 and 5.08, respectively A fish bioconcentration study with spiroxamine has therefore 0 been conducted and has been summarised below

The Log Pow of sparoragene-desethyl M01) is 2.41, 1.97 and >3.64 at pH 4, 7 and 9, respectively. The Log Pow of spinoxamine-despropyl. (1402) in 1.95, 1041 and 3.44 at pH 4, 7 and 9, respectively. The Log Pow of spiroxamine-N-oxide (1003) is 2.82, 2.59 and 2.63 at pH 4, 7 and 9, respectively. The Log Pow of spiroxamine-carboxylic acid (1066) is 9.45, 9.25 and 0.10 at pH 4, 7 and 9, respectively. Thus, an assessment of the potential risk from bioconcentration of spiroxamine-desethyl (M01) and spiroxaminedespropyl (M02) also needs to be addressed in the risk assessment.

Executive Summary

The objective of the bioconcentration study was to measure uptake and depuration of $[^{14}C]$ -KWG 4168 in bluegill surfish by determining, if possible, its uptake rate constant (K_1) , depuration rate constant (K_2) and steady state proconcentration factor (BCF).

KWG 4768 was accumulated by bluegill sunfish with a maximum total residue bioconcentration factor of 87 for whole fish Kinetic modelling yielded BCF values of 31 and 24 (edible parts) for the 20 µg [¹⁴G-KWG4168/L and 200 µg [¹⁴C]-KWG 4168/L groups, respectively and BCF values of 87 and 71 (whole f(h)) for the 20 µg [¹⁴C]-KWG 4168/L and 200 µg [¹⁴C]-KWG 4168/L groups, respectively.

The kinetic BCF values for edible parts and whole fish corresponded well with the respective average steady-state (days 7 - 28) bioconcentration factors of 25.8 (edible parts) and 86.2 (whole fish) for 20 µg



[¹⁴C]-KWG 4168/L and of 22.3 (edible parts) and 64.6 (whole fish) for 200 μ g [¹⁴C]-KWG 4168/L, respectively.

When exposure ceases, the residues are depurated very quickly with a half-life of approximately 13 to 19 hours (0.55 to 0.78 days, respectively). э. Д

I. **Materials and Methods**

٨ Matariala

A. Materiais	
Test Material	KWG 4168
Lot/Batch #:	Lager Nr. 8686/B
Radiochemical purity:	>99%
Description:	Clear, early liquid
Stability of test compound:	KWG 4168 Lager Nr. 8686/B >99% Clear, early liquid Not reported Not reported 20 and 200 ttg a.s./L Triethyleneglycet Exposure phase days: 0, 1, 3, 7, 10, 14, 21, and 28 Depuration phase days: 29, 31, 35, 38 and 42 Bluegill sumfish (<i>Lepomis Glacrochirus</i>)
Reanalysis/Expiry date:	Not reported to a star of the
Density:	Not reported
Treatments	
Test rates:	20 and 200 0 a.s./K
Solvent/vehicle: 😽	Triethyleneglycol
Analysis of test 🖉 🖉	Exposure phase days 0, 1, 3, 7, 10, 14, 24, and 28,
concentration	Deparation phase days: 29, 31, 35, 38 and 42
Test organisms	
concentrationer Test organismer Species:	Bluegill summish (Lepomis macroshirus)
Source:	
Acclimatisation	6-hour day high photoperiod and observed for at least 14 days prior
Acclimatisation	to testing. No mortality was noted 14 days prior to the test initiation
period:	and all unsuitable fish (<i>i.e.</i> , injured, deformed, <i>etc.</i>) were eliminated from inclusion in the test prior to assignment to test groups During the acclimation and test periods, the fish received once daily
	During the acclimation and test periods, the fish received once daily
	a Winitian a standard fish food (Vronon ED 50E monufactured by
A STA	Theinkrone, D-4230 Wesel as batch no. WAJ 1). The feed was analysed for unwanted contaminants
Treatment for	
disease:	Not reperted
Test design	
Testvessel	000 litre test aquaria
Feeding: Treatment for disease: Test design Test vessel Test water: Replication:	Aerated reconstituted water
Replocation:	Single
No. of	
animals/vessel:	56



28 days exposure and 13 days depuration phase

conditions	
Temperature:	21 – 23°C
Dissolved oxygen:	82 - 118%
pH:	6.7 – 7.5
Photoperiod:	16-hour daylight photoperiod

B. Study Design

Duration of test:

Environmental test

The objective of the bioconcentration study was to measure uptake and pepuration of C-KWG 4/68 by determining, if possible, its uptake rate constant (K1), deputation rate constant (K2) and steady state K. bioconcentration factor (BCF).

The uptake phase was initiated by transferring groups of 5 grandowly selected and prevously acclimated fish to each of the control and test chambers. The initial loading was 29 g fish/L and 0.48 g fish/D day (calculated from the mean bodyweights of sampled, ashes over the whole, exposure period). The fish were observed initially and every 24 hours on working days thereafter during the exposure period of 28 days for mortality and/or adverse behaviour. At the same intervals pH, temperature and dissolved oxygen were measured in all aquaria. Additionally the daily temperature fluctuation was controlled continuously in the control tark by a mercury-minimum-maximum-thermometer for detection of technical defects.

On day 28 of the exposure period, the addition of the [14C]-KWG #168 test material ceased. At the beginning of the depuration phase, the aquaria were deaned mechanically, emptied by suction to a water height of ca. 5 cm, and filled with uncontaminated and temperated (22%C) different water. During that procedure the fish remained in the aquara. The fish were then exposed to flowing uncontaminated diluent water for by day The chemical and physical water parameters during depuration phase were recorded as described for the uptake phase.

Fish were sampled dofing the uptake phase on days 0, 1, 5, 7, 10, 14, 2, and 28 and during the depuration phase on days 29, 31, 35, 38 and 42. On each occasion, four thish from each chamber were collected and processed individually. The first were dissected into edible, (body, muscle, skin, skeleton) and viscera / nonedible (head, fins, internal organs). Samples were transferred into weighed polystyrene vials suitable for further handling. After determining the weight of the samples they were frozen, lyophilised, reweighed, and homogenised.

On each sampling day, three samples of m of water were removed from each aquarium. The concentrations of ¹⁴C calculated as [¹⁴C KW 4168 m water were calculated by liquid scintillation counting of triplicate 7 mL samples pipetted directly from each control and test tank. To each sample 7 mL scinffilation cocktar (United Technologies Packard Instant Scint. Gel) were added.

The term uptake rate constant (K) as used in this report is the mathematically determined value that is used to define the uptake of test material by exposed test organisms. The depuration rate constant (K₂) is defined as the mathematically determined value of the depuration of test material from previously exposed test animaly when placed in untgated dilution water. The steady-state bioconcentration factor (BCF) is the ratio of the less substance concentration in the whole fish (C_F) and the concentration in the test water (Cw) of steady-state (apparent plateau) or the ratio of K_1 and K_2 .

Results and Discussion

The stude was conducted to the HAMELINK, J.L., "Current Bioconcentration Test Methods and Theory In: Aquatic Toxicology and Hazard Evaluation, ASTM publication STP 634, 149 -161, 1977. Validity criteria under the guideline used at the time that this test was conducted are not available



therefore the study has been assessed against the criteria in the current OECD 305 test guideline (2012) which have all been met: Q_{μ}°

- The water temperature variation is less than $\pm 2^{\circ}$ C, because large deviations can affect biological parameters relevant for uptake and depuration as well as cause stress to paimals
- The concentration of dissolved oxygen does not fall below 60% saturation
- The concentration of the test substance in the chambers is maintained within $\pm 20\%$ of the mean measured values during the uptake phase
- The concentration of the test substance is below its limit of solubility in water taking into account the effect that the test water may have on effective solubility (Actual: yes)
- The mortality or other adverse effects/disease in both control and treated fishes less than 10% at the end of the test; where the test is extended over several week or months, death or other adverse effects in both sets of fish should be test that 5% per month and not exceed 30% in all. Significant differences in average growth between the test and the control groups of sampled fish could be an indication of a toxic effect of the test chemical.

A control analysis of stock-solutions of taining 211 μ g as L and 1666 μ g a.s/L showed that KWG 4168 was stable over a period of 4 weeks in triethylonegicol which was used as solved. Therefore, the stability of the ¹⁴C-labelled test substance in the stock solutions can be assumed.

Water concentrations ranged through 28 days of the bioconcentration (uptake) phase from 17.7 μ g a.s./L to 20.9 μ g a.s./L for the nominal concentration of 20 μ g a.s./L and from 181.3 μ g a.s./L to 196.7 μ g a.s./L for the nominal concentration of 209 μ g a.g./L. The average water concentration (using the mean value for each sample) during the uptake phase was 18.9 (± ± 0) μ g a.s./L for the nominal concentration of 20 μ g a.s./L for the nominal concentration of 20 μ g a.s./L for the nominal concentration of 20 μ g a.s./L for the nominal concentration of 20 μ g a.s./L for the nominal concentration of 20 μ g a.s./L for the nominal concentration of 20 μ g a.s./L for the nominal concentration of 20 μ g a.s./L for the nominal concentration of 20 μ g a.s./L for the nominal concentration of 20 μ g a.s./L and 186.9 (± 5.7) μ g a.s./D for the nominal concentration of 200 μ g a.s./L. These concentrations compared well with the expected nominal concentrations of 20 μ g a.s./L and 200 μ g a.s./L for [¹⁴C]-KWG 4168.

The specific radioactivity of $[{}^{14}C]$ -KWG 4468 was 5416.8 dpm ug test substance for the 20 µg a.s./L level and 548.4 dpm ug test substance for the 200 µg a.s./L level.

	Control group 2000g test substance / L 200 µg test substance / L										
Sampling day	dpm 7 ml	in wate sample	per ,	dpm in mL sai	water p	S.	, μg a.s./L water	dpm in mL sar	water p nple	er 7	μg a.s./L water
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	1	20	3	<b>4</b> 0 ⁹	50	Č)	Mean	7	8	9	Mean
Exposure	Exposure										
Exposure A	1.4~		20.5	676.2		670.7	17.73	761.7	774.2	732.8	196.70
1	-2.0	-3,6	-4,5	726,4	697.1	687.6	18.65	684.9	691.2	709.0	181.97
3	-2.8	67"	-4,8	799.5	∕¶73.4	711.6	19.13	707.9	693.3	729.7	185.15
7	\$3.2	-4.5	¶.4 (	726.3	739.7	716.8	19.19	731.5	703.8	734.0	188.36
10	-2.0 <	-3.40	^{\$} -4.5	704.Q	734.7	716.6	19.04	752.3	735.4	734.9	193.85
14 O	40	-18	-0.5	702.0	690.8	672.7	18.21	698.6	699.9	683.3	181.28
14 O 21	3.3	Ø.8	<b>¥6</b> .9	898.4	706.0	683.6	18.31	726.2	693.3	673.8	183.07
28 0 0	0.4		¥.5	803.4	782.9	796.0	20.88	719.7	722.1	694.7	184.87
Deputation 29	A	Ş									
29 🖉 🦉	15	14	-1.0	43.5	21.9	22.6	<1	22.9	8.0	5.7	12.2
	-1.5	\$2.6	-3.6	-0.1	13.4	-4.6	<1	-3.7	2.7	0.3	<1
35 38	-1.4	-4.6	-2.9	6.7	-3.7	-2.9	<1	-1.6	-0.4	-1.3	<1
38	-2.3	-4.0	-2.9	-3.8	-2.8	3.5	<1	-5.0	-1.5	-3.6	<1
42	-4.5	-4.7	-4.5	0.0	-3.7	7.3	<1	0.6	-0.2	-3.2	<1

### Table CA 8.2.2.3/01-1 Measured concentrations of KWG 4168 in test medium



10.1 5.2 5.1 3.2 4.5 3.5	10.0     3.9       3.0     0       4.1     0       2.9     0
5.1 3.2 4.5 3.5	3.0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
3.2 4.5 3.5	4.1 0 ⁴ · · · · · · · · · · · · · · · · · · ·
4.5 3.5	
3.5	A.3 2 2 2
( )	
3.9	
5.4	S 5.7
6.6	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
5.8	A A. A A
9.4 @	7.5 4 0 0
74 ° 5	4,70 0 2
18 O V	
y in whole fish (ppm/g fresh wei	ight) A A A A A A A A A A A A A A A A A A A
	$ \begin{array}{c} 3.9 \\ 5.4 \\ 6.6 \\ 5.8 \\ 9.4 \\ 7.4 \\ 7.4 \\ 3.8 \\ 9.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 \\ 7.4 $

Table CA 8.2.2.3/01-2 Fresh weight of whole fish (edible parts and viscera) (g)

Table CA 8.2.2.3/01-3	Radioactivity in	whole fish	ì*(dpm	/g fresh	weight).

Sampling day	Control mean         20 µg/L mean         200 µg/L mean           15.6         7700.5         6101.6
1	15.6 7700.50 9 9101 6 9 9.6 911767?3 82762 5
3	
7	40.2 % Ø 8842.3 Ø Å S 9 8904.7 Ø %
10	38.7 Q 5 6460.7 7 Q 4102.3 4
14	88.2i 39175,4 5463,1 O
21	280.9 280.9 11784.0 0 499.5 0
28	105.8 ~ 7,59.1 ~ ~ ~ 5,59.3.7 ~
29	$62.8$ , $62.8$ , $6208.5$ , $0^{\circ}$ ( $4575.10^{\circ}$
31	910 0 V 14400 V 0 V7643V
35	196.0 1 288.1
38	5121.5 0 7 889.1 7 9 7.3
42	89.1°°°°°°°°°°°°°°°°°°°°°°°°°°°°°°°°°°°°
×	

Table CA 8.2.2.3/01-4 Bioconcentration factors for whole fish (calculated from edible part and viscera)

	×.		
Sampling day	<u>گ</u> ] 2	20 jpg/L mean	200 μg/L mean
1		1840 4 1 A 1	\$%I.8
5	9″ 1	17.3 2 2 2	#82.5
7	J 8		87.6
10		53.20 E A A	<b>40.0</b>
14 🔊	0 8		53.2
21	Ď		61.4
28	<del>ک</del> (7		80.5
29	<u>مَ</u>		44.1
31 🔊	J A	<b>3</b> .2 0 0 27	6.6
35 🏷	\$ ⁹	9.8 ° ° °	1.8
38	7	1.5 Å V Å	1.9
42	A Z		1.2

Table CA \$2.2.3/407-5 Uptake and depuration of radioactivity, whole fish (calculated from edible part and viscera / all values related to steady-state mean values)

Sampling 20 µg/L mean 200 µg/L mean day					% Radioactivity relative to dry weight				
Sampling 20 µg/L mean		200 μg/L mean		20 μg/L mean		200 μg/L mean			
uay	~0¥	Uptake	Depurated	Uptake	Depurated	Uptake	Depurated	Uptake	Depurated
1	0	65.4	-	68.7	-	64.7	-	66.9	-
3		100.0	-	93.2	-	98.5	-	93.3	-
7		74.9	-	100.0	-	73.3	-	100.0	-



C	% Radio	oactivity relat	ive to fres	h weight	% Radioactivity relative to dry weight				
Sampling	20 μg/L mean		200 μg/L mean		20 µg/L r	nean	200 μg/L mean		
day	Uptake	Depurated	Uptake	Depurated	Uptake	Depurated	Uptake	Depurated	
10	54.6	-	45.8	-	52.3	-	44.4	- 🏹 🕯	
14	77.3	-	60.6	-	75.6	-	₫9.2	- 0 6	
21	97.8	-	70.0	-	100.0	-	70.4	-4 . 4	
28	66.8	-	92.9	-	67.1	- 4	94.2		
29	-	47.7	-	49.1	-	49.1	- °C	51.4Q x	
31	-	88.5	-	92.4	Ô	92.4	- 🔊	928 S	
35	-	91.4	-	97.9	<u> V</u>	97.5	- 0	SP8.2 🔬	
38	-	93.5	-	97.8 🗸	-	<u>9</u> 0.8	-%	97.8 🛇 🔇	
42	-	93.5	-	98.6	- 4	98.6		98.7	

		05	· 🗸 🛛	// 1	$\backslash \bigcirc$		w.
Table CA 9 2 2/01 C	Cummon of dominad	walnes grow the	I in atis ma Stal	ling (maing	DIAT	( M) (	$\sim 0$
Table CA 8.2.2.3/01-6	Summary of derived	values from the	: KINCERC INOCIEN		ØΓUΓ A	▲≪ /∠)) °	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
				8 W P 8	0		2 2

Parameter	20 µg [ ¹⁴ C]-KWG 41		200 prg [14C)-KWG	4168/L
rarameter	Edible parts	Whole fish	Edible parts	Whole fish 🗸
Bioconcentration factor (BCF)	31 (± 9.2)		$24^{v}(\pm 10)$	71 (± 23)
Time to reach 90% of steady state (days)				2.64 0.59)
$t(\frac{1}{2})$ for clearance (days)		0.55 = 0.05		0.78 (± 0.18)
Uptake rate constant K1 (1/day)	34 (± 7.5) (c)	(±6)	15 (± 4 A)	64 (± 15)
Clearance rate constant $K_2$ (1/day)			0.6 (± 0.18)	* 0.9 (± 0.20)

The BIOFAC calculated BCF values for edible parts and whole fish corresponded well with the respective average stead state mays 7-28) bioconcentration factors of 25.8 (edible parts) and 86.2 (whole fish) for 20  $\mu$ g [^AC]-KWG 4468/L and of 22.3 (edible parts) and 64.6 (whole fish) for 200  $\mu$ g [¹⁴C]-KWG 408/L, respectively.

In viscerathe following data were calculated:

BCF: 165 (± 48); Time to reach 90% of steady state: 26 (± 0.52) days, t(1/2) for

Clearance:  $0.78 (\pm 0.160 \text{ days})$  ptake Rate Constant (K1) A7 (± 30) 1/days,

Clearance Rate Constant ( $(\Omega)$ ): 0.89 ( $\pm 0.78$ ) 1/days.

- 200 ug 14q-KWG 4168/1 (nominal):

BCF 122 ( $\pm$  33); Time to reach 90% of steady state: 2.5* ( $\pm$  0.46) days, t(1/2) for

Clearance: 0.75  $\neq 0.14$ ) days Uptak Rate Constant (K1): 112 (± 23) 1/days,

Clearance Rate Constant (12):  $0.92' (\pm 0.07)$  1/days.

After 14 days in uncontaminated water for 20 ug [14C]-KWG 4168/1 (nominal) 92, 94 and 94 percent of the mean plateau radioactivity was depurated from edible portions, nonedible portions and whole fish, while for 200 ug [14C]-KWG 4168/1 (nominal) these were 99, 99 and 99 percent of the mean measured plateau radioactivity.

III, O Conclusion

The objective of the bioconcentration study was to measure uptake and depuration of  $[^{14}C]$ -KWG 4168 in bluegill sunfish by determining, if possible, its uptake rate constant (K₁), depuration rate constant (K₂) and steady-state bioconcentration factor (BCF).



KWG 4168 was accumulated by bluegill sunfish with a maximum total residue bioconcentration factor of 87 for whole fish. Kinetic modelling yielded BCF values of 31 and 24 (edible parts) for the 20  $\mu$ g [¹⁴C]-KWG 4168/L and 200  $\mu$ g [¹⁴C]-KWG 4168/L groups, respectively and BCF values of 87 and 71 (whole fish) for the 20  $\mu$ g [¹⁴C]-KWG 4168/L and 200  $\mu$ g [¹⁴C]-KWG 4168/L groups, respectively.

The kinetic BCF values for edible parts and whole fish corresponded well with the respective average steady-state (days 7 - 28) bioconcentration factors of 25.8 (edible parts) and 86.2 (whole fish) for  $20 \ \mu g$  [¹⁴C]-KWG 4168/L and of 22.3 (edible parts) and 64.6 (whole fish) for  $200 \ \mu g$  [¹⁴C]-KWG 4168/L and of 22.3 (edible parts) and 64.6 (whole fish) for  $200 \ \mu g$  [¹⁴C]-KWG 4168/L and of 22.3 (edible parts) and 64.6 (whole fish) for  $200 \ \mu g$  [¹⁴C]-KWG 4168/L and of 22.3 (edible parts) and 64.6 (whole fish) for  $200 \ \mu g$  [¹⁴C]-KWG 4168/L and of 22.3 (edible parts) and 64.6 (whole fish) for  $200 \ \mu g$  [¹⁴C]-KWG 4168/L and of 22.3 (edible parts) and 64.6 (whole fish) for  $200 \ \mu g$  [¹⁴C]-KWG 4168/L and  $200 \ \mu g$ 

When exposure ceases, the residues are depurated very quickly with half-life of approximately 13 to (19 hours (0.55 to 0.78 days, respectively).

#### Assessment and conclusion by applicant:

The study was conducted in 1995 to an ASTM test gaideline. The current version of the OECD 305 test guideline requires that BCF values be reported which have been corrected to a kpid content of 5%. At the time of study conduct, measurement of fish fipid content was not a requirement and was not therefore conducted as part of this test. Thus, lipid data are not available to be able to calculate BCF values which have been standard sed to a 5% lipid content. Under the current guideline, whet is BCF values can also be corrected for growth dilution and expressed in addition to the standard kinetic BCF values, however, growth corrected kinetic BCF values have also not been determined.

The study was conducted to the HAMELINK, J.L., "Cuffent Borconcentration Test Methods and Theory" in: Aquatic Toxicology and Hazard Evaluation, ASTM publication STP 634, 949 -161, 1977.

Validity criteria according to the Current test guideline OECD 305 (2012) were met.

- The water temperature variation is less than  $\pm 2^{\circ}$ C, because large deviations can affect biological parameters relevant for uptake and depuration as well as tause stress to animals
- The concentration of displiced oxygen does not fall below 60% saturation
- The concentration of the test substance in the chambers is maintained within ± 20% of the mean measured values during the uptake phase
- The concentration of the test substance is befow its limit of solubility in water, taking into account the effect that the test water may have on effective solubility (Actual: yes)
- The mortality or other adverse effects/disease in both control and treated fish is less than 10% at the end of the test, where the test is extended over several weeks or months, death or other adverse effects in both sets of fish should be ess than 5% per month and not exceed 30% in all. Significant differences in average growth between the test and the control groups of sampled fish could be an indication of a toxic effect of the test chemical

Taking the above points into consideration, the bioconcentration study is still considered to be acceptable on the basis that it was conducted to a recognised test guideline at the time of conduct. The data are considered to be adequate to demonstrate the low potential of spiroxamine to bioaccumulate in aquatic organisms with a kinetic BCF value of only 87. Furthermore, to conduct a new bioconcentration study would be considered to be unnecessary vertebrate testing therefore it is considered acceptable to use the available that to confirm the low bioconcentration potential and short clearance time of spiroxamine.

The study is therefore considered acceptable.

A BCE value of 87 has been taken for the risk assessment.



Data Point:	KCA 8.2.2.3/02
Report Author:	
Report Year:	1997
Report Title:	[Cyclohexyl-1-14C] KWG 4168: Metabolism in the edible parts of bluegill sunfish
Report No:	PF4215
Document No:	<u>M-006169-01-1</u>
Guideline(s) followed in study:	US EPA 165-4 Pesticide Accumulation in Fish
Deviations from current test guideline:	Not conducted to a specific guideline
Previous evaluation:	yes, evaluated and classified DAR (1997), RAR (2010) RAR (2017) Validation not possible (The study as such is acceptable considering the respective EPA test method. However, the circal test is not designed to determine a BCF.)
GLP/Officially recognised testing	Yes, conducted under GLA Officially recognised testing facilities
facilities:	
Acceptability/Reliability:	Supportive ally the second sec

#### **Executive Summary**

The purpose of the study was togain information on the native of the residue in the edible parts of the fish and to quantify the metabolites to the extent possible. "

A 42-day study was conducted to evaluate the Goconcentration of [Syclopexyl-1, C] KWG 4168 by bluegill sunfish (Lepomis macrochirus)." Mean water concentrations of 20 and 200 µg KWG 4168/L, respectively, were maintained for a 28-day exposure period.

#### I. Study Design

A 42-day study was conducted to evaluate the bioconcentration of [cyclohexyl-1-14C] KWG 4168 by bluegill sunfish (Leponis macrochaus). Mean water concentrations of 20 and 200 µg KWG 4168/L, respectively, were maintained for a 28-day exposure period. Please refer to M-006479-01-1 for a full summary of the test.

#### Extraction and sample processing

Ô A Ľ For the high dose group the combined fish samples (2802 g) were given to a suction filter. After addition of the respective extraction solution for sample was homogenised for three minutes using a Polytron homogeniser. Then the solution was filtered by applying a controlled vacuum. The volumes of the filtrates were determined and their radioactivity content was measured by liquid scintillation counting of aliquots. The extraction respine was dried under vacuum and the radioactivity determined after combustion. The further purification procedure was conducted by liquid partition and elution via a hydrophobic polyarophatic resin (Amberline XAD-4, Sigma).

For the low dose group the copusined sample of the low dose group (33.34 g) were extracted in close analogy to the procedure described for the digh dose group (see above). However, some of the liquid partition stepOwere onittee since they proved to be unnecessary.

#### Measurement of radioactivity

Liquid samples were readioassayed by the following liquid scintillation counters; Beckman LS 6000 LL and 15 650 Quench correction using the "H-number" Philips PW 4700, Quench correction using the "ESCR-number" LKB Rack Beta 1219 Spectral, Quench correction using the "SQP(E)-number".

Solid samples like extraction residues were weighed and combusted in an oxygen atmosphere using a Packard Instruments oxidiser.



#### II. **Results and Discussion**

The concentration of the total radioactivity in the edible parts of the fish after administration of 20 or 200 µg/L [¹⁴C] KWG 4168 is given in the report on the bioconcentration and also summarised in the table below. Since the overan concentration period (*i.e.* from day 1 to uay 42) for the bioconcentration and the 14-day depuration period (*i.e.* from day 1 to uay 42) for the two dose groups to gain as much material as possible for the metabolite identification. The metabolism was only investigated in the edible parts of the fish. The mean fissue residues at steady state follows: table below. Since the overall concentration is very low, all available samples from the 28-day

	(mean values corre	cted for outliers)	Q' 6° Å	°, °°, , °
	Dose level 20 µg/L	Q.	Dose level 200 µg/L	
Time after dosing		Equivalent 🖉 🔬		
[days]	DPM/g dried fish	concentration 📈	DPN/g drigd fish	
				[HE] 7 4
1	10215	KJ.89 ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	5139 A 6	<b>HQYS</b> 0.95 ↓ 1.52 ↓ ▲
3	18702	¥3.45 9 0 4	8250 2	₩1.52 KU KE
7	13293 Q	2,45	17223 8	3.18
10	7657	AT N N	17223 2 2 4535 2 5	0.84 .
14	9699 Q	1.79	37631	A.41 %
21	14932	2.76 2	7893 ~ ~	1.46
28	7984 🖓 😽	1,47 0 4	7807 B	1.4
29	7045 🔍 🌾	030 Sy m	4819	0.89
31	2142Q O	0.40	725	0.13
35	1515 🔊	0.28	603 🖤 🔊	0.11
38	1355 5	0.25 2 3	545 & 20	0.10
42	Ø97	Q.18 3 3	418 0 4	0.08

Table CA 8.2.2.3/02-1	[ ¹⁴ C]KWG 4168: Radioactivity concentra	tion in the edible	parts of the	fish samples
	[ ¹⁴ C]KWG 4168: Radioactivity concentra (mean values corrected for outpers)			

Table CA 8.2.2.3/02-2 [NC]KWG 4168: Extraction yields of the total radioactivity from the edible tissues and percentage of the radioactivity subjected to chromatographic analysis

0			0	
Dose [µg/I	Æxtr	action rate [%] 🔗		% of organ radioactivity subjected
		ý v		6 HPLC
20	<u>م</u> ر م م 84.20	) <u>``</u> `		80.02
200	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	5 Ö 4 K	A I	86.39
	S i i i		~	

Table CA 8.2.2.3/02-3 JC KVG 4168 Relative distribution and concentration (µg a.s. equiv/g) of the dentified metabolites in the edible parts of the high dosed Bluegill sunfish

Metaboline	% of HPLO	Sámple . KNO 2493B	% of edible tissue	Concentration (ppm)
KN@01634A 🔬	715.1 2 0	U13.5 X	18.4	0.83
EC₩ 8046/ECW 80511	16.8 0	265	15.4	0.70
KWG 4168	28.8 × ×	-~	22.2	1.00
Totally identified	60.7	^{100.0}	56.0	2.53

Table CA8.2.2.302-4  $[^{14}C]$ KyG 4168: Relative distribution and concentration (µg a.s. equiv/g) of the distribution and concentration (µg a.g. equiv/g) of the distribution and concentration (µg a.g. equiv/g) of the distribution (µg a.g. equiv/g) of

Metabolites	% of HPLC	% of edible tissue	Conc. (ppm)
	Sample KNO 2404A		
KNO 22302	6.8	5.4	0.031
ECW 8046/ECW 80511	15.5	12.4	0.072
KWG 4168	10.5	8.4	0.049



Metabolites	% of HPLC	% of edible tissue	Conc. (ppm)
Totally identified	32.8	26.2	0.152
III. Conclusion			

#### III. Conclusion

A 42-day study was conducted to evaluate the bioconcentration of [cyclohexy@1-14C] KWG@4168 by bluegill sunfish (Lepomis macrochirus). Mean water concentrations of 20 and 200 µg KWG 4169L. respectively, were maintained for a 28-day exposure period. The purpose of the experiments reported herewith was to gain information on the nature of the residue in the edible parts of the fish and to quantify the metabolites to the extent possible.

The mean tissue residues at steady state were as follows:

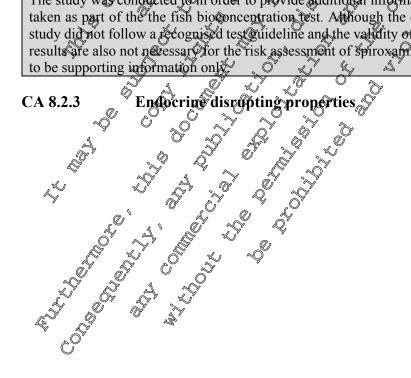
	20 μg [ ¹⁴ C] Κ ₄ G 41	68/L	_200 μg,[ ^Ω C] Ι	<b>KWĞ 416</b>	\$/L _©
Edible parts:	0.58 mg/kg 0		4.52 ng/kg (	<u>~</u> .~	
Non edible parts:	3.12 mg/kg		22.8 mg/kg	. \$	Ž,
Whole fish:	1.64 mg/kg		134 mg/kg	Ň	

The radioactivity was extracted almost completely from the edible tissues with mixtures of acetonityle and tetrahydrofurane. After purification, Interabolites besides the unchanged parent compound were identified. К 1

Metabolite     % of total radioactive residue       Higt dose     Low dose       KNO 22302 (alcohol-sulphate)     28.4       KNO 1634A (glucuronide)     48.4       ECW 80511/8046 (Carboxylic acid)     15.4       KWG 4168     220
High dose     Low dose       KNO 22302 (alcohol-sulphate)
KNO 1634A (glucuronide)     Image: State of the state of
ECW 80511/8046 (Carboxylic and) 15.4
ECW 80511/8046 (Carboxync aeid) 15.4
KWG 4168 22, 22, 22, 27 7 7 8.4, 2
Identification rate
Extraction rate $31.4$ $91.4$ $84.2$ $84.2$

#### Assessment and conclusion by applicants

The study was conducted toin order to provide additional information on the residues of the samples taken as part of the the fish bioconcentration test. Apprough the data are considered to be valid, the study did not follow a recognised test guideline and the valuatity of the results cannot be assessed. The results are also not necessar for the risk assessment of spiroxamine. As such the study is considered





Data Point:	KCA 8.2.3/01
Report Author:	
Report Year:	2008
Report Title:	Spiroxamine - fish screening assay (FSA) with fathead minnow
Report No:	EBKWX094
Document No:	<u>M-304833-01-1</u>
Guideline(s) followed in study:	OECD FSA test protocol (ENV/JM/TG/EDTA (2004) 1 REV2), including and standard operating procedures and guidance documents
Deviations from current test guideline:	Yes OECD 229 (2012) Fish were older than recommended (32 weeks instead of 20+2) The mean measured concentrations ranged from 29 to 84 percent of nominal for all test levels Each experimental unit was composed of four female and two male minimows instead of five of each however four experimental units were used instead of tw
Previous evaluation:	yes, evaluated and accepted RAR (2010), RAR (2017)
GLP/Officially	Yes, conducted under GLP/Officially recognised testing facilities
recognised testing	
facilities:	
Acceptability/Reliability:	Yes y 'o' 'y

In order to address potential concerns over possible endocrine disruption, a fish screening assay (equivalent to a Fish short-term reproduction assay (FSTRA)) and a *Xenopus* eleuthercembryo thyroid assay (XETA) using spiroxamine have been conducted and have been submitted here. Full details are provided below. Please reter to the ED assessment for discussion of the results and an assessment against the ED criteria.

#### Executive Summary

This study was conducted in order to identify potential impacts of spiroxamine on endocrine biomarkers of reproductive performance in sexually mature fathead minnows over a 21-day exposure period. The assessment was based on the following biomarker endpoints: secondary sexual characteristics, vitellogenine plasma concentration and gonad histopathology. Fecundity and fertility of eggs were also evaluated and growth, expressed as full length and weight, was measured as a non-endocrine-specific endpoint.

Mean measured test concentrations were 1.6, 63, 18.0 56.4 and 58.8 µg a.s./L, along with a control.

A statistically significant and dose-dependent decrease of vitellogenin concentration in female blood plasma was observed in the 56.4 and 58.8  $\mu$ g as /L test concentrations. All other endocrine-specific biomarkers, secondary sex characteristics and gonad histopathology showed no treatment-related effects up to and including 58.8  $\mu$ g as /L, then ighest dose tested.

No effects on growthas a non-endoerine pecific endpoint were observed.

Fecundity could not be valuated in the study due to the very low control egg production during exposure. Measures of fertility were not affected by exposure to the test substance.

I. Materials and Methods A. Materials 5 4 4 Test Material 4 Spiroxam

 t Material
 Spiroxamine technical (KWG 4168)

 Lot/Batch #:
 EDTH004650

 Purity:
 95.1%

 Description:
 Light brown clear oily liquid



Stability of test compound:	Not reported
Reanalysis/Expiry date:	14 August 2007
Density:	Not reported
Treatments	
Test rates:	Not reported 14 August 2007 Not reported Nominal: 2.50, 7.50, 22.5, 67.5 and 203 ug a.s./L Measured: 1.6, 6.3, 18.9, 56.4 and 58.8 ng a.s./L None Yes, mean measured concentrations 29, 84% of nominal Yes, mean measured concentrations 29, 84% of nominal
Solvent/vehicle:	None $\mathcal{Q}^{\mathcal{V}}$ $\mathcal{Q}^{\mathcal{V}}$ $\mathcal{Q}^{\mathcal{V}}$ $\mathcal{Q}^{\mathcal{V}}$ $\mathcal{Q}^{\mathcal{V}}$ $\mathcal{Q}^{\mathcal{V}}$
Analysis of test concentrations:	None Yes, mean measured concentrations 29 84% of nominal Adult fathead minnow ( <i>Rimephotes promelas</i> ) appress. 32 weeks ofd at day 0
Test organisms	
Species:	Adult fathead minnow (Rinephotes promelas) approx. 32 weeks our at day 0
Source:	
Acclimatisation	Acclimated under similar conditions to the test for at least two weeks
period:	priooto testimitiation
Feeding:	prior test mitiation Feel ad libitum Frine shrimp muplic and ground flace food twice and Since daily, respectively
Treatment for disease:	None reported
Test design 🌮 🦨 🧹	
Test vessel:	None reported 22 x 35 cm glass aquaria, with a water depth of 21 cm and approx. Solume of 16 L Reconstituted water
Test medium	Reconstituted water &
Replication A	Four replicates per concentration
No. of $\mathcal{O}^{\mathbb{V}}$	Twomales and four females
animals/vessel:	
Environmental test	Polumes 16 L Reconstituted water Four replicates per concentration Two males and four females 21  days $3.2 + 25.1^{\circ}$ 93 - 106 saturation 66 - 6.9 16 h light : 8 h dark at 63 - 241 lux (mean 131 lux)
conditions v	
Discoled overland	$22.2 \times 25.1^{\circ}$
Dissorted oxygen:	$9_{X_{-}}$ 106 saturation
	0.6 - 6.9
	$\approx$ 10 ii iigiii . 8 n dark at 03 – 241 iux (mean 131 iux)
<b>B.</b> Study Design	

This study was conducted in order to identify potential impacts of spiroxamine on endocrine biomarkers of reproductive performance in sexually mature fathead minnows over a 21-day exposure period. The assessment was based on the following biomarker endpoints: secondary sexual characteristics,



vitellogenin plasma concentration and gonad histopathology. Fecundity and fertility of eggs were also evaluated, and growth, expressed as final length and weight, was measured as a non-endocrine-specific endpoint.

Test fish were adult male and female fathead minnow (*Pimephales promelas*), with clear secondary sexual characteristics visible. Fish were approximately 32 weeks old at the beginning of the exposure phase on day 0. The fathead minnow had not been held under spawning conditions prior to the test, and so went through the first spawning cycle during the study period.

Glass aquaria used in the study were 22 x 35 cm, with a water depth of 21 cm, yielding an approximate chamber volume of 16 L. Test water was a reconstituted water actated to oxygen saturation and periodically analysed for impurities. The test system was delivered at a flow rate of L/h, approximately 10.5 water changes daily. Two stainless-steel 100 x 75 mm spawning substrates were added to each chamber.

Two males and four females were placed in one aquarium including two spawning substrates, consisting of two single half tiles constructed of stainless steel. They were exposed to various test item concentrations and a water control under continuous flow-through conditions for 21 days with four replicates for each test level. Nominal test concentrations were 2.63 (2/50), 7.88 (7.50), 23.7 (22.5), 71.0 (67.5) and 213 (203) µg test item (a.s. pL. Mean measured concentrations of spire amine in the aquaria over the exposure period were 1.6, 6.3, 18.9, 56.4 and 58.8 µg a.s./L. equivalent to 64, 84, 84, 84 and 29% of nominal.

Before start of the test a 'pre-exposure' period over 15 days under 'non-exposure' conditions was initiated in the same aquaria as used in the test to check spawning activity of the selected breeding pairs. As a result, equilibration of the test system with the test compound was not possible prior to test start.

Fish were examined daily over the exposite period for mortality and behavioural or physical abnormalities. Observations of spawning activity in each test vessel were made daily. Eggs laid on the spawning substrate were removed, consted and discarded Representative egg clutches were also checked for fertilisation occess.

Fish were fed *a libitum* during the course of the test with fresh hatched brine shrimp (*Artemia* spp.) nauplii and ground take food (Tetramin[®]). Nauplii were added twice daily and ground flake food was added once daily. Food was withheld from fish for 12 hours prior totest termination on day 21.

The standard length was determined for each individual fit by measuring from the tip of the mouth to the tip of the caudappedurcle using an electronic digital caliper. Individual wet weight after blotting was also determined.

Observations of secondary sexual characteristics (SSCs) were made on working days of the course of exposure. Recorded characteristics were colouration patterns, specialised SSCs (size of dorsal nape pads, number of nuptial tubercles in males and ovisositor in females) and territorial aggressiveness (assessed semi-quantitatively) Afterwards gross morphology including secondary sex characteristics

was assessed. The monthal therefore as male secondary sex characteristics located around eyes, between nares and/or around the mouth were counted in each individual fish, using a stereo microscope. Nuptial tubercle size was ranked as follows: (4) present = tubercle having a single point whose height is nearly equivalent to its diameter, (5) enlarged = tissue resembling an asterisk in appearance, having a large radial base with grooves of furrows emerging from the center and (3) pronounced = quite large and rounded with less definition in structure.

The measurements of blood plasma vitellogenin levels were made using a commercially produced enzyme-linked immunoalsorbant assay (ELISA) for fathead minnow VTG.

Three transverse sections of each fish were processed. The thickness of each slice and the distance between the three slices was dependent upon individual body anatomy (varying size of female and male fish). The dorsal fin was used as orientation. The sections were performed in a way that they contained the gonads (testes and deferent ducts or ovaries and oviducts) and the liver. The material was embedded



in Paraplast and cut at an approximate thickness of about 4 micrometers. The slides were stained with Hematoxylin and Eosin (H&E).  $Q_{2}^{\circ}$ 

#### Analytical method

Samples of water were analysed using the validated analytical method 00629, report reference 8 031628-01-1 (see Doc MCA Section 4).

#### II. Results and Discussion

Validity criteria according to the OECD draft protocol were met:

- Control mortality or fish with signs of disease to not exceed 10% at test termination (actual: 0% mortality, 0.2% (one fish) was missing an eye, however this could be due to aggressive spawning behaviour of males rather than asymptom of the a.s.
- Dissolved oxygen concentration to be 60% in all test vessels throughout the test (actual 93 106%)

One validity criterion was not met:

Water temperature to not differ by more than £1.0°C between test vessels of any one time during the test (actual: a difference of 1.1°C was observed between test concentrations 1.6 and 6.3 μg a.s./L on study day 20, however this was the only incidence of exceedance of the cruterion)

Mean measured concentrations of spiroxamine in the aquada over the exposure period were 1.6, 6.3, 18.9, 56.4 and 58.8  $\mu$ g a.s./L, equivalent to 64, 84, 84, 84, and 20% of nominal. The mean measured values ranged from 29 to 84 percent of nominal for all test levels. If the lowest test level the mean measured concentration was at 64 percent of nominal, in the three mid test levels at 84 percent of nominal each, respectively, and in the highest test level only 29 percent of nominal was found. Therefore, the mean measured concentrations of the 2 highest level test level nearly in the same concentration.

No malfunctions of the dosing and diluter system were observed during the exposure, so the nonhomogeneous malytical recoveries also in the newly prepared stock solutions may indicate that the test item could not been sufficiently dissolved. Adsorptivity of the test item may also had an influence on test media concentrations showing inconsistent results with high variations especially during the first two days of the test (conflictently dissolved). Adsorptivity of the test item may also had an influence on test media concentrations showing inconsistent results with high variations especially during the first two days of the test (conflictently dissolved). Adsorptivity of the test item concentrations especially during the first two days of the test (conflictently dissolved). Adsorptivity of the system was not possible before start of exposure). The lowest and the highest test level were both throughout the whole exposure far too low and clearly deviated from the nominal values. According to the current OECD draft FSA guideline the test item concentrations should have been maintained within a 20 % of the mean measured values, which was not fulfilled for all test levels in this test due to high variabilities. But the mean concentrations of

the three mid test levels are approximately reflecting the nominal values and the effect threshold, was within the concentration range. The results of the study have been presented based on the mean measured test concentrations.

Nominal concentration (µg a.S.L)											
Control 2.50	@ 7.50	22.5	67.5	203							
Measured test concentration (µg a s./L)	Ŷ										
Mean (2492 ) 66	6.3	18.9	56.4	58.8							
Min X & A XI.1	4.7	10.8	29.9	31.5							
	7.1	23.0	80.6	84.5							
Max Q- 7 2.1 % of 7 - 8 64	84	84	84	29							
nomina											

Table CA 8.2.3/01-1	Measured concentrations of	f spiroxamine during the test

SD Standard deviation

LOQ Limit of quantification: 0.8 µg/L



No substance-related observations on mortality, behaviour or secondary sexual characteristics in either sex were observed over the 21-day exposure period.  $Q_{\mu}^{\circ}$ 

Table CA 8.2.3/01-2	Effects of spiroxamine exposure on the mortality	of fathead minnow	at test 🔊
	termination	ð	, O

	u	1 11111141	ion						L.	<i>y</i>	L	'		
Mean	Contr	ol			1.6 µg	1.6 μg a.s./L				6.9 µg a.s./L				
measured concentration						<i>i</i>		*	<b>,</b>					
Replicate	Α	В	С	D	Α	B	С	D	Α	B	C ~	D		
Mortality (%)	0	0	0	0	0	0	16.7	Ø	0	Ŵ	Ø	<i>A</i>	] 🔊	
Mean (%)	0				4.2	a y		4	0	$\tilde{\mathbf{O}}$	Q ,	Ő S		
SD	0				8.4	<b>b</b>	Ą		0	i L	C		1	
Mean measured concentration	18.9 µ	ıg a.s./l			5 <b>64</b> µ	ig a.s./L			58.8⁄µ	/ ig a.9./L		J.		
Replicate	Α	В	С	D «			C	DÖ	A	B 🔊	C	<b>D</b> 🔊		
Mortality (%)	16.7	0	0	0			Q v	01	05	16.7	1467	Q		
Mean (%)	4.2				0×		L.	Å.	<b>≈</b> §.4	Z Z	K)	A. C.		
SD	8.4				× 0 ×		/ %		9.6	Ŭ Ó	y (	C		
			0	78 X/	- C						Ro		-	

The baseline fecundity determinations during the pre-exposure phase without the test item (from day - 15 until the start of the exposure phase on day 0 gave to indications of spaceting follows in most cases. Fish were exchanged due to no or low egg production ( $\sqrt{4}$  egg per female and day before start of exposure in only five of the total 24 replicates. Afterwards spaceting success was improved in four of these replicates.

The number of eggs produced per female and day during xposure to spiroxappine did not indicate a statistically significant effect. Interpretation of this endpoint is difficult due to the very low egg production in the control group. By production in the two highest test levels was very low, however due to the comparably low control egg production, a definitive evaluation of this endpoint is not possible. No significant effect of the fertilisation rate was observed in the assessed egg clutches of all test levels

No significant effect on the fertilisation rate was observed in the assessed egg clutches of all test levels relative to the control fertility during the exposure phase.

Table CAS.2.3/01-3 Effects of spirosamine exposure on egg production of fathead minnow during the

	- <u>~</u> 0″		r		Lí –	<u> </u>	A'		-				
Mean	Sontrol σ σ σ σ σ σ								6.3 μg a.s./L				
measured													
concentration	O V		, O		, O ^v	°°							
Replicate 🔊	A	<b>B</b> O	×~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	<b>B</b> ×	é v	°₿⁄	С	D	Α	В	С	D	
Mean 🕰	0.2	2.0	90.6 <i>j</i>		ð.6	Ø2.1	0.9	7.8	4.4	0	0.8	6.7	
eggs/female					°~	V							
SD ~	1,0,7	6.8	2	0	7.0	4.6	2.1	11	11	0	2.1	13	
Mean	<i>б</i> .Л	Ž]		ð	<u>8</u> .3				3.0				
SD ²		2.4 ° (0 6.4							6.4				
Mean	18,9 µ	ig a.s.fr	- L	Q,	56.4 μg a.s./L				58.8 μg a.s./L				
measured O	A		K) ^v	@,*									
concentration	<i>لال</i> ۳	S.	L) *	Ő									
Replicato	A (	B ^	°C	Ď	Α	В	С	D	Α	B	С	D	
Mean 🔗 🔊	10		5.5	2.4	0.9	0.1	0.2	0.1	0.3	2.1	1.1	0	
eggs/temale	S.	, K											
Replicato Mean Sp Méan	21	¥.7	11	9.1	2.8	0.4	0.6	0.3	1.2	5.2	3.9	0	
Mean N	4.8	-			0.3				0.9				
1.1.00011 (())													

No substance-related significant effects on fathead minnow growth, expressed as male or female final standard length or wet weight, were detected after exposure to spiroxamine.



Mean	Males					^ ^				
measured concentration	Control	1.6 µg a.s./L	6.3 μg a.s./L	18.9 µg a.s./L	56.4 µg a.s./I	58.8 μg a.s./L				
Mean length (mm)	49.6	49.5	49.3	49.4	49.5	50.4				
SD	4.1	1.9	2.0	1.7	1.8	5.0 5				
Mean wet weight (g)	2.71	2.56	2.54	2.69	92.48 ×	2.69				
SD	0.64	0.29	0.34	0.30	0.28	Q. 97 2 A				
	Females									
Mean	Control	1.6 μg a.s./L	6.3 μg-a.s./L	18.9 µg	°56.4 sig 🛴	58.8 µg				
measured concentration					m s	58.8 µg a.s./L				
Mean length (mm)	40.1	39.0	39.4 J	39.9	\$40.7 §	39.7				
SD	2.0	0.8	2.2	1,2 * ,1	1,9	1.5				
Mean wet weight (g)	1.23	1.08			°4,23 ↔	1.18				
SD	0.24	0.09 0 %	0.18 🖓 🍂	0.200	0.20	0.16				

Table CA 8.2.3/01-4	Effects of spiroxamine exposure on measures of fathead growth
---------------------	---------------------------------------------------------------

At test termination the quantitative and qualitative assessment of nupbal tubercles is a specialised male secondary sexual characteristic in fathead mithows served as measurable biomarker endpoints. After exposure to spiroxamine, the number and score of nuptial tubercles in males was not affected in any test level, when compared to the control. In febrales for nuptial tubercles were observed as

# Table CA 8.2.3/01-5 Mean number/score of nuptial dubercos in male fathead monows at test

	01			0	~~	
Mean	Mares 🖌				<i><b>Q</b></i> ₁	
measured	Control	Д.6 µg a.s./L ∧	6.3 μg a.s./L .	🖉 🕺 کھیر 8.9آر	>56.4 μg	58.8 μg
concentration		· ~ ~ ~ ~		a.s.	a.s./L	a.s./L
Number of O	13	8.7	9.59 B	1108 2	10.3	7.8
nuptial 🔬 🖗	J.,	X9 A	S. O	a õ		
tubercles	Ô 1					
SD 🔊	6.5 %	Ø3.5 ×	5.5 0 🖤	2.5	5.3	4.9
Score of		10.3 . O'	1409 54	144	14.1	9.9
nuptial			φ ο .			
tubercles*	Ŷ Q .	¢ v ×				
SD	ن ^ک ر 8.8 ^ک ر 8.8	5.1°~~~~~	7.3	3.4	10.1	8.6

* score formula = (number of tubercles tanked as present x 1)+(number of tubercles ranked as enlarged x 2)+(number of tubercles ranked as pronoticed x 3)

The vitellogenin concentration in plood plasma showed a significant reduction in females at the two highest test levels (56.4 and 58.8 ng a.s.q.). With regard to vitellogenin contents in male blood plasma, detected at concentrations four orders of magnitude below the control female level, only a slight and insignificant decrease was observed in the inghest test level.

## Table CA \$2.3/01 Effects of spiroxamine exposure on vitellogenin (VTG) concentration in blood

Mean	Males					
	Control	1.6 μg a.s./L	6.3 μg a.s./L	18.9 µg	56.4 μg	58.8 μg
concentration				a.s./L	a.s./L	a.s./L
VTG (ng/mL)	1140.9	930.6	524.0	1172.7	656.2	142.1
SD	969.0	393.4	769.1	1548.4	856.7	66.7



	Females					
Mean measured concentration	Control	1.6 µg a.s./L	6.3 μg a.s./L	18.9 µg a.s./L	56.4 μg a.s./L	58.8 μg a.s./L
VTG (ng/mL)	10237556.2	10338738.8	5361114.7	4500759.3	1988 65.4*	2612292.5*
SD	3275125.5	4058132.3	2890049.9	2574270.9	1949671.1	1645951.8

* Statistically significantly different to the control ( $\alpha$ =0.05) (Dunnett's test)

The histopathological investigation of male and female gonads showed that they we for unaffected by exposure to spiroxamine up to and including 58.8  $\mu$ g as /L, regarding the developmental stage and degenerative changes.

Eggs or debris in the oviduct were frequently observed, indicating pawning activity. The incidence of inflammatory changes of the oviduct was slightly force ased at 56,4  $\mu$ g  $\alpha$ s./L.

The histopathological investigation of the liver tissue  $\sigma e^{\circ}$  vealed also no concentration related effects up to and including 58.8 µg a.s./L.

#### Table CA 8.2.3/01-7 Summary of histopathological findings in males

Mean measured	Control 1.6 4 46.3 7 18.9 50	58.80 57 57
concentration		
(µg a.s./L)		$\sim$ $\sim$
Total number of males		
Number of males a	t testis stage: 0 0 2 0	ř 🖇
Juvenile		2
1 (early spermatogenic)		
2 (mid spermatogenic) 🛸		× 4
3 (late spermatogenic) 🔬		2
	vitilestis observations: S	
Increased spermatogonia		-
Increased interstitial cells		-
Decreased spermatozoa		2
in sperm (deferent) dort		
Number of males v	vith liveRobservations 7 0 g	
Increased Depatic		-
Increased Prepatic		

Table CA 8.2.3/01- Summary of historathological findings in females

Car i d				10.0		
Mean measured concentration	Control &	1.6	6.3	18.9	56.4	58.8
concentration Ö		$\sim$ $\sim$				
(µg a.s./L)						
Total number of females		14	45	15	16	14
Total number intersex	V ″ ヘ		0	0	0	0
Number of fomale	s at ovar fostage	e.O X				
2 (mid development)	¥5 č	NY ON	7	7	5	5
3 (late development)	8 4	9	6	6	10	7
4 (very late development)		1	2	2	1	2
Number of female	Swith ovary ob	servations:				
Vacuolation of overian c strome S Oocyte atresionmature	2 5	) S	2	2	2	5
stroma A						
Oocyte atresionmature	25	1	-	-	-	-
Oocyte atresta mature	75	4	5	4	8	6
Increased postovulatory	8	6	8	6	6	6
follicles						
Egg debris in oviduct	11	9	9	8	9	9
Inflammation in oviduct	-	-	-	-	3	1
Number of males	with liver obser	rvations:				



Mean measured concentration (µg a.s./L)	Control	1.6	6.3	18.9	56.4	58.8
Decreased hepatic basophilia	-	-	-	-	2	
Increased hepatic basophilia	4	2	4	5	, F	

#### III. Conclusion

A statistically significant and dose-dependent decrease of vitellogener concentration in ternale blood  $\bigcirc$  plasma was observed in the 56.4 and 58.8 µg a.s./L dest concentrations. All other endocrine specific biomarkers, secondary sex characteristics and gonat histopathology showed no treatment-related effects up to and including 58.8 µg a.s./L, the highest dose tested.

No effects on growth as a non-endocrine-specific endpoint were observed

Fecundity could not be evaluated in the study one to the very low control egg production during exposure. Measures of fertility were not affected by exposure to the test substance of t

#### Assessment and conclusion by applicant;

The study was conducted to the DECD PSA test protocol (ENV/JO/TG/EDTA (2004) J REV2), including all standard operating procedures and guidance documents.

Validity criteria according to the OECD draft protocol were met

- Control mortality or fish with signs of disease to not exceed 10% at test termination (actual: 0% mortality, 0.2% (one fish) was missing an eye however this could be due to aggressive spawning bekaviour of males rather than asymptom of the a.s.)
- Dissolved oxygen concentration to be ≥60% in all test vessel@throughout the test (actual: 93 106%).

One validity coterior was not met:

• Water temperature to not differ by more than  $\pm 1.0^{\circ}$ C between test vessels at any one time during the test tactual a difference of 1.1°C was observed between test concentrations 1.6 and 6.3 µg as /L on tudy day 20 however this was the only incidence of exceedance of the criterion)

The study has been re-assessed against the current OECD 229 test guideline validity criteria which are as follows:

- The mortality in the water (or solvent) controls should not exceed 10 per cent at the end of the exposure period;
- The dissolved oxygen concentration should be at least 60 per cent of the air saturation value (ASV) throughout the exposure period
- The water temperature should not driver by more than  $\pm 1.5$  °C between test vessels at any one time during the exposure period and be maintained within the range of  $25 \pm 2$ °C for fathead minnow
- Explence should be available to demonstrate that the concentrations of the test substance in solution have been satisfactorily maintained within 20% of the mean measured values;
- Evidence that fish are actively spawning in all replicates prior to initiating chemical exposure and in control replicates during the test.

The first three validity criteria from OECD 229 are essentially the same that were used for the acceptance criteria in the study report. The measured test concentrations have been discussed in the report and summary; measured concentrations were not maintained within 20% of the nominal value (particularly at the highest and lowest test concentrations) but the middle three treatment



concentrations were considered to have been maintained at levels around the nominal value. As the results have been based on mean measured test concentrations, the results are considered to accurately reflect the actual concentrations that fish were exposed to.

A 15-day pre-exposure period was included as part of the test and demonstrated that the fise were actively spawning prior to initiating chemical exposure.

Taking these points in to account, it is believed that the validity criteria of the current OECD 229 test guideline have been fulfilled therefore the study is considered to be valid.

A statistically significant and dose-dependent decrease of vitellogenip concentration in female bloc plasma was observed in the 56.4 and 58.8  $\mu$ g a.s./L test concentrations.

The data from this screening assay have been used for the assessment of EB potential only. As a result, it is not considered necessary to perform  $EC_{10}$  and  $EC_{20}$  calculations for the parameters assessed in this study as these values will not be used in the convertional rest assessment.

Data Point:	KCA 8.2.3/02
Report Author:	$\begin{array}{cccccccccccccccccccccccccccccccccccc$
Report Year:	
Report Title:	Zenopus eleuthergembryonic thyroid assay (XETA) analysis report -
	Spiroxangine & & & & O & S
Report No:	P-20(2)-01(22) 0 2 0 0 0 0 0 0
Document No:	$M_{2}^{*} \delta 2327 - 0 1 - 1 \sim 0^{*} \delta 2327 - 0 1 - 1 \sim 0^{*} \delta 2327 - 0 0$
Guideline(s) followed in	OECD 16 248 (2019) 7 7 7 7
study:	
Deviations from current test guideline:	None, Star Contraction of the star of the
	No, not previously submitted S
Previous evaluation:	
GLP/Officially	No, not conducted under GLP/Officially recognized testing facilities
recognised testing facilities:	
facilities:	
Acceptability/Reliability:«	Ves < ~~ ~~ ~~ ~~ ~~ ~~ ~~ ~~ ~~ ~~ ~~ ~~ ~
Executive Summary	$\begin{array}{cccccccccccccccccccccccccccccccccccc$

## Executive Summary

The objective of the *Xenopus* Electheroeonbryonic Thyroid Assay (XETA) assay was to detect potential activity of the test item on the thyroid axis. According to ØECD TG 248, the XETA detects thyroid active molecules action through various mechanisms of action including TH receptors agonists and antagonists, modulators of TH metabolism (including deiodinase inhibitors), modulators of TH clearance (including UDPGT modulators), and potentially modulators of TH transport *via* interaction with TH plasma binding proteins.

The purpose of this test was to measure the capacity of spiroxamine to activate or inhibit the transcription of a genetic construct (TFb/ZIP GFP X. *Vaevic* eleutheroembryos) either directly through binding to the thyroid receptor (TR) or modifying the binding of thyroid hormones (TH) to the TR, or indirectly by modifying the amount of TH available to activate the TR and thereby transcription of the TH/bZIP-GFP construct.

Nominal concentrations of 0.025, 0.1, 0.4 and 1.6 mg a.s./L were evaluated for fluorescence. The highest test concentration was selected based upon the results of solubility and survival pre-tests showing that this concentration, corresponding to the LC₅, results in 5% mortality, and potentially corresponds to the Maximum Tolerated Concentration (MTC). The exposure levels of the nominal concentrations were verified analytically. Geometric mean measured concentrations were 3.52, 19.35, 102.90 and 434.15 µg a.s./L for nominal test concentrations 0.025, 0.1, 0.4 and 1.6 mg a.s./L, respectively, corresponding to a mean recovery of 21.5%.



No toxicity was observed in the eleutheroembryos in the controls. In the definitive test, the highest concentration of 434.15  $\mu$ g a.s./L showed 33.3% cumulated mortality over the three runs in both unspiked and spiked modes; and therefore exceeds the MTC estimated from the survival pre-tests. The second highest nominal concentration (102.9  $\mu$ g a.s./L) showed no sign of toxicity over the three runs.

Spiroxamine showed no indication of endocrine activity for the thyroid modelity at the geomean test concentrations of 3.52, 19.35 and 102.90 µg a.s/L. A statistically significant increase in fluorescence was observed at the highest nominal test concentration, 1.6 mg a.s./L, however as this test concentration, was above the MTC, it cannot be concluded that the increase in fluorescence is indicative of T-mediated endocrine activity.

It is therefore concluded that XETA.	t spiroxamine does not show endocrine activity on the thyroid axiolin the Spiroxamine technical AE 1344093-04-09 92.3% Colourless to pale brown liquid Not reported 31 March 2022 Notreported Nominal: 0.025, 0.1, 0.4 and 1.6 mg a.s./L Measured 32, 1995, 102-90 and 434.15 µg a.s./L Ethanot (0.01%)
Materials and Methods	
Materials	
Test Material	Spiroxamine techningal
Lot/Batch #:	AE 1344293-04-09
Purity:	
<b>Description:</b>	Colourlessto pale brown liquid
Stability of test compound:	Spiroxamine technical AE 1344293-04-09 92.3% Colourless to pale brown liquid Not reported 31 March 2022 Not reported Not reported 0.025, 0, 1, 0.4 and 1.6 ng a.s./L
	31 March 2022
date:	
date:	31 March 2022
Treatments	
Test rates: 🖉 🔬	Nominal: $\sqrt[4]{0.025}, 0.1, 0.4$ and 1.6 mg a.s./L
Softent/vehicle:	Measured $3,32,19,55,102.90$ and $454.15 \ \mu g a.s./L$
Analysis of test	$\sqrt{2}$ Vas $\sqrt{2}$ $2$
concentrations:	$\int \frac{1}{2} \int $
concentrations:	Nominal: $0.025, 0.10.4$ and $1.6$ rag a.s./L Measured $3.52, 1995, 102.90$ and $434.15 \ \mu g$ a.s./L Athanot $(0.01\%)$ Yes, geometric mean recovery of $14.2 - 27.2\%$ (mean 21.5%) <i>Xanopus laevis</i> ejeuthercembryos, stage 45
Species:	Xenopus laevis eleuthersembryos, stage 45
Source:	D-house culture
Source: Acclimatisation period:	Yes geometric mean receivery of 14.2 – 27.2% (mean 21.5%) Xenopus laevis eleuthercembryos, stage 45 Di-house culture None
Treatment for disease	Nong & Q L Q
Test design 🖉 🖉	
Fest vessel:	Plastic 6-well plates containing 8 mL test solution
Fest medium?	Evian [®] water
<b>Replication:</b>	Three runs
No. of animals/run:	20 eleutheroembryos (split evenly over 2 wells)



Duration of test:	72 hours
Environmental test conditions	
Temperature:	21°C
Dissolved oxygen:	Not reported
Loading:	15.75 g/L (mean fresh weight 12.6 mg) $\frac{1}{2}$
pH:	7-8
Photoperiod:	Constant darkness
Study Design	

This study was conducted in order to detect potentia activity of spiroxamine technical on the thyroid axis of Xenopus laevis eleutheroembryos. Test concentrations were selected based on the results of solubility and survival pre-tests, and were obtained via serval dipution of a 16 gd stock solution, prepared daily. Ø i

Nominal concentrations were 0.02500.1, 0.4 and 0.6 mg/a.s./10 and the respective geometric mean measured concentrations were 3.52, 19.35, 102.90 and 434 3 µg S./L, corresponding to a mean recovery of 21.5%. Endpoints are therefore based on geometric mean measured concentrations.

The exposure solutions were prepared by secial dilution of the 16 g/L stock solution with 100% ethanol in the Evian® water to reach the final concentration of Colvent of 0.07%. For the enspiked mode, the exposure solutions were prepared by dilution of the 4.6 mg Solution into the test medium (Evian® water + 0.01% ethanol), For the spiked mode, 100 mL of each exposure solution were spiked with 20 µL of a 16.25 mg/L solution of T3 bormone. Each spiked exposure solution contained 3.25 µg/L of T3 hormone in the exposure medium. There were additionally a megative control, a solvent control, a T3 (triiodothyronine at 3.25 µg/L) control and a 14 (thyroxine at 10 mg/L) separation control.

The test media was repewed@fter 2@and 48 hours exposure.

Test vessel@were plastic_6-well_plates of a chonicallyInert material containing 8 mL test solution per well. Each test group contained 20 Xeropus laevis Quether combry os per run, with three runs per group for a total of 60 eleutheroembryos. To each well was added 10 eleutheroembryos.

Eleutheroembryos in the "unspiked" test condition received no supplementation of thyroid hormones, whereas those in the "spiked mode were supplemented with 3.25 µg/L of the thyroid hormone T3 (trijothyroning)

Mortality of the eleutheroembry and abservations of gross morphology and behaviour were recorded after 24, 248, and 72 hours exposure. The pH of the exposure solution containing the highest concentration of the test item was measured at the start of the test, and temperature was measured S. continually.

Anaesthetised elevitheroembryos were then shared and transferred to a 96-well plate, one per well, and imaged using a robotised in agery system to assess the fluorescence. Data were then analysed using Microsoft Escel and Graphpad Prism.

All the tested groups, stock solution and Evian® water + 0.01% Ethanol control groups were sampled in order to perform chemical analysis of the test item.

## Results and Discussion

Validity Criteria according to the OECD 248 (2019) "Xenopus Eleutheroembryo Thyroid Assay (XETA)" guideline were met:

Control mortality to not exceed 10% at the end of the test (actual: 0%)



- Malformed organisms in the control to not exceed 10% (actual: 0)
- Initial pH of the exposure solutions to be between 6.5 and 8.5 for each renewal (actual: 7 8)
- Mean fluorescence of the T3 control group to be at least 20% higher than the mean fluorescence of the test medium control (actual: ≥58.8%)
- There should be a significant induction of fluorescence of at least 70% in the T4 control group compared to the test medium control (actual: ≥119.9%)
- The coefficient of variation of the fluorescence intensity in the test medium control on the fluorescence intensity in the test medium control on the fluorescence intensity in the test medium control on the fluorescence intensity in the test medium control on the fluorescence intensity in the test medium control on the fluorescence intensity in the test medium control on the fluorescence intensity in the test medium control on the fluorescence intensity in the test medium control on the fluorescence intensity in the test medium control on the fluorescence intensity in the test medium control on the fluorescence intensity in the test medium control on the fluorescence intensity in the test medium control on the fluorescence intensity in the test medium control on the fluorescence intensity in the test medium control on the fluorescence intensity in the test medium control on the fluorescence intensity in the test medium control on the fluorescence intensity in the test medium control on the fluorescence intensity in the test medium control on the fluorescence intensity in the test medium control on the fluorescence intensity in the test medium control on the fluorescence intensity in the test medium control on the fluorescence intensity in the test medium control on the fluorescence intensity in the test medium control on the fluorescence intensity in the test medium control on the fluorescence intensity in the test medium control on the fluorescence intensity in the test medium control on the fluorescence intensity in the test medium control on the fluorescence intensity in the test medium control on the fluorescence intensity in the test medium control on the fluorescence intensity in the test medium control on the fluorescence intensity intensity in the test medium control on the fluorescence intensity intensintensity intensity intensity intensity intensity intensity

Geometric mean measured concentrations of test concentrations 0.02 20.1, 0.4 and 0.6 ms a.s./L were 3.52, 19.35, 102.90 and 434.15 µg a.s./L, respectively corresponding to a mean recovery 0.21.5 0.14.2 to 27.2%). The mean percent recovery in the stock solutions was 91.6%. The results of the test should therefore be considered with regard to the geometric mean measured concentrations. Addition of To in the "spiked" mode had no effect on the exposure concentrations.

Nominal test concentration	Mean me	easur	ed conce	ntrati	. 🖌	.s.7b) ¹	[°]	Ĵ.		Ç,		Ç,
(mg a.s./L)	0 h		24 h		48 h 2 2 2		72 h O					
	Fresh	%	Spent	%©	Frest	%	Spent	<b>N</b>	Fresh		Spent	%
0.025	19.2	77	@.967 *	Ă	109.2	77	Ø.00		0 <b>r</b> 8.1 ₀ 0	72	1.08	4
0.1	75.4	7 <b>S</b> Û	3.68	4	76.0	76	5.68	60	685	68	8.87	9
0.4	304 💊	<i>9</i> 6	2 <b>9</b> .8	Ĩ	270	s 68	41,3	M0	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	<i>68</i>	55.6	14
1.6	1063 🔬 🖉	66	£129	8	۵ ³ 176 @	74	Ĵ ³³⁵³ ≰		M038	65	151	9

Table CA 8.2.3/02-1 Mean measured concentrations of spiroxamine in sest medium

¹ Figures rounded to 1 d

Table CA 8.2.3/02-2 Gometric mean measured concentrations of spirox anine in test medium

Nominal test	Geomean (zig a, s. () ) (+ Standard for ror	Recovery (%)
Nominal test concentration		• ( )
(mg a.s./L)		
0.025	3.52	14.2
0.1 ~ ~ ~	1995 S S S S S S S S S S S S S S S S S S	19.0
0.4	1260	25.7
1.6	434.10° (191.00°	27.2
	5 0 x 5 6 Mean:	21.5

#### Toxicity:

No morphological abnormalities were observed in any of the eleutheroembryos at any point during the test.

All cleutheroembryos exposed to the highest test concentration of 434.15  $\mu$ g a.s./L died in both the unspiked and spiked modes of the first run. While there was no mortality in the second and third runs, toxic effects were nevertheless recorded at this same concentration after 24 hours of exposure (reduced mobility). Curvulated mortality over the three runs was 33.3%.

No signs of toxicity were observed at the second highest test concentration of 102.9  $\mu$ g a.s./L, and one dead elevatheroembryo was recorded at the test concentration of 19.35  $\mu$ g a.s./L (spiked mode) in the second run. Overall, the survival rate was greater than 90% in all groups in all runs and in the pooled date as required in the OECD TG 248, except for the 1.6 mg a.s/L nominal concentration which exceeds the Maximum Tolerated Concentration (MTC) and is therefore not included in the assessment for potential T-mediated effects.



Group (geomean)	Survival	of eleuthero	embryos ¹		Q
	24 h	48 h	72 h	Survival (%)	Malformations
Test medium	100	100	100	100	- "Ű é
Test medium + solvent (Ethanol)	100	100	100	100	- 🔦 🔍 🖓
T3 3.25 $\mu$ g/L + solvent (Ethanol)	100	100	100	100 🐧	- \$ ~
T4 10 mg/L + solvent (Ethanol)	100	100	100	100	-~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
Spiroxamine – 3.52 µg/L	100	100	<b>(2)</b> 00	100	
Spiroxamine – 19.35 µg/L	100	100	100	1000	
Spiroxamine – 102.9 µg/L	100	100 🔬	100	0ð0 🔊	- 0. 5
Spiroxamine – 434.15 µg/L	100	0	$0$ $\hat{Q}$	$0^2$ $\sim$	
Spiroxamine – $3.52 \ \mu g/L + T3$	100	1000	100 ~ '	1969 - 2,	
Spiroxamine – 19.35 µg/L + T3	100	100 .	1000	× ~ 00¢	\ K ^a . Ka
Spiroxamine – 102.9 µg/L + T3	100	M00 🔗	1,00	100 2 ~	- ~ ~
			ð Ø		A. 2
20 eleutheroembryos introduced	0× %	ter 24 hours of eleut	of exposure	os – test run	
Spiroxamine $-434.15 \ \mu g/L + T3$ ¹ 20 eleutheroembryos introduced ² Lethal concentration. 2 less mobile <b>able CA 8.2.3/02-4</b> Survival an Group (geomean)	embryos af d observat	ter 24 hours of ions of eleut	of exposure	os – test run g	
¹ 20 eleutheroembryos introduced ² Lethal concentration. 2 less mobile <b>able CA 8.2.3/02-4</b> Survival an <b>Group (geomean)</b>	embryos af d observat Survival 24 k	ter 24 hours ions of eleut of eleuthero	bf exposure heroembry embry os ¹	os – test run g	
¹ 20 eleutheroembryos introduced ² Lethal concentration. 2 less mobile <b>able CA 8.2.3/02-4</b> Survival an <b>Group (geomean)</b>	embryos af d observat Survival 24 fr 100	ter 24 hours of tons of eleut of eleuthero 48 h	bf exposure heroembry embry os ¹	os – test run g	
¹ 20 eleutheroembryos introduced ² Lethal concentration. 2 less mobile ² Lethal concentration. 2 less mobile <b>able CA 8.2.3/02-4</b> Survival an         Group (geomean)         Test medium         Test medium + solvent (Ethanol)	embryos af d observat Survival 24 fe 100	ter 24 hours of tons of eleut of eleuthero 48 h 100	bf exposure heroembry embry os ¹ 72 h 400 0 100	Survival (%)	
¹ 20 eleutheroembryos introduced ² Lethal concentration. 2 less mobile <b>able CA 8.2.3/02-4</b> Survival an <b>Group (geomean)</b> Test medium Test medium + solvent (Ethanol) T 3 3.25 μg/L + solvent (Ethanol)	embryos af d observat Survival 24 fr 100 100 100	ter 24 hours of tons of eleut of eleuthero 48 h 100 150 400	bf exposure heroembry embry s ¹ 729 100 100	Survival (%)	
¹ 20 eleutheroembryos introduced ² Lethal concentration. 2 less mobile <b>able CA 8.2.3/02-4</b> Survival an Group (geomean) Test medium Test medium + solvent (Ethanol) T 3.25 μg/L + solvent (Ethanol) T4 10 mg/L + solvent (Ethanol)	embryos af d observat 24 f 100 100 100	ter 24 hours of tons of eleut of eleuthero 48 h 100 100 100 100	of exposure heroembry embry os ¹ 72% 100 100 50 60	Survival (%)	
¹ 20 eleutheroembryos introduced ² Lethal concentration. 2 less mobile <b>able CA 8.2.3/02-4</b> Survival an <b>Group (geomean)</b> Test medium Test medium + solvent (Ethanol) T3 3.25 μg/L + solvent (Ethanol) T4 10 mg/L + solvent (Ethanol) Spiroxamine – 3.52 μg/L	embryos af d observat Survival 24 fe 100 100 100 100	ter 24 hours of tons of eleut of eleuthero 48 h 100 100 100 100 100	bf exposure heroembry embry os ¹ 72-0 100 100 200 200 200 200 200 20	Surviyal (%)	
¹ 20 eleutheroembryos introduced ² Lethal concentration. 2 less mobile <b>able CA 8.2.3/02-4</b> Survival an Group (geomean) Test medium + solvent (Ethanol) T3 3.25 μg/L + solvent (Ethanol) T4 10 mg/L + solvent (Ethanol) Spiroxamine - 3.52 μg/L Spiroxamine - 19.35 μg/L	embryos af d observat Survival 24 fr 100 100 100 100 100 100	ter 24 hours of eleut of eleuthero 48 h 100 100 100 100 100 100 100 10	of exposure heroembry embry os ¹ 72-0 100 100 100 100 100 100	Survival (%) 100 100 100 100 100 100 100 10	Malformations
¹ 20 eleutheroembryos introduced ² Lethal concentration. 2 less mobile <b>able CA 8.2.3/02-4</b> Survival an <b>Group (geomean)</b> Test medium Test medium + solvent (Ethanol) T3 3.25 µg/L + solvent (Ethanol) T4 10 mg/L + solvent (Ethanol) Spiroxamine – 3.52 µg/L Spiroxamine – 19.35 µg/L Spiroxamine – 1029 µg/L	embryos af d observat 24 fr 100 100 100 100 100 100 100	ter 24 hours of tons of eleut of eleuthero 48 h 100 100 100 100 100 100 100	of exposure heroembry embry os ¹ 72-0 100 100 100 100 100 100	Surviyal (%) 106 106 100 100 100 100 100 100	Malformations
¹ 20 eleutheroembryos introduced ² Lethal concentration. 2 less mobile <b>able CA 8.2.3/02-4</b> Survival an <b>Group (geomean)</b> Test medium Test medium + solvent (Ethanol) T3 3.25 μg/L + solvent (Ethanol) T4 10 mg/L + solvent (Ethanol) T4 10 mg/L + solvent (Ethanol) Spiroxamine – 3.52 μg/L Spiroxamine – 19.350 g/L Spiroxamine – 1029 μg/L Spiroxamine – 40.15 μg/L	embryos af d observat 24 f 100 100 100 100 100 100 100 100 100	ter 24 hours of tons of eleut of eleuthero 48 h 100 100 100 100 100 100 100	of exposure heroembry embry os ¹ 729 100 100 100 100 100 100 100 10	Survival (%) 109 100 100 100 100 100 100 100	Malformations
¹ 20 eleutheroembryos introduced ² Lethal concentration. 2 less mobile <b>able CA 8.2.3/02-4</b> Survival an <b>Group (geomean)</b> Test medium Test medium + solvent (Ethanol) T3 3.25 μg/L + solvent (Ethanol) T4 10 mg/L + solvent (Ethanol) T4 10 mg/L + solvent (Ethanol) Spiroxamine – 3.52 μg/L Spiroxamine – 10.35 μg/L Spiroxamine – 10.25 μg/L Spiroxamine – 44.15 μg/L Spiroxamine – 45.25 μg/L + T2	embryos af d observat Survival 24 F 100 100 100 100 100 100 00 100 00	ter 24 hours of ions of eleut of eleuthero 48 h 100 100 100 100 100 100 100 10	f         exposure           beroembry         embry           embry         embry           100         100           100         100           100         100           100         100           100         100           100         100           100         100           100         100	Survival (%) 109 109 100 100 100 100 100 100	Malformations
¹ 20 eleutheroembryos introduced ² Lethal concentration. 2 less mobile <b>able CA 8.2.3/02-4</b> Survival an Group (geomean) Test medium Test medium + solvent (Ethanol) T3 3.25 $\mu$ g/L + solvent (Ethanol) T4 10 mg/L + solvent (Ethanol) T4 10 mg/L + solvent (Ethanol) Spiroxamine – 19.35 $\mu$ g/L Spiroxamine – 1029 $\mu$ g/L Spiroxamine – 404.15 $\mu$ g/L Spiroxamine – 19.35 $\mu$ g/L + T3 Spiroxamine – 19.35 $\mu$ g/L + T3	embryos af d observat Survival 24 k 100 100 100 100 100 00 00 00 00 00 00 0	ter 24 hours of tons of eleut of eleuthero 48 h 100 100 100 100 100 100 100 100 100 10	of exposure heroembry embry os ¹ 72-9 100 100 100 100 100 100 100 10	Survival (%) 109 109 100 100 100 100 100 100	Malformations
¹ 20 eleutheroembryos introduced ² Lethal concentration. 2 less mobile <b>able CA 8.2.3/02-4</b> Survival an <b>Group (geomean)</b> Test medium Test medium + solvent (Ethanol) T3 3.25 μg/L + solvent (Ethanol) T4 10 mg/L + solvent (Ethanol) T4 10 mg/L + solvent (Ethanol) Spiroxamine – 3.52 μg/L Spiroxamine – 10.35 μg/L Spiroxamine – 10.25 μg/L Spiroxamine – 44.15 μg/L Spiroxamine – 45.25 μg/L + T2	embryos af d observat Survival 24 10 100 100 100 100 100 100 100	ter 24 hours of tons of eleut of eleuthero 48 h 100 100 100 100 100 100 100 100 100 10	brook of exposure beroembry embry os ¹ 72-0 100 100 100 100 100 100 100 1	Sur (%) 5 5 5 5 5 5 5 5 5 5 5 5 5	Malformations

Table CA 8.2.3/02-3	Survival and observations of eleutheroembryos – test run 1
	Sur vivar and obser vacions of creatmer demoty ds test run r

 Table CA 8.2.3/02-5
 Survival and mervations of eleuther oembryos – test run 3

			0*		
Group (geomean)	[*] Surviyal o	of eleuthero	embryos ¹		
	24 h	∲48 <b>ի</b> _©	72 h	Survival (%)	Malformations
Test medium	100	1007	100	100	-
Test medium + solvent (Ethanol)	100	*190	100	100	-
T3 $3/25$ µg/L + solvent (Ethanol)	100	M00	100	100	-
T4 10 mg/L + solvent (Ethanol) Spiroxamine - 5.52 μg/L Spiroxamine - 19.35 μg/L	100 5	100	100	100	-
Spiroxamine - \$.52 µg/L 🦉 🕺	9100 $9$	100	100	100	-
Spiroxamine 19.35 pg/L	100@	100	100	100	-
Spiroxamine – 1029 µg/LO	100	100	100	100	-
Spiroxanume – 484.15 µg/L O	100	100	100	100 ²	-
Spiroxamine $5.52 \text{ yg/L} + 73^{\circ}$	100	100	100	100	-
Spiroxamine 53.52 µg/L + 73 Spiroxamin@- 19.35 µg/L */T3	100	100	100	100	-
Spiroxanonie – 102.9 $\mu$ g/L ⁺ T3	100	100	100	100	-
Spiroxaprine – 434.15 $\mu$ g/L + T3	100	100	100	100 ²	-

¹ 20 eleutheroembryos introduced
 ² Toxic concentration. All embryos less mobile after 24 hours of exposure



#### Fluorescence:

#### Unspiked

The highest test concentration,  $434.15 \,\mu\text{g/L}$  resulted in a statistically significant increase of fluorescence, at 46.2%.

No statistically significant variation of fluorescence greater than 12% was induced by the test item at any of the test concentrations showing no mortality or sub-lethal toxic effects.

#### Spiked

The highest test concentration,  $434.15 \,\mu g/L$  resulted in a statistically significant increase fluorescence, at 22.1%.

No statistically significant variation of fluorescence greater than 12% was induced by the test i any of the test concentrations showing no mortality or sub-lethal toxic effects.

## Table CA 8.2.3/02-6 Normalised mean fluorescence of the control groups

Group	Normalised mean SEM ¹ A D Induction (%)
	fluorescence in the second sec
Test medium + solvent (Ethanol)	
Test medium	
T3 3.25 $\mu$ g/L + solvent (Ethanol)	M.72 0 0 0.05 0 70+** V
T4 10 mg/L + solvent (Ethanol) $\mathbb{Q}$	2.35 0 235 0 4 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0

¹ Standard Error of the Mean

*** Significantly different to the control (Marn-Whitney, p@.001)

#### Table CA 8.2.3/02-7 Normalised mean fluorescence of the Sunspiked" groups

Cuoun (geomeon)		The second secon	× N	/
Group (geomean)	a sormaused	mean SEM ² O	O' ⁴ y'	Induction (%)
	Normalised fluorescence			
Spiroxamine $-3\beta^2 \mu g/D$	1,03	`_`Y` 0092_0°	Å Å	3
Spiroxamine 🔊 🕼 .35 μOL	1,04 60	× × 0.02		4
Spiroxamine _102.9 gg/L &	1.08	Q Q 0.020		8.3**
Spiroxamine – 434.15 µg/L	1.46	<u>, 000000000000000000000000000000000000</u>	,	46.2***

¹ Pooled and normalised to the solvent control

² Standard Error of the Mean

** Significantly different to the control (Dunnett's post-how test, p = 0.01)

*** Significantly different to the Control Dunnet post-hoc test p<0.001)

## Table CA 8.2.342-8 Normalised mean foresconce of the "spiked" groups

Group (geomean)	SEM ²	Induction (%)
Spiroxamine – $3.52 \mu g L$ $4,67 L$ $0^{\prime}$	0.03	-2
Spirovamine – 19.3 Jg/L 3 11.77	0.03	3
Spiroxamine – 102.9 μg/ΙΦ & 1.83 O	0.03	6.7*
Spiroxamine – 424.15 µg/L 2 99	0.03	22.1***

¹ Pooled and normalised to the T3 control

² Standard Kiror of the Mean

* Significantly different to the control (Dunnett's post-hoc test, p < 0.05)

*** Significantly different to the control (Dunnett's post-hoc test, p < 0.001)

#### Conclusion

Mortality and sub-lethal effects of the test item was observed at the highest test concentration of 434.15  $\mu$ g a.s./L in both the unspiked and spiked modes during the definitive test (33.3% cumulated mortality), which excludes this concentration for T-mediated endocrine evaluation in this test system. The second highest test concentration of 102.9  $\mu$ g a.s./L (nom) showed no sign of toxicity or lethality.



No statistically significant variation of fluorescence greater than 12% was induced by the test item at any of the relevant test concentrations in both the "spiked" and "unspiked" modes, indicating, no activation or inhibition of the thyroid axis.

It is therefore concluded that the test item spiroxamine (technical substance) does not show any activity on the thyroid axis at nominal concentrations of 0.025, 0.1 and 0.4 mg a.s. (2, and geometric mean measured concentrations of 3.52, 19.35 and 102.9 µg a.s./L in the Xenopus eleutheroembryonic theroid assay (XETA).

#### Assessment and conclusion by applicant:

The study was conducted to the OECD test guideline 248, "Xenopuls Eleutheroendry (XETA)", adopted 18 June 2019.

Validity criteria according to the OECD 248 (2019) guidelin@were met:

- Control mortality to not exceed 10% at the ond of the test facture 0%)
- Malformed organisms in the control to not exceed 10% factual: 0)
- Initial pH of the exposure solutions to be between 65 and \$5 for each renewal (actual) 8)
- Mean fluorescence of the 13 control, group to be at least 20% bigher than the mean fluorescence of the test medium control (actual:  $\geq 58,8\%$ )
- There should be a significant induction of fluorescence of at least 70% in the T4 control group compared to the test medium control (actual:  $\geq 119.9\%$ )
- The coefficient of variation of the fluorescence intensity in the testomedium control to no exceed 30% (actual:  $\leq 12\%$ )

The study is therefore considered acceptable.

Lable Conclusion Laion lested did Love the MPIC at Laion effects. The stur-M-7315501-1) Let toxicity to Daphila magna Acute toxicity toxicity to Daphila magna Acute toxicity The results of the and to the condusion that spiroxamine does not show any activity on the thyroid axis. The highest conceptration tested did show a significant increase in the fluorescence but this concentration was well above the MYC and therefore the results are not included in the assessment of potential T-mediated effects. The study dras been used as part of the ED assessment

cute to picity to aquatic invertebrates 🤇



Data Point:	KCA 8.2.4.1/01
Report Author:	
Report Year:	1994
Report Title:	Acute toxicity of KWG 4168 (tech.) to waterfleas (Daphnia magna)
Report No:	HBF/DM 122
Document No:	<u>M-006245-01-1</u>
Guideline(s) followed in	OECD 202 (1984)
study:	
Deviations from current	Yes OECD 202 (2004) Daphnids were 10/vessel instead of the recommended 5/vessel and only 3
test guideline:	OECD 202 (2004)
	Daphnids were 10/vessel instead of the recommended 5/vessel and only 3
	replicates instead of A is a set of A is a s
	Temperature was measured in only one vessel and at the ord of the study
Previous evaluation:	1  ves evaluated and accepted
	DAR (1997), RAR (2010), RAR (2010)
GLP/Officially	Yes, conducted under GLP/Officially recognised testing facilities (
recognised testing	
facilities:	
Acceptability/Reliability:	Yes of the transformed and

Available study data for the paren material clearly demonstrate that algaes are the most sensitive organism group by at least two orders of magnitude therefore acute studies with the metabolites using aquatic invertebrates are not considered necessary. A non-GPP study is available for spiroxamine-N-oxide (M03) but acute *Daphyta* data are not available for the other relevant metabolites because these data are not considered to be required.

#### **Executive Summary**

The 48-hour acute toxicity of KWG 168 to Daphoia magna was studied under static conditions. Test organisms were exposed to measured test concentrations of 0.23, 0.60, 0.82, 2.2, 4.4, 8.3 and 22 mg a.s./L for 48 hours immedilisation and sub-lethal effects were observed after 24 and 48 hours. The 48-hour EC₅₀ was 64 mg a.s./L. The 48-hour NOEC based on immedilisation was 2.2 mg a.s./L.

hour EC₅₀ was 69 mg a.s./L. The 48 hour NOEC based on immobilisation was 2.2 mg a.s./L. Materials and Methods I. A. Materials **Test Material** Lot/Batch **Purity:** Description: olourless hauid Stable for the duration of the test, as shown by the results of the 48-hr Stability of test analytical determination compound: Reanalysis/ January date: ot reported Dens Treatmen ominal: 0.32, 1.00, 1.78, 3.16, 5.62, 10.0 and 31.6 mg a.s./L Measured: 0.23, 0.60, 0.82, 2.2, 4.4, 8.3 and 22 mg a.s./L Solvent/vehicle: Not reported Amalysis of test Yes, mean initial measured concentrations 46.2 to 83.0% of nominal concentrations:



Test organisms	
Species:	Daphnia magna, first instar (6 – 24 hrs old)
Source:	In-house culture, originally from Bundesgesundheitsamt, Berlin, A Germany
Acclimatisation period:	None reported, however culture was raised in the laboratory
Feeding:	Not fed during the test
Treatment for disease:	None reported
Test design	
Test vessel:	100-mL beakers containing 50 mL test solution, sovered with plexi glass plates
Test medium:	M7-medium $\sqrt{2}$ $\sqrt{2}$ $\sqrt{2}$ $\sqrt{2}$ $\sqrt{2}$ $\sqrt{2}$ $\sqrt{2}$
<b>Replication:</b>	Three replicates 2 2 2 2 2 2 2
No. of animals/vessel:	None reported, however culture was raised in the laboratory Not fed during the test None reported 100-mL beakers containing 30 mL test solution, sovered with plexi glass plates M7-medium Three replicates Ten daphnids per vessel
<b>Duration of test:</b>	As hours of the of o
Environmental test conditions	Daphnia magna, first instar (6 – 24 hrs old) In-house culture, originally from Bundesgesundheitsamt, Berlin, Germany None reported, however culture was raised in the laboratory Not fed during the test None reported 100-mL beakers containing a0 mL test solution, covered with plexi glass plates M7-medium Three replicates Ten daphnid per vessel A8 hours Test end: 19.9°C Test start, 8.7 – 8.8 mg/L (approx. 95.46, – 96.56% saturation) Fest end:
Temperature:	Frest end: 19.9°C
pH: 7 5 4	Test start: $\sqrt[4]{7.98} - 8.0$ Test start: $\sqrt[4]{7.98} - 8.0$ Test end: $A$ $\sqrt[4]{73} - 8.0$ $\sqrt[6]{6}$ h light : 8 h dark $\sqrt[6]{7}$
Photoperiod:	Test end: $A$
B. Study Design	passess the acute toxicity of KWG 4168 to the water flea Daphnia magna
This study was conducted to	assess the acute toxienty of KWG 4168 to the water flea Daphnia magna
over 48 nours I ne design of	the study was based on the results of a preliminary non-GLP test.

over 48 hours 1 he design of the study way based on the results of a preliminary non-GLP test. First instar *Daphnia magna* were used in the test from an in-house culture, aged 6 to 24 hours. First instar daphnids were separated from older daphnid by sequential mesh screening.

Test vessels were 100-mL beakers commining 50 mL test solution, covered with a plexi glass plate. Beakers were held in a climatic chamber for 49 hours at  $20 \pm 1^{\circ}$ C under a 16 hours light to 8 hours dark photoperiod.

Nominal concentrations of the test substance were prepared by dilution of stock solutions. Stock solutions bad been treated in an ultrasonic bath for five minutes and stirred using a magnetic stirrer for 10 minutes. Nominal concentrations were 0.32, 1.00, 1.78, 3.16, 5.62, 10.0 and 31.6 mg a.s./L. Measured concentrations were 0.23, 0.60, 0.82, 2.2, 4.4, 8.3 and 22 mg a.s./L.

To each test concentration were added ten first instar *Daphnia magna* using a pipette. Three replicates were used per concentration.



After 24 and 48 hours, water fleas were assessed visually for survivors, *i.e.* animals with swimming movements within 15 seconds of gentle agitation of the test vessel, and any uncertainty was checked using a stereomicroscope.

Temperature, oxygen content and pH of the test water was determined using electronic measuring equipment. Temperature was determined at test end, and oxygen content and pH were determined both at test start and test end.

EC50 values were manually determined using probit analysis after the maximum-likelihood method

#### Analytical method

Samples of water were analysed using the validated malytical method 00252 MO01, report reference <u>M-008490-02-2</u> (see Doc MCA Section 4).

#### II. Results and Discussion

Validity criteria according to the OECD 202 guideling were met:

- Mortality/immobilisation in the control to not exceed 10% (actual: 0.4%)
- Dissolved oxygen concentration at test xermination to be >30 mg/L in all test vessels (actual: 8.6 to 8.8 mg/L)

Test concentrations were determined at test start in all test concentrations. Mean measured concentrations.

Stability analysis of three test concentrations confirmed that test concentrations remained approximately constant throughout the test, with 48-hour mean measured concentrations 85.0 to 18.2% of the 0-hour concentrations.

Nominal test 🖉	Measured	test conce	ntration (m	g als./L) 🏾 🔊		$\mathcal{A}$		
concentration	Teststart	( <b>0</b> hr) °	Ŵ,		Test end	48 hr)		
(mg a.s./L)	Rep 1	Rep 🏖	Mean	* % <b>0</b>	Rep 1 🖑	Rep 2	Mean	% of 0-
	$\mathbb{P}_{\mathbb{Q}}$			nominal				hr concs
0.32	0.225	0335	0.23	F.9 🔊	·0		-	-
1.00	0.610	<b>%</b> .590 👟	0.60	≫60.0 _{&amp;}	0,479	0.549	0.51	85.0
1.78	Q5724	0.92	0,82 ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	46.20	-		-	-
3.16	\$ <b>1</b> .99	2,20	2.2 💭	62.6	2.41	2.83	2.6	118.2
5.62 🖉	4.320	A39 ° Û	4.40	08.3 0	-		-	-
10.0 ^	8.19	08.50~	83	₹83.0	7.63	7.63	8.2	98.8
31.6	21.4 0	22.30	AZ Q	69,0	-		-	-
Mean 🖉	, Q	Q.		68.4				100.7
		· ¥0.05		$\cap$				

## Table CA 8.2.4.1/01 Measured concentrations of KW6 4168 during the test

LOD Limit of determination: 0.05 µg/L

The results of the measured concentrations taken at 48 hours confirm that the measured concentrations were within 20% of the initial measured concentration. Thus, the results of this study have been based on initial measured test concentrations.

After 48 hours expersure to KWG4168, Qumulative immobility of *Daphnia magna* was 0, 0, 0, 0, 3, 23, 73 and 100% in the control and 0.23, 0.60, 0.82, 2.2, 4.4, 8.3 and 22 mg a.s./L test concentrations, respectively.



Measured concentration		ative immo te after 24		Cumulat replicate	ive 0° ty (%) 48			
(mg a.s./L)	1	2	3	1	2	3	24 hours	48) Hours (5)
Control	0	0	0	0	0	0	0	0 . 5
0.23	0	0	0	0	0	0 1	0	
0.60	0	0	0	0	0	0%	0 3	Q × ×
0.82	0	0	0	0	0	Ø ^r	0	N O
2.2	0	0	0	0	1	<u>\$0</u>	00	3 🖉
4.4	1	1	1	1	3 a 🔍	3	510 Q	230
8.3	3	4	1	8-4	8 a 🖓	6 ^{ao}	§ 27 L	19
22	10 ^b	10 ^b	10 ^b	0 b	10%	Ø10 b 🖓	· 100°	©100 ©

 Table CA 8.2.4.1/01-2
 Immobility of Daphnia magna after 48-hr exposure to KWG 4168

b Animals observed in clusters at the water surface

The resulting NOEC and LOEC values after 48 hours exposure were 2 .2 and 4,4 mg a.9/ The 24- and 48-hour EC50 values were 2.3 and 6.1 mg/a.s./ respective confidence intervals of 7.8 to 11.2 and 1 to 7.2 mg/as./Ls

Table CA 8.2.4.1/01-3	Summary of endpoints afte	r 48-hour	exposure	tospiroxamine	S.
-----------------------	---------------------------	-----------	----------	---------------	----

		4	Sa ia-	Ro	· 💫			. (C.		
Endpoint	NOEC	a	LQEC	<i>w</i>			EC2	~Õ~	EC ₅₀	
mg a.s./L	2.2	Ź.	Å?¥∕	$\sim$	Ø <u>2</u> .9 K	Ĩ	4,1	0ř	<b>6</b> .1	
				Y L		Ş		Ô,		

#### Conclusion 🔊 III.

After 48 hours exposure to KWG 4168, the seute 48 hour SC 50 to Daphita magna was 6.1 mg a.s./L, with 95% confidence intervals of 5 to 7.2 mg as./L. The 48 four WOEC and LOEC were 2.2 and 4.4 mg a.s./L, respectively

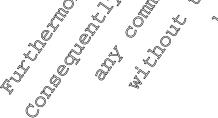
#### Assessment and conclusion by applicant

The study was conducted to the OECD test guideline 202 (1984), the post up-to-date version of which is the "Daphnia sp., acute immobilisation test" adopted 13 April 2004.

Validits criteria according to the most up-to-date OECD 202 guideline (2004) were met:

- Mortality/immobilisation in the Control to not exceed 10% (actual: 0.0%)
- Dissolver oxygen concentration at test termination to be  $\geq 3$  mg/L in all test vessels (actual: 8.6 to 8.8 mg (1)P

Only three replicates were tested instead of the recommended four replicates but groups of ten individuals, were tested instead of groups of five, thus, the total number of organisms tested was greater than the guide me recommendation but fever replicates were used. However, the results are





Data Point:	KCA 8.2.4.1/02
Report Author:	Q° 🏷
Report Year:	1996
Report Title:	Acute toxicity of 14-C-KWG 4168 (tech.) to water fleas (Daphnia magna)
Report No:	HBF/DM 148
Document No:	<u>M-006476-01-1</u>
Guideline(s) followed in	OECD-Guideline No. 202 'Guideline for Testing of Chemicals', 'Daphnia sp 🖉
study:	Acute immobilisation Test and Reproduction Test, Part I, Adopted April 984'
	and EPA FIFRA Guideline 72-2 of of of of of of of other
Deviations from current	Yes R A A A
test guideline:	OECD 202 (2004)
	Daphnids were 10/vessel instead of the recommended 5/vessel and only 3
	replicates instead of 4 and a second and a second and a second a second a second a second a second a second a s
Previous evaluation:	yes, evaluated and accepted of the transformed and accepted of the transformed of the transformed and the
GLP/Officially	Yes, conducted under GL®Officially recognised esting facilities
recognised testing	Yes, conducted under GLK@fficially recognised Esting facilities
facilities:	
Acceptability/Reliability:	Yes a way to way of a o
Executive Summary	

#### **Executive Summary**

The 48-hour acute toxicity of ¹⁴C-KWC 4168 to Dapania magna was studied under static conditions. Test species were exposed to nominal concentration of 0.56, 1.0 R8, 3.2, 5.6, 10 and 8 mg a.s./L and ¢, a control and solvent control. K, Õ Ø

Based on mean measured concentration with  $E_{50}(24 \text{ pours})$  was 10% mg as 1/L (5% confidence limits 8.9 - 12.7 mg a.s./L) and the EQ50 (48 hours) was 6.8 mg a.s./L (95% confidence limits 5.7 - 8.1 mg S  $\bigcirc$ Ś L, Ň a.s./L). O K)  $\bigcirc$ Ø

Based on mean measured concentrations, the no-observed-effect-concentration (NOEC) after 24 hours was 3.7 mg a.s./C and after 48 hours 2.1 mg a.s./L the lowest-observed effect-concentration (LOEC) after 24 hours was 6.5 mg a. O/L an Dafter 48 hours 3.7 nor a.s./ , Ø

I. Materials ar	nd Methods A A A	, de la companya de l
I. Materials an A. Materials		
		Labelled test substance
Test Material		¹⁴ C-KWG 4168
Lot/Batch/#: .		KML 2216
Active substance		NA
content: Specific Vadioactivity:	KW (24168) 10 00 00 00 00 00 00 00 00 00 00 00 00	4.3 MBq (116 µCi)/mg
Radiochenrical purity:	Nor Co Q	99%
Description	Colourless liquid	Not reported
Stability of test	Mean recoveries of 66.6 – 21.8%	Not reported
Reanalysis/Expiry date:	Not reported	Not reported
Density:	Not reported	Not reported



#### Treatments

Treatments	
Test rates:	Nominal: 0.56, 1.0, 1.8, 3.2, 5.6, 10 und 18 mg a.s./L
	Mean measured: 0.43, 0.68, 1.3, 2.1, 3.7, 6.5 and 19.7 mg a.s./L
Solvent/vehicle:	Nominal: 0.56, 1.0, 1.8, 3.2, 5.6, 10 und 18 mg a.s./L Mean measured: 0.43, 0.68, 1.3, 2.1, 3.7, 6.5 and 19.7 mg a.s./L Acetone Yes (mean measured concentrations 66.6 – 99.2% of nominal Daphnia magna < 24 hours old In-house culture, originally from the Bundes resundbeits and, Berlin, Germany This strain was manutained in the laboratory for more than ten years (2 litre containers), the water in which they are kept is changed weekly (dilution water, see below). The organisms were kept in an environmental chamber under the test conditions 20 ± 1° C, 16/: 8 hour light-dark sycle, the animals were fed with single cett green algae
Analysis of test	Yes (mean measured concentrations 66.6 – 99.2% of nominal
concentrations:	
Test organisms	
Species:	Daphnia magna < 24 hours old
Source:	In-house culture, originally from the Bundesgesundbeitsanit, Berlin,
	Germany
Acclimatisation	This strain was manitained in the laboratory for more than ten years (2
period:	(dilution water see below). The organisms were kept is changed weekby
	environmental chamber under the test conditions $20 \pm 4^{\circ}$ C, 16: 8 hour
	Scenedesmus subspicitus and occasionally some commercial ornamental fish food (TegraMing) (aquoous suspension)
Feeding:	Noted during the test
Tuestment for	Not row that a start of the sta
disease:	
Test design 🔬	Scenedesmus staspicatus and occasionally some commercial ornamental fish food (TetraMints) (aqueous sespension) Not fed during the test Not reported 100 mb beakers containing 50 mL test solution, covered with plexi glass
Test vessel:	100 mb beakers containing 0 ml@test solution_covered with plexi glass
	plates
Test medrum:	MJ7-medaim & J D J
Replication:	Not reported Not reported 100 mb beakers containing 50 mL/test solution, covered with plexi glass plates M7-medium Three per concentration Tes 48-hours $20 \neq 1^{\circ}C$ 3,1 to $86$ mg/L = 99%) 7.9206 8.28 He'h light': 8 h dark at 700 lux
No. of	TO S S J O
animals/vessel	
Duration of test:	48-hours 2 2 2
Environmental test	
conditions	
Température:	
Dissolved oxygen:	1  to  6  mg/ (8.6  mg/ L = 99%)
pH:	³⁷ 7.9200 8.28 ³
Photopersiod:	20 $\neq$ 1°C $=$ 99%) 3.1 to 8.6 mg/L = 99%) 7.92 to 8.28 16 h light : 8 h dark at 700 lux
B. Study Design	
The even wine where	$x = \frac{140}{100} \text{ (Logh)}$ was conducted for 40 hours under static

The exposure of Daphnia mona to ¹⁴C-KWG 4168 (tech.) was conducted for 48-hours under static conditions in order to assess the acute toxicity of the active substance. The test consisted of 100 mL glass beakers (DIN 12332), labelled with study number, concentration and series number. Each test vessel contained 50 mL of the test solutions with ten animals per vessel, three replicates per concentration. The beakers were covered with a plexi glass plate and placed in a environmental chamber for 48 hours at  $20 \pm 1$  °C and a 16:8 light-dark cycle. Light intensity



has been measured with a LMT "pocket-lux", type PO 449, and was about 700 lux. The water fleas were not fed and the test solutions were not aerated during the test.  $Q_{\mu}^{\circ}$ 

Test concentrations were chosen based on the results of earlier acute toxicity tests. *Daphnia magna* (<24) hours old) were used in the test from an in-house culture.

Test vessels were held under a 16-hour light to 8-hour dark at 700 lux photoporiod at  $20\pm1^{\circ}$  C. The medium was deionised water reconstituted to M7-medium.

Ten Daphnia magna were introduced into each test versel, and there were three replicates concentration.

Nominal test concentrations were 0.56, 1.0, 1.8, 3.2, 5.6, 10 und 18 mg a.s./L along with a control and solvent control groups. Measured test concentration of the freshly prepared test medium gave recoveries of 66.6 to 99.2% of nominal, whereas, recoveries at 48 hours ranged from 66.7 to 12.8% of nominal.

Assessments of mortality and sub-lethal effects were made at 24 and 48 hours. Dophnia were taken as live when swimming movements were observed within approximately 15 seconds.

#### Analytical method

Samples of water were analysed using the validated analytical method 09252 N2001, report reference  $\underline{M-008490-02-2}$  (see Doc MCA Section 4)  $\xrightarrow{\sim}$   $\xrightarrow{\sim}$ 

#### II. Results and Discussion

Validity criteria according to the OECD 202 guideline were met:

- Mortality in the control and solvent control does not exceed 10% at the end of the test (actual = 0 and 0%)
- The dissolved exygen concentration at the end of the test should be  $\geq 3$  mg/L in control and test vessels (actual = 8.1 8.6 mg/L)

The study is therefore considered acceptable

The mean active substance contents analysed at the beginning and the end of the test were 66.2 to 109.5 % of the nominal concentrations (for an average 74.7%). Therefore the results of this test are reported as mean preasured concentrations.

Moreover, the stability analyses at the end of the study showed, that 87.3 % to 98.4 % of the active substance was detected as KWG 168 and WAK 6300 (KWG 4168-N-oxide), which is supposed to be in equilibrium with the active substance in water (for an average 92.9 %).

Table CA 8.2.4.1/02	-₽Anal	<b>Sed</b> concent	rations of ⁹	4 <b>C-KWG 4168 in</b> 1	test solutions at d	lay 0 according to
radioactively measurements (1443945.34 dpm/L = 19.3 mg/L						
	<i>R</i> a	N° Qi			_	
N						

Nomina	<u> </u>	Mean 🖉 🧹	Calculated	Calculated	Percent of
Concentrations	Radioactivity	ameasuzed ~~~	radioactivity	concentration	nominal
(mg a.s./L)	dpm/5mL	radioactivity	dpm/L	day 0	concentration
Control		dpm/5mL		(mg a.s./L)	
Control	^	<u>-</u> 6,20 Q	<dl< td=""><td>-</td><td>-</td></dl<>	-	-
Solvent control		-0.17	<dl< td=""><td>-</td><td>-</td></dl<>	-	-
0.56	221.5 0 ×	/ 161.19	32220	0.43	76.9
	397.5	266.10	53220	0.71	71.1
1.8 2 6	709.8	507.93	101586	1.4	75.4
3.2 5 Q	<b>2</b> 77.7°	836.50	167300	2.2	69.9
5.6	2214.	1437.77	287554	3.8	68.6
10 °O	3946.8	2491.03	498206	6.7	66.6
18	7098.5	6678.73	1335746	17.9	99.2

<DL Lower than detection limit (53.6 dpm)



#### Table CA 8.2.4.1/02-2 Analysed concentrations of ¹⁴C-KWG 4168 in test solutions after 48 hours according to radioactivity measurements (1443945.34 dpm/L = 19.3 mg/L)

Nominal	•	Mean	Calculated	Calculated	Percent of
Concentrations (mg a.s./L)	Radioactivity dpm/5mL	measured radioactivity dpm/5mL	radioactivity dpm/L	concentration day 0 (mg a.s./E)	nominal concentration
Control	-	2.4	<dl< td=""><td>- 2</td><td></td></dl<>	- 2	
Solvent control	-	0.73	<dl td="" 🔊<=""><td>- 2</td><td></td></dl>	- 2	
0.56	221.5	172.93	3458	0,46	82.6 Y O
1.0	397.5	274.17	54834	Ø.73 🔬	73 5 0
1.8	709.8	505.43	10/1086	1.4 °O	75.4
3.2	1277.7	825.30	€ \$65060	2.200	<b>4</b> 8.9
5.6	2214.7	1403.93	280786	-3.8°	67.0
10	3946.8	2495.80 🔬	4999160 🔊	6.7 🖉 🖉	66: 🖓 📣
18	7098.5	8203.63 O	1640726 Å	21.20 8	121.8

<DL Lower than detection limit (53.6 dpm)

# Table CA 8.2.4.1/02-3 Mean measured concentrations @¹⁴C-KWG 4008

Nominal	Calculated $O^{>}$	Corrected	Mean of day 0	% of nominal
concentrations	concentrations	<b>concentrations</b> 48		concentrations
(mg a.s./L)	day 0 🕺 🖉	hours 🖉 🖉	(mg <b>g.s</b> ./L)	U V
	(mg a.s./Ly	hours (mga.s./L		
0.56	0.43	10©#2 °	0,45 . 9	76.¥
1.0	0.71	0.64	0.68	<b>, 6</b> 7.6
1.8	1.4~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	1.2	N1.3 1.3	72.0
3.2	2.2 5 0	1.2		66.9
5.6	Q.8 0 ⁷ 2 ¹	$\frac{2.6}{3.6}$	3.9	66.2
10	46.7 x, 6 ² ~	×6.3 × ×	Q6.5	64.9
18	17.9	21.67		109.5
			Average: 🖉	74.7

The control mortalities were below the 10 % value which is regarded as the limit for natural mortality. As the physico-chemical measurements show, the composition of the test water corresponds to the nominal values. The  $BC_{50}$  of the reference substance lies within the required range. Thus, the study conditions correspond to the standard.

# conditions correspond to the standard.

Nominal conventrations (ng a.s./L)	Mean numbe animals after	n of living	Number of im (%) after:	mobilised animals
	24 hours	48 hours	24 hours	48 hours
Control ~	39	, <b>39</b> .	0	0
Solvent control	30 0 ~	≫30	0	0
0.43"	30 <b>30</b>	29	0	$3\pm 6$
0.68		30	0	0
1.3 0 2 2	×29 0,	28	$3\pm 6$	$7 \pm 12$
2.1 1 2.1	, 30 ∧Q	30	0	0
3.7 0 5 0 0	<i>2</i> )	26	$3\pm 6$	$13 \pm 15$
6.5 5 2 2 2	29 [1] [3]	18 [3] [2] [1]	$3\pm 6$	$40 \pm 20$
19.7	1 [1]	0	$97 \pm 6$	100

Number of living animals with symptoms, if observed Symptoms:

^[1] quide trembling antennae movements [2] frequency of antenna movements clearly increased

^[3] frequency of antenna movements clearly decreased



Endpoint	24-hours	48-hours	
NOEC	3.7 mg a.s./L	2.1 mg a.s./L	
LOEC	6.5 mg a.s./L	3.7 mg a.s./L	<u>6</u> , 10,
EC ₅₀	10.6 mg a.s./L	6.8 mg a.s.L	
			8

Table CA 8.2.4.1/02-5	Summary of endpoints after 48-hour exposure to spiroxamine
	Summary of enupoints after to nour exposure to spiroxumme

#### III. Conclusion

Based on mean measured test concentrations, the EC₅₀ (24 hours) was 19.6 mg a.s./L 495% confidence limits 8.9 - 12.7 mg a.s./L) and the EC₅₀ (48 hours) was 6.8 mg a.s./L 495% confidence limits 5.748.1 mg a.s./L).

Based on mean measured concentrations, the no-observed-effect-concentration (NOEC) after 24 hours was 3.7 mg a.s./L and after 48 hours 2.1 mg a.s./L, the lowest observed-effect-concentration (LOEC) after 24 hours was 6.5 mg a.s./L and after 48 hours 3 Jang a.s./L.

#### Assessment and conclusion by applicant:

The study was conducted to the OECDitest grideline 202, the most up-to-date version of which was adopted 13 April 2004

Validity criteria according to the QFCD 292 guideline (2004) were met.

- Mortality in the control and softent control does not exceed 10% at the end of the test (actual = 0 and 0%)
- The dissolved oxygen concentration at the end of the test should be 3 mg/L in control and test vessels (actual = 8. -8.6 mg/L)

Only three replicates were tested instead of the recommended four replicates but groups of ten individuals were tested instead of groups of five. Thus, the total number of organisms tested was greater than the guideline recommendation but fewer replicates were used. However, the results are still considered to be sufficiently reliable for use in the risk assessment.

The study is therefore considered acceptable.

The 48-hour EC5	was determined	to be 6.8	ing a.s./

Data Point:	KCA 8,2,4.1/030 × ×
Report Author:	
Report Year:	1999 6 7 6 8
Report Titles	Acute toxicity of I4C-KWG 4168 (tech.) to water fleas (Daphnia magna) under
	flow-through test conditions
Report Nor Co	HBF DM 184 N N
Document No:	<u>M-006523-01-1</u>
Guideline(s) followed in	DECD-Quidelige No. 202 "OECD-Guideline for Testing Chemicals", 4 April
	1984 Daphnia spec Acute Immobilisation Test and Reproduction Test
Deviations from current	Yest
test guideline	QECD 202 (2004)
	Top concentration was not in solution, however, it was excluded from statistical
	analysis. Therefore, this had no impact on the study
Previous evaluation:	yesDevaluated and accepted
GLEOfficially	DAR (1997), RAR (2010), RAR (2017)
GLR Officially	Yes, conducted under GLP/Officially recognised testing facilities
recognised testing	
facilitie	
Acceptability/Reliability:	Yes

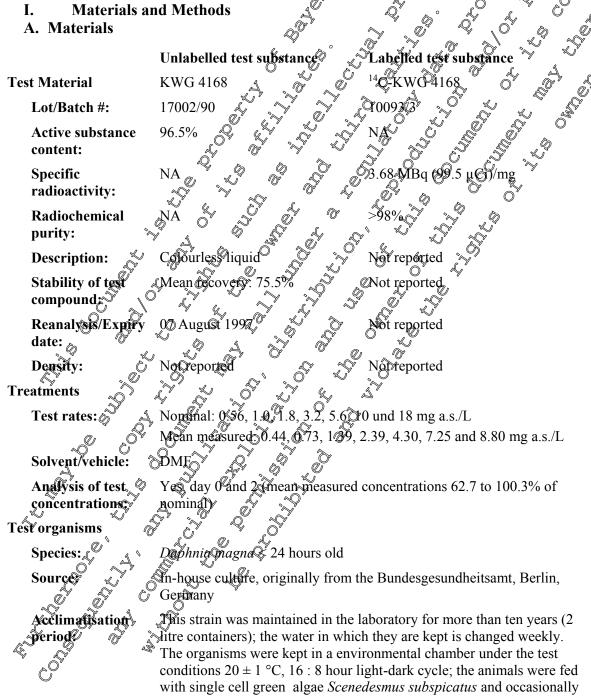


#### **Executive Summary**

The 48-hour acute toxicity of ¹⁴C-KWG 4168 to *Daphnia magna* was studied under flow-through conditions. Test organisms were exposed to nominal concentrations of 0.56, 1.0, 1.8, 3.2, 5.6, 10 and 18 mg a.s./L and a control and solvent control.

Based upon the mean measured concentrations, the EC₅₀ (24 hours) for ¹⁴C-KWG 4168 (tech.) was 3.8 mg a.s./L (95% confidence limits 3.4 - 4.3 mg a.s./L) and the EC₅₀ (48 hours) was 3.0 mg a.s./L (95% confidence limits 3.1 to 4.4 mg a.s./L).

Based upon mean measured concentrations, the no-observed-effect-concentration (NOEC) obter 24 and 48 hours was 1.4 mg a.s./L, the lowest-observed-effect-concentration (LOEC) was 2.4 mg a.s./L





	some commercial ornamental fish food (TetraMin®) (aqueous suspension)
Feeding:	Not fed during the test
Treatment for disease:	some commercial official first food (retraining) (aqueous suspension) Not fed during the test Not reported 100 mL beakers containing 50 mL test solution, covered with plexi glass
Test design	
Test vessel:	100 mL beakers containing 50 mL test solution, covered with plexi glass plates M7-medium Four per concentration
Test medium:	M7-medium
<b>Replication:</b>	M/-medium Four per concentration
No. of animals/vessel:	Ten $\begin{pmatrix} & & & & \\ & & & & \\ & & & & \\ & & & & $
<b>Duration of test:</b>	48-hours $\sqrt{2}$ $\sqrt{2}$ $\sqrt{2}$ $\sqrt{2}$ $\sqrt{2}$ $\sqrt{2}$ $\sqrt{2}$
Environmental test conditions	100 mL beakers containing 50 mL test solution, covered with plexi glass M7-medium Four per concentration Ten 48-hours $20 \pm 1^{\circ}$ 8.700 8.9 mg/L (8.7 mg/L = 96%) 7.9 to 8 Y 16 hAlight ; 8 h dark at ~700 lux 5 magna to ¹⁴ C-KWG 4168 thech twas conducted for 48-hours under flow-
<b>Temperature:</b>	$20 \pm 1^{4} \mathcal{C} \qquad \qquad$
Dissolved oxygen:	8.700 8.9 mg/L (8.7 mg/ $D = 96\%$ )
pH:	7.9 to 84 5 0 0 1 5 0 0
Photoperiod:	$\sqrt[3]{16}$ by light ; 8 h dark at $\sim$ 700 lux $\sqrt[3]{16}$ , $\sqrt[3]{16}$ , $\sqrt[3]{16}$
B. Study Design	$\frac{16 \text{ h}}{5}$ h dark at ~700 lux $5^{\circ}$ $5^{\circ}$ $5^{\circ}$
B. Study Design The exposure of Baphnic through conditions in order	magny to ¹⁴ CKWG 4168 tech was conducted for 48-hours under flow- er to assess the acute toxicity of the active substance.

The test vessels consisted of 00 mL glass beakers, labelted with study number, concentration and series number. Each test vessel, contained 50 mL of the test solutions with ten animals per vessel, four replicates per concentration. The beakers were covered with a plexi glass plate and placed in a environmental chamber for 48 hours at 20 ft i °C and a 16.8 light-dark cycle. Light intensity was about 700 lux. The water peak were not ped and the test solutions were not aerated during the test.

Test concentrations were chosen based upon historical teacity information for the active substance. Daphnia maged (<24 Plours old) were used in the jest from an in-house culture.

Ten Daphria magna were introduced into each tesevessel, and there were four replicates per test concentration.

Nominal test concentrations were 0.56, 10, 1.8, 3.2, 5.6, 10 and 18 mg a.s./L along with a control and solvent control groups. Ateasured test concentration of the test medium gave recoveries of 62.7 to 100.3% of nominal.

Assessments of mortanity and sub-lethal effects were made at 24 and 48 hours. *Daphnia* were taken as live when wimming movements were observed within approximately 15 seconds.

#### Analytical method

Samples of water were analysed using the validated analytical method 00252 M001, report reference  $M_{008490}^{-00252}$  (see Doc MCA Section 4).

## II. Results and Discussion

Validity criteria according to the OECD 202 guideline were met:



- Mortality in the control and solvent control does not exceed 10% at the end of the test (actual = 2.5 and 2.5%)
- The dissolved oxygen concentration at the end of the test should be  $\geq 3 \text{ mg/L}$  in control and test vessels (actual = 8.7 - 8.9 mg/L)

The study is therefore, considered acceptable.

mina Measured concentrations of ¹⁴C-KWG 4168 were 73 to 78 % (for an average of 75.5 concentrations.

Nominal		Measured Radioactivity (dpm/10 mL) & A &						
Concentrations (mg a.s./L)	Radioactivity dpm/10mL	Day 0 (average)	Day 1 (aværage)	Bay 2 (average)	Percept of nominal concentration			
Control	-	9.1	9.1	7.80	h' a' 4			
Solvent control	-	10.3 5	7.9	<u>816</u>	- & _			
0.56	200	199.1	206.4	\$196.4 ~	100,3			
1.0	200	1830	185.9 × V	184.0 0	9 <b>2</b> .1 O			
1.8	200	199.8	192.3	1907	95.8 0			
3.2	200	1886	180.0	199.3 D A	91.3			
5.6	200	197.2	105.3	187.2	26.3			
10	200	1904	1997.1 🗸 🎊	180,1 0	\$4.6			
18	200	\$33.2 × 4	118.7, 🗸	124.6	62.7			

Table CA 8.2.4.1/03-1 Measured radioactivity in the test solutions

Detection limit (50@ cpm, Corresponding 15/55.5 dpm) LOD

The control mortalities, were 2,5% which was well below the 10% value which is regarded as the limit for natural mortality as the physice chemical measurements show, the composition of the test water corresponds to the bominal values. The EC50 of the reference substance (K2Cr2O7) lies within the required range. Thus, the study conditions and culture health correspond to the standard.

## Table CA 8.2.41/03-2 Water lea toxicity of 4C-KWG 4168 (tech) using Daphnia magna

<u> </u>			"0"	<u>n</u>	
Mean measured	concentration	Mean number o	of <b>h</b> iving 🚫 🤺	Ámmobilised or d	ead water fleas
(mg a.s.∕Ľ)	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	animals after:	0' ^w v	(%) after:	
		🖓 4 hours 🔬	48 kours 🔊	24 hours	48 hours
Control	<del>o A E</del>	×40 × ×	39 ⁰ 🗞	0	$2.5 \pm 5.0$
Solvent control		400		0	$2.5 \pm 5.0$
0.44		×40 ~ ~ ~	040 °	0	0
0.73		X40 Q Q	37 0	0	$7.5 \pm 9.6$
1.4		38 7 2	325	0	$7.5 \pm 9.6$
<u>1.4</u> 2.4	N Q	36 ^{[1] 6,4}	376	$5.0 \pm 5.8$	$15.0 \pm 5.8$
4.3 🔬 🖉	S A	20 [3] [9] [176,4,7,8	9 [3] [1] [3] 7,8	$10.0 \pm 8.2$	$77.5 \pm 22.2$
7.3			0	100	100
8.8**	<u> </u>	00 2	0	100	100

[] Number of living animal with symptoms if observed

These concentrations were used to calculate the EC50

** In this concentration on oily haver of the test substance was observed on the water surface ^[5] swimming movements show coordination

Symptons:

[1] quick trempting antennae novements

^[2] frequency of antenna movements clearly increased

^[3] frequency of antenna movements clearly decreased

^[4] hardly any movements perceivable

^[6] animals lie at the bottom

disturbances

^[7] animals cling to the water surface

^[8] animals cling together in clusters

The highest test concentration (8.8 mg a.s./L) was observed to have an oily layer on the water surface. The KWG 4168 was not soluble at this concentration. Therefore, the highest concentration was excluded from statistical calculations.



Endpoint	24-hours	48-hours	
NOEC	1.4 mg a.s./L	1.4 mg a.s./L	
LOEC	2.4 mg a.s./L	2.4 mg a.s.(L	<u>6</u> 7 10 ⁷
EC ₅₀	3.8 mg a.s./L	3.0 mg a.s.s.	

Table CA 8.2.4.1/03-3	Summary of endpoints after 4	48-hour exposure to spiroxamine

#### III. Conclusion

Based upon the mean measured concentrations, the EC₅₀ (24 hours) for  $\frac{1}{10}$ -KWG 4168 (tech.) was 3.8 mg a.s./L (95% confidence limits 3.4 - 4.3 mg a.s./L) and the EC₅₀ (48 fours) was 3.0 mg a.s./L (95% confidence limits 3.1 to 4.4 mg a.s./L).

Based upon mean measured concentrations, the no-observed-effect concentration (NOEC) after 24 and 48 hours was 1.4 mg a.s./L, the lowest-observed offect-concentration (COEC) was 2.0 mg as./L.

Assessment and conclusion by applicant: The study was conducted to the OECD test guideline 202, the most up-to-date version of which was adopted 13 April 2004
The study was conducted to the OECD test guideline 202, the most up to-date version of which was
adopted 13 April 2004. $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + 1)^{2}$ $(1 + $
<ul> <li>adopted 13 April 2004.</li> <li>Validity criteria according to the OECD 202 guideline (2004) were met</li> <li>Mortality in the control and solvent control does not exceed 10% at the end of the test (actual = 2.5 and 2.5%)</li> </ul>
• Mortality in the control and solvent control does not exceed 10% at the end of the test (actual
• The dissolved oxygen concentration at the end of the test should be >> mg/L in control and
test vessels (actual $\approx 8.7 - 8.9 \text{ mg/L})$
The study is therefore condidere Discount the
The study is therefore considered acceptable.
The 48-hour EC ₅₀ was determined to be 3.00mg as L.
Metabolites
<u>KWG 4168-N-ocide (M03)</u> $\sqrt{2}$ $\sqrt{2}$ $\sqrt{2}$ $\sqrt{2}$ $\sqrt{2}$
Data Point:
Report Author:
Report Vear: 1998
Report Title: Orientating waterflea texicity of N-oxide-KWG 4168
Report No: A HBF ODM 102 A
Document No: $M_{\pm}$
Guideline(s) followed in GECD-Guideline No. 202
study: ² ² ² ² ² ² ²
Deviations from current, test guideline:
Previous evaluation, S Acs, evaluated and accepted
⁽¹⁾ (1) (1) (1) (1) (1) (1) (1) (1) (1) (1)
When compared to respective data on the active substance spiroxamine, the
KWG-4rG-N-Oxde is definitely less toxic. KWG 4168-N-oxide (WAK 6301) is
CL D/Officially irrelevant.
GLP/Officially recognised testing facilities
facilities. A A

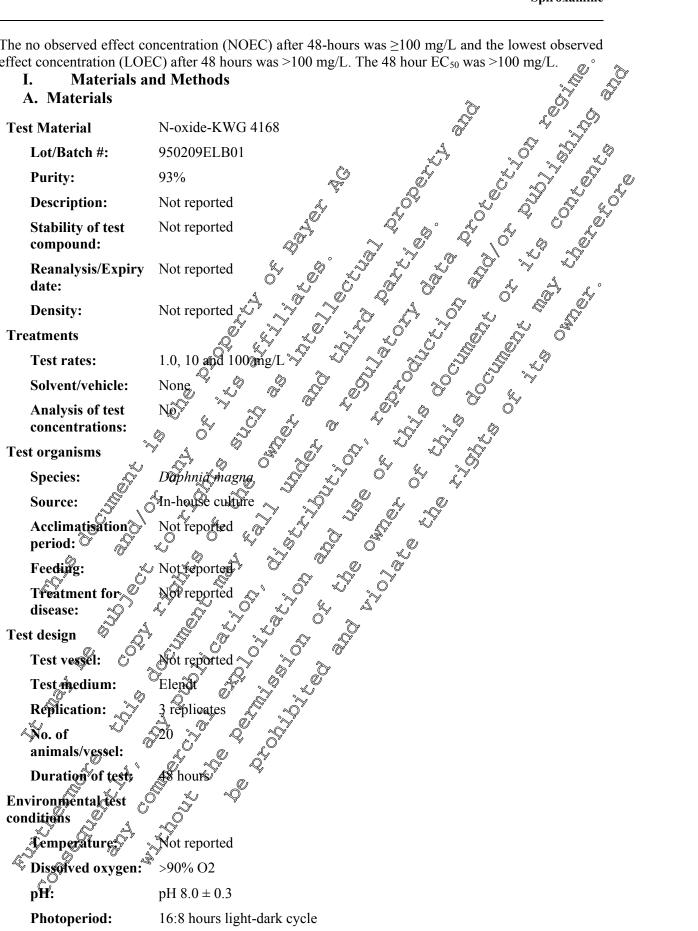
Acceptability Reliability: Supportive only

## Executive Summary

A non-GLP 48-hours acute toxicity test to *Daphnia magna* with KWG 4168-N-Oxide was conducted.



The no observed effect concentration (NOEC) after 48-hours was  $\geq 100 \text{ mg/L}$  and the lowest observed effect concentration (LOEC) after 48 hours was >100 mg/L. The 48 hour EC₅₀ was >100 mg/L.





100

#### B. Study Design

The test was conducted according to the OECD Test Guideline 202. Test water (synthetic test water according to ELENDT, >90% O2, pH 8.0  $\pm$  0.3) and test substance dispersions were prepared at the desired concentrations. 50 mL dispersion each were filled into a 100 mL beaker with three beakers per concentration. Each beaker was stocked with 20 young Daphnia magna. After 48 hours exposure at 20 °C and 16:8 hours light-dark cycle the dead animals were counted. 

#### II. Results

Table CA 8.2.4.1/04-1    Survival of the pare	ent water fleas	Ő	<b>\$</b>	
Concentration (mg/L)	Morta	ality (%)́∛	s á	8
Control		× .	ذ V	, 10
1.0	( 10°		, ⁽ )	Ĩ)
10	o V	\$ \$		

#### III. Conclusion

100 mg/L and the lowest observed The no observed effect concentration (NOEC) after 48 effect concentration (LOEC) after As hours was >100 mg/I The 48 -bour ECG wa ۵100 mg/L

#### Assessment and conclusion by applicant:

C The study was conducted to the QECD test guideline 202. The results have been re-assessed to the current test guideline for *Daphnia magna* acute toxicity tests OECD 202 (adopted; 13 April 2004)

Validity criteria according to the OEGD 200 guideline (2004) were met:

- In the control including the control containing the solubilising agent, not more than 10 percent of the daphnids should have been in mobilized (actual: 0%);
- The dissolved oxygen concentration at the end of the test should be  $\geq 3 \text{ mg/L}$  in control and test orssels

The test would appear to have met the validity criteria but as insufficient test details are available in the report and because no analytical verification of test, concentrations was conducted, the study is therefore considered to be supporting information only.

A new GLP acute Daphnia study has not been conducted using KWG 4168-N-Oxide (M03) as this was not considered to be necessary. Available study data for the parent material clearly demonstrate that algae are the most sensitive organism group by at least two orders of magnitude therefore acute metabolite studies with quatic invertorates are no considered necessary. Furthermore, available algal data show that this metabolite is far less foxic than the parent material. Thus, this non-GLP study is considered sufficient to demonstrate the low toxicity of KWG 4168-N-Oxide (M03) to aquatic invertebrates.

468-N Oxide (M03) was determined to be >100 mg/L. The 48-hour EC 50 for KWG

#### Acute Exicity to a additional aquatic invertebrate species CA 8.2.4.2

No acute data with an additional aquatic invertebrate species are available. Spiroxamine is a fungicide and does not display insection dal activity therefore acute data with an additional species of aquatic invertebrate are not required.



#### CA 8.2.5 Long-term and chronic toxicity to aquatic invertebrates

#### CA 8.2.5.1 Reproductive and development toxicity to Daphnia magna

Data Point:	KCA 8.2.5.1/01
Report Author:	
Report Year:	1994
Report Title:	Influence of KWG 4168 (techn.) of the reproduction rate of water fleas
Report No:	
Document No:	<u>M-006401-01-1</u>
Guideline(s) followed in	EEC XI/681/86 (1987)
study:	EEC XI/681/86 (1987)       OECD 202 (II) (1984), pow OECD 211 (2012)
Deviations from current	
test guideline:	Yes OECD 211 (2012) No analysis of aged test media. Only fresh test media was sampled
	No analysis of aged test media. Only fresh lest media was sampled a sub-
Previous evaluation:	yes, evaluated and accepted share a star way a
	yes, evaluated and accepted DAR (1997), RAR (2010), RAR (2017)
GLP/Officially	Yes, conducted under GLP/Officially recognised testing facilities
recognised testing	
facilities:	
Acceptability/Reliability:	Yes y y y y y
Executive Summarv	

#### utive Summary

The 21-day chronic toxicity of KWG 4168 to Daphnia magna was studied under semi-static conditions. Test organisms were exposed to nominal test concentrations of 0.032, 0.10, 0.48, 0.32, 0.56, 1.0 and 3.2 mg a.s./L and a control. A delay an time to first brood was observed at concentrations ≥0.32 mg a.s./L. The most sensitive endpoint was reproduction. The 21-day NOEC based on reproduction was 0.10 mg a.s./L.

```
Materials and Methods
  I.
  A. Materials
Test Material
   Lot/Batch #:
    Purity:
                                                   · and
   Description
                            olourless liqui
   Stabilit of test
                                                   5% of initial concentrations in parallel
                           Mean recover
    compound:
                             ot reporte
    Reamalysis/E
   date:
    Density
                           Vot reporte
Treatment
                    Nominal: 9.032, 0.10, 0.18, 0.32, 0.56, 1.0 and 3.2 mg a.s./L
                          None
    Sólvent
            cehicle
               test
                           Yes (mean measured concentrations 76 – 122% of nominal)
    concentrations:
Test brganisms
   Species:
                          Daphnia magna, approx. 6 – 24 h old
```



Source:	In-house culture, originally from the Bundesgesundheitsamt, Berlin, Germany
Acclimatisation period:	None reported
Feeding:	0.2 mg TOC-content green alga
Treatment for disease:	None reported
Test design	
Test vessel:	100-mL beakers containing 50 mL dest solution covered with plexi
Test medium:	M7-medium & g° 5° 4° 4° 4° 4° 4°
<b>Replication:</b>	Ten replicates
No. of animals/vessel:	Individually held
Duration of test:	21 days $\mathcal{F}$ $\mathcal{F}$ $\mathcal{F}$ $\mathcal{F}$ $\mathcal{F}$ $\mathcal{F}$ $\mathcal{F}$ $\mathcal{F}$
Environmental test conditions	
Temperature:	19.6 ⁴ /20.3 ²
Dissolved oxygen: 📎	Fresh:
	In-house culture, originally from the Bundesgesundheitsamt, Berlin, Germany None reported 0.2 mg TOC-content green alga None reported 100-mL beakers containing 50 mL test solution, covered with plexi glass plates M7-medium Ten replicates Individually held 21 day 19.6 - 20.3 Fresh: 87 - 949 mg/L (approx. 94:89 - 109:5% saturation) Speff: 80 9.3 tog/L (approx. 94:89 - 109:5% saturation) Speff: 80 9.3 tog/L (approx. 94:89 - 109:5% saturation) Speff: 81 0 - 8.15 Speft: 88 - 618 16 b Hight: 9 h dack at 1000 lux
pH:	Fresh, $8.00 - 8.15$ , $7$ , $7$ , $7$ , $7$ , $7$ , $7$ , $7$ , $7$
	Spent: 7 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
Photoperiod	$16 \text{ hIght} : \otimes \text{ h dark at 1600 lux}$
B. Study Design	

This study was conducted in order to assess the reproductive toxicity of KWG 4168 to the water flea *Daphnia magna* under semi-static conditions. Test concentrations were chosen based on the results of earlier acute toxicity tests.

First instar *Daphnia magna* were used in the test from an in-house culture, aged 6 to 24 hours. First instar daphnids were separated from older daphnids by sequential mesh screening. Test *Daphnia* were the young of an approximately 21-day old synchronous culture.

Test vessels were 100-mL glass beakers containing 50 mL test solution and covered with plexi glass plates. These were held order a 16-hour light to 8-hour dark 1000 lux photoperiod at  $20 \pm 1^{\circ}$ C. The test medium was deponised water reconstituted to M7-medium.

One tomale *Daphma magna* was introduced into each test vessel, and there were ten replicates per test concentration. Daphnids were transferred three times per week to freshly prepared test medium using a pipette and fed the green alga (*Scenedemus subspicatus*) to a quantity of 0.2 mg TOC-content.



Nominal test concentrations were 0.032, 0.10, 0.18, 0.32, 0.56, 1.0 and 3.2 mg a.s./L along with a control. Measured test concentration of the freshly prepared test medium gave recoveries of 76 to 122% of nominal.

Assessments of mortality and reproduction were made thrice weekly, at each test medium pansfer. *Daphnia* were taken as dead when no swimming and/or antennae movements were observed within 15 seconds. Body length of the adult daphnids was determined at the end of the 21-day test. This was done by measuring from the head to the base of the spine using a binocular microscope with an expine expine graticule.

Temperature was measured in one vessel of the control

#### Analytical method

Samples of water were analysed using the validated analytical method 00252 M00 report reference M-008490-02-2 (see Doc MCA Section 4).

#### II. Results and Discussion

Validity criteria according to the test guideline were me

- Mortality of the parent animals in the control to not exceed 20% at the and of the test. (actual: 0%)
- Mean number of living offspring produced per parentonimal surviving at the end of the test ≥60 (mean control value: 943)

Analytical results, corrected by the recovery rates, show measured test concentrations 76 to 122% of nominal. The stability of the active substance was ascertained with two parallel studies, with mean recoveries of 51 to 75% of initial concentrations. The results have been presented based on nominal concentrations.

			· 🖌 🏑				
Nominal test	Analysed c	oncentration	is (mg a.s./L)				Mean % of
concentration	Day 2 🐇	% of	Day 9	% of nominal	Day 16	% of	nominal
(mg a.s./Ļ)	*	nominal 🎢		nominal	. °	nominal	
Contro	<lodo ,<="" td=""><td>F S</td><td><lod< td=""><td>¥- 2,5 ,</td><td>LOD</td><td>-</td><td>-</td></lod<></td></lodo>	F S	<lod< td=""><td>¥- 2,5 ,</td><td>LOD</td><td>-</td><td>-</td></lod<>	¥- 2,5 ,	LOD	-	-
0.032	0.063	×195 ×	<b>0</b> .026	82, Á O &	0.029	90	122
0.10	<b>9</b> .125			× 65	0.076	76	89
0.18	0.154	^{C85}	~0,128 ~~ 0		0.128	71	76
0.32	0.321		0.333	J04	0.308	96	100
0.56	0.615	110		108	0.679	121	113
1.0 >	0.935	97,~~ ~	©0.91	92	0.826	83	91
3.2	2.91	Ø J	2 @ "	91	2.91	91	91
Mean 🖉	**	115 ~	<u>0</u>	88	-	90	97
LOD Limito	fedetection 0	1	ý				

## Table CA 8.2.5.1/01-1 Measured concentrations of KWG 4168 during the test

LOD Limit of detection: 0.1 10/L

No motiality of daphnids was observed in the control, 0.032, 0.10, 0.18, 0.32 and 1.0 mg a.s./L test concentrations after 21 days. When exposed to 0.56 and 3.2 mg a.s./L, mortality of 10 and 60% was observed, respectively. Compared to the control, the number of offspring produced was significantly reduced p < 0.05) at test concentrations of 0.18, 0.32, 0.56, 1.0 and 3.2 mg a.s./L.



1.0

3.2

0

0

0

0

0

0

At test termination, parent animals in the control had a body length of 4.34 mm indicating good female development. Adult body length was significantly reduced (p<0.05) at test concentrations of 1.0 and 3.2 mg a.s./L.

Nominal	Cumu	lative m	ortality (%	6) by day				100	~ í	
concentration (mg a.s./L)	0	2	5	7	9	12		. 16	196 ³	
Control	0	0	0	0	0	0	00	0 (		
0.032	0	0	0	0	0 ,	0	R	0 _ @	0 3	
0.10	0	0	0	0	0	0	0	0 0	0 🖓	۵° ۵
0.18	0	0	0	0	ćθ,	0 🖓	°0 ©°	05	A)	$9$ $\checkmark$
0.32	0	0	0	0	0	0 🕎	<u>_0</u> @*	°™ \		0
0.56	0	0	0	0 6	0 🔊	0		10 🔊	10~	10

	Table CA 8.2.5.1/01-2	Mortality of <i>Daphnia magna</i> over 21-day exposure to KWQ4168	
--	-----------------------	-------------------------------------------------------------------	--

A delay in time to first brood was observed at test concentrations 0.32, 0.56 and 1.0 kmg a s/L, with first brood observed 2, 2 and 7 days later than in the control. No offspring were observed at any time in the highest test concentration, 3.2 mg a, 0.2 mg as 1.0 mg mg s 1.0 mg mg

**≇**0

40

×60

Table CA 8.2.5.1/01-3	Number of off	spring of Day	hniq nagna over	· 21-day	exposure to KWG 4168

 $0 \bigcirc$ 

0

				~~	0			d( ))	0	- Nor	<u> </u>	
Nominal	Numb	oer of o	ffspring	g by da		- <del>O</del> Ž	~	Û,	Ô		$\bigcirc^{\nu}$	
concentration	0	2	້5 🖇	7 🔍	بر ©_	12 🦷	r∕14 '	×16	M19 🔬	21 ₍₎	<b>Total</b>	% of
(mg a.s./L)			O	20	, C	al a		J.		Ś	ų.	nominal
Control	0	0 *	Ø.	0	AS .	<u>1012</u>	267	57	186	29	943	-
0.032	0			JO	Ŭ "	938 J	<b>≈2</b> 69	<u> 2</u> 5	\$193	2×291	916	97
0.10	0	0	0 🗇	0	10 🔊	138	182	48 (	217 6	¥254	849	90
0.18	0	.00%	0~	0	0	45Q	200	0 🎸	22	244	724	77
0.32	Ô,	Q	<u>م</u>	¢0	$0^{\prime}$	Û, ^y	197	,5¥	230	207	646	69
0.56	0	0 C	0 0	)0 ((		<u>6</u>	38 🛓	ðð.	155	211	404	48
1.0	0 0	$0 \ll$	0	0 4	0,%	0 🔊	0 C	5 🔬	20	52	77	8
3.2	0	<b>X</b>	Ø	0	00	0	00	QO	0	0	0	0
			N 7			18	A 8					

The mean body length of surviving adults was 1020, 101.3, 100.6, 102.2, 101.7, 92.3 and 44.7% of the control at test conceptrations 0.032, 0.10 0.18, 0.32, 0.56, 1.0 and 3.2 mg a.s./L.

# Table CA 8.2.5.1/01-4 Rody length of Surviving adult Daphina magna after 21-day exposure to KWG

~~~		$0 \sim 0$						
Nominal 🔬	Control [《]	0.03	A .10 🖉	0.18	0.32	0.56	1.0	3.2
concentration		Â,						
(mg a.ś.)L)				~Q′				
Number	106,5	\$10 . ?		<i>710</i>	10	9	10	4
Mean (mm)	4.34 0	4.43	4.40 O	4.38	4.44	4.42	4.01	1.94
SD	20.100	0.068	0.105	0.124	0.132	0.203	0.211	0.165
CV (%)	2.31	£.53 √	2.42 📎	2.83	2.97	4.6	5.28	8.50
% of control	-~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	\$102 . 0	JO1.3	100.6	102.2	101.7	92.3	44.7

SD: Standard Destation; CV: Coefficient of Variation

The resulting NOEC and LOEC values based on reproduction after 21 days exposure were 0.10 and 0.18 mg a.s. U, respectively.

Table GA 8.2.5.1/01-5 Effects of spiroxamine exposure on the juvenile growth phase of the F₀ generation

Endpoint	NOEC	LOEC
mg a.s./L	0.10	0.18



III. Conclusion

The 21-day chronic toxicity of KWG 4168 to *Daphnia magna* was studied under semi-static conditions. A delay in time to first brood was observed at concentrations ≥ 0.32 mg a.s./L. The most sensitive endpoint was reproduction. The 21-day NOEC based on reproduction was 0.10 mg a.s./L.

Assessment and conclusion by applicant:

The study was conducted to the OECD test guideline 202 (II), the most up to-date version of which is the OECD 211 "Daphnia magna reproduction test", acopted 02 October 2012.

Validity criteria according to the OECD 211 guideline (2012) were net:

- Mortality of the parent animals in the control to not exceed 20% at the end of the test: (actuals 0%)
- Mean number of living offspring produced per parentanimal surviving at the end of the test ≥60 (mean control value: 94.3)

It is noted that analytical samples were only taken on freshly prepared test media on three occasions during the study. No samples of old test media were taken for analysis but the report does reference other parallel studies in which the stability of spiroxamine in test conditions has been demonstrated. The results of this study have been based on nominal concentrations which may therefore overestimate the concentrations that were achieved in the test, thereby potentially over-estimating the NOEC value.

The study is considered acceptable but the results should be treated with caution due to the lack of chemical analysis for the old test media. Additional Daphnia reproduction studies using Spiroxamine are available and have determined slightly more conservative NOEC values than this study therefore the results of this study have not been used directly in the lisk assessment.

The data have been subjected to statistical re-evaluation and the results have been presented in the following study summary.

Data Point KCA82.5.104
Report Author:
Report Year: $\sqrt{2}$ $\sqrt{20}$ $\sqrt{2}$ $\sqrt{2}$ $\sqrt{2}$
Report Title: Calculation of EC10 and EC20 values for Daphnia magna with spiroxamine TG
Report No: \mathcal{O}^{\vee} \mathcal{O}^{\vee} \mathcal{O}^{\vee} \mathcal{O}^{\vee}
Document No. 1.76 $46-01$ 0
Guidelinets) followed in Anney to Com. Reg. 283/2013
Deviations from current None 2
test guideline:
Previous evaluation: No, not previously submitted
GLP/Official@ (So, not conducted under GLP/Officially recognised testing facilities
recognised testing to the second seco
Acceptability/Reliability: Yes

The 21-day NOEC based on reproduction was determined to be 9.10 mg a.s./L

Executive Summary

The report <u>M-006401-01-1</u> on the effects of Spiroxamine TG on the reproduction of water fleas (*Daphnia magna*) study did not provide estimates of EC_{10} or EC_{20} values. Therefore, these values have been calculated in accordance with the Annex to Com. Reg. 283/2013. Effect concentrations with a 10% and 20% effect on survival, length and reproduction when compared to the control were re-calculated.



The resulting EC₁₀ and EC₂₀ values for survival at 21 d were 1.11 (95% CL: 0.58 - 2.11) and 1.62 (95% CL: 1.01 - 2.58) mg a.s./L, respectively. The resulting EC₁₀ and EC₂₀ values for length at 21 d were 1.13 (95% CL: 1.06 - 1.21) and 1.57 (95% CL: 1.49 - 1.64) mg a.s./L, respectively. The resulting EC₁₀ and EC₂₀ values for reproduction at 21d were 0.12 (95% CL: 0.06 - 0.17) and 0.20 (95% CL: 0.26 - 0.26) mg a.s./L, respectively.

I. Methods

The statistical evaluation was performed with statistical software ToxRat. Standard v3.3.

Effect concentrations with 10 and 20% from the test item treatment when compared to the powed controls were determined for survival, length and reproduction. A Problem function using linear maximum of likelihood regression was used along with 95% EC_x confidence limits for length and a Werbull function using linear maximum likelihood regression was used along with 95% EC_x confidence limits for length and a Werbull function reproduction.

II. Results and Discussion

An explanation is given for regression analysis entroints for length, reproduction and survival. These details can be found below.

Length at 21 days

Regarding the calculation of EC_{10} and EC_{20} values for length at 2 (d, the criteria for goodness of fit were met as the P(Chi²) value was 1 (0), showing no significant deviation between fit and data, and a statistically significant concentration/response was found (p(F) < 0.001) for this parameter.

The resulting EC_{10} and EC_{20} values and the respective confidence intervals are represented in the following table.

Table CA 8.2.5.1/04-1 Results of the Frobit analysis max. likelihood regression) with length at 21 d: Selected effective concentrations (EC_x) of the test item and their 95%-confidence limits (according to Fieller's theorem)

× ¥		
	Y V & Y & Uei	ngto 🗸
Daramator Q		EC20
I al alletel	(95% confidence interval)	√ (95 % confidence interval)
Ô	\mathcal{L} \mathcal{L} $[mg a.s. \mathcal{P}]$ \mathcal{O}	© [mg a.s./L]
		mgas/[]

The resulting EC_{10} and EC_{50} values of 1. O (95% CL: 1.06 - 1.21) and 1.57 (95% CL: 1.49 - 1.64) mg a.s./L, respectively, meet the goodness of fit criteria and therefore the estimated EC_{10} value is considered reliable for use within Orisk assessment.

Reproduction at 21 d

Regarding the calculation of EC₁₀ and EC_{20} values for reproduction at 21 d, the criteria for goodness of fit were met as the P(Cn^2) value was 1.00 showing no significant deviation between fit and data, and a statistically significant concentration/response was found (p(F) < 0.001) for this parameter.

The resulting EC₁₀ and EC_{20} values and the respective confidence intervals are represented in the following table below \sim

Table CA \$2.5.1/042 Results of the Weibull analysis (max. likelihood regression) with reproduction at 21 d: Sefected effective concentrations (ECx) of the test item and their 95%

Aconfidence	limits (according to	o Fieller's theorem)
	(0	,

	Repro	luction
	EC 10	EC ₂₀
Par@meter	(95 % confidence interval)	(95 % confidence interval)
9	[mg a.s./L]	[mg a.s./L]
Effect on	0.12 (0.06 - 0.17)	0.20 (0.13 – 0.26)
reproduction at 21 d	0.12(0.00 - 0.17)	0.20(0.13 - 0.20)



The resulting EC₁₀ and EC₂₀ values of 0.12 (95% CL: 0.06 - 0.17) and 0.20 (95% CL: 0.13 - 0.26) mg a.s./L, respectively, meet the goodness of fit criteria and therefore the estimated EC10 value is considered reliable for use in the risk assessment.

Survival at 21 d

Regarding the calculation of EC₁₀ and EC₂₀ values for survival at 21 d, the coveria for goodness of fit were met as the P(Chi²) value was 0.677, showing no significant deviation between fit and data and a statistically significant concentration/response was found (p(F) = 0.013) for this parameter.

Table CA 8.2.5.1/04-3	Results of the Weibull (n	nax. likelihood	regression with	survival at 2	1 de Select	éd 🦨
	Results of the Weibull (reffective concentrations normal approximation)	(ECx) of the tes	t item and their	95%-confide	Oce limits	(bv)
	normal approximation)	(,		de la	0	

	normal approximation)	~~			, A
	Q~	Survival 🦻			~~?
Parameter	EC10 🐇		É C EC	ð °>	$\mathcal{A}_{\mathbf{x}}$
rarameter	(95 % confidence interva	$\tilde{0} \approx \tilde{0}$	(95 % configer	ice intervaß	
	[mg a <u>.s</u> ./L]	. Ø Q	[mg a.s		
Effect on	1 11 (0(58 - 27))			⊈ 2.58	<u>A</u>
reproduction at 21 d	1.11(0.90 - 2.13)	V. A Ö	, <u>1,02 (1.01</u>	= 2.3.0J	N. C. C.
					9

III. Conclusion

The resulting EC₁₀ and EC₂₀ values for survival at 20 d were $1.1\sqrt{95\%0L}$: 0.68 - 2.11 and 1.62(95%CL: 1.01 – 2.58) mg a.s. \mathbb{Q} , respectively. The resulting EC of and EC₂₀ values for length at 21 d were 1.13 (95%CL: 1.06 – 1.21) and 1.57 (95%CL: 1.49 – 1.64) mg a. 2L, respectively. The resulting EC10 and EC20 values for reproduction at 21d were 0.12 95%CL: 0.06-0.15) and 0.20 (95%CL: 0.13

The statistical re-valuation of the data has determined reliable EC_{10} and EC_{20} values for survival, length and reproduction. However, the lowest endpoint remains the NOPC of 0.10 mg a.s./L based on reproduction therefore this value shall be taken as the most critical endpoint from this study.

The values determined in the recovaluation work are considered to be fully valid.

12'05 uatchas determined relia ver, the lowest endpoint remain as value shall be taken as the most of <u>a in the resovaluation work are considered to</u> <u>a in the resovaluation work are considered to <u>a in the resovaluation work are considered to <u>a in the resovaluation work are considered to <u>a in the resovaluation work </u></u></u></u>



Data Point:	KCA 8.2.5.1/02
	KCA 6.2.5.1/02
Report Author:	
Report Year:	1998
Report Title:	Influence of 14C-KWG 4168 (technical) on the reproduction of water fleasonder
•	flow-through test conditions
Report No:	HBF/RDM 61
Document No:	<u>M-006555-01-1</u>
Guideline(s) followed in	OECD-Guideline No. 202 "OECD-Guideline for Testing Chemicats", 4 April
study:	1984: "Daphnia spec., Acute Imageoilisation Test and Reproduction Test."
Deviations from current	None R A A
test guideline:	
Previous evaluation:	yes, evaluated and accepted $Q' \circ A' A' O' C'$
	$ \mathbf{D} \mathbf{A} \mathbf{P} (1007) \mathbf{P} \mathbf{A} \mathbf{P} (2016) \langle \mathcal{O} \mathbf{P} \mathbf{A} \mathbf{P} (2017) \mathbf{e} \langle \mathcal{O}_{\mathbf{n}}^{S} \rangle \langle \mathcal{O}_{\mathbf$
GLP/Officially	Yes, conducted under GLP/Officially recognised testing facilities
recognised testing	
facilities:	
Acceptability/Reliability:	Yes A A A A
Executive Summary	

Executive Summary

Executive Summary The 21-day chronic toxicity of ¹⁴C WG 4168 to Daphylia magna was studied under flow-through conditions. Test organisms were exposed to nominal concentrations of 0,0056, 0,010, 0,018, 0.032, 0.056, 0.10 and 0.18 mg a.s./L plus a control and solvent control. ð ×, Ø

Test water samples were collected from affrest concentrations including controls on Seven occasions during the study and the radioactivity was measured by Liquid Scintillation Counts. The exposure concentrations were calculated from the measured dpm and the ratio of radiolabeled a.s. to unlabeled a.s. as determined in the corresponding stock solutions. The actual concentrations were 102.5 to 115.5 % (for an average: 198.1 %) of the pominal concentrations. L O

The no observed effect concentration (NOEC) with regard to coproduction was 0.034 mg a.s./L, whereas, the body length NOEG was 0.11 mg a.s./L and the body weight NOEC was 0.11 mg a.s./L. 0 B

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Materials and Methods I. Materiale Ô

A. Materials		, °
	U O Vabelled test substance	Labelled test substance
Test Material	spiroxamine	cyclohexyl-1-14C]-KWG 4168
Lot/Batch #:	717692/96	10039/3
Activesubstance	$ \begin{array}{c} 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 $	-
content:		
Specific y		3.68 MBq (99.5 uCi)/rag
radioactivity;		
Radiochemical	$\dot{\gamma} = \chi \dot{\rho} (\eta) \dot{\gamma} \chi \dot{\rho} (\eta)$	>98%
purity:		
Description:	Sufficient based on expiration	Not reported
Stability of test		Sufficient based on expiration date
compound:	date	
Reanalysis/Expiry	07 Aug 1997	Not reported
V dater		
Density:	Not reported	Not reported
Treatments		



Test rates:	Nominal: 0.0056, 0.010, 0.018, 0.032, 0.056, 0.10 und 0.18 mg a.s./L
	a.s./L
Solvent/vehicle:	DMF
Analysis of test concentrations:	Nominal: 0.0056, 0.010, 0.018, 0.032, 0.056, 0.10 und 0.18 mg a.s./L Mean measured: 0.0065, 0.011, 0.020, 0.034, 0.057, 0.11 and 0.19 mg a.s./L DMF Yes, day 0, 2, 7, 9, 14, 16 and 21 (mean measured concentrations 102 9 to 115.5% of nominal) Daphnia magna < 24 hours old In-house culture, originally from the Bundesgesundheitsamt, Berlin, Germany This strain was maintained in the faboratory for more than ten years (2 litre containers); the water in which they are topt is changed weekty.
Test organisms	
Species:	Daphnia magna < 24 hours old O^{4} z^{4} O^{4}
Source:	In-house culture, originarily from the Bundesgesundbritsamt, Berlin, Germany
Acclimatisation period:	The organisms were kept in a environmental character under the test conditions 20 ± 1 °C 16 : 8 hour light-dark cycle, the animals were fed with single cell green algae <i>Scenedesmus subspicatu</i> and occasionally some commercial ornamental tish food (TetraMing) (aqueous suspension)
	The food suspension was freshly prepared two times per week from the algae stock suspension. During the study, the water fleas were fed with each cycle of test solution preparation. The food was laving single cell green algae (<i>Scenegesmus subspicatus</i>). The total organic carbon (TOC) content of this food suspension was determined photometrically. The daphnids were fed with 0.5 tog TOC per test vestel at each cycle of the test during system. The food suspension was continuously stirred to ensure homogeneity and equal delivery of food to all test chambers
Treatment for S	Not reported
Test design	
disease:	The test beak of had holes of 3 cm diameter at the water level of 250 mL (water height, about 6 cm). Stanless steel screens (200 um mesh size) were secured to the outside of the beakers to prevent the loss of water fleas as the test solutions exerflowed at each renewal.
Test medium:	M7-pedium
Replication:	Four per concentration
ANo. of animals/vessel:	Four per concentration
Duration of test;	days S
Environmental test conditions	5
Dissolved oxygen	6.7 to 9.0 mg/L (8.9 mg/L = 98%)
p [©]	7.5 to 8.0
Photoperiod:	16 h light : 8 h dark at ~700 lux



B. Study Design

The exposure *of Daphnia magna* to ¹⁴C-KWG 4168 (technical) was conducted for 21-days under flow- through conditions in order to assess the chronic toxicity of the compound.

The test vessels consisted of beakers which had holes of 3 cm diameter at the vater level of 250 mb, stainless steel screens (200 um mesh size) were secured to the outside of the beakers to prevent the boss of water fleas as the test solutions overflowed at each renewal. Each test vessel contained five an malso per vessel, four replicates per concentration. The beakers were placed in a environmental chapter for 21 days at 20 ± 1 °C and a 16:8 light-dark cycle. Light intensity was about 700 lux. The water fleas were fed on media renewals days.

Test concentrations were chosen based upon historical toxicity information for the compound. Daphna magna (<24 hours old) were used in the test from an in-house culture.

Nominal test concentrations were 0.0056, 0.010, 0.018, 0.032, 9.056, 0.10 und 0.18 mg a.s./L alorg with a control and solvent control groups. Mean measured test concentrations were 0.0065, 0.011, 0.020, 0.034, 0.057, 0.11 and 0.19 mg a.s./L.

Parent daphnids in the test vessels were observed daily, with exception of Saturday and Sunday of the first week (days 3 and 4). Observations for parent survival, neonate survival, and sublethal and behavioral effects were made and recorded. Starting with day 8 heonates were counted and removed daily. Prior to counting neonates the parent daphnids were carefully removed from the beaker and placed in a quantity of the appropriate test solution. The test solution contaming the neonates was then strained through a 0.20 mm plastic mesh. The neonates were retained on the mesh. The number of neonates was counted, the count recorded and the neonates discarded. The test solution, without neonates, and the parent daphnids were then returned to the original test beaker.

The stock solutions used for the flow-through test were prepared once per week; three were used for the definitive study. The new stock solutions were put into use if the diluter system on day -2, day 7, and day 14. The stock solutions were sampled for measured concentration on day 0, 7, 14 and 21. Both new and old stock solutions was analysed to confirm stability during the course of the study. The measured concentrations in the stock solutions, were determined by GLC (Gas Liquid Chromatography). Additionally the amount of 14C-KWG 168 was measured by Liquid Scintillation Counts.

Test water samples were collected from all test levels including controls on seven occasions during the study. The exposure concentrations were calculated from the measured dpm and the ratio of radiolabeled a.s. to unlabeled a.s. to unlabeled a.s. as determined in the corresponding stock solutions.

Analytical method

Samples of water were analysed ising the validated malytical method 00252 M001, report reference M-008496 02-2 (see Doc MCA Section 4).

II. Results and Discussion

Validity criteria according to the test guideline were met:

- Mortality of the parent animals (female *Daphnia*) does not exceed 20% at the end of the test (actual: 5% and 0% for the control and solvent control, respectively)
- The mean number of bying offspring produced per parent animal surviving at the end of the test $\sqrt{s} \ge 60$ (actual) ≥ 110

The study is therefore considered acceptable.



Nominal		Average measured radioactivity (dpm/10 mL)* and Calculated								
Concentrations	Radioactivity	Day 0		Day 2		Day 7 🔊		Day 9 🔊		
(mg a.s./L)	dpm/10mL	*	**	*	**	*	***	*		
Control	-	5.4	-	5.7	-	7.2	10 ^v	5.6		
Solvent control	-	6.7	-	8.1	-	4.6 🚄	-	4.3	- 🛇 🧯	
0.0056	200	193.8	0.00535	211.4	0.00584	219	0.00605	21/8.3	Q.0070Q	
0.010	200	186.9	0.0096	220.6	0.0113	202.2	0.0104	204.2	×0.011	
0.018	200	194.5	0.0181	195.5♥	0.0182	198.0	0.0185	216	>0.011₫) 0.02¥1	
0.032	200	187.7	0.0263	204.5	0.0286	231.3	0.032	19Q3	00321	
0.056	200	191.5	0.0496	£15.8	0.0559%	213,5°	0.0553	A99.3	9.0544	
0.10	200	192.2	0.094	212.7	0.104	2 @ .5	0.098	D210.70	0.107	
0.18 Mangurad radioa	200	191.8	0.163	210.3	0.198	204.8	@.174~	180.9	0.166	

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Table CA 8.2.5.1/02-1 Summary of analytical results of water analyses

* Measured radioactivity (dpm/10 mL). Average 64 replicates

** Calculated concentration (mg a.s./L)

Calculated concentration (ing d.s./L)	A	. 0	۵ 🔍	Q	4	R.
Table CA 8.2.5.1/02-2 Continued sum	×.	\sim	~×.	ð.	A.	. Õ
Table CA 8.2.5.1/02-2 Continued sum	imary of a	nalytic	al resul	ts of wat	er anal	yses

Nominal	Average measured radioactivity (dpm/10 mL)* and Calculated concentration (mg a.s./L)*								
Concentrations (mg a.s./L)	Radioactivity dpm/10mL@	Day 14	à à	Day 16		D ay 21 [°] ^	**		
Control		[™] 9.3 ~♡	- "	¥9.3 °	-0	9.1 0	-		
Solvent control	- 🔉 🖄	8.3	a v	8.5 🗡 💧		7 🕰	-		
0.0056	200	2002.5 K	Q0.007 1 4	21⊽.8 ≪	0.00	20 9.5	0.00680		
0.010	200	199.9	0.01 0	0209.3 <u>k</u>	0.0122 8	204.4	0.0119		
0.018	200 200	197.8	0,0193	₹210.Ø [™]	Ø:9224 ×	206.5	0.0220		
0.032	200	212,1	0.0357 S	203.4	0.0406	207.4	0.0414		
0.056	200 0 20	XV5.2 ∾	0.0588	2 12.9	0.06	213.3	0.0640		
0.10	200 🔧 🖗	210.1	0.107	217.8	0:424	212.8	0.121		
0.18	200 0	209%8	0, 193 0	214	P .210	217.2	0.212		

* Measured radioactivity (dpm/10 mb). Average of 4 replicates

** Calculated concentration (mg a s/L)

The results of the study have been presented in terms of mean measured concentrations.

No mortalities higher than 5 % were observed in the parent animals of the control and solvent control. There was no mortality of any test concentration exceeding a mortality rate of 20% which is considered acceptable backgroun@mortality. \bigcirc Ô

Survival of the parent water fleas Table CA 8.2.5.1/02-3

	\square								
Mean measured	Study d	NAY A	/ .~~	7					
concentration		r 1 🖉	2	5	6	7	8	9	10
(mg/a.s./L)		Ŷ							
Control	20	2Ø	32 0	20	20	20	20	19	19
Solvent control			20	20	20	20	20	20	20
0.0065	\$20	20	20	20	20	20	20	20	19
	20 5	20 👻	20	20	20	20	20	20	20
0.020	20	20	20	20	20	20	19	19	19
0.034	20	20	20	20	20	20	20	20	20
0.057	¥20	20	20	20	20	20	20	20	20
0.M \$	20	20	20	20	20	19	19	19	19
0.19	20	20	19	19	19	19	19	19	19



Mean measured	Study	y day									Ű
concentration	11	12	13	14	15	16	17	18	19	20	A A
(mg a.s./L)								A			б ^у Ю
Control	19	19	19	19	19	19	19	19	² 19	19	19 0
Solvent control	20	20	20	20	20	20	20	20 🔊	20	20	20
0.0065	19	19	19	19	19	19	19	19	19	19	SP9 ô
0.011	20	20	20	20	20	20	20	×20	20 🕺		\$20 ×
0.020	19	19	19	19	19 🚿	<u>9</u> 19	19 🖉	19	19 🔊	19	19
0.034	20	20	19	19	19	⁸ 19	19	19	190	19	19
0.057	20	20	20	20	20	20	20	20	Žť	-20	20
0.11	19	19	19	19	49	19	Qý (19	<u>ب</u> 19	19	0 19
0.19	19	19	19	19 🐇	2 ⁰ 19	19 🔊	19 🖉	19 🖓	19,0	190	190

A delay in time to first brood release was observed only in the highest test concentrations of 0.11 and 0.19 mg a.s./L. No neonates were observed in this study before days in any test concentration including control and solvent control. No dead offspring or aborted eggs were found in any test revelsationughout the study. Also, no abnormal behaviors of adult or juvenile organisms were observed.

In the control, the mean number of & offspring per parent per reproduction day (corresponding to a sum of offspring per parent of 116) represents an acceptable reproduction rate. Compared to the pooled control data, there was no biologically or statistically significant reduction of the number of offspring per parent per reproduction day@t test concentrations from @0065.60 0.03@mg a S./L (Qunnett's-test, p = 0.05). Concentrations from 0.057 to 0.19 mg a.s./L reduced the number of offspring per parent per reproduction day significantly compared to the pooled control data. Therefore, the NOEC for reproduction is 0.034 mg/g/s./L.

		Ø "	AY	<u>~ ~ ``</u>	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	n.			
Day	Mean me	åsurediconc		ng a.s./L)	~~ (
	Control	Solvent	0.0065/	0.0011	0.020	0.094	[∞] 0.057	0.11	0.19
	ų į	Scontrol				0. 6 54	,		
7	, Ø					0 0	0	0	0
8	23	R15 .0	4.25	7.75 .	5.75 🔊	3.5	6.25	1.5	0.5
9	43	8.75	76 9 5 `^	9.5	8 8	8.25	5	3.25	0.5
10	38	4025	§46.75¢	46.5	\$ 4 2.75	47	20.75	24.25	18.5
11	16Q75	G16.750	4	¥3.5 ×	2.5	14.75	10.25	7.25	2.25
12	4.5	19	38.75 J	28:5	B.5	59	10.5	27	15
13	95	ĴØ8.5	^{\$} 99.5	94.75 °C	73.5	58.75	45.5	53.75	50.5
14	27	38.75	36.75 <	48.23	8	18.75	19.75	13.75	5
15	26.2	27.25	¥24.75	4825	29.5	39.75	18.75	26.5	20.5
16	625 Å	99.5	93.75	©9 4.25	86.25	75.5	64.75	74.75	49.75
17	57.75	523	Å 1 3.5	48.75	54.75	12.25	31.25	25.75	29.25
18	335	Å7 . S	34.75	46	48.25	38	34	22	28.75
12	Ø56.5 O	1005	75.5	88.5	57.25	107.5	53.5	100	19.5
20 0	109	51.25	59.75	61.25	61	19.75	53.5	22	57.25
21	48.25	21.75	39	41.75	42.5	29.75	36.5	29	7

Table CA 8.2.5.1/02-5 Number of juveniles produced



Since the body length data of control and solvent control were not significantly different, the statistical comparison of the lengths of parent animals at the end of the study were performed with pooled control and solvent control data. The statistical comparison of the length of parent animals showed no significant reduction of body lengths at all concentrations except for the 0.19 mg a.s./L treatment where body length was reduced compared to the control (Dunnett's-test, p = 0.05). Therefore, the NOEC for the body length of the parent animals was 0.11 mg a.s./L.

Animal No.: Mean measured concentration (mg Control 0.0065 0.01 1 4.20 4.40 4.55 4.20 2 4.50 4.60 4.25 4.30 3 4.30 4.55 4.45 4.40	11 0,020 0 4 .65 0 4 .50	0.034 4.55 4.55 250	, ,	9.11 2 4.2%	0.197 C
control control 1 4.20 4.40 4.55 4.20 2 4.50 4.60 4.25 4.30	0 4.65 4.50	4.55	, C	1 26 11 2 1 1 2 1 1 2 1 1 2 1 1 2 1 2 1	N O
1 4.20 4.40 4.55 4.20 2 4.50 4.60 4.25 4.30	0 4.65 4.50	4.55	A.30) ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	
2 4.50 4.60 4.25 4.30	0 @ 4.50	4.55	A.30	1 200/	
	0 0 4.50	1 2×0 5		4.30/	3.95 😽
3 4.30 4.55 4.45 4.40	a		4.35	4,55	3.95 4.00 3 \$0
5 4.50 4.55 4.45 4.40	0% 4050 *	\$¥.45 ≪		Q4.65 📎	3.30
4 4.60 4.30 4.50 4.30	Ø Vio č	4.50	4030 🔊	4.50	<u>A</u> 10 。
5 * 4.40 4.35 433	5 74.100	4.95	4.25	4.05°	
6 4.50 4.50 4.35		¥.55 A	4.00	<u>4</u> .35 ×	3.75 3.80
7 4.40 4.35 4.25 2 4.30		¥ 4.550 [°]	475 4	, .	5,00
8 4.55 4.45 4.20 44	¥ A.50 ~~	4.35	¢4.20 🔊	4.30	3.80
9 4.25 4.30 3.80 4000		4,20 🚿	4.10	4\$0 1	² 3.90
10 4.80 4.20 * 2 4.35	5 0 4 2 5	€ ¥.30 °	4.00	QA.35 🛯 🥎	3.60
11 4.30 4.30 Ø.15 V 4.50	60° (4,00 (0)		4960 2	4.30	3.85
12 4.50 4.45 4.60 4.60	0 4.30	4,000 €	a4 60	4.40"	3.90
13 4.55 4.55 4.30 4.30	5 4.400	4.35 4.50	4.30	A 55	3.75
14 4.45 4.55 4.40 4.35	5, 4, 55	∖4.50√	4.65	Å.30	3.80
			4.25	4.20	3.75
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	5 4.10 7	400 %	4.55°	4.80	3.70
17 4.25 4.45 4.5 4.40	0 2 4.20	4.50 O	4.50	4.30	3.45
18 4.25 \$ 4.35 4.00 4.26	8 <u>\$</u> 4975 Q	4.65	4.40	4.40	3.00
19 4.55 4.30 4.25% 4.6	Ø & A.55 ~		¥.65	4.45	3.65
	5 4.550	×	4.05	*	3.85
Mean 4.429 04.410 4289 4.35	53~ 4.359	4.392	4.303	4.405	3.732
	21 0.170	0.15%	0.199	0.158	0.256
% of 2 - 99.6 96.9 98.2	3 ∂*98.9≼∦	900 37	97.1	99.5	84.3
control		A Y			
* Animal died before the end of the study	à Ó è	~ .			

Table CA 8 2 5 1/02_6	Body length (mm) of adult	Danhnia oftor 21	days of woosure	to KWC- 1168
1 able CA 0.2.5.1/02-0	body length (mm) of adult	<i>Daphnia</i> after 21	uays of exposure	10 K WG 4100

Since the body weight data of control and solvent control were significantly different, the statistical comparison of the dry weights of parent animals at the end of the study were performed with the solvent control data only. The statistical comparison of the dry weights of parent animals showed no significant reduction of body weights at the concentrations from 0.0065 to 0.11 mg a.s./L, except at 0.020 mg a.s./L. This exception was not considered in the assignment of the NOEC since higher concentrations did not show a reduction in body weight (0 unnet s-test, p = 0.05). The highest test concentration 0.19 mg a.s./L reduced the body weights significantly compared to the solvent control data. Therefore, the NOEC for the dry weight of the parent animals s 0.11 mg a.s./L.

Table CA 8,2,5.1/02-7	Dry weight (mg) of adult Da	<i>uphnia</i> after 21 days of exposure to KWG 4168

Animal	Meanme	asured con	centration	(mg a.s./L	.)				
No.: 🔊	Control	Solven	0.0065	0.011	0.020	0.034	0.057	0.11	0.19
14									
14	0.712	1.200	1.284	1.148	0.810	1.166	1.228	1.112	0.730
2 🔊	0.714	0.952	1.084	1.208	1.060	1.242	1.206	1.050	1.012
3	1.072	1.224	1.048	0.378	1.086	1.134	1.234	1.310	0.650
4	0.874	1.178	1.344	1.126	0.884	1.172	1.086	1.022	0.786
5	*	1.156	1.042	1.104	0.726	1.180	1.038	1.052	*



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Animal	Mean me	easured con	ncentration	(mg a.s./L)				
No.:	Control	Solvent	0.0065	0.011	0.020	0.034	0.057	0.11	0.19
		control							
6	1.220	1.168	1.140	1.212	0.958	1.014	0.758	1.082	0,322 6
7	0.876	1.102	1.038	1.250	1.198	1.020	0.970 🏷	1.218	Ø,694 🚕
8	0.826	1.218	1.060	1.208	0.874	0.684	1.176	1.076 4	0.754
9	1.020	1.030	0.776	1.248	0.762	1.082	0.862	1.034 🔊	0.566
10	0.850	0.718	*	1.204	1.146	1.002	Q.622	1.1800	00078 ~
11	1.016	1.104	1.002	1.228	0.730	1.014	§ 1.096	1. 10 Å	0.702 0.798
12	1.116	1.240	1.250	1.126	0.800	1.152	0.998	1,966 ~	0.798
13	1.000	1.294	0.826	1.170	1.040	1.154,0♥	1.046	J.326	0.696
14	0.994	1.250	1.154	1.180	sf.120	1.246	1.036	1.170 📎	Ø.762 🖉
15	0.936	1.330	0.958	1.130	*	0.852	@1.022Q	0.848	0.532
16	0.738	0.976	0.932	0.992	0.742	A.224 ~	1.404	0.910 🔬	0.532 0.806×
17	1.002	1.044	1.178	1.218	19032	1.216	0.904	§1.046 [°] ≫	0.666
18	0.656	0.718	1.054	0.906	J.072 👸	1.202	338 0	1.154	428 °
19	1.024	1.154	1.114	_4 . 264 _ 0	1.174	1.1994	0.85	1.964	0.734
20	0.938	1.162	0.990	1.268	1:076		0.898	star and a star and a star a sta star a star a sta	0.866
Mean	0.925	1.111	1.067 🖉	1.128	0963 🔬	* 1.100°	£037 â	1.107	0สูโ7
SD	0.150	0.167	0.143	0498	0.164	0.148	Ø.195 S	0 1 8	0.123
% of	-	120.0	1159	121.9 🔊	104.0	J¥9.5 Ó	112.0	1006 1	77.5
control			l ^Q ∂) Ô	ð é	S .O		$\tilde{\mathcal{O}}$	
* Anii	nal died be	fore the end	l & the stud	y O	S O	Q	°, °,		

Table CA 8.2.5.1/02-8 Effects of KANG 4168 expositre on the Daphnia Ô

Endpoint	Reproduction - number Growth Body length Growth Body dry weight of offspiring (mg a.s.)
_	of offspering a long a solution of the second s
	(mg_{A})
NOEC	
LOEC	

Ш. Conclusion 🚽

K, The 21-day chronic toxicity of C-KMG 4108 to Daphnia magna was studied under flow-through conditions? Test organisms were exposed to nominal concentrations of 0.0056, 0.010, 0.018, 0.032, 0.056, 0.10 and 0.18 mg a.s. I plus a control and solvent control.

The no observed effect concentration (NOEC) with regard to perioduction was 0.034 mg a.s./L, whereas, the body length NOEC was 0.11 mg 4.8./L and the body weight NOEC was 0.11 mg a.s./L.

Assessment and conclusion by applicant:

The study was conducted to the OE D test guideline 202 (1984), therefore, the test was assessed to the current test guideline for Daphnia magna reproduction tests OECD 211 (adopted: 02 October 2012).

Validity criteria according to the OECD 211 guideline (2012) were met:

- Mortality of the papent aritmals (female Daphnia) does not exceed 20% at the end of the test (actual: 5% and 5% for the control and solvent control, respectively)
- The mean number of Fiving offspring produced per parent animal surviving at the end of the test is≥60 (actual:©116)

The study is therefore considered acceptable.

The da@ have been subjected to statistical re-evaluation and the results have been presented in the following study summary.

The NOEC with regard to reproduction was determined to be 0.034 mg a.s./L.



Data Point:	KCA 8.2.5.1/05
Report Author:	
Report Year:	2020
Report Title:	Calculation of EC10 and EC20 values for Daphnia magnewith 14C-spiroxamine
	TG in a reproduction study
Report No:	0471836-ECO6
Document No:	<u>M-760409-01-1</u>
Guideline(s) followed in	None
study:	
Deviations from current	None A A A
test guideline:	
Previous evaluation:	No, not previously submitted
GLP/Officially	No, not conducted under GLE Officially recognised jesting facilities
recognised testing	
facilities:	
Acceptability/Reliability:	Yes y y y y y y
Executive Summery	

Executive Summary

The report <u>M-006555-01-1</u> on the effects of ¹⁴C-Spiroxamine TG on the reproduction of water fleas (*Daphnia magna*) study did not provide estimates of $4^{\circ}C_{10}$ or $4^{\circ}C_{20}$ values. Therefore, these values have been calculated in accordance with the Annex to Com. Reg. 283/2013. As there were no effects > 5% on the parameter survival, it was not possible to determine reliable EC values

Due to a scattering of data around the lower coses of the response curve. The low amount of variance explained by the models, and nor-fitting linear and non-linear regression models, it was not possible to calculate reliable EC values for any of the parameters tested.

I. Methods 🖉

The statistical evaluation was performed with statistical software oxRat Professional v3.3.0.

Concentrations with 10 and 20% effects from the test item treatment when compared to the pooled controls were calculated for reproduction, but due to lack of a dose response, these could not be determined for length Although ECX values and 95% confidence interval were calculated for the parameter dry weight, as contridence intervals were large, spanning more than 2 concentrations and the dose response curves is not covered by the data it was not possible to determine reliable ECx values.

A Logit function using Onear maximum liketihood regression was used along with 95% EC_x confidence limits (calculated using normal approximation) for dry weight.

II. Results and Discussion

An explanation is given for regression analysis endpoints for length, dry weight and reproduction. These details can be found below A

Length at 21 days

Due to the significant amount of data scattering around the lower doses of the response curve and due to the low arount of variance (<30%) explained by all tested regression models, no reliable EC₁₀ and EC₂₀ can be calculated.

Dry weight at 21 d

Regarding the calculation of EC_{10} and EC_{20} values for dry weight at 21 d, the criteria for goodness of fit were met as the P(Chi²) value was 1.00, showing no significant deviation between fit and data, and a statistically significant concentration/response was found (p(F) = 0.047) for this parameter.

The resulting EC_{10} and EC_{20} values and the respective confidence intervals are represented in the following table and figure below.



Ď

 Table CA 8.2.5.1/05-1
 Results of the Logit analysis (max. likelihood regression) with reproduction at 21

 d: Selected effective concentrations (ECx) of the test item and their 95%-confidence limits (by normal approximation)

Parameter	Dry weight	
	EC10	EC ₂₀
	(95 % confidence interval)	(95 % confidence interval)
	[mg a.s./L]	[mg a.s./L] 🔬 🔊 🔊
Effect on dry	0 117 (0 075 0 191)	0.152 (0.44 - 0.203)
weight at 21 d	0.117 (0.075 – 0.181)	

The resulting EC₁₀ and EC₂₀ values of 0.117 (95% CL c 0.075 – 0.180), 0.152 (95% CI: 0.914 – 0.203), \bigcirc mg a.s./L, respectively, meet the goodness of fit criteria. However, with a p(F) value of 0.047, the dose response is only marginally significant and only 57.8% of the variance is explained by the dose/response function (r² = 0.578). Along with a poor-fitting concentration curve (see below), the estimated \bigcirc CL c 0.075 – 0.180), 0.152 (95% CI: 0.914 – 0.203), \bigcirc response is only marginally significant and only 57.8% of the variance is explained by the dose/response function (r² = 0.578). Along with a poor-fitting concentration curve (see below), the estimated \bigcirc Cl c 0.047, the dose value is therefore not considered reliable for use in the risk assessment.

Reproduction (cumulative offspring per survived parent) of 21 d

Regarding the calculation of EC₁₀ and EC₂₀ values for reproduction at 21 d. the criteria for goodness of fit were met as the P(Chi²) value was 0.999, showing no significant deviation between for and data, and a statistically significant concentration response was found ($p(E) \neq 0.026$) for this parameter

The resulting EC_{10} and EC_{20} values and the respective confidence intervals are represented in the following table and figure below.

 Table CA 8.2.5.1/05-2
 Results of the Probit (max dikelihood regression) with reproduction at 21 d:

 Selected effective concentrations (ECx) of the test item and their 95%-confidence limits (by normal approximation)

, ,	Reproduction & S S O A
Parameter	ECO
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	(mg a.Q/L) O (mg a.s./L)
Effect on reproduction at 21 d	$0.032(0.01)^2 - 0.093$ $0.068(0.036 - 0.132)$
reproduction at 21 d	$\frac{1}{1} 0.032 (0.016 - 0.095) - 0.005 (0.036 - 0.132) - 0.005 (0.036 - 0.132)$

The resulting  $EC_{10}$  and  $EC_{20}$  values of 0.032 (95% CL: 0.011 - 0.095) and 0.068 (95% CI: 0.036 - 0.132) mg a.s./L, respectively, meet the goodness of fit criteria. However, as the confidence intervals are large, spanning more than 6 concentrations and the obtained endpoints do not visually fit with the raw data from the study it was concluded that the estimated  $EC_{10}$  value is not considered reliable for use within a risk assessment.

#### III. AConclusion

It was not considered possible to determine teliable ECx values for any parameter.

### Assessment and conclusion by applicant:

The statistical pe-evaluation of the data has determined  $EC_{10}$  and  $EC_{20}$  values for dry weight and reproduction but these values were not considered to be reliable. The lowest  $EC_{10}$  and  $EC_{20}$  values of 32 and 68 dg a.s./L, respectively were determined for reproduction. Thus, the  $EC_{10}$  of 32 µg a.s./L is lower than the NOEC of 34 µg a.s./L for this parameter but as the  $EC_{10}$  is not considered reliable, the NOEC of 34 µg a.s./L shall be main the critical endpoint determined from this study.

The NOE Value determined in the re-evaluation work are considered to be fully valid.

C



Data Point:	KCA 8.2.5.1/03
Report Author:	
Report Year:	1996
Report Title:	Influence of 14C-KWG 4168 (techn.) on the reproduction rate of water Fleas
Report No:	HBF/RDM 55
Document No:	<u>M-006466-01-1</u>
Guideline(s) followed in	OECD 202 (II) (1984), now OECD 211 (2012)
study:	EPA FIFRA 72-4
	EEC XI/681/86 (1987)
Deviations from current	None R None
test guideline:	
Previous evaluation:	yes, evaluated and accepted, Q & A
	$  RAR(2010) RAR(2013) O' \land O' $
GLP/Officially	Yes, conducted under GLP/Officially recognised testing facilities
recognised testing	
facilities:	
Acceptability/Reliability:	Yes A Y A Y
Executive Summary	

#### **Executive Summary**

The 21-day chronic toxicity of ¹⁴COKWC magna was studied under comi-static Jo Daphnia conditions. Test organisms were exposed to mean measured test concentrations of @015, 2027, 0.047, 0.085, 0.15, 0.27 and 0.47 mg a.s./L plus a control and solvent control. The 21 day NOEC based on reproduction was determined to be 0.047 mg a.s./L.

- Materials and Methods I.
- A. Materials





<b>Treatment for</b>	None reported
disease:	
Test design	
Test vessel:	250 mL glass beakers containing approx. 200 mL test solution at ab depth of approximately 8 cm
Test medium:	M7-medium
<b>Replication:</b>	Mortality: three replicates Reproduction: ten replicates
No. of animals/vessel:	Mortality: five animals Reproduction: individually held
<b>Duration of test:</b>	21 days $(x_{\mu}, y_{\mu}) = (x_{\mu}, y_{\mu}) + (x_{\mu}, y_{\mu}$
Environmental test conditions	
Temperature:	$19.7 - 202^{\circ}C$
Dissolved oxygen:	$\operatorname{Freshr}^{O^{\vee}} \overset{\mathcal{V}}{\to} $
	8.4
	5.2 - 10.4  mgg (approx. 56.8 - 1) 4.8% saturation)
pH:	None reported 250 mL glass beakers containing approx. 200 mL test solution at a depth of approximately 8 cm M7-medium Mortality: three replicates Reproduction: ten replicates Mortality: five animals Reproduction: individually held 21 days 19.7 – 202°C Freshr 8.4 & 8.9 mg/L (approx. 91.8 – 98/2% saturation) Spent: 3.2 – 10.4 mg/L (approx. 56.8 – 114/8% saturation) Freshi: 7.35 8.70 He h light : 8 h dark at approx 700 lift 5 – 5 – 5 – 5 – 5 – 5 – 5 – 5 – 5 – 5 –
Photoperioù:	Spents 7.32 8.70 5 5 5 6 7 6 7 6 7 6 7 6 7 6 7 6 7 6 7 6
Photoperlog:	Ron light : 8 Moark at approx 700 tex
B. Study Design 🔍	
This study was conducted in	a order to assess the reproductive toxicity of 14C-KWG 4168 to the water

This study was conducted in order to assess the reproductive toxicity of ¹⁴C-KWG 4168 to the water flea *Dapphia magna* under semi-static conditions. Dest concentrations were chosen based on the results of earlier toxicity tests.

Nominal test concentrations were 0.01%, 0.032, 0.056, 0.10(0.18, 0.32 and 0.56 mg a.s./L along with a control and solvent control. Mean measured concentrations were 0.015, 0.027, 0.047, 0.085, 0.15, 0.27 and 0.47 mg as./L, respectively, equivalent to \$2/2 to \$5.2% of nominal.

Test vessels were 250 mL glass beakers containing approximately 200 mL test solution at a depth of approximately 8 cm. Test solutions were reviewed every 48 hours during the week, and after 72 hours over weekends. Beakers were covered with plexi glass plates and placed in an environmental chamber for 27 days at  $20 \pm 1^{\circ}$ C under a 16 hour light 8 hour dark photoperiod at approximately 700 lux.

Daphnia were difst instar at toss that 24 hours old. Three replicates of five animals were assessed for the mortality assessment and ten replicates of individually held daphnids were used for the reproductive and sub-lethal effects as a sessment. Test animals were transferred to newly prepared test media with one or two drops of the old test solution.

Immediately after removal of the parent animal, neonates from each reproductive vessel were counted by straining the test solution through a 0.20 mm mesh. Observations for survival, sub-lethal and behavioural effects to the parent daphnids were made daily. At test termination, the body lengths and dry weights of the parent animals were determined.



Body length measurements were made under a binocular microscope from the apex of the helmet to the base of the posterior spine using an eyepiece graticle. Body weight was then determined individually using a microbalance after 48-hours drying at 60°C.

Temperature was measured in one vessel of the control. Oxygen content and pH values in the control and test concentrations were determined in freshly prepared test solutions.

Samples of the test solution were taken four times during the study and analysed for the content of the active substance. A stability assessment of the test substance was also made on days 7, 44 and 21.

#### Analytical method

Samples of water were analysed using the validated analytical method 00252 Mo01, report reference <u>M-008490-02-2</u> (see Doc MCA Section 4).

#### II. Results and Discussion

Validity criteria according to the test guideline were wet

- Mortality of the parent animals in the controls to not exceed 20% at the end of the test: (actual: 0 and 7% in the control and solvent control, respectively)
- Mean number of living offspring produced per parent animal surviving at the end of the test in the controls to be ≥60 (actual: mean total offspring 1397 and 43.3 in the control and solvent control, respectively)

Analytical results, measured of four occasions in the fresh solutions, ranged from 969 to 112.4% of nominal, with a mean recovery of 104.7% After 72 hours of exposure measured radioactivity was 98.8 to 110.1% of nominal, with a mean recovery of 104.7%. The results achieved in this study have been presented in terms of mean measured test concentrations.

Nominal test	Analysed	concentrat	tions (mg a	.s./12) 🔿	? <u>_</u> 07	<u>~</u>		
concentration O	Day 0	% of ∕≫	Dax 4 🔬	3% of		% of	Day 18	% of
(mg a.s./L) ^{©*}	8 V	nominal			0,0	nominal		nominal
Control 🔬 🖗	-	-23	A- ">>"	- 0		-	-	-
Solvent control	- 0		-	¢, Ý		-	-	-
0.018		98.6	0@188 ~	D104.3	0.0202	112.4	0.0199	110.7
0.032	@.0323 <i>\{</i> /	100.8	@.0339%	1059	<i>4</i> <b>0</b> .0341	106.6	0.0354	110.7
0.056	0.05 <u>6</u> 1	100.2 ĸ		107.2 🗞	0.0607	108.3	0.0629	112.4
0.10	0.0986	98.6 p	0.105	9705.0 103.2	0.105	104.8	0.109	109.2
0.18	Ø,178 C	98.9~	A 86 %	103.2	0.190	105.5	0.201	111.4
0.32	0.310	26.9	Q0.331, Q	10.9.5	0.333	104.2	0.346	108.2
0.56	0.546	D.4 🔊	0.5%	<b>₩Ø</b> 2.0	0.557	99.4	0.587	104.9
		Q r						

## Table CA 8.2.5.1/03 @ Measured concentrations of spirosamine in fresh solutions during the test

Table/CA 8.2.5.1	1/03-2 Measure	d concentrati	ons of spiroxamin	e in test solutions after 7	2 hours
			one of open on an in	e in test solutions arter :	= nours

Nominal test	Analysed con	centrations (m	g a.s./L)			
concentration (mg a.s./L)	Day 7	% of O	Day 14	% of nominal	Day 21	% of nominal
Control 🖉 🐇	V- 8 «	,- ~	-	-	-	-
Solvent control		-	-	-	-	-
0.018	0_0188	104.5	0.0193	107.0	0.0191	106.2
	D:0336	104.9	0.0340	106.2	0.0352	109.8
	0.05	104.2	0.0597	106.6	0.0617	110.1
0.10	0.1033	103.3	0.105	104.9	0.106	106.3
0.18 Ŭ	0.184	102.5	0.189	104.8	0.195	108.5
0.32	0.320	100.0	0.329	102.7	0.342	107.0
0.56	0.553	98.8	0.557	99.4	0.568	101.4



Table CA 8.2.5.1/03-3	Mean measured concentrations of ¹⁴ C-KWG 4168 calculated from the 0-hour,	72-
	hour and stability analysis solutions	<i>°</i>

	nour and stabil	ity analysis solutions		Øř 🐆
Nominal test concentration	Mean 0-hr concentrations	Mean 72-hr concentrations ¹ ,	Mean analysed concentrations	% of nontrial
(mg a.s./L)	(mg a.s./L)	(mg a.s./L)	(mg a.s./L)	
Control	-	-	- 7	- ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
Solvent control	-	-	- 1	- 5 5
0.018	0.0192	0.0104	0.015 🔊	*\$2.2 ***
0.032	0.0339	0.0199	0.027	84.1 × 0× 4
0.056	0.0599	0.0349	0.047	84.67 2
0.10	0.1044	0.0651	0.085	8428 0
0.18	0.189	0.118	0.98 0 4	85.2
0.32	0.330	0.215	∧Ø.27 Q	85.20
0.56	0.565	0.369	Q 0.47 % , & &	83.4
Mean:			4.88	.84.2
¹ Corrected by	stability analysis			A A L

There was no mortality observed in the control in the solvent control and all test concentrations no mortality >20% was observed which is considered acceptable background mortality. In the control, the mean number of 140 newborn waterbeas/adult was high compared to the profied control data, there was no biologically and statistically significant reduction of the sum of offspring per parent and the number of offspring per parent and reproduction day at the control of the sum of offspring per parent and the number of offspring per parent and reproduction day at the control of the sum of offspring per parent and the number of offspring per parent and reproduction day was significantly reduced (p=0.05) compared to the pooled controls.

Table CA 8.2.5.1/03-4	Mortality of Daphn	ia magna over 2	21-Bay exposu	re to KWG 4168

	d V	· ·	408	$\sim$	-	ć	(	1.	$\bigcirc$	6	<u> </u>	<i>c</i> ″				
Mean	(Qu'n	nulati	ve mø	talit	<b>v (%</b> )	by đầ	íy "	5	a	C	)	$\sim$				
measured	Ĩ	ů,	2	3	(Å	~	8	9 ू	\$10		14	15	16	17	18	21
concentrations 🖒	Ĩ ?		~	K,	4	Ň	Ľ,	Ň	r A	8	J.					
(mg a.s./L)	, C		D	$\bigcirc^{\vee}$	&"(		Ç,	ð			-					
Control	00° [™]	$0 \ll$	0	ຸ 0	^س 0		0	$\mathbb{S}$ 0	Ø	0	0	0	0	0	0	0
Solvent control	0	Ŕ	0	0	<del>,</del> 0	١	0		۵ ا	, Ø	7	7	7	7	7	7
0.015	~ (//	G0	<i>A</i>	0		0	<b>A</b>	0	0 0	$\widetilde{0}$	0	0	0	0	0	0
0.027 **	0	0 🛸	×0	<b>0</b>	05	0 🌾	$ $	0	$0^{\circ}$	0	0	0	0	0	0	0
0.047	D.	$0 \gtrsim$	0	0	200	7	7 0	₹7	7	7	7	7	7	7	7	7
0.085	$\widetilde{0}$	Å.	0	0	JO °	×0°	0		٥٧	0	7	7	7	7	7	7
0.15		≪0 <u></u>	Ŵ.		0 _	$\gg 0$	ð	0	0	0	0	0	0	0	0	0
0.27 ~	00	$0 \otimes 0$	0 &	Ĭ.	$\langle \rangle$	0	0	0	0	0	13	13	13	13	13	13
0.47	0	<u> </u>		0	<u>j</u> e	0,13 Ø	0 0	0	0	0	0	0	0	0	0	0
@. ¥		6	~ 12	$\bigcap$	V ·	× n	\$									

Table CA 8.2.5.1/03-5 Number of offspring of Dap Dia magna over 21-day exposure to spiroxamine

Mean measured	Total offspring ±SI		Number of offspring/adult/reproductive				
concentration	1. Q1	<u> </u>	day				
(mg a.s./L)	Mean ± SD 🔗	% of controls	Mean ± SD	% of controls			
Control O (	<b>3</b> 39.7 <b>2</b> 6.9	Ð	$10.5 \pm 2.0$	-			
Solvent control	143, <b>©</b> ≟25.4⁄ ^	<b>9</b> 02.6	$10.9 \pm 1.5$	103.4			
0.015	135.5 ± 25,3	97.0	$10.1 \pm 1.5$	96.2			
0.027	453.2 ± \$7.9	109.7	$11.4 \pm 1.6$	108.0			
0.047	£124.1 → 38.1	88.8	$9.5 \pm 2.5$	90.5			
0.085	99.7 22.4	71.4*	$8.1 \pm 1.8$	76.4*			
0.15 0	$76.1 \pm 23.9$	54.5*	$6.7 \pm 2.2$	63.8*			
0.27 U	$51.1 \pm 13.4$	36.6*	$6.4 \pm 1.6$	60.8*			
0.47	$29.6 \pm 15.6$	21.2*	$7.2 \pm 3.3$	68.5*			

* Significantly reduced compared to the pooled controls (Dunnett's t-test,  $\alpha$ =0.05)



At the end of the study, the parent animals in the control had a body length of 4.89 mm indicating well developed females of *Daphnia magna*. Compared to the pooled control data, at the concentrations from 0.015 to 0.047 mg a.s./L no significant decrease in body length was recorded. In the concentrations higher than 0.047 mg a.s./L the body length was significantly reduced (p=0.05) compared to the pooled control data.

	Dody longth of Danhuig magua of	ter 21-day exposure to spiroxamine
1 XDIE C.A. A.Z.J. 1/03-0	<b>BOOV PERSIN OF <i>DUDNNID MUSHU</i> AT</b>	ier zi-dav exposure 10 spiroxamme*

	-	-	-	-	-			, O	0 J.S	4
Mean	Control	Solvent	0.015	0.027	<b>0:0</b> 47	0.085	0.15	0.27	9.47	0
measured		control			T.	, O'	/	Ĉ.		Å
concentration				L		Ő	Ŵ		S a	$\bigcirc^{\nu}$
(mg a.s./L)				"Q"		Å.	_0	~~		1
Number	10	10	10	10	10	M &	10 A		10 5	
Mean (mm)	4.89	4.90	4.86	4.86	4.77	4.58	4.52	¥.24 🔊	3.90	
SD	0.08	0.10	0.09	KØ.11 🖉	0.17	0.27 2	<b>()</b> .20 🔊	0.17	0.80	
% of control	-	100.2	99.5 C	99.5 [©]	97,5	@3.9*~~	* 92.5 <b>%</b> *	86,7*	<b>1</b> 81.7* 。	
SD Standard de	eviation		2	. 0	<u>v</u> 4			0		
* 0' '0' 1	1 1	1.								

* Significantly reduced compared to the pooled controls (Dunnett's t-test,  $\alpha = 0.05$ )

The statistical comparison of the dry weights of the parent mimals at the end of the study showed no significant reduction of body weights at the concentrations from 0.015 to 0.085 and a.s./L the body weights were significantly reduced compared to the pooled control data (Dunnett's test, p=0.05).

Table CA 8.2.5.1/03-7	Dry weight of	L Daphrid magn	a after 21-day	expositore to s	paroxamine
		Y 1 6 97.		1. O.	•.* (m) ·

	là.	h $O'$	Ň	.0	•	AC Y		2	
Mean	Control	Solvent	0.015	<b>0.027</b>	0.047 [^]	0.985 🦼	90.15 N	0.27	0.47
measured	ĸ	control	ð Ő						
concentration	9 50 . 6	ON X		S 1	J C		L'Y'		
(mg a.s./L)	Å.	, ÔŠ			r a		» •		
Number	$\mathfrak{F} $ $\mathcal{O}'$	10 3	19	10	10,2	<b>1</b> 9 ~	<i>2</i> 10	10	10
Mean (mm)	1.20	1.288	¥1.21,7°	1,113	1.212	≫1.139 ^{©©°}	0.992	0.815	0.635
SD Ö	0,199	0.160	0.178	0¢173 "	0.111	0.187	0.104	0.175	0.202
% of control		106,90	101.1	<b>9</b> 2.4 O	100.7	94.6	82.4*	67.7*	52.7*
SD Standard d	eviation		<u>, O, i i i i i i i i i i i i i i i i i i</u>			$\searrow$			

* Significantly reduced compared to the pooled Ontrols (Dunnett's t-test, α=0.05)

The resulting NOE and LOEC salues are summarised in the table below:

Table CA 8.2.54/03-8 Summary of endpoints of Dephnia magna after 21 days exposure to spiroxamine

× i

Endpoint NOEC (mg a.s./La)	LOEC (mg a.s./L)
Sum of offspring/parent Q 0.047	0.085
Number of offspring arent/day	0.085
Body length of parent animals $\sqrt{0.04\mathcal{Q}}$	0.085
Dry weight of parent animals 0.085	0.15

# III. Conclusion &

The 21-day chronic toxicity of ¹⁴C-KWG 4168 to *Daphnia magna* was studied under semi-static conditions. Test organisms were exposed to mean measured test concentrations of 0.015, 0.027, 0.047, 0.085, 0.15, 0.27 and 0.47 mg a.s./L plus a control and solvent control. The 21-day NOEC based on reproduction was determined to be 0.047 mg a.s./L.



#### Assessment and conclusion by applicant:

The study was conducted to the OECD test guideline 202 (II), the current version of which is the OECD 211 "Daphnia magna reproduction test", adopted 02 October 2012.

Validity criteria according to the OECD 211 guideline (2012) were met:

- Mortality of the parent animals in the controls to not exceed 20% at the end of the set: (actual: 0 and 7% in the control and solvent control, respectively)
- Mean number of living offspring produced per parent animal priviving at the end of the test in the controls to be ≥60 (actual: mean total offspring 1389 and 143.3, if the control and solvent control, respectively)

The study is therefore considered acceptable.

The 21-day NOEC based on reproduction was determined to be 0.647 mg/a.s./L

The data have been subjected to statistical re-evaluation and the results have been presented in the following study summary.

Data Point:	KCA 8.2.5 106 & 2 & 2 & 2 & 2 & 2 & 2 & 2 & 2 & 2 &
Report Author:	
Report Year:	
Report Title:	Calculation of EC10 and EC20 values for Daphnia magna with 14C-spiroxamine
	Tổ in a teproduction study 💦 😽 🖓
Report No:	(0471836)ÉCOA
Document No:	M-76 544-01-1 & A A A
Guideline(s) followed	Annex to Com. Reg. 283/2013
study:	
Deviations from current	
test guideline:	
Previous evaluation:	No not previously osubmitted
Previous evalution:	No, not previously osubmetted
recognised@sting	soft applicable
facilities:	
Acceptability/Reliability:	Yes in the second
Executive Summary	

#### Executive Summar

The report M-006466 Q1-1 of the effects of ¹⁴C-Spiroxanine TG on the reproduction of water fleas (*Daphnia magna*) study did not provide estimates of EC₁₀ or EC₂₀ values. Therefore, these values have been calculated in accordance with the Annex to Com. Reg. 283/2013. Effect Concentrations with a 10% and 20% effect on dry weight and reproduction when compared to the control were re-calculated. As there were no effects on the parameter survival was not possible to determine reliable ECx values. The resulting EC₁₀, and EC₂₀ values for ength at 21 d were 0.194 (95% CL: 0.168 – 0.219) and 0.523 (95% CL: 0.453 – 0.629) mg a S./L, respectively. The resulting EC₁₀ and EC₂₀ values for reproduction at 21d were 0.039 (95% CL: 0.028 – 0.049) and 0.065 (95% CL: 0.052 – 0.077) mg a.s./L, respectively.

### I. Methods

The statistical evaluation was performed with statistical software ToxRat Professional v3.3.0.

Effect concentrations with 10 and 20% effects from the test item treatment when compared to the pooled controls were calculated but due to lack of a dose response, these could not be determined for survival. A Probit function using linear maximum likelihood regression was used along with 95%  $EC_x$  confidence



limits for length and reproduction while a Logit function using linear maximum likelihood regression was used along with 95% confidence limits for dry weight.  $Q_{p}^{\circ}$ 

#### II. Results and Discussion

An explanation is given for regression analysis endpoints for length, dry weight and reproduction.

#### Length at 21 days

Regarding the calculation of  $EC_{10}$  and  $EC_{20}$  values for length at 21 d, the criteria for goodness of fit were met as the P(Chi²) value was 1.00, showing no significant deviation between fit and that, and a statistically significant concentration/response was found (p(F) < 0.001) for this parameter.

The resulting  $EC_{10}$ , and  $EC_{20}$  and values and the respective confidence intervals are represented in the following table.

#### Table CA 8.2.5.1/06-1 Results of the Probit analysis (max. likelihood regression) with length at 21 d: Selected effective concentrations (EQ) of the test item and their 95%-confidence limits (according to Dieller Stheorem)

Parameter	A95 % confidence interval)
Effect on length at 21 d	0.523 $0.453$ $0.629$

The resulting  $EC_{10}$  and  $EC_{20}$  values of 0.04 (95% CL 0.168  $\neq$  0.21%) and 0.523 (95% CL: 0.453 – 0.629) mg a.s./L, respectively, meet the coordinate of fit criteria and therefore the estimated  $EC_{10}$  value is considered reliable for use within a risk as essented.

#### Dry weight at 21 d

Regarding the calculation of EC₁₀ and EC₂₀ values for ary weight at 21 d, the criteria for goodness of fit were met as the  $\Psi(\text{Chi})$  value was k00, showing to significant deviation between fit and data, and a statistically significant concentration response was found (p(F)  $\approx 0.004$ ) for this parameter.

The resulting  $EC_{10}$  and  $EC_{20}$  values and the respective confidence intervals are represented in the following table below.

#### Table CA 8.2.5.1/06? Results of the Logic analysis (max likelihood regression) with reproduction at 21 d:Selected effective concentrations (EO₃) of the test item and their 95%onfidence limits (according to Fieller's theorem)

~~	Dry weight	
Parameter	EC10 S A C	EC20
	(93 % confidence interval)	(95 % confidence interval)
~	[mg a.s./L] > 4 . 9	[mg a.s./L]
Effect on dry	0.075(0.036-0.119)	0.150 (0.099 - 0.192)
weight at 21 d		0.150 (0.099 0.192)

The resulting  $C_{10}$  and  $EC_{10}$  values of 0.0% (95% CL: 0.036 – 0.111), and 0.150 (95%CI: 0.099 – 0.192) mg as /L, respectively, meet the goodness of fit criteria and therefore the estimated  $EC_{10}$  value is considered reliable for use in the risk assessment.

Reproduction council at 21 d

Regarding the calculation of  $EC_{10}$  and  $EC_{20}$  values for reproduction at 21 d, the criteria for goodness of fit were that as the P(Chi²) value was 1.000, showing no significant deviation between fit and data, and a statistically significant concentration/response was found (p(F) < 0.001) for this parameter.

The resulting  $EC_{10}$  and  $EC_{20}$  values and the respective confidence intervals are represented in the following table and figure below.



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 Table CA 8.2.5.1/06-3
 Results of the Probit (max. likelihood regression) with reproduction at 21 d: Selected effective concentrations (ECx) of the test item and their 95%-confidence of limits (according to Fieller's theorem)

	, s		Ç7
	Reproduction		p ^r
Parameter	EC ₁₀ (95 % confidence interval)	EC ₂₀ (95 % confidence interval)	
	[mg a.s./L]	[mg a.s./L] 🔬 🔊 🔗 🔗	2
Effect on	0.039 (0.028 – 0.049)	0.065 (0.052 - 0.077)	?
reproduction at 21 d	0.039 (0.020 0.049)		,O
	- W		al a

The resulting EC₁₀ and EC₂₀ values of 0.039 (95% CL: 0.028 - 0.049) and 0.065 (95% CI: 0.052 - 0.049) and 0.065 (95% CI: 0.052 - 0.049) mg a.s./L, respectively, meet the goodness of fit criteria and the estimated EC₁₀ value is considered reliable for use within a risk assessment.

#### III. Conclusion

The resulting EC₁₀, and EC₂₀ values for length at 21 a were 0.194 (95% at : 0.168 – 0.219) and 0.523 (95% CL: 0.453 – 0.629) mg a.s./L, respectively. The resulting EC₁₀ and EC₂₀ values for dry weight at 21d were 0.076 (95% CL: 0.036 – 0.111) and 0.750 (95% CE 0.099 – 0.192) mg a.s./L, respectively. The resulting EC₁₀ and EC₂₀ values for reproduction at 21d were (0.039 (95% CE 0.028 – 0.049) and 0.065 (95% CL: 0.052 – 0.077) mg a S.L, respectively.

#### Assessment and conclusion by applicant:

The statistical re-evaluation of the data has determined  $E_{C_{10}}^{(0)}$  and  $E_{C_{20}}^{(2)}$  values for length, dry weight and reproduction and these values are considered to be reliable. The lowest  $E_{C_{10}}^{(1)}$  and  $E_{C_{20}}^{(2)}$  values of 39 and 65 µg a.s./L, respectively were determined for reproduction. Thus, the EC₁₀ of 39 µg a.s./L is lower than the NOEC of 47 µg a.s./L for this parameter. The EC₁₀ of 39 µg a.s./L shall therefore be taken as the critical endpoint determined from this study.

The values determined in the re-evaluation work are considered to be fully valid.

# CA 8.2.5.2 Reproductive and development toxicity to an additional aquatic

No reproductive data with an additional aquatic invertebrate species are available. Spiroxamine is a fungicide and does not display insecticidal activity therefore chronic data with an additional species of aquatic invertebrate are not required. However, sediment-water toxicity studies with *Chironomus* and *Lumbriculus* are available and have been summarised and expoint CA 8.2.5.4.

## CA 8.2.5.3 @ Development and emergence in Chironomus riparius

Spiroxamine is not an insecticite nor seit an GR therefore a chronic study with *Chironomus* is not triggered However, in accordance with the Aquatic Guidance Document (EFSA, 2013), a study with a sediment-dwelling organism has been triggered based on the environmental fate water-sediment study which demonstrates that sonoxamine is present in the sediment at  $\geq 10\%$  of the applied radioactivity on Day 14. Furthermore, the chronic *Daphnia* NOEC is <0.1 mg a.s./L therefore, taking these two points together, a study with a sediment-dwelling organism is automatically required. A water-sediment toxicity stud with *Chironomus* has been summarised under point CA 8.2.5.4.



Data Point:	KCA 8.2.5.4/01
Report Author:	
Report Year:	1998
Report Title:	Influence of 14C-KWG 4168 (techn.) on development and emergence of Marvae of
	Chironomus riparius in a water-sediment stystem
Report No:	HBF/CH 21
Document No:	<u>M-006549-01-1</u>
Guideline(s) followed in	Proposal for a BBA-Guideline: "Effects of planoprotection products of the $\ll$   $\ll$
study:	development of sediment-dwelling larvae of Phironomus riparius in water and a sediment-dwelling larvae of Phironomus riparius in a water and a sediment-dwelling larvae of the sediment of the
	sediment system" (1995)
Deviations from current	Yes Q Q Q Q
test guideline:	$1 \text{ UECD } 219(2004)$ $\mathbb{Y}$ $\mathbb{Z}^{2}$ $\mathbb{Z}^{2}$ $\mathbb{Z}^{2}$
	Replication of vessels not as for current guidance which recommends at least four
	replicates per controland test group
	Three-litre glass brakers were used as test vessels, however, 600mL glass
	beakers are recommended by the current guidance. However, the larger vessels
	were not likely to have a negative impact on the organisms, based on space
	available per arvae
	The composition of the artificial sediment is not as per surrent surdance
	however, it was prepared following OFCD 200 guidance at the time of testing.
<b>D</b> 1 1	Based on the control omergence rate this did not impact the Validity of the test
Previous evaluation:	yes Evaluated and accepted
	DAR (1997), RAD (2016), RAB (2017)
GLP/Officially	The second ucted under GLP/Officially recognised testing facilities
recognised testing	
facilities:	
Acceptability/Reliability:	$\begin{array}{cccccccccccccccccccccccccccccccccccc$

CA 8.2.5.4	Sediment dwelling	organisms
CI1 0.2.3.T	Scullicht uwening	of gamons

A water-sediment study using *Chironomus* is available and has been summarised below. According to the Aquatic Gundance Document (FFSA, 2013) the preferred species for fungicides is *Lumbriculus* therefore a new study using *Lumbriculus* has been conducted and has also been summarised below.

dwelling life stage O Chironomus riparius. Test organisms were exposed to nominal concentrations of 0.1, 0.18, 0.32, 0, 6, 1.0, 1.8, 3, and 3, 6 mg & S./L and a control and solvent control.

Three times during the study fon days 0, 7 and 280 the radioactivity was measured in all test solutions. Based on the ratio between analysed concentration of KWG 4168 technical and the radioactivity measurements in the stock solutions, the concentrations of test compound in the overlying water were calculated as a.s.-equivalents. The day 0 apprytical samples were taken 1 hour after application. These results indicated that \$9 to 105 % (for an average \$9 %) of the nominal concentration was parent KWG 4168 The initial test concentrations were prepared correctly as verified by the measured concentration analysis. The pointial initial concontrations were used to calculate EC-values. On day 7 after application, the percent parene KWG 4168 in the test solutions was 52 to 65 % (for an average 58 %) of the initial concentrations. On Day 28 after application, the percent parent KWG 4168 was 38 to 49 % (for an average 44%) of nominal concentrations.

The EQS with regard to development of male and female midges ~5.6 mg initial nominal a.s./L (EC50 > 5.6 mg/L) The NDEC with regard to emergence rate was determined to be 5.6 mg a.s./L.

Materials and Methods ÆĨ. A. Materials

**Test Material** KWG 4168 (tech.)



Lot/Batch #:	07002/96
Active substance content:	96.3%
Specific activity:	3.68 MBq or 99.5 $\mu$ Ci/mg (radiochemical purity = $\sqrt{8\%}$ )
<b>Description:</b>	Clear yellow liquid
Stability of test compound:	96.3% 3.68 MBq or 99.5 μCi/mg (radiochemical purity = 98%) Clear yellow liquid Sufficient based on expiration date 10 March 1998 Not reported 0.10, 0.18, 0.3240.56, t0, 1.8 3.2 and 5.6 mg a.s. 4. Dimethylformamide (DMF) Day 0, 7 and 28 Chironamus riparius Obtained from a culture maintained at the University of Sheffield (UK) Foo the breading phase, the midges are kept in plastic tages (60 x 60 x 55
Reanalysis/Expiry date:	10 March 1998 $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4)$ $(4$
Density:	Not reported
Treatments	
Test rates:	0.10, 0.18, 0.3240.56, 400, 1,803.2 and 5.6 mg a.s. 4
Solvent/vehicle:	Dimethylformamide (DMF)
Analysis of test concentrations:	Day 0, 7 and 28 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2
Test organisms	
Species:	Churonomus riporius 2
Source:	Obtained from a culture maintained at the University of Sheffield (UK)
Acclimatisation period:	ciff) with plastic gauge on the sides. A basin (45 \$ 55 x 10 cm) made of inert plastic is secured to the bottom of each breeding cage. The bottom of the basins are covered with a thin layer of "Kieselgur" (silica). Reconstructed water M2 (Elendt, 1999) was added to the basin to a depth of approximately 3 cm. The water in the basin was aerated gently. To start
Replication:	Three per control and solvent control, single replicate for the test
Kephtauon:	concentrations



No. of animals/vessel:	25 ذ &
<b>Duration of test:</b>	28 days
Environmental test conditions	
Temperature:	$20 \pm 2^{\circ}C$ (measured = 18.8 to 20.7°C) 6.7 to 10.0 mg/L 6.6 to 7.7
Dissolved oxygen:	6.7 to 10.0 mg/L
pH:	6.6 to 7.7
Photoperiod:	6.7 to 10.0 mg/L 6.6 to 7.7 16:8 hours light-dark cycle (including half hoar dusk and dawn) mean light intensity – 3800 fax
B. Study Design	
KWG 4168 (tech.) was test life stage of <i>Chironomus n</i>	sted to assess the potential impact on the maturation of the sedimented welling

Each test vessel contained twenty-five mimals, three teplicates were prepared for the control and solvent control, whereas, single replicate vessels were prepared for the test concentration groups. The test was conducted at 18.8 to 20.7°C and a to 8 light-dark cycle (including half four dusk and dawn). Mean light intensity was about 3800 lux.

The bottom of the test containers (3L glass beakers) were covered with a 2-cm deep layer of sediment. Prior to adding the test water, the sediment was covered with a plastic sheet. This was done to avoid a separation of the sediment ingredients when the water was poured into the test vessel. The test water was slowly poured into the beaker (the beakers were filled with 2.65 L water) and then the sheet was carefully removed. The height of the water was 20 cm. Gentle aeration was provided through a glass Pasteur pipette situated about 2. Scm above the sediment layer. Test beakers were covered by clear plastic plates to prevent evaporation and prevent emerged nordges from escaping.

The range of text concentrations were selected to determine the  $\mathbf{SC}_{15}$ . The following initial nominal test concentrations were chosen 0.10 0.18 0.32 0.56, 60, 1.8, 3.2 and 5.6 mg a.s./L. For biological evaluations, three replicates were prepared of the control and solvent control and one replicate for each test concentration. For chemical analysis of the active substance additional parallel replicates were prepared for analytical putposes only ( $\mathbf{OI8}$ ,  $\mathbf{k0}$ , and 5.6 mg a.s./L: 2 replicates). These analytical replicates were prepared and mantained under identical conditions to the biological test chambers; the analytical replicates also contained organisms and got food Each of these extra beakers was used for all analyses (water, port water, and sediment).

Dimethylformamide (DMF) was used to prepare the stock solutions and the solvent control. The solvent load in the solvent control and all test concentrations was 0.1 mL/L. The dilution water control consisted of water only; no solvent and no compound.

Nominal test concentrations were 0.10, 048, 052, 0.56, 1.0, 1.8, 3.2 and 5.6 mg a.s./L along with control and solvent control groups.

The test vessels were observed at least three times per week to make a visual assessment of any behavioural differences compared to the control. The sex, time to emergence and number of emerged or not fully emerged adults was becorded daily during the period of emergence. As only fully emerged adults are relevant for the empoints of this study, larvae which did not mature were not evaluated.

To determine number and sex of emerged adults, the covering plates of each test container were carefully removed. The midges, which mostly stayed at the sides of the vessels, were enumerated and the sex of each was determined (male midges have feathered antennae). The midges were removed and discarded from the test vessels at the end of each observation period.



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Average:

Min:

Max:

44.0

37.8

48.7

#### **Analytical method**

Samples of water were analysed using the validated analytical method 00252 M001, report reference M-008490-02-2 (see Doc MCA Section 4).

#### II. **Results and Discussion**

The study was deemed to be valid in the report as 90% of the larvae inserted into the control and control groups had emerged.

Three times during the study (on days 0, 7 and 28), the refroactivity was measured in all test volutions Based on the ratio between analysed concentration of KWG 4168 technical and the ordioactivity measurements in the stock solutions, the concentrations of test compound in the overlying water were calculated as a.s.-equivalents. The day 0 analytical samples were taken & hour after application. These results indicated that 89 to 105 % (for an average 99 %) of the nominal concentration was parent KWG 4168. The initial test concentrations were prepared correctly as verified by the measured soncentration analysis. The nominal initial concentration were used to calculate C-vanies. On day 7 after application, the percent parent KWG 4168 in the test solutions was 52 to 65 % (for an average 58 %) of the initial concentrations. On Day 28 after application, the percent parent KWG 4168 was 38 to 99 % (for an average 44 %) of nominal conceptrations.

Table CA 8.2	2.5.4/01-1 S	ummary o	fanalysis		G 4168 (tê	sh.) in the	e overlying v	water 🖉	
Nominal	Day 0	a	* 2	Day 7	ş _Q	Å	Day 28	la la	
concentrati on (mg/L)	Measured radioactiv ity (dpm/10 mL)	Meast ed ass equivale mi (mg/L 4)	%% nomin Al ¹⁴ C KW 4168 (toch.) Conc.	(dpm/10 /	s (	Albert nomin al 40 KWG 4168 (tech.K Conc.	Measured radioactiv ity dopm/10 mL)	Measur ed a.s. equivale nt (mg/L)	% of nomin al ¹⁴ C- KWG 4168 (tech.) Conc.
Control	5.8	ð ~	)- ₁ , 7	54	¥- 00	- 4	<u>JØ</u>	-	-
S. control	5.40	<u>∖- ∜</u>	-%	4.5 L	- ~		2.4	-	-
0.10	1561.9	0.1	<b>O</b> 1.3 §	949.4	0055 4	\$54.6	818.0	0.047	47.0
0.18	1806.1 0	0.19	105.2	950.4	9.10	55.2	836.0	0.088	48.7
0.32	1829.9	KØ.33 🔊	1045	10\$6.7	0.19	<i>59</i> 92	803.6	0.15	45.9
0.56	1739.4	0.50	889	1014.8	0.29	<b>⊘</b> 1.8	789.0	0.23	40.2
1.0	1864.3	1.0%	≪ <b>1</b> 02.3	1095,4%	Q0.60 A	60.1	834.4	0.46	45.8
1.8	1841	1.7	95.1 🕎	1105.6 (	1.03	57.2	850.9	0.79	44.0
3.2	18.28.2	8.1	975	s1159.7 _{€®}	1.98	61.8	799.6	1.36	42.6
5.6	1861.9	5.4	\$ <del>9</del> .0	01241,30	3.62	64.6	725.1	2.11	37.8

Table CA 8.2.5.4/01-1 Summa	y of analysis of	¹⁴ C- <b>R</b> WG 4168	(tech.) ir	n the overlying	g water 🔬
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Pore water samples were taken from concentrations 0.18, 1.0 and 5.6 mg/L and the radioactivity was measured in these samples. The pore water samples were taken from the parallel replicates at these test concentrations, The parallel replicates were oncluded in the test system to provide samples for pore water and sediment analysis on Des 7 and Day 28. These replicates did also contain test organisms. The results are given in the table below.

Average:

Min:

Max:

58.1

51.8

64.6

Table (X 8.2.5 701-2 Analytical results of pore water

Average

Min:

Max

.68 (

88

105.2

Initial Nominal concentratio n (mg/L)	Volagn e (mL) day 7	Measu	ctivity o	on day 7 averag e	Total radioactivit y (Bq)	Volum e (mL) Day 28			on day 28 averag e	Total radioactivit y (Bq)
0.18	155	161. 8	159. 9	161	42	115	267. 0	246. 7	257	49



1.0	140	58.9	50.9	55	13	115	262.	269.	266	51		1
							7	4			0	
5.6	100	32.6	31.5	32	5	100	110.	114.	112	19	<u>N</u>	5
							3	5				Ş
The total radi	ionotivity Do	Deguer		alaulatadi	average dpm/10	m I v volu	$\frac{3}{10}$	5			<u>×</u>	¥.

The total radioactivity Bq (Bequerel) was calculated: average dpm/10 mL x volume / 10 / 60

ø) The wet weights of the sediment before vacuum filtration, and the dry weights of the sediment after vacuum filtration were recorded. Dry weights were calculated from the wet weight and the volume of filtered pore water. The total radioactivity in the sediment was calculated

	~ -	~		~	-	<u> </u>			$\sim ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~$
Initial	Stud	Sedimen	Volume	Calculate _	Measured		vity in	Averag 4	TotaO 2
Nominal	y day	t weight	of pore	d A	, the sedim	ent 🍫	¢ ô	e 🛴	TotalO' radioactivit
concentratio		before	water	sediment [®] weight		$\sim$ (	7, ``≶	、O″	v∕≱in the <i>@</i> ∥
n		vacuum	remove	weight		6°, °N	1 and the second	s s	«sediment»
(mg/L)		filtration	d from	after	Cdpm/g~	(dpm/g	(djpun/g		V(Bq)
		(g)	the	vacuum 🔬		)	(dipun/g	d' L	A
			sedimen	filtration	, ©			$\bigcirc^{\nu}$	
			t (mL)	K(g) °∕Y		× A		2	
0.18		444.7	155	₹ 289 Ž 🎽	Z90.5 🔊	351.4	319.9	2 <b>2</b> 0.6 火	1548 کې 1548 ر
1.0	Day 7	436.6	140 0	296,6 😤	411.4	32*6	291.4	Ø374.8 🖓	1853
5.6		412.2	100	2.2 ×	69 <b>0.9</b>	چ 0.006.0	Q688.7 S	795	4@8
0.18	D	410.9	1Q'	295.9		3651.5	637	677.8 2	3343
1.0	Day 28	409.5	J15 🔊	2945	Ç611.9	674	700.1	<b>6</b> 63.0	3254
5.6	20	379.8 🔊	7 100 📎	279.8	624.8	672.8	634.3	643.9	3003

The total radioactivity Bq (Bequerrel) calculated: @erage dpm/g/60x dry weight (g)

At study days 0, 7 and 28, samples of the overlying water were malysed with Thin Layer Chromatography (TLQ) in order to differentiate between the radioactivity of the active substance and metabolites. The results are calculated from the amount of radioactivity applied to the beakers in the concentrations 0.18 1.0 and 5.6 mg/L and the percentage of active substance and metabolites. The analyses were performed in two different ways: the results obtained by the first method (silica gel) are reported here results obtained by the reversed phase method are very similar and confirm these results very well.

Table CAC8.2.5.4/01-4	<b>Measurement</b>	of radioactivity in	the sediment
	a cubu culture	A manoquer in the	cit & spannen y

Initial	Study dax	Measured	Calculated	Percentage	Calculated	Calculated
nominal	S a	radioactivity		of a.s.	radioactivity	radioactivity
	<i>"</i> Q. ""	(faveræge) 🔬	radioactivity	obtained	as a.s.	as
(mg a.s./L)	õ ^v oa	(dpm/10 _ Oʻ	in water 🛷	from the	(Bq)	metabolites
		mal N	in vater 🔊 (Ba) 🏷	thin layer		(Bq)
4	Or .	S AS .		analysis		
a de la constante de la consta				(%)		
0.18		1820,9	80×9	100	8042	0
1.0	Day 0	1926.0	<b>&amp;50</b> 7	100	8507	0
5.6 *	, O	₫900.2 👻	<b>\$</b> 392	96.84	8127	265
0.18		925.2 0	4089	36.91	1509	2580
1.0	Day 7	11114.1	4920	40.23	1979	2941
5.6	Daty 7	¥208.6 ~	5338	61.96	3307	2030
0.18	Ş Ö	336.0	3692	1.73	64	3628
1.0	Day 28	834.4	3685	4.98	184	3502
	Day 28	725.1	3203	27.34	876	2327

The total redioactivity Bq Bequerel) was calculated: average dpm/10 mL x 265 / 60

The resolts achieved in the study have been presented in terms of the nominal overlying water concentrations.



The %-emergence of midges in the control and solvent control fulfilled the guideline requirements: 90 % of the inserted larvae maturated to adults. The x2-test established no difference of sex in emerged midges at any test concentration (p = 0.05). Because it was not possible to introduce the same number of female and male organisms as larvae into each test beaker, the emergence rates of male and female mumbers were pooled for the statistical analysis.

Since the rate of development (male and female midges) was not influenced at any test concentration, except for the delay of 17.5 % (related to the pooled control results) at the highest test concentration of 5.6 mg a.s./L, the EC₁₅ for numbers the development rate was ~5.6 mg initial nominal a S./L ( $EC_{50} > 5.6$  mg/L).

The NOEC with regard to emergence rate and development rate was determined to be > 96 mg@s

	1	, [°]		
Initial nominal	Number of	Emergence (%) of	🕅 % mate 🖉 🔬	% female
concentration (mg	emerged midges	inserted larvae 🖉	emorgence 0	
a.s./L)		ANT		
Control	68	90.7* ~ ~ .	©#2.6 , , O ^v	\$7.4 \$ \$50.7 \$
Solvent control	67 🖉	89,3*	49.30° 🔊 á	¥ 30. / 🔊 👘
0.10	23	92,0 ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	52,72 ,0 ,5	47.8
0.18	22	<b>808</b> .0 × ×	A\$.5 8 A	54.5 J
0.32	22 👋 õ	88.0 0 0	45.5 <u>0</u> <u>0</u>	C54.5 🔭
0.56	25	1000 5	56.QO O A	¥ 44. <b>©</b>
1.0	19	7620	52.6 0	47.9
1.8	19	\$6.0 ° °	47.4 ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	<i>\$</i> 2.6
3.2	24 5	96.0	∿54.2∜ <u>~</u> \$* a	45.8
5.6	20, ~ ~	80.5 20 0	5060	50.0

Ø1

	Summary of numbers of	
Table CA 8 2 5 4/01 5	Summary of numbers of	f approad midgas
1 abic CA 0.2.3.4/01-3	Summary of numbers of	i chagoigeu innuges 🖇

*related to three beakers with 2 larvae/each; in all other cases related to 1 beaker

Table CA 8.2.5.4/01-6	Man development	time of Chiran	omus riparius ex	posed to spiroxamine
A				1. 1

Initial nomina Concerdination	Replicate 2	Mean development time	Mean development
Initial nomina Conceptration (mg a.s./L)	Replicate 2	(days) O	rate (1/d)
Control		1978 a.	0.056
		16.2 ×	0.061
Control Control		16.1	0.062
Control Contro	Mean 🗸 🗸	\$6.7 ± 0.91	$0.060 \pm 0.003$
		16.1	0.062
Solvent control		16.4	0.061
Solvent control Solvent control		16.4	0.061
	Mear	$6.3 \pm 0.20$	$0.061 \pm 0.001$
		16.1	0.062
0.18	8	16.2	0.062
0.32		17.1	0.058
	7 - ~ ~~	16.3	0.061
		16.6	0.060
1.8		16.8	0.060
		16.6	0.060
1.0     1.0       1.8     1.0       3.2     1.0       5.6     1.0		19.4	0.052

The results of this study are summarised in the table below (mg a.s./L, based on nominal initial concentrations):

 Table C 8.2.5.4/01-7
 Effects of KWG 4168 exposure on Chironomus riparius

Endpoint	EC ₁₅	95% confidence limits	EC50
Emergence rate (mg a.s./L)	> 5.6	Not calculated	> 5.6



Development rate (mg a.s./L)	~ 5.6	Not calculated	> 5.6

#### III. Conclusion

¹⁴C-KWG 4168 (tech.) was tested to assess the potential impact on the maturation of the sediment dwelling life stage of *Chironomus riparius*. Test organisms were exposed to nominal concentrations of 0.1, 0.18, 0.32, 0.56, 1.0, 1.8, 3.2 and 5.6 mg a.s./L and a control and solvent control.

The EC₁₅ with regard to development of male and female midges ~5.6 mg/initial nominal a.s.  $\mathcal{L}$  (EC >5.6 mg/L). The NOEC with regard to emergence rate was determined to be 5.6 mg as  $\mathcal{L}$ .

#### Assessment and conclusion by applicant:

An assessment has been made against the validity criteria in the current QECD 219 (2004) test guideline:

- The emergence in the control and solvent control must be at least 70% at the end of the test (actual: 90.7 and 89.3%, respectively)
- *C. riparius* emergence to adults from control vessels should occur between 12 and 23 days after their insertion into the vessels (actual: between days 57 and 21)
- At the end of the test, pH and he dissolved oxygen concentration should be measured in each vessel. The oxygen concentration hould be at least 60% of the air saturation value (ASV) at the temperature used, and the pH of overlying water should be in the 69 range in all test vessels (actual: Dissolved oxygen: 90 to 100 mg/2, pH; 6.6 7.9)
- The water temperature should not differ by more than +4.0°C The water temperature could be controlled by isothermal room and in that case the room temperature should be confirmed in an appropriate test vessel (actual: 4.9°C however, the control emergence would suggest that this temperature variation had to impact on the organisms)

The study was conducted in 1998 and therefore followed the BBA test guideline in place at the time. Several differences exist between this test guideline and the current @ECD 218 and 219 test guidelines, most notably the number of repricates used and the size of the test vessels. This study tested only a single replicate of 25 organisms at each test substance concentration as opposed to the four replicates of 20 organisms (total: 80 organisms per treatment). The artificial sediment is also different to that currently recommended.

All these points taken into consideration the results are still considered to be suitable for use in the risk assessment as the study met the requirements of the test guideline at the time and the results largely fulfil the validity criteria of the current OECD test guideline. The study is therefore, considered acceptable

The EC₁₅ with regard to development of nale and fencele midges  $\sim$ 5.6 mg initial nominal a.s./L (EC₅₀ > 5.6 mg/t)). The NOEC with begard to emergence rate was determined to be 5.6 mg a.s./L.

This study used an overlying water spike test method. However, due to the potential concern for spiro amine to affect sedment dwelling organisms, a study in which the test vessels were dosed *via* the sediment may have been preferable. I should be noted that a recent water-sediment study conducted using *Lumbriculus* is available and has been summarised in KCA 8.2.5.4/03 below.

The data have been subjected to statistical re-evaluation and the results have been presented in the following study summary.

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Data Point:	KCA 8.2.5.4/02	
Report Author:		
Report Year:	2020	, Or
Report Title:	Calculation of EC10 and EC20 values for Chironomus riparius with 14C-	F
Report No:	0471836-ECO5	
Document No:	<u>M-760403-01-1</u>	»
Guideline(s) followed in	None V V	2
study:		a
Deviations from current	None 🕅 🖉 🖉 🖉	Ľ
test guideline:		0°
Previous evaluation:	No, not previously submitted	1
GLP/Officially	No, not conducted under GLP/Officially recognised testing facilities	
recognised testing		
facilities:		
Acceptability/Reliability:	Yes A & Q Q A O A O' A' A'	

#### **Executive Summary**

The report <u>M-006549-01-1</u> on the effects of C-Spiroxanone TG on the development and emergence of the non-biting midge (*Chironomus ripartus*) study did not provide estimates of EG₁₀ or EC₂₀ values. Therefore, these values have been calculated in accordance with the Annex to Com Reg. 283/2013. Due to a lack of dose response, it was not possible to calculate reliable EQx values for other of the parameters tested.

#### I. Methods

The statistical evaluation was performed with statistical software ToxRat Professional v3.3.0.

Effect concentrations with 10 and 20% from the test stem treatment when compared to the pooled controls were calculated for cumulative emergence and development rate, but due to lack of a dose response, these could not be determined for either parameter.

#### II. Results and Discussion

An explanation is given for regression analysis endpoints for cumulative emergence and development rate. These details can be found below

#### Cumulative emergence at 28 days

Due to the lack of a significant dose response on the emergence, when compared to the pooled control, it was not possible to calculate  $EC_{10}$  and  $EC_{3}$  values.

#### Development rate at 28 d

Due to the lack of a significant dose esponse on the development rate, when compared to the pooled control,  $\Re$  was not possible to calculate EQF and  $\Re C_{20}$  values.

#### Development rate for males at 28 d

Due to the lack of a significant dose desponse on the development rate in males, when compared to the pooled control it was not possible to calculate  $EC_{10}$  and  $EC_{20}$  values.

#### Development rate for fepales at 28 d

Due to be lactor a significant dose response on the development rate in females, when compared to the project control, towas not possible to calculate  $EC_{10}$  and  $EC_{20}$  values. Sex ratio at 28 d

According to the obtained results due to the  $p(Chi^2)$  being above the chosen alpha, no effects were detected on sex ratio differences at the study termination.



#### III. Conclusion

Due to a lack of dose response, it was not possible to calculate reliable  $EC_x$  values for any of the parameters tested.

#### Assessment and conclusion by applicant:

The statistical re-evaluation of the data confirmed that due to a lack of a significant dose response it was not possible to determine reliable  $EC_{10}$  and  $EC_{20}$  values for emergence and development rate.

The NOEC of 5.6 mg a.s./L shall remain the critical endpoint determined from this study.

The values determined in the re-evaluation work are considered to be fully valid

Data Point:	KCA 8.2.5.4/03
Report Author:	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$
Report Year:	
Report Title:	Spiroxamine technical - Effects on Lumbriculus variegaus in a sediment-wate
	system - exposed via spiked settiment O 47 20 20 20
Report No:	
Document No:	M-68812701-1 5 5 5 5 5 5
Guideline(s) followed in	Regulation 1107/2009 (Europe)
study:	Regulation 1107/2009 (Europe)
	Lumbriculus Foxicity Test bring Spiked Segiment', adopted October 16, 2007
Deviations from current	Neine A A A A
test guideline:	
Previous evaluation:	No, not previously submitted where the second s
GLP/Officially recognised testing	Yes, conducted under GLP/Officially recognised testing facilities
recognised testing	
Acceptability/Rehability:	
ð st	

#### **Executive Summary**

The 28-day chronic toxicity of spiroxanine technical to *Lumbriculus variegatus* was studied under static exposure conditions. Test organisms were exposed, to an intreated control, solvent control and spiroxamine technical at nominal concentrations of 6.25, 12.5, 25, 50 and 100 mg a.s./kg dry sediment. The test concentrations correspond to the weighted average concentrations of 4.44, 8.88, 16.7, 35.5 and 74.2 mg a g/kg dry sediment.

The endpoints of this study were the total number of light individuals per replicate and their biomass.

The 28-day  $EC_{10}$ ,  $EC_{50}$ , NOEC and LOEC values for total number of worms were determined to be 27.6, 74.2, 35.5 and 74.2 mg a.s. kg dry sediment, respectively. For weight based on the dry weight of the worms, the  $EC_{10}$ ,  $EC_{50}$ , NOEC and LOEC values were determined to be 20.4, 40.3, 16.7 and 35.5 mg a.s./kg dry sediment, respectively. For individual weight, the  $EC_{10}$ ,  $EC_{50}$ , NOEC and LOEC values were determined to be 20.4, 40.3, 16.7 and 35.5 mg a.s./kg dry sediment, respectively. For individual weight, the  $EC_{10}$ ,  $EC_{50}$ , NOEC and LOEC values were determined to be 7.12, 41.5, 66.7 and 35.5 mg a.s./kg dry sediment, respectively.

The analytical determination showed that the test item was not stable during the 28-day exposure period. Since the recovery deviates from the nominal concentrations by more than  $\pm 20\%$  of the nominal concentrations, the effect concentrations refer to time weighted average concentrations.

I. Materials	and Methods
Test Material	Spiroxamine technical

Lot/Batch #: AE 1344293-01-07



Purity:	97.0%
<b>Description:</b>	Light-yellow liquid
Reanalysis/Expiry date:	04 June 2019
Treatments	
Test rates:	Light-yellow liquid 04 June 2019 Nominal: 6.25, 12.5, 25, 50 and 100 mg a.s./kg dry sediment TWA: 4.44, 8.88, 16.7, 35 and 74.2 mg a.s./kg dry sediment Acetone Yes, 60 -79% of nonanal
Solvent/vehicle:	Acetone
Analysis of test concentrations:	Yes, 60 -79% of nominal
Test organisms	
Species:	Lumbriculus pariegous, 14 days of at test initiation $O'$ $O'$
Source:	In-house aboratory culture
Acclimatisation period:	Acetone Yes, 60 - 79% of nominal <i>Lumbriculus bariegeous</i> , 14 days old at test initiation In-house laboratory culture 2 days None, because the sediment contained a food source <i>e. Urtica</i> powder Glass beakers of 250 mL volume covered with a pierced lid
Feeding:	None, because the sediment contained a food souce O.e. Urtica powder
Test design	
Test vessel:	Glass beakers of 200 mL volume covered with a pieteed lid
Test medium: 🔬	Formulated settiment and reconstituted water
Replication:	4 replicates per test concentration and the control, 6 replicates for the solvent control $\sim$
No. animals/vessel:	10 animals per replicate
DUTABED OF LEST.	28 days A SY B Q A
Environmental test	4 replicates per test concentration and the control, 6 replicates for the solvent control $\sqrt{200} - 24$ for $\sqrt{200} - 2$
Temperature.	$200^{\circ} - 210^{\circ} C_{\odot} O^{\circ} O^{\circ} O^{\circ}$
Dissolved oxygen?	83.0,-99.0% of the ar saturation value
pH:	$7.35 8.3 Q^{7} Q^{7} Q^{7} Q^{7}$
Photoperiod:	to hour light & hours dark (light intensity 210 to 400 lux)
<b>B</b> . Study Design	be hours light & hours dark (light intensity 210 to 400 lux)
This study was conducted	in order to assors the potential impact of spiroxamine technical on the

This study was conducted in order to assess the potential impact of spiroxamine technical on the endobenthic ordinent ingesting of gochaete *Lumbriculus variegatus*. Test organisms were exposed to spiroxamine technical for 28 days to assess the impact on reproduction and biomass of adult worms.

*Lumbriquus vallegatus* were exposed to spiroxamine technical at nominal test concentrations of 6.25, 12.5, 25, 50 and 100 ing as kg dry sediment, an untreated control and a solvent control. The worms had been through a synchronisation phase of 14 days so that at test initiation, they were of the same reconstituted stage.

Test vessels comprised of glass beakers of 250 mL covered with a pierced lid to allow for aeration and prevention of organism escape. Each beaker was filled with moist, formulated sediment which consisted of 75% quartz sand, 20% kaolinite clay, 7.5% sphagnum moss peat and 0.75% CaCO₃ to a depth of 1.5



cm (approximately 60 g wet weight and 43 g dry weight). Test water was then added to each vessel to a depth of 6 cm.  $Q_{\mu}^{\circ}$ 

Ten synchronised oligochaetes were allocated randomly to each test vessel at test initiation. Observations of intoxication (*e.g.* leaving sediment unusual swimming) were made on days 1, 4, 8, 11, 12, 14, 18, 20, 21, 22, 25 and 28. The total dry weight of the living worms per replicate was determined at test termination after placing the worms in a drying oven at 60°C to dry. Observations of mortality and reproduction were also made at test termination by determining the total number of 10 ing and dead individuals per replicate. Missing worms and worms that were unresponsive to gentle mechanical stimulus were considered dead.

The test vessels were held in a controlled environment foom maintained at a temperature within 00.3 to 21.2 °C. At test initiation, test termination and once a week during the test pH, dissolved Oxygen content and water temperature were measured. pH in the overlying test water ranged from 7.3 to 8.3, oxygen content ranged from 83 to 99% of the air saturation value in the overlying test water and the water temperature was maintained within 19.3 to 20.7 °C. The test vessels were held order a photoperiod of 16 hours light, 8 hours dark (light intensity 210 to 400 lug).

The worms were not fed during the test because the sediment contained a food source i.e. Urtica powder.

The 28-day EC₅₀, EC₂₀, EC₁₀ and the 5% confidence limits for worm number, de weight and individual dry weight were calculated by problem analysis. For the determination of the SOEC and LOFC values, Student t-tests were performed to compare the intreated control with the solvent centrol (two-sided,  $\alpha = 0.05$ ). Both controls were possed and the Williams t-test (one-folded smaller,  $\alpha = 0.05$ ) was used to determine the NOEC and LOFC values for worm number, dry weight and individual dry weight after 28 days.

#### Analytical method

Samples of water and sediment were analysed using the validated analytical method <u>M-688127-01-1</u>, report reference <u>M 888127-01-1</u> (see Doc MCA Section 4).

### II. Results and Discussion

Validity criteria according to the QECD 225 guideline (2007) were met.

- The average number of living worms per replicate in the controls should have increased by a factor of at least 1.8 at the end of exposure compared to the number of worms per replicate at the start of exposure (actual: 2.6 m untreated control and 2.1 in the solvent control)
- The pH of the overlying water should be between 6 and 9 throughout the test (actual: 7.3 8.3)
- The oxygen concentration in the overlying water should not be below 30% of air saturation value at test temperature during the test (actual: ≥91%)

Analytical results are summarised in the table below.

Treatment @	Overlying water	Pore water	Sediment	Total
group	% of nontinal ¹	% of nominal ¹	% of nominal ¹	recovery
(mg a.s. kg				
Test start (Day 0)				
Control 🖓	n.a ₄	n.a.	n.a.	n.a.
Solvent control	ncar, w	n.a.	n.a.	n.a.
25 5 6	D' L'	0.1	77	79
100	4	0.2	81	85
Test end (Day 28	)			
Control	n.a.	n.a.	n.a.	n.a.
Solvent control	n.a.	n.a.	n.a.	n.a.
25	<loq< td=""><td>0.04</td><td>60</td><td>60</td></loq<>	0.04	60	60

Table CA 8.2.5.4/03-1 Summary of analytical results



Treatment	Overlying water	Pore water	Sediment	Total
group	% of nominal ¹	% of nominal ¹	% of nominal ¹	recovery
(mg a.s. kg)				(C
100	2	0.11	60	62 🔊 🖓

¹: mean value of all measured samples per treatment group

²: sum of recovery in overlying water (if applicable), pore water (if applicable) and sectionent LOQ: Limit of Quantification (=  $100\mu g$  test item/L)

n.a.: not applicable

W X

For the 25 mg a.s./kg treatment the time weighted average recovery was 7% and for the 100 mg a.s. treatment the time weighted average recovery was 74%. This gave an overall time weighted average recovery of 71%. The value of 71% was used to determine the TWA concentrations for the 50, 105 and 6.25 mg a.s./kg treatments. The test concentrations, therefore correspond to time weighted average concentrations of 4.44, 8.88, 16.7, 35.5 and 74.2 mg a.s./kg dry sediment.

The number of worms after 28 days was not statistically significantly different between the untreated control and the solvent control (Student t-test, two sided,  $\omega = 0.050$  so both controls were pooled. The number of worms after 28 days was not statistically significantly different compared to the pooled control up to and including the test concentration of 3%5 mg a.s./kg dry sediment. At 74,2 mg a.s./kg dry sediment, the number of worms was statistically significantly reduced compared to the pooled control (Williams t-test, one-sided spaller, y = 0.05). Ð 

TWA test concentration	Mean number of worms		% of pooled control
(mg a.s./kg dry	0 days after exposure	28 days after exposure 🗞	
sediment) 🗞			
Control		Q6 0 [°] (k	Mean of the pooled
Solvent control	10 2	21.2 0 4	controls = 23
4.44			^{\$\%} 76.8
8.88	10 2 4 ~	29.5 0 24	110.4
16.7	10 % & %		121.2
35.5		21.20	92
74.2	10 6 A 27	1,6,5 4	45.5*

Table CA 8.2.5.4/03-2 Summary of reproduction data

* statistically significantly different compared to the pooled controls (W Hiams t-test,  $\alpha = 0.05$ )

The dry weight of worths after 28 days was not statistically significantly different between the untreated control and the solvent control (Student Ptest, two sided,  $\alpha = 0.05$ ) so both controls were pooled. The dry weight after 28 days was not statistically significantly offerent compared to the pooled control up to and including the test conceptration of 16 mg test item of dry sediment. At 35.5 and 74.2 mg a.s./dry sediment, the dry weight of worms was statistically significantly reduced compared to the pooled control (Williams tetest, one-side O smaller,  $\alpha = O(05)$ ).

	Q. 4	a			
Table C 3 9 2 5 4/0	2 2 🔊	maileafhi	marchinta	A	dava avnaanna
Table CA 8.2.5.4/0	J-J⊚ ⊘um	mary or on	Jillass uata	auter 20	uavs exposure
		4	× × ×	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	

TWA test concentration (mg a.s./kg drw sediment)	Mean number	Mean dry weight (mg)	% of pooled control	Mean individual dry weight (mg)	% of pooled control
	<u>\$6 0 x</u>	30. <b>5</b>	Mean of pooled	1.184	Mean of pooled
Solvent Control	21.2 ° ~	32.3	control (mg):	1.632	control (mg):
	A N		31.6		1.453
4.44	14.8 .	31	98.2	1.859	128
8,88 2 16.7 0 ⁹	25.5 2	28.1	88.9	1.108	76.3
16.7	28	31.8	100.7	1.141	78.6
35.5 0	21.3	18.1	57.4.*	0.858	59.1
74.2	10.5	4.5	14.3*	0.434	29.9



TWA test	Mean number	Mean dry	% of pooled	Mean	% of pooled
concentration	of worms (28	weight (mg)	control	individual dry	control o
(mg a.s./kg dry	days after			weight (mg)	
sediment)	exposure)				

* statistically significantly different compared to the pooled controls (Williams t-test, me-sided smaller, a = 0.05)

The results achieved in the study have been summarised in the table below for each of the parameters assessed.

	J		
Parameter	Number of worms	Weight (biomass)	Individual weight a grad
	[mg a.s./kg dry sediment]	[mga.s./kg dry sediment]	(bionass)
			[mg a.s./kg dry.sediment]
28-day EC ₅₀	74.2	×40.3 0 5 ×	41.5 ° '~ ~
	(n.d.)	D(27.3 61.1)	(24.0) >74.2)
28-day EC ₂₀	38.8	25 B Q	
	(n.d.)	(6,46-1 <u>3</u> ,4) ~ A	(A.44 x 22.8)
28-day EC10	27.6	20.4 @ O .	(©4.44 (22.8) ~ (7) 7.12 ~ (7) ~ (7)
	(n.d.)	¥ (<4,47-29,4) × 0	(<4,44-14)
28-day NOEC	35.5	16.9 ^x ~ ~ ~	169 2 2
28-day LOEC	74.2	25.5 m 2 0	35.5 E x
n.d. not determinal	ble 🖉 🗸	TO ST OF OF	Š ₂ 0 _k ,

Table CA 8.2.5.4/03-4	Summary of biomass	data after	28 days	exposu
-----------------------	--------------------	------------	---------	--------

Values in parentheses refer to 95% confidence limits Values refer to time weighted average concentrations

#### III. Conclusion

The influence of spirovamino technical on the development of the freshwater origochaete Lumbriculus variegatus was assessed in a static cose-response test.

For total number of worms, the 28-day  $EC_{50}$  was estimated to be 74.2 mg as ./kg dry sediment. The 28day NOEC and OEC values were determined to be 35.5 and 74.3 mg a.s./kg dry sediment, respectively. The  $EC_{10}$  was 27.6 mg a.s./kg dry sediment.

For weight based on dev weight of the worms, the  $EC_{50}$  was determined to be 40.3 mg a.s./kg dry sediment. The 28-day NOEC and LOEC values were determined to be 16.7 and 35.5 mg a.s./kg dry sediment, respectively. The  $EC_{10}$  was 20.0 mg as /kg dry sediment.

For individual weight, the ECS was determined to be 41  $\beta$  mg a.s./kg dry sediment. The NOEC and LOEC values for individual weight were determined to be 16.7 and 35.5 mg a.s./kg dry sediment, respectively. The EC₁₀ was 7.12 mg a.s./kg dry sediment.

### Assessment and conclusion by appricant

This is a new study that has not been pressiously submitted or evaluated.

Validity criteria according to the OECD 225@uideline (2007) were met.

- The average number of living works per replicate in the controls should have increased by a factor of at least 1.8 at the end of exposure compared to the number of worms per replicate at the start of exposure (actual: 2.6 in untreated control and 2.1 in the solvent control)
- The photo the overlying water should be between 6 and 9 throughout the test (actual: 7.3 8.3)  $6^{3}$ 
  - The oxygen concentration in the overlying water should not be below 30% of air saturation value at test temperature during the test (actual:  $\geq 91\%$ )

The study is therefore considered acceptable.



T-LL CA 93(1

The most sensitive parameter in the test was biomass which gave a NOEC of 16.7 mg a.s./kg dry sediment. However it is noted that the EC₁₀ of 7.12 mg a.s./kg dry sediment, determined for individual weight biomass, is lower than the NOEC value therefore the  $EC_{10}$  value of 7.12 mg a.s./kg/dry sediment has been taken as the critical endpoint determined from this study.

#### CA 8.2.6 Effects on algal growth

For procedural reasons studies listed in the Table 8.2.6-1 below are included in the current dossier as available data or information previously submitted but not necessarily evaluated. However, these reports have been fully superseded by newer studies. Consequently, no summaries of the reports have been included in the dossier.  $\bigcirc$ 

I able CA 8	.2.6-1: Studi	es prev	viously	submitted and	mot re	lied upo	n for 👧	e risk a	psessm	ent 🔊	a)"
Data	Document	Date	Title	~~	× 0	"Oʻ	ĴŅ.	. O	»		<u>_</u>
Point	No.			^S	Ŵ	×	Š.	di a	S	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	4
KCA	M-296921-	2009	Evalua	tions in aquatic	fisk as	sessmen	s base	Don stuc	lies with	aquatio	plants;
8.2.6/01	01-5		choice	of biomass or g	growth	rate	A	, Oya	≪		R
					a,°	d'	OY Y	~~		×1	410

		, ⁶ %				, ¢
CA 8.2.6.1	Effects on gr	ewth of g	reen alga	¢		

Data Point:	K & A 8.2, 6.1/01
Report Author:	
Report Year:	⁷ 1994 ³ ³ ³ ³ ⁴ ¹ ³ ³
Report Title:	Influence of KWG 4168 of the growth of the green alga, Scenedesmus
	subspicatures of the subspicature of the subsp
Report No:	AJO/122694 ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~
Document No: 🖉 🔊	<u>M-066228601-1</u> × x x x x
Guideline(s) for towed on	EC Directive 79/831/E Annex V, C.3 Algal Inhibition Test, Revised Version
study:	No. L383 A/179 (1992)
	ISO Guideline 8692: 0989 (E) Wate Quality - Fresh Water Algal Growth
	Inhibition Test with Scenedesmus subspicatus and Selenastrum capricornutum"
	(4989)
\$\$ [°]	DECD Guideline 201 Alga Growth Inhibition Test" (1984)
Deviations from corrent	None V V
test guideline:	
Previous evaluation: 🖒	yes, evaluated and accepted
₩ 4	DAR (1997), RAR (2010), RAR (2017)
GLP/Officially	Yes conducted under GLP Officially recognised testing facilities
recognised testing	
facilities:	$A \sim \phi = \phi = \phi$
Acceptability/Reliability:	Yes in Q Q
Executive Summary	

In a 72-hour oxicity study cultures of Scenedesmus subspicatus were exposed to KWG 4168 at mean measured test concentrations of 0.00023, 0.00038, 0.00084, 0.0012, 0.0028, 0.0042, 0.0068, 0.014 and 0.024 mg a.s./L under static conditions.

The  $\text{MOE}_{r}$  C and  $\text{E}_{r}$  C and  $\text{E}_{r}$  values for growth rate were 0.0012 and 0.012 mg a.s/L, respectively. The percent growth inhibition in the treated algal culture as compared to the control ranged from 1.7 to 65.4%. The NOE_bC and E_bC₅₀ values for biomass were 0.0012 and 0.0032 mg a.s/L, respectively. The percent growth inhibition in the treated algal culture as compared to the control ranged from 1.9 to 93.3%.





This study was conducted in order to assess the growth of the green alga *Scenedesmus subspicatus* when exposed to KWG 4168 over 72 hours.



Test concentrations were prepared from a stock solution and 150 mL of the solution was used in 300-mL Erlenmeyer flasks during the test. Nominal test concentrations were 0.00032, 0.00056, 0.0010, 0.0018, 0.0032, 0.0056, 0.010, 0.018 and 0.032 mg/L. Corresponding measured test concentrations were 0.00023, 0.00038, 0.00084, 0.0012, 0.0028, 0.0042, 0.0068, 0.014 and 0.024 mg/L.

Test media were inoculated with enough 2 or 3-day old pre-culture to give an apritial cell density of  $\mathbb{P}^4$  cells/mL.

Incubation was at  $23 \pm 2^{\circ}$ C and under continuous light at 8000 lux. Sedimentation of the cells or test substance was prevented by intermittent turning of the pole on which test flasks were suspended.

Cell numbers were determined microscopically on eack day of the tes as a Thoma counting chamber

#### Analytical method

Samples of water were analysed using the validated analytication 00252 M001, report reference <u>M-008490-02-2</u> (see Doc MCA Section 4).

#### II. Results and Discussion

Validity criteria according to the OECD 201 guideline in place at the time of study conduct were achieved because the cell density in the control cultures increased by a factor of at least 16 by the end of the test.

The recoveries of treated, cell-free test vossels during the study were 68.2 to 88.5% of nominal, with a mean recovery of 76.2%. The results of the study have been precented based on mean measured test concentrations.

Table CA 8.2.6.1/01-1	4	A-		6 · · · ·	
Nominal concentration		Mean measur	ed Concentration	% of noimin	al

Nominal concentration	Mean measured concentration	% of nominal
(ug a.s./L) (ug test item/L)	Qug a set 5	O' 4
0.31 (0.32)		, 72.9 Ø
0.55 (0.56)		68 ² /2 ³
0.98 (1.0)		<u>86.0</u>
1.76 (1.8)		\$8.8
3.12 (2.58)		88.5
5 16 (1.200)	4	76.2
9.75 (7.17)	6.79 S in in	69.6
17.6 (14.7)	14.2	80.7
31.2 (25.2)		75.3
-	23 2 3 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	76.2
TI I AR A CAR AND		

Limit of Detection (LOD): 0.001 mg/Ly

After 24 hours exposure, some cells were spherical and enlarged in the 0.014 and 0.024 mg a.s./L test concentrations. After 48 hours, some cells were spherical and enlarged in the 0.0042 mg a.s./L test concentration, and 40 cells were spherical and enlarged in the 0.0012, 0.0028, 0.0068, 0.014 and 0.024 mg a.s./L test concentrations.

Mean measured 📣	Number of cells (x104/mL)	¹ by time (h)	
concentration (mg a, s, L)	Q4 ~~	48	72
(mg a 3 L) > _			
Control ON	4,06) ≟ 1.00	$27.42 \pm 4.40$	$149.00 \pm 11.90$
0,00023 0	$347 \pm 1.00$	$29.67 \pm 3.70$	$136.50 \pm 4.90$
0.00038	$5.67 \pm 1.00$	$25.17 \pm 2.40$	$125.33 \pm 13.20$
0.000	$3.83 \pm 0.80$	$34.50 \pm 7.90$	$131.33 \pm 10.20$
0.0012	$4.17 \pm 0.30$	$25.50 \pm 4.80$	$127.33 \pm 6.70$
0.0028	$4.33 \pm 1.00$	$16.67 \pm 5.40$	$94.67 \pm 2.90$
0.0042	$4.33 \pm 0.30$	$11.50 \pm 3.10$	$55.75 \pm 3.20$

#### Table CA 8.2.61/01-2 Cell density during the toxicity phase



 $\bigcap$ 

Mean measured	Number of cells (x10 ⁴ /mL) ¹ by time (h)				
concentration (mg a.s./L)	24	48	72		
0.0068	$3.50 \pm 1.50$	$6.00 \pm 1.50$	$16.17 \pm 4.60$	N O	
0.014	$2.67 \pm 1.80$	$3.17 \pm 1.90$	7. 🕅 ± 0.80		
0.024	$2.83 \pm 0.60$	$4.67 \pm 0.80$	$5.67 \pm 0.80$	4 6	

Mean of two samples of three replicates (six replicates for the control)

Statistically significant inhibition of area under the growth curve (biomass) could be observed after 48 hours in the 0.014 and 0.024 mg a.s./L test concentrations. After 72 hours, significant inhibition could be observed in the 0.0028, 0.0042, 0.0068, 0.014 and 0.024 mg a.s./L test concentrations.

ai

Table CA 8.2.6.1/01-3	Area under the growth	curve and inhibition	of treated cu	iltures 🖉
	mea under the growth	cui voașu minoritori	or il caggu cu	inear co

Mean measured	24 h		48 h 。		72 h	N N
concentration	Area	%	Area		Area	×%
(mg a.s./L)		inhibition igodot	× O	inhibition 🐎		inhibition °
Control	36	-	389	- ~ 4	2 <b>4</b> 84 O	-2 0
0.00023	26	27.8	398 🕎	, T.8 💭 🗸	2346 ≪	5.6
0.00038	56	-55.60	402	∀-3.3 ° ≪	2184	12.1
0.00084	34	5.64	47.0	-2008	2426 2	1.9
0.0012	38	5.6 0	370	4.9 0	21,80	¥Q.2
0.0028	40	- <u>11.1</u>	\$268 D	§1.1 ( °	Q 580 0	<b>*%</b> 6.4*
0.0042	40	-11 1	206	47.00 [°]	9995 🖇	59.8*
0.0068	30 🔊	16.7	120	69.2	362 0	85.4*
0.014	20	Å4.4 S	66 O	83.0*	×1,66 Ø	93.3*
0.024	22	38.9	88 4	y7.4 🖉 🦼	§188	92.4*

* Statistically significantly different to the control (Bunnett Otest, 1=0.05, one-sided)

Statistically significant inhibition of growth rate could be observed after 24 hours in the 0.00023, 0.0068, 0.014 and 0.024 mg a.s./L test concentrations. After 49 hours, significant inhibition could be observed in the 0.0028, 0.0042, 0.0068, 0.014 and 0.024 mg a.s./L test concentrations. After 72 hours, significant inhibition could be observed in the 0.0028, 0.0042, 0.0068, 0.014 and 0.024 mg a.s./L test concentrations. After 72 hours, significant inhibition could be observed in the 0.0028, 0.0042, 0.0068, 0.014 and 0.024 mg a.s./L test concentrations.

Mean measured 🔹	@24 h 🎸 ]		≪448h ≪y	S.	72 h	
concentration	Rate	~% L L	Rate	, %	Rate	%
(mg/L)		inhibition	105	inhibition		inhibition
Control	4,36 V	$\sim$	×.05	-	1.67	-
0.00023	1.11	¥8.2*,Q		-2.5	1.64	1.7
0.00038	1.72	-26 5	1.6	2.4	1.61	3.5
0.00084	1:33	2.1	1.78	-6.8	1.63	2.5
0.0012	^¶.43 , <b>_</b>	¥.9 V	24,61	2.3	1.62	3.1
0.0028	1.45	≽-6.6 Q ~	\$1.39	16.0*	1.52	9.0*
0.0042	1.46	-7.8	1.21	26.7*	1.34	19.6*
0.0068	k19 🖉	j2\$8* ~~	0.89	46.4*	0.92	45.0*
0.014	1.19 9.81	40.6* Ø	0.48	70.8*	0.66	60.7*
0.024	1.03	₽24.3*	0.77	53.6*	0.58	65.4*

#### Table CA 8.2.6.1/01-4 Growth ate and inhibition Freated cultures

* Statisticall C significantly different to the control (Dunnett's test, p=0.05, one-sided)

The endpoints derived from the results of this study have been summarised below:

 Table CASE.2.6.1/01-5
 Summary of derived endpoints

Biomass		Growth rate	
$E_bC_{50}$	0.0032 mg a.s./L	$E_rC_{50}$	0.012 mg a.s./L
(95% CI)	(0.0020 to 0.0052 mg a.s./L)	(95% CI)	(0.0081 to 0.020 mg a.s./L)



LOE	C 0.0028 mg a.s./L	LOErC	0.0028 mg a.s./L	7
NOE		NOE _r C	0.0012 mg a.s./L	
III.	Conclusion		0.0012 mg a.s.r.L	- S
T 70 1		1 .		-

#### III. Conclusion

In a 72-hour toxicity study, cultures of Scenedesmus subspicatus were exposed to KWG 416 at mean measured test concentrations of 0.00023, 0.00038, 0.00084, 0.0012, 0.0028, 00042, 0.0068, 0.014 and 0.024 mg a.s./L under static conditions.

The NOErC and ErC₅₀ values for growth rate were 0.0012 and 0.012 mg als./L, respectively. The percent growth inhibition in the treated algal culture as compared to the control ranged from \$27 to \$3.4% The NOE_bC and  $E_bC_{50}$  values for biomass were 0.0012 and 0.0032 mgOa.s./L, respectively. The percent. growth inhibition in the treated algal culture as compared to the control ranged from 1.9 to 93.8%.

#### Assessment and conclusion by applicant:

The study was conducted to the OECD test guideline 200 (1984), the current version of which is the OECD 201 "Freshwater alga and cyanobacteria, growth inhibition test", adopted 28 July 2011. Validity criteria according to the OEC 20 kguidekne (201) have been te-assessed and the offcome presented below.

- 1) The cell density increase in the control culture to be at least a factor of 16 (actual 149);
- 2) The mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2, and 2-3) in the control culture to  $\mathfrak{be} \leq 35\%$  (actual 24.4%);
- 3) The coefficient of variation of the average specific growth rates over the whole test period should be  $\leq 7\%$  (actual 1.61%)?

The validity criteria according to the current test grideling have been met but please note that the two coefficient of variation criteria are only met if replicate 2 (out of the 6 replicates in the control) is excluded from the analysis. Replicate Thas been excluded due to a technical error with this replicate

The validity criteria have been achieved therefore the study is considered to be acceptable.

The data have been subjected to statistical re-evaluation and the results have been presented in the

wed therefore the sh wed to be 0012 mg a s.L. ojected to statistical re-evaluation a <u>many</u> of the shift of t



Data Point:	KCA 8.2.6.1/10
Report Author:	
Report Year:	2020
Report Title:	Calculation of EC10, EC20 and EC50 values for Scenedesmus subspicatus with
	KWG 4168 in analgal growth inhibition test
Report No:	0471836-ECO24
Document No:	<u>M-761401-01-1</u>
Guideline(s) followed in	None
study:	
Deviations from current	None 🕅 🖉 🖉 🖉 🗸
test guideline:	
Previous evaluation:	No, not previously submitted
GLP/Officially	No, not conducted under GLP/Officially recognised testing facilities
recognised testing	
facilities:	
Acceptability/Reliability:	Yes $\Delta \mathcal{D} \mathcal{Q} \mathcal{Q} \mathcal{Q} \sim \mathcal{O}^{\prime} \mathcal{Q}^{\prime} \mathcal{A}^{\prime}$

#### **Executive Summary**

The report <u>M-006228-01-1</u> on the effects of exposure to KWG 4068 on the powth of algae (*Scenedesmus subspicatus*) did not provide estimates of  $EC_{10}$  of  $EC_{20}$  calues. Therefore, these values as well as  $EC_{50}$  values have been calculated in accordance with the Annex to Con. Ref. 283/2013 for yield and growth rate.

The resulting  $EC_{10}$ ,  $EC_{20}$  and  $EC_{50}$  values for yield at 72 h were 0.84, 644 and 3.28 µg a.s./L, respectively. The resulting  $EC_{10}$ ,  $EC_{20}$  and  $EC_{50}$  values for growth rate at 72d were 0.56, 3.51 and 11.90 µg a.s./L, respectively.

#### I. Methods

The statistical evaluation was performed with statistical software ToxRatPro v3.3.0.

Effect concentrations with 10, 20 and 50% from the test item treatment when compared to the control were determined for yield and growth rate after 72 hours exposure. A Weibull regression was performed, with confidence limits for the EC2 values estimated according to Figher's theorem.

#### II. Results and Discussion

#### Yield at 72 hours

Regarding the calculation of  $EC_{10}$ ,  $EC_{20}$  and  $EC_{50}$  values for yield at 72 h, a statistically significant concentration/response was found (p(F) <0.001) for this parameter. The resulting  $EC_{10}$ ,  $EC_{20}$  and  $EC_{50}$  values and the respective confidence intervals are represented in the following table below.

Å

# Table CA/8.2.6.1/10-1 Results of the Woibull analysis of yield at 72 h: Selected effective concentrations

		Yield	
	CEC10 CO	<b>EC</b> ₂₀	EC ₅₀
Parameter	(95% confidence)	(95 % confidence	(95 % confidence
jor v	ja interval)	interval)	interval)
	🔊 [µg, a.s./LQ	[µg a.s./L]	[µg a.s./L]
Effect on yield \$72	ల్ ని 0.84	1.44	3.28
<u> </u>		(1.11 - 1.73)	(2.91 – 3.66)

The resulting  $EC_{10}$   $EC_{20}$  and  $EC_{50}$  values of 0.84 (95%CL: 0.57 – 1.09), 1.44 (95%CL: 1.11 – 1.73) and 3.28 (95%CL: 2.91 – 3.66) µg a.s./L, respectively, meet the goodness of fit criteria showing a significant concentration/response relationship, and therefore the estimated ECx values are considered reliable.



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#### Growth rate at 72 hours

Regarding the calculation of  $EC_{10}$ ,  $EC_{20}$  and  $EC_{50}$  values for growth rate at 72 h, a statistically significant concentration/response was found (p(F) < 0.001) for this parameter.

The resulting EC10, EC20 and EC50 values and the respective confidence intervals are represented in the following table below.

#### Table CA 8.2.6.1/10-2 Results of the Weibull analysis of growth rate at 72 perfected effective concentrations (ECx) of the test item and their 95% confidence limits

		Growth rate	
	<b>EC</b> ₁₀	EC20	
Parameter	(95 % confidence	(95 % confidence (2)	(95 % confidence
	interval)	🧳 interval)	mteryal)
	[µg a.s./L]	« [μg æs./L] « γ	🖉[μg a.s./L] 🗸
Effect on growth	1.56 (	) <u>0</u> 29.51 2 0	¢ 11.90
rate at 72 h	(0.97 – 2.18)	(258 - 409)	(1032 - 1337)

The resulting EC₁₀, EC₂₀ and EC₅₀ values of 1,56,95% EL: 0.90-2.18, 3.51,95% EL: 2,58-4.39 and 11.90 (95%CL: 10.32 – 13.77) µg a.s. L, respectively, meet the goodness of fit riteric by showing a significant concentration/response relationship, and therefore the estimated EC values are considered reliable.

#### III. Conclusion

The resulting  $EC_{10}$ ,  $EC_{20}$  and  $EC_{50}$  values for yield at 72-hours were determined to be 0.84, 1.44 and 3.28  $\mu$ g a.s./L, respectively. The resulting  $EC_{10}$   $\mathcal{C}_{20}$  and  $EC_{50}$  values for growth rate at 72-hours were determined to be 1.56, 3.5 and  $11.90 \mu$  a.s./

### Assessment and conclusion by applicant:

 $\bigcirc$ The statistical re-valuation of the data has determined reliable EG10, EC2, and EC50 values for both growth rate and yield. The ErQ50 determined in this re-evaluation ork of 1.9 µg a.s./L is considered to be the same as the  $E_rC_{50}$  determined in the original study report of 0.012 mg a.s./L (12 µg a.s./L) therefore the original ErCs, from the study report remains the critical endpoint determined from this study.

study. The values determined in the re-evaluation work are considered to be fully valid.



Data Point:	KCA 8.2.6.1/02
	KCA 8.2.0.1/02
Report Author:	
Report Year:	1998
Report Title:	Toxicity of 14C-KWG 4168 to the green alga Selenastrum capricornutum
Report No:	108058
Document No:	<u>M-006533-01-1</u>
Guideline(s) followed in	American society for testing and materials (ASTM), 1990. Standard guide for
study:	conducting static 96-hour toxicity tests with microalgae. ASTM Standard E1218
	Philadelphia, PA.
Deviations from current	None & Q V X X
test guideline:	
Previous evaluation:	yes, evaluated and accepted, Q & A
	<b>RAR</b> (2010) <b>RAR</b> (2017) $\sim \sim \sim$
GLP/Officially	Yes, conducted under GLP/Officially recognised testing facilities
recognised testing	
facilities:	
Acceptability/Reliability:	Yes A A A A A A A
Executive Summary	

#### **Executive Summary**

The objective of the study was to determine the growth of the green alga, Selenastrum capricornutum. °

WG 468 minitial measured test The cultures of Selenastrum copricornatum were exposed to concentrations of 0.55, 1.05, 201, 4.12 and 1.14 µg a.s./L under static conditions.

The 96-hour EC₅₀ and E $Q_{25}$  for growth rate were calculated to be µs a.s./L, the highest concentration tested. Ć C

The 96-hour EC₅₀ and EC₂₅ for area under the growth cutve were calculated to be 5.5 and 2.1 µg a.s./L, The 96-hour EC₅₀ and EC₂₅ for cell density were calculated to be 5.7 and 2.5 μg a.s./L, respectively. I. Materials and Methods

Test Material Lot/Batch vity 29.6 mCi/mMole) **Purity:** opecifi Description: reported Stability of test Not reporte compound: 🔏 Reanalysis/E t reporter date: Densi Treatmen Nominal: 0.5, 1.0, 2.0, 4.0 and 8.0 µg a.s./L Initial measured: 0.55, 1.05, 2.01, 4.12 and  $8.14 \ \mu g \ a.s./L$ Methanol ehicle: Analysis of test Yes, measured concentrations 94 - 111% of nominal concentrations:

**Test organisms** 



Species:	Green alga, Selenastrum capricornutum (now Raphidocelis subcapitata)
Source:	Carolina Biological Supply
Test design	
Test vessel:	Carolina Biological Supply 250-ml borosilicate glass culture flasks filled with approximately 100 mL of test solution and capped with starile glass closures Freshwater Nutrient Media ASTM, 1990 Three replicate vessels were prepared for each concentration and used
Test medium:	Freshwater Nutrient Media ASTM, 1990
<b>Replication:</b>	to determine daily cell density. The highest test concentration had four are replicates: 3 replicates for cell density determinations and one additional replicate that was only used to provide a sufficient volume
Initial cell density:	$1 \times 10^4 \text{ cells}$
<b>Duration of test:</b>	96 hours of the second se
Environmental test conditions	
<b>Temperature:</b>	2474 to 250°C & 57 57 57 57 57 57 57 57 57 57 57 57 57
<b>Conductivity:</b>	×85 - 87 μmhos/cm ζ ζ ζ ζ
pH:	
Photoperiod: 🔬	Continuous lighting & 4300 lux & &
B. Study Design This study was conducted Selenustrum cupricorputum	n order to determine the growth affects of ¹⁴ C4XWG 4168 to the green alga,
250-mL Entenmeyer flask a 8.0 μg & s./L. Correspondir μg a.s./L.	epared from a stock solution, and 100 mK of the solution was added to each t the start of the test. Nominal test concentrations were 0.5, 1.0, 2.0, 4.0 and agonitial measured test concentration were 0.55, 1.05, 2.01, 4.12 and 8.14 with enough 3-day old pre-culture to give a density of 1 x 10 ⁴ cells/mL.
Incubation was at 24 Pto 2 substance was prevented (rpm).	50°C under continuous light at ~4300 lux. Sedimentation of the cells or test placing test vessels on a shaker table set at 100 revolutions per minute mined in three teplicates at each test concentration using a light microscope
and an Improved Neubauer	
the report.	Discussion be valid at the time of conduct but no specific criteria have been cited in area test vessels during the study was 94 to 105% of nominal. The results of

Analytical recoveries of treated test vessels during the study was 94 to 105% of nominal. The results of the study have been presented in terms of the initial measured test concentration.



cup	locornatam			
¹⁴ C-KWG 4168 Nominal	inal Measured concentration (µg/L)			
concentration	Day 0	Percent of	Day 4 ^a	Percent of
(µg/L)		nominal		nominal 🖉 🔊
Control	< 0.08	-	<0.08	
Solvent control	< 0.08	-	<0.08	- 5 5
0.5	0.55	111	0.47 🔊	94~ ~ ~
1.0	1.05	105	1.01	
2.0	2.01	101	1.91	. 696 37 20 27
4.0	4.12	103	3.98	<u>99 Q 07 X</u>
8.0	8.14	102	~ <b>&amp;</b> 07 ⊘° ∧	
Lab recovery ¹	1.66	104	¥ 1.67 🖉 👋	

 Table CA 8.2.6.1/02-1
 Measured test concentrations based upon LSC during the exposure of Selenastrum capriocornutum

¹ Lab recovery based upon a lab spike of 1.6  $\mu$ g ¹⁴C-KWG 416%L media

^a These values indicate that 94 to 101% of the nominal amount of total radioactivity was still present in the test solutions after 4 days. It was determined by radio PLC that the radioactivity was present in the compound (32%). The KWG 4168 was not stable under test conditions

The growth curves clearly show decreased growth in the 442 and 8.14 µc.a.s./L test levels as compared to the controls. The controls, 0.55, 1.05 and 2.01 µc a.s./L level exhibits similar growth through Day 4.

Table CA 8.2.6.1/02-2 Measure Calgal cell densities during the ¹⁴C-KOVG 4168 Selanastrum

				2
Measured concentration	Alean Cell density	cells/mL) x 10 ⁴		<u> </u>
(µg/L) 🗞	Day 4 🖉		Day 3 🖉 🛷	Day 4
Control 🔬	3.08 0	Day 2 2 2	\$7.83 <u>5</u>	195.63
Solvent control	3.21 ~ 0	18.64 🗸	94.25 4	222.75
0.55	\$3.29 S	20.11	100.33	219.50
1.05	3.16	14.09° 5	82.33 ~ T	225.33
2.01	3,10 ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~	11.60	\$1.58	159.67
4.12	∠3,15 ×	11909	°48.8∰	127.50
8.14	3.17	HØ.40 ° 🥡	37,96	127.50

The endpoints derived from the results have been symmatized bedow:

# Table CA 8.2.6.1/02 Summary of derived endpoints

Biomass (Area under the growth curve)				
LQEC 442 µg as./L		EC ₂₅ (95% CI)	2.1 μg a.s./L	
NOEC 2.01 up a.s./LQ	R Q	EC ₅₀ (95% CI)	5.5 μg a.s./L	
Growth write & & &	y . D			
LOÈC 4,12 µg.a.s./L	A 18	EC ₂₅ (95% CI)	>8.14 µg a.s./L	
NOEC 201 µg@r.s./L		EC ₅₀ (95% CI)	>8.14 µg a.s./L	
Cell density	$O^{\mathbf{v}}$			
[©] LOEC 4.1 ² μg a st Ω	1	EC ₂₅ (95% CI)	2.5 μg a.s./L	
Ο NOTC 201 μg a.s./L		EC ₅₀ (95% CI)	5.7 μg a.s./L	

### III. Concrusion

The objective of the study was to determine the growth effects of ¹⁴C-KWG 4168 to the green alga, Selengstrungeaprices nution.

The cultures of *Selenastrum capricornutum* were exposed to  14 C-KWG 4168 at initial measured test concentrations of 0.55, 1.05, 2.01, 4.12 and 8.14 µg a.s./L under static conditions.

The 96-hour EC_{50} and EC_{25} for growth rate were calculated to be >8.14  $\mu g$  a.s./L, the highest concentration tested.



The 96-hour EC₅₀ and EC₂₅ for area under the growth curve were calculated to be 5.5 and 2.1  $\mu$ g a.s./L, respectively.

The 96-hour EC50 and EC25 for cell density were calculated to be 5.7 and 2.5 µg a.s./L, respectively

#### Assessment and conclusion by applicant:

The study was conducted to the American society for testing and materials (ASTM). 1990, Standard guide for conducting static 96-hour toxicity tests with microalgae, ASTM Standard 19218, Philadelphia, PA.

The study has therefore been assessed against the validity criteria according to the guideline (2011).

Validity criteria according to OECD 201 (2011) were not consistently pre-

- Cell density of control cultures to increase by at least 16x (actual: 209)
- Mean coefficient of variation for section-by section specific growth racks in control oultures. to be ≤35% (actual: 46.2% in the control and solvent control, respectively)
- Coefficient of variation of average specific growth rates in control cultures over the test period to be ≤7% (actual: 6.18% in the control and solven control, respectively)

The control growth rate data do not most the mean coefficient of variation criterion of  $\leq 35\%$ . However, it is noted that if Replicate 1 is excluded for being an output then the validity criteria are all met.

The results have been based on initial measured concentrations whereas it would have been expected to base the results on nominal concentrations on the basis that the recoveries remained within 80 - 120% of nominal for the duration of the test. The initial measured concentrations are very similar to the nominal concentrations free fore this is not considered to be a significant deviation from the OECD test guideline recommendation.

On balance the study is considered to be acceptable and provides the lowest endpoint for a green algal species. The 96-hour  $C_{50}$  was determined to be  $\frac{98.14}{9}$  ug a.s./C, the linguistic concentration tested.

The data have been subjected to statistical re-evaluation and the results have been presented in the following study summary.

K ^y . U	
Data Point:	4 CA & 3.6.1/1 O & A
Report Author:	
Report Year:	
Report Title:	Galculation of EC10, EC20 and EC50 values for Selenastrum capricornutum with
¥ - ,	4C-KWG 4168 in an algal growth inhibition test
Report No	04713836-EGQ927
Document No:	<u>M-%1427-01-1</u>
Guideline(s) followed in	Apne of Or Sy
study:	
Deviations from current	None Q
test guideline: 🗸 🔪	
Previous evaluation.	No, not previously submitted
GLP/Officially	No, not conducted under GLP/Officially recognised testing facilities
recognised testing	Ő
facilities:	
Acceptabilit@Reliability:	N es

#### Executive Summary

The report <u>M-006533-01-1</u> on the effects of exposure to ¹⁴C-KWG 4168 on the growth of algae (*Selenastrum capricornutum*) did not provide estimates of  $EC_{10}$  or  $EC_{20}$  values. Therefore, these values, as well as  $EC_{50}$  values, have been calculated in accordance with the Annex to Com. Reg. 283/2013.



The resulting  $EC_{10}$ ,  $EC_{20}$  and  $EC_{50}$  values for yield at 96 h were 1.29, 2.18 and 5.90 µg a.s./L, respectively. For growth rate after 96 h, the  $EC_{10}$  and  $EC_{20}$  values were 4.93 and 10.51 µg a.s./L, respectively. An  $EC_{50}$  value could not be reliably determined due to value being beyond the tested concentrations.

#### I. Methods

The statistical evaluation was performed with statistical software ToxRatPro v3.3.0.

Effect concentrations with 10, 20 and 50% from the test item treatment when compared to the pooled control were determined for yield and growth rate after 96 hours exposure. A Probat regression was performed with confidence limits for the EC_x values estimated according to Fieller's theorem.

#### II. Results and Discussion

#### Yield at 96 hours

Regarding the calculation of EC₁₀, EC₂₀ and EC₃₀ values for growth rate at 96h, a statistically significant concentration/response was found (p(F) < 0.09).

The resulting  $EC_{10}$ ,  $EC_{20}$  and  $EC_{50}$  values and the respective confidence intervals are represented in the following table and figure below.

Table CA 8.2.6.1/11-1	Results of the Probit analysis of yield at 96 fr. Selected effective concentrations
	(ECx) of the test item and their 95%-confidence limits

Parameter	Yield S S S
	EC ₄₀ EC ₂₀ EC ₅₀ EC ₅₀ 95 % confidence (95 % confidence (95 % confidence
	interval) interval) interval) Sug a.s./L] Sug a.s./L] [µg a.s./L]
Effect on yield at 96	

The resulting EG10, EG20 and EC50 values of 1.29 (95% CL: 0.82 - 1.72), 2.18 (95% CL: 1.62 - 2.66) and 5.90 (95% CL: 5.01 - 7.95) µg a.s./L; respectively, meet the goodness of fit criteria and therefore the estimated ECx values are considered reliable.

#### Growth cate at 96 hours

Regarding the calculation of  $EC_{10}$   $EC_{20}$  and  $EC_{30}$  values for growth rate at 96 h, a statistically significant concentration/response was found (p(R) < 0.00  $\beta$ ).

The resulting  $FC_{10}$ ,  $EC_{10}$  and  $FC_{50}$  values and the espective confidence intervals are represented in the following table and figure below  $\sqrt{2}$   $\sqrt{2}$ 

 Table CA 8.2.6.1/11-2. Results of the Probit analysis of growth rate at 96 h: Selected effective concentrations (EC sof the test item and their 95%-confidence limits

	1	
	Growth rate	
	EC20	EC50
Parameter (95% confidence interval)	(95 % confidence	(95 % confidence
interval) @	interval)	interval)
$\sqrt{2}$ $\sqrt{2}$ $\sqrt{2}$ $\mu ga.s./L$	[µg a.s./L]	[µg a.s./L]
Effect on growth 0 4.93	10.51	n.d.
Effect on growth $4.93$ rate at 960 $4.29 - 5.55$ )	(9.04 - 13.08)	

n.d.: for determined since value is beyond the tested concentrations

The resulting EC₁₀ and EC₂₀ values of 4.93 (95%CL: 4.29 – 5.55) and 10.51 (95%CL: 9.04 – 13.08)  $\mu$ g a.s./L, respectively, meet the goodness of fit criteria and therefore are considered reliable. The EC₅₀ value could not be reliably determined since it is beyond the tested concentrations and was therefore considered to be >8.14  $\mu$ g a.s./L.



#### III. Conclusion

The resulting EC₁₀, EC₂₀ and EC₅₀ values for yield at 96-hours were determined to be 1.29, 2.18 and 5.90  $\mu$ g a.s./L, respectively. For growth rate after 96 h, the EC₁₀ and EC₂₀ values were 4.93 and 70.51  $\mu$ g a.s./L, respectively. An EC₅₀ value could not be reliably determined due to value being beyond the tested concentrations and was therefore considered to be >8.14  $\mu$ g a.s./L.

#### Assessment and conclusion by applicant:

The statistical re-evaluation of the data has determined reliable  $EC_{10}$ ,  $EC_{20}$  and  $EC_{50}$  values for yield and reliable  $EC_{10}$  and  $EC_{20}$  values for growth rate. A reliable  $EC_{50}$  value for growth rate could not be determined.

The  $E_rC_{50}$  determined in this re-evaluation work is the same as that determined in the original study report. Thus, the  $E_rC_{50}$  of >8.14 µg a.s./L shall be taken as the ortical endpoint determined from this study.

The values determined in the re-evaluation work are considered to be fully valid.

Data Point:	KCA 8.2.6.1.03 ( ) ( ) ( ) ( ) ( ) ( )
Report Author:	
Report Year:	
Report Title:	Desmodesmus subspicatus growth in publicion test with Spirocamine
Report No:	EBKWX077
Document No:	<u>M-273962-01-1</u> 2 4 m 4 2 2
Guideline(s) followed in "	Praft Proposal for Updating QECD Guideline 201: Freshwater Alga and
study:	Cyanobacteria. Growth Inhibition Test (October 22 2004)
Deviations from current	Yest 2 O D w & k w
test guideline:	OFCD 201 guid@ine (2011) The inclume was approximately 1 x 64 cells/mL, more than the recommended
Č.	The inoculum was approximately 1 x 104 cells/mL, more than the recommended
	12-5  g/l03 cells/ml
Previous evaluation:	yes, evaluated and accepted RAR (2010), RAR (2012)
Previous evalution:	$\mathbf{R}^{A}$ R (2010), RAR (2019) $\mathbf{R}^{a}$ $\mathbf{O}^{a}$ $\mathbf{O}^{a}$
GLP/Officially	Yes, conducted under GLP/Officially recognised testing facilities
recognised testing	
recognised testing	
Acceptability/Reliability:	
Executive Summary	Ayes a co co co co

#### Executive Summary

In a 72-hour oxicite study cultures of *Desmodesmus subspicatus* were exposed to spiroxamine at nominal test concentrations of 9.53, 30, 97.7, 313 app 1000 µg a.s./L under static conditions.

The growth rate NOE @ and  $@_rC_{50}$  values were >9.53 and 175 µg a.s./L, respectively. The percent inhibition of average specific growth rate in the treated algal cultures compared to the controls ranged from 16.9 to 68.1% after ? hours exposure.

I. Materials and Methods A. Materials Test Material Lot/Batch#: Purity Description: Stability of test compound: Hot reported



Reanalysis/Expiry date:	09 December 2006
Density:	Not reported 9.53, 30.5, 97.7, 313 and 1000 μg a.s./L None Yes, measured concentrations 93 – 108% of nominal (mean 193%) on
Treatments	
Test rates:	9.53, 30.5, 97.7, 313 and 1000 μg a.s./L
Solvent/vehicle:	None
Analysis of test concentrations:	day 0 and 95 – 104% of prominal (mean 99.0%) on day 3 2
Test organisms	
Species:	Desmodesmus subspicatus
Source:	Göttingen, Gérmany
Test design	
Test vessel:	300-mb Erlemmeyer Basks containing 150 mL test medium
Test medium:	Prepared according to OECD 200 (2000)
<b>Replication:</b>	Three per test vessel, sa per control
Initial cell density:	10,000 cells and by or by the by
Duration of test: 🥍	72 hours 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2
Environmental test	300-mJ Erlenmeyer dasks containing 150 mL test medium Prepared according to OECD 20 $V(2006)$ Three per test vessel, as per control 10,000 cells mL 72 hours 22,2 - 23.3 °C 8.0 - 8.5 Under continuous illumination at 6990 - 5620 lux n order to assess the effects of exposure to spiroxamine on the green alga n static vest over 72 hours.
Temperature:	24,2-23.3°C 3 3 2 5
рН: 🏷 🖧 🗸	
Photoperiod:	Under continuo illumination at 6990 – 5620 lux
B. Study Design	
This study was conducted i	n order to assess the effects of exposure to spiroxamine on the green alga
	n Astatic lost over /2 hours.
Tast vascale appra 200 mL I	Manmarar flocks antrining 150 mL tast madium. These were placed on a

Test vessels vere 300-mL briennever flasks containing 150 mL test medium. These were placed on a tablet rotating at 100 rpm to precent sedimentation of the cells while preventing further aeration. The test media were prepared to the OECD 201 guideline, sterilised by membrane filtration and aerated with sterile air. Test media were inoculated with approximately 10,000 cells/mL, from an exponentially-growing pre-culture prepared four days before the start of the test and cultivated under the same conditions as used in the test.

Nominal concentrations were 9.53, 30.5, 97.7, 313 and 1000 µg a.s./L, along with a control and solvent control, with three replicates per test condition and six replicates per control.

Morphological examination of cells were made over the exposure period on each study day by a microscope. Cell numbers per volume were estimated photometrically as a surrogate for biomass per volume.

Temperature was determined by one continuous measurement of an additional glass vessel filled with an equivalent amount of de-ionised water as in the test vessels. The pH was measured daily in all test levels and the control. Samples were analysed for spiroxamine concentration at test start and end.



#### **Analytical method**

Samples of water were analysed using the validated analytical method 00623, report reference 031628-01-1 (see Doc MCA Section 4).

#### II. **Results and Discussion**

Validity criteria according to the OECD 201 guideline were met.

- Biomass of control cultures to have increased exponentially by a factor of at heast 16 increased by a factor of 42.4) increased by a factor of 42.4)
- Mean coefficient of variation for section-by-section specific growth rates in the control curtu to not exceed 35% (actual: 26.4%)
- The coefficient of variation of average specific growth rates over the whole test period between the replicate control cultures should not exceed 7% (actual: 2.4%) <

The mean recoveries of treated, cell-free test vessels at test start ranged from 93 to 108% of nominal, with an overall mean recovery of 103%. A measured concentration 173% of prominal was observed in the 9.53 µg a.s./L test concentration, however this was deened unrealistic and likely due to handling errors, and therefore excluded as an outlier, xt test end, mean recoveries ranged from 95 to 104% of nominal, with an overall mean recovery of 99.0%. The results of this study have there fore been presented based on nominal test concentrations.

Table CA 8.2.6.1/03-1 Nominal and measured concentrations in treated, cell-free vessels at day 0

Nominal concentration	Mean measured concentration
(µg a.s./L)	$(\mu\sigma \mathscr{R}_{S}/L)$
Control	167* 0 $4$ $4$ $4$ $4$
Solvent control	
9.53	
30.5	32.9 1080
97.7	91/2 0 4 93
313	327 2 2 2 2 2 204
1000	1060 2 106
	Mean: 103

Limit of Quatification (LQQ): 1.25 µg/L

Measured concentrations in the control the considered as not being evident in the exposure vessels and were most likely caused by handling errors sifter comparable amounts of spiroxamine were not found in 3-day samples of the same vessels of For the same reasons this value is deemed anreal since and therefore excluded as an outlier

#### Table CA 8.2.6.1/03-2 Nominal and measured concentrations in treated, cell-free vessels at day 3

Nominal concentration	Mean measured concentration	% of nominal
(µg st.s./L)	μg a.@/L)	
Control	<loq< td=""><td>-</td></loq<>	-
Solvent control $\mathbb{A}^{\mathbb{N}}$		-
9.53	§9.10	95
30.5	29.7 [©]	97
97.7 9 2 2	93.5	96
313	324	104
	1033	103
	Mean:	99.0

Limit of Quatification (LOQ): 1.25 µg/L

The following table details the effects of exposure on biomass (cell density):



 $\bigcirc$ 

Nominal	Number of cells (x1	0 ⁴ /mL) ¹ by time ± SD		¢ `گ
concentration	24 h	48 h	72 h	
(µg a.s./L)				6 6
Control	$5.0 \pm 0.844$	$13.6 \pm 0.806$	42, <b>4</b> € 2.087	
Solvent control	$4.7 \pm 0.516$	$14.7 \pm 0.505$	3809 ± 3.994	
Pooled controls	$4.9 \pm 0.695$	$14.2 \pm 0.850$	<u>40.7 ± 3.546</u>	S S
9.53	$4.9 \pm 0.330$	$12.7 \pm 0.540$	Ž1.7 ± 0.563	
30.5	$4.3 \pm 0.446$	$10.4 \pm 0.4$	() 14.4 ± 1.243	
97.7	$4.0 \pm 0.535$	$6.8 \pm 0.514$	$7.2 \pm 0.802$	<u> </u>
313	$1.3 \pm 0.214$	5.5 ± \$33	4.8±\$330	
1000	$2.0 \pm 0.201$	2.6 ± 0.206	² 3.3 ± 0.209,€	

Mean of three replicates (six replicates for the gortrol) ± standard deviation

z, Ô Statistically significant inhibition of the 0-24 hour growth rate was observed at test concentrations 97.7 and 1000 µg a.s./L compared to the pooled controls. For the 0-48 hour growth rate significant inhibition to the controls could be observed at test concentrations 9,93, 30.9, 313 and 1,000 µg a.s./L Significant inhibition to the controls for the 0-72 hour growth rate could be observed in all test concentrations. Ø

#### X Table CA 8.2.6.1/03-4 Growth rates and inhibition of treated cultures

Nominal	0 - 24 h		0 - 48 h			°~~
concentration	Growth	2% &	Growth rate		Growth	°⁄0
(µg a.s./L)	rate 🔗	inhibition		inhibition	rate o	inhibition
Pooled controls	1.576	-& 0	1.\$25	- ~ ~	1.234	-
9.53	1.587 🔊 🖗	-Q.7 ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	272*	4.0	പ്.025*്	16.9
30.5	1.444 🗡 🚄	8.4	1.171 ×	ÿ11.6 ≪	0.88	28.1
97.7	1.384*	13.02	0.997	27 🖉 候	0.655*	46.9
313	1,463	70	0.856*	35.4 Oʻ	0.524*	57.5
1000	\$.706* ×	S8.2 J ~	0.485*0	53.4 L	Ø.394*	68.1

L

* Statistically Significantly different to the coptrol (Welch-trest for Schomogeneous Variances with Bonferron Adjustment, a=0.05, one-side smaller)

A summary of the	¥	Л	4			O
A summary of the	roulto in	procontad	instha	tak	hala	~
A summary of the	1050115 15	preserveu	Thermo	Layle	UCIUM.	0
	s de la companya de l	* «J	n y	O'	-	Ø
	.*~	~ ~	<i>U //</i> 14			~~

# Table (\$ 8.2.6.1/03-5, Summary of results after 72. Dour exposure to spiroxamine

Nominal concentration	Cell number per 0-72 haverage mLafter 2 h	Inhibition of	Doubling time
concentration	mLafter 2 h $\sim$ specific growth $\sim$	average specific	(days)
(μg a.s./L)	Reperday S	growth rate (%)	
Control 🔊	A24,32	-	-
Solvent control	389,00 0 5 - 6 0	-	-
Pooled controls	496,800 ~ 0 1.234 4	-	0.562
9.53	246,670 1925	16.9	0.676
30.5%	7143,800 ° 20.888 7	28.1	0.781
97.7	71,630 2 0.65	46.9	1.06
313	48,240 4 0 24	57.5	1.32
1000	\$2,640 \$ 0.394	68.1	1.76

A summary of the relevant endpoints is presented in the table below:

Table 6 8.2.6 103-6 Summary of derived endpoints

Growth rate 0	
[∞] E _r C ₂ (95% CI):	175 μg a.s./L (118 to 273 μg a.s./L)
EC20 (95% CI):	11.4 μg a.s./L (4.31 to 21.0 μg a.s./L)
$E_{r}C_{10}$ (95% CI):	<9.53 μg a.s./L
LOE _r C:	<9.53 μg a.s./L
NOE _r C:	<9.53 μg a.s./L



#### III. Conclusion

In a 72-hour toxicity study, cultures of *Desmodesmus subspicatus* were exposed to spiroxamize at nominal test concentrations of 9.53, 30.5, 97.7, 313 and 1000 µg a.s./L under static conditions. Ô

The growth rate NOE_rC and  $E_rC_{50}$  values were <9.53 and 175 µg a.s./L, respectively. The percent inhibition of average specific growth rate in the treated algal cultures compared to the controls ranged from 16.9 to 68.1% after 72 hours exposure.

#### Assessment and conclusion by applicant:

The study was conducted to the OECD test guideline 201 (2004), the current version of which is the OECD 201 "Freshwater alga and cyanobacteria, growth inhibition west", adopted 28 July 2011

Validity criteria according to the current OECD 201 guideline (2011) were met

- Biomass of control cultures to have increased exponentially by a factor of at least 16 (actual: S increased by a factor of 42.4) Ô
- Mean coefficient of variation for section-by section specific growth rates in the control cultures to not exceed 35% (actual: 26.4%) Ò,
- The coefficient of variation of average specific growth gates over the whole test period between the replicate control cultures should not exceed? (actual: 24%)

It is noted that the report has based the validity criteric on the pooled control and solvent control data. Therefore the validity criteria have been re-assessed for the control data only and presented below:

- Biomass of control cultures to have increased exponentially by a factor of at least 16 (actual: increased by a factor of  $\mathfrak{P}.4$ )
- Mean coefficient of Agriation for section by-section specific growth rates in the control cultures to not exceed 35% factual: 26.7%
- The coefficient of variation of average specific growth rates over the whole test period between the reportate control cultures should not exceed 7% (actual: 1.36%)

All validity crueria have been met therefore the study is considered acceptable.

The data have been subjected to statistical re-evaluation and the results have been presented in the

the state of the s



Data Dainti	
Data Point:	KCA 8.2.6.1/12
Report Author:	
Report Year:	
Report Title:	Calculation of EC10, EC20 and EC50 values for Desmodesmus subspicatus with
	KWG 4168 in an algal growth inhibition test
Report No:	0471836-ECO30
Document No:	<u>M-761457-01-1</u>
Guideline(s) followed in	Noone
study:	
Deviations from current	None V O O V V
test guideline:	
Previous evaluation:	No, not previously submitted $\mathcal{O}$
GLP/Officially	No, not conducted under GLP/Officially recognised testing facilities
recognised testing	
facilities:	
Acceptability/Reliability:	Yes $\Lambda \approx 0^{\circ} Q$ $\sim 0^{\circ} 2^{\circ} 2^{\circ}$

#### **Executive Summary**

The report <u>M-273962-01-1</u> on the effects of exposure to KWG 4068 on the prowth of algae (*Desmodesmus subspicatus*) did not provide estimates of  $EC_{10}$  of  $EC_{20}$  alues based of yield. Therefore, these values have been calculated alongside the EC in accordance with the Annex to Com. Reg. 283/2013.

The resulting  $EC_{50}$  value was 10.52 µg a CL.  $EC_{10}$  and  $EC_{20}$  values could not be determined for yield due to values being beyond the tested concentrations.

#### I. Methods

The statistical evaluation was performed with statistical software Tox RatPro \$3.3.0.

Effect concentrations with 10, 29 and 50% from the test item treatment when compared to the control were determined for yield after 72 hours exposure. A linear Probit regression was performed in order to determine EC values, with confidence limits estimated according to Fieller's theorem.

#### II. Results and Discussion

## Yield at 72 hours

Regarding the calculation of the  $EC_{50}$  value for yield, a statistically significant concentration/response was found (p(F)  $\mathcal{D}(01)$  for this parameter.

The resulting  $BC_{50}$  value and confidence intervals are represented in the following table below.

#### 

, ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~		Yield [µg a.s./L]	
Parameter	<b>EC10</b>	EC20	EC 50
	(95% confidence	(95 % confidence	(95 % confidence
<u> </u>	🔪 🦉 interval) 🚿	interval)	interval)
Effect on yield at $\int 2y^2$	n.d. O	n.d.	10.52
			(8.83 - 13.31)

n.d.: not determined since value @beyond the tested concentrations

The resulting  $EC_{50}$  value of 10.52 (95%CL: 8.83 – 13.31) µg a.s./L meets the goodness of fit criteria showing a significant concentration/response relationship, and therefore the estimated  $EC_{50}$  value is considered reliable. The resulting  $EC_{50}$  value was 10.52 µg a.s./L.  $EC_{10}$  and  $EC_{20}$  values could not be determined for yield due to values being beyond the tested concentrations.

#### III. Conclusion

The resulting EC₅₀ values for yield at 72-hours was determined to be 10.52  $\mu$ g a.s./L.



#### Assessment and conclusion by applicant:

 $EC_{10}$ ,  $EC_{20}$  and  $EC_{50}$  values for growth rate were calculated in the study report therefore only yield endpoints have been determined in this statistical report.

The statistical re-evaluation of the data has determined a reliable  $EC_{50}$  value for yield. Reliable  $EC_{20}$  values for yield could not be calculated.

The  $E_rC_{50}$  determined in the original study report of 175 µg a.s./L shall remain as the critical endpoints determined from this study.

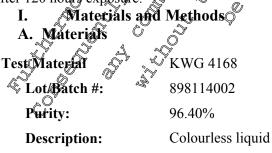
The values determined in the re-evaluation work are considered to be fully valid

	KCA 8.2.6.1/04
Data Point:	KCA 8.2.6.1/04
Report Author:	
Report Year:	
Report Title:	Influence of KWG/4168 on the growth of the green alge Selenastrum
Report No:	AJO/129595
Document No:	
Guideline(s) followed in	M-00651869Y-1 EEC Directive 79/831/E, Annex V, C.3 Algal Onibition Test Bevised Version
study:	No. L389 A/179 (1992)
	No. L383 A/179 (1992) EPA Guideline 540/9-86-13 Growth and Reproduction of Aquatic Plants, Tiers 1 and 2 (1986)
	1 and 2 (1986)
	ISO Guideline 8692: 1989 (E) "Water Quality, Fresh Water Abgal Growth
2	Inhibition Test with Scenedermus subspicators and Schenastriph capricornutum"
L L	(1989) OFCD Grudeline 201 "Area Growth Inhibition Fest" (1984)
Deviations from current	
test guideline:	None of the second seco
Previous evaluation:	yes, evaluated and accepted DOR (1997), RAR (2010), RAR (2017) Yes, conducted under CLP/Officially recognized testing facilities
GLP/Officially	DOR(1907), RAP(2010), RAP(2017), RAP(2017), RAP(1907), RAP(1907)
	Yes, conducted under GLP/Officially recognised testing facilities
recognised festing facilities	
Acceptability/Reliability:	Supportive only 2 2

**Executive Summary** 

In a 120-hour poxicity study Eultures of Sclenas film capricornutum were exposed to KWG 4168 at nominal test concentrations of 0.18, 0.32 (9.56, 1.00, 1.80, 3.20, 5.60, 10.0, 18.0 and 32.0 µg a.s./L under static conditions.

The NOE_bC and  $E_bC_{\pi}$  values were 0.32 and 5.42 µg a.s./L, respectively. The percent inhibition of biomass in the treated algal culture as compared to the control ranged from -4.6 to 99.5% after 120 hours exposure. The NOE_rC and  $E_rC_{\pi}$  values were 1.80 and 19.43 µg a.s./L, respectively. The percent inhibition of growth rate in the treated algal culture as compared to the control ranged from -0.8 to 100% after 120 hours exposure.





Stability of test compound:	Not reported
Reanalysis/Expiry date:	07 August 1995
Density:	Not reported
Treatments	
Test rates:	Not reported 07 August 1995 Not reported Nominal: 0.18, 0.32, 0.56 1.00, 1.80, 320, 5.60, 10.0 18.0 and 32.0 μg a.s./L None Yes, mean measured concentrations 121 and 96% of nominal at test start and end, respectively. Green alga Selenastrum capricoonutum fnow Raphidocelis wheemit fine strum capricoonutum fnow Raphidocelis
Solvent/vehicle:	None Yes, mean measured concentrations 121 and 96% of nominal at test start and end, respectively. Green alga Selenastrum capricoonutum fnow Raphidocelis
Analysis of test	Yes, mean measured concentrations 121 and 96% of nominal at test
concentrations:	start and end, respectively.
Test organisms	
Species:	Green alga Selenastrum capricornutum Thow Raphidocelis
I	subcapitoura), strain 61 H
Source:	Collection of Algal Cultures Universität Göttingen Göttinger
	Germany & & & &
Test design	Green alga Selenastrum capricomutum Thow Raphidocelis subcapitotu), stram 61.81 Collection of Algal Cultures, Universität Göttingen, Göttingen Germany
Test vessel:	
Test medium:	Nutrient solution prepared with slight modification that described in
ž.	QECD 201 0 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2
Replication:	Nongreported 5 5
Initial cell density	$3\xi^{2}10^{3}$ cells/mL $\beta^{2}$ $\beta^{2}$ $\beta^{2}$
Dunation of tort	0120 hours 4 5 5 6 6
Environmental test	
Environmental test conditions	
Temperature	$\frac{120 \text{ hours}}{\sqrt{23}} = \frac{120 \text{ hours}}$
рН:	300 ML Erleameyer flask seontaining 150 mL solution Nutrient solution prepared with slight modification to that described in OECD 201 None reparted $3x 10^{3}$ cells/mL 120 hours $23 \pm 2^{\circ}C$ 799 - 925 Continuous behting at 8000 lux
Photoperiod: 200	Continuous fighting at 8000 lux
A Ö	
B. Study Design	
This study was conducted in	order to assess the growth of the green alga Selenastrum capricornutum

This study was conducted in order to assess the growth of the green alga *Selenastrum capricornutum* when exposed to KWG 4368 over a duration of 120 hours.

Test concentrations were prepared from a stock solution and 150 mL of the solution was used in 300mL Erlenme of flasts during the test. Nominal test concentrations were 0.18, 0.32, 0.56, 1.00, 1.80, 3.20, 5.60, 10.0, 18.0 and 2.0 pg a.s./L. Mean measured concentrations were 121 and 96% of nominal at test stat and test end, respectively.

Test media were inoculated with enough 2 or 3-day old pre-culture to give a density of 3 x  $10^3$  cells/mL. Incubation was at  $23 \pm 2$ °C and under continuous light at 8000 lux. Sedimentation of the cells or test substance was prevented by intermittent turning of the pole on which test flasks were suspended.

Cell numbers were determined microscopically on each day of the test using a Thoma counting chamber.



#### Analytical method

Samples of water were analysed using the validated analytical method 00252 M001, report reference <u>M-008490-02-2</u> (see Doc MCA Section 4).

#### II. Results and Discussion

Validity criteria according to the OECD 201 guideline in place at the time of study conduct were achieved because the cell density in the control cultures increased by a factor of at least 06 by the end of the test.

The mean recoveries of treated, cell-free test vessels at test start ranged from 77 to 157% of nominal, with an overall mean recovery of 121%. At test end, mean recoveries ranged from 40 to 152% of nominal, with an overall mean recovery of 96% Results have been presented based on nominal concentrations.

		~~		~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	$\sim$	1. 7	4	
T-LL CA 01(1/0/1	M	1	al	•1	· · Worth · · · ·		- 4 -1 0 .	
Table CA 8.2.6.1/04-1	Nominal and m	easured concer	Invations™	¥n treated.	cen#tree	vessels a	M dav u«	
1 4 5 10 10 10 10 11 0 1 1		east ea conce				1000010		

Nominal concentration	Mean measured concentration % of notinnal (μg ats/L) / / / / / / / / / / / / / / / / / /
(µg a.s./L)	
Control	
0.18	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$
0.32	
0.56	
1.00	
1.80	$\begin{array}{cccccccccccccccccccccccccccccccccccc$
3.20	3.87 5 Q Q Q Q Q
5.60	1169 AV AL AN W77 AV A
10.0	
18.0	27.2 a 2 1 0 by
32.0	36.4 × × × × × × × × × × × × × × × × × × ×
18.0 32.0 -	636.4 ℃ 118 Mean:

Limit of Quatification (LOQ): 0.09 µg/by 60 x

Table CA 8.2.6.1/04-2	Nominal and	measured	concentrations	in treated, cell-free	vessels at day 5
		Con		/ · · · · · · · · · · · · · · · · · · ·	

Nominal concentration	Mean measure concentration	% of nominal
(µg a.s./L)	$(\mu g a.s)$ $( ( ( ( ( ( ( ( ( ( ( ( ( ( ( ( ( ( ($	
		-
0.18	0.425	72
0.32		40
0.56 ~ 0 ~	0.635	118
1.00 1		113
1.80	2.20	127
3.20	N3,89 L . O	126
5.60 [×]	73.2 0 ~ ~	58
	7.8 5 0	81
18.0	13.4 Q	89
32.0 × × ×	40.8 g	132
	Mean	96

Limit of Quatification (LOQ): 0.69 µg/L

Cell densities determined during the study are presented in the table below. In the 0.56  $\mu$ g a.s./L test concentration, some cells were slightly swollen after 48, 72 and 96 hours. In the 1.00  $\mu$ g a.s./L test concentration, some cells were slightly swollen after 48 hours and some cells were smaller than normal after 72 and 96 hours. In the 1.80  $\mu$ g a.s./L test concentration, some cells were slightly swollen after 48 hours and some cells were cylindrical in form after 48, 72, 96 and 120 hours. In the 3.20, 5.60, 10.0 and 18.0  $\mu$ g a.s./L test concentrations, some cells were deformed after 24 hours, and after 48, 72, 96 and 120 hours exposure cells were cylindrical in form and



were swollen. At the highest test concentration,  $32.0 \ \mu g a.s./L$ , all cells were deformed after 24 hours, and after 48, 72, 96 and 120 hours exposure cells were cylindrical in form and were swollen.

Nominal	Number of cells (x10 ⁴ /mL) ¹ by time (h)		e (h)	Â,	
concentration	24	48	72	96 🗭	120
(µg a.s./L)				4	
Control	$1.42 \pm 0.58$	$12.83 \pm 0.93$	84.42 = 7.51	$240.33 \pm 61.52$	Å91.33 <b>≿</b> 26.22
0.18	$2.50 \pm 0.50$	$11.50 \pm 1.00$	87.32 13.70	$250.67 \pm 40.46$	518.67 ± 27.28
0.32	$1.50 \pm 1.00$	$11.67 \pm 2.25$	$83_{0}33^{\circ} \pm 9.02$	262.00 ± 4.00 °	486.67 ± 12.22
0.56	$1.50 \pm 1.00$	$9.50 \pm 1.00$	$6500 \pm 11.76$	190.00 ± 11.6	48\$,33 ± 02.86
1.00	$1.83 \pm 0.29$	$9.67 \pm 2.52$	₹ <b>3</b> 6.50 ± 3.91	$244.67 \pm 20.43$	$476.00 \pm 4.00$
1.80	$1.33 \pm 0.58$	7.17 ± 1.76	$60.50 \pm 3.12$	222.67 ± 18.58	$9521.39 \pm 30.02$
3.20	$1.67 \pm 0.58$	10.50 ± 1.00	$4550 \pm 259$	¥ 158.67 @ 10.6	$413.33 \pm 37.17$
5.60	$1.33 \pm 1.26$	$7.83 \pm 0.76$	40.33 ± 0.76	140.09 ± 18.03	<i>364.00</i> ± 32.74
10.0	$2.00 \pm 0.50$	6.33 ± 1.61	21.83 1.04	$68.93 \pm 4.48$	200.67 11.37
18.0	$1.50 \pm 0.50$	2.50 ± \$50 ~	4.50 ¥ 0.87	283 ± 0.56	8.50 20.50
32.0	$0.83 \pm 0.58$	1.50 \$ 0.50	$1 / 7 \pm 0.76$	0.50 ± 9.50	$0.00 \pm 0.00$

Table CA 8.2.6.1/04-3 Cell density during exposure to KWG 4168

Mean of two samples of three replicates (styreplicates for the control) ± standard deviation

Statistically significant inhibition of area under the growth curve (biomass) could be observed after 96 hours at test concentrations 3.20 µg a.s. and above After 620 hours, significant inhibition could be observed at test concentrations 0.56 µg a.s./L, and additionally at 320 µg a.s./L and above. Ş

	w ^v «.		L	~ Å	-	Ô.
Table CA 8.2.6.1/04-4	Areaunderche	growth c	u 🛿 e and	inhibition	of treated	coltures?

					$\sim$				
24 h	V	⊿48h	, in the second s			96 h 🚿		120 h	
Area		Ärea /	%	Area	**	Area	%	Area	%
	aphibition		inhorition	S i	Inhibition	°"	inhibition		inhibition
13	S- 🗸	177	. T. V	1332	·	5227	(T)	14000	-
26	-97,02	187	-5.6	1366	-2:2		≫-3.6	14640	-4.6
. H	-75	165 🖔	6.8 0	1298	2.9	5435	-4.0	14412	-2.9
		139 O	21.4	چ) 026	23.3	4079	22.0	12176	13.0*
18	©-37.3 ∞	149	15.8	°936 🖓		<b>¥</b> 45¥3	13.1	13184	5.8
12	7.5			912	31.	4303	17.7	13224	5.5
16	-22.4	X155 🗟		820	\$ <b>7</b> .7	3263	37.6*	10120	27.7*
12	×5 ×	115	35	~ <b>7</b> 34 .	45.1 🔊	2939	43.8*	8980	35.9*
20 🔍	₹-32.2 ¥	140	86 ×	″ 444 👝 ″	66.8	1525	70.8*	4752	66.1*
14	-7.5	S :	~¥00.0 🗶 j	132	900	225	95.7*	366	97.4*
6	520	27	84.7	52	26.0	65	98.8*	64	99.5*
	Area 13 26 74 18 12 16 12 20 6	Area 26 -97 0 -97 0 -	$\begin{array}{c c c c c c c c c c c c c c c c c c c $	$\begin{array}{c c c c c c c c c c c c c c c c c c c $	$\begin{array}{c c c c c c c c c c c c c c c c c c c $	$\begin{array}{c c c c c c c c c c c c c c c c c c c $	$\begin{array}{c c c c c c c c c c c c c c c c c c c $	$\begin{array}{c c c c c c c c c c c c c c c c c c c $	24 h       48 h       72 h       96 h       120 h         Area       %       Area

Statistically significantly different to the control (Dunnett's test, p=0.05, one-sided)

Statistically significant inhibition of growth rate could be observed after 24 hours in all test concentrations except for the 1.80 µg a.s./L test concentration. After 48 hours exposure, significant inhibition was observed in all test concentrations. After 72 hours exposure, statistically significant inhibition could be observed at test concentrations 0.56 µg a.s./L and above. Statistically significant inhibition in growth rate was observed after be hours exposure at test concentrations 0.32 and 0.56 µg a.s./L, and additionally at concentrations of 3.20 µg a.s./L and above. After 120 hours exposure significant indibition could be observed in test concentrations 3.20 µg a.s./L and above. .Ô Ľ

Table CAS.2.6.104-5 Growth ate and inhibition of treated cultu	res
----------------------------------------------------------------	-----

Ĵ.

Nominal	24 h		48 h		72 h		96 h		120 h	
concentration	Rat	%	Rate	%	Rate	%	Rate	%	Rate	%
(µg/a.s./L)		inhibition		inhibition		inhibition		inhibition		inhibition
Control O	1.45	-	1.88	-	1.88	-	1.66	-	1.48	-
0.18	2.11	-44.8*	1.82	2.9*	1.89	-0.5	1.68	-1.1	1.49	-0.7
0.32	1.41	2.8*	1.82	2.8*	1.87	0.2	1.69	-1.9*	1.48	0.1
0.56	1.41	2.8*	1.73	8.1*	1.79	4.8*	1.61	6.0*	1.48	0.2



Nominal	24 h		48 h		72 h		96 h		120 h	
concentration	Rate	%	Rate	%	Rate	%	Rate	%	Rate	% 。
(µg a.s./L)		inhibition		inhibition		inhibition		inhibition		inhibitten
1.00	1.80	-23.8*	1.72	8.1*	1.75	7.1*	1.68	-0.8	1.47	0.4
1.80	1.44	1.3	1.58	16.0*	1.77	5.9*	1.65	0.6	1.49	-005
3.20	1.67	-14.5*	1.78	5.4*	1.67	10.9*	1.57	5.7*	1.45	<u></u>
5.60	1.24	14.5*	1.63	13.2*	1.67	11.4*	1.53	7.6%	1.42	4.1*
10.0	1.88	-28.9*	1.51	19.4*	1.43	23.9*	1.36	18.2*	1.30	1240
18.0	1.57	-7.9*	1.05	43.9*	0.90	52.2*	0.63	<b>6</b> 7.9*	0.67	54 <b>.8</b> * x
32.0	0.88	39.7*	0.79	58.2*	0.40	786*	0.14	91.4*	\$0.00	100.0*

Table CA 8.2.6.1/04-6	Summary of derived	endpoints

* Statistic	Ily significantly different to the control (Dunnett's test $p=0.05$ , one-sided)
The endpoir	s derived from the results of this study have been summarised below
	.6.1/04-6 Summary of derived endpoints
Biomass	Spowth rate w w w w
$E_bC_{50}$	5.42 μg a.s./L
(95% CI)	(2.265 to 11.87 μg a.s./L) A Q Q Q Q Q Q Q Q Q Q Q Q Q Q Q Q Q Q
LOE _b C	0.56 μg a.s./L LOFrC O 3.20 μg a SOL L
NOE _b C	0.32 μg a.s./L

#### III. Conclusion

In a 120-hour toxicity study, cultures of Selenfastrum Capricornutum were exposed to KWG 4168 at nominal test concentrations of 0,48, 0.32, 0.56, 1.00, 1.80, 3,20, 5.60, 10.0, 18.0 and 32.0 ag a.s./L under static conditions. m

The NOE_bC and  $E_bC_{50}$  values were 0.82 and 42 µg a.s./L respectively. The percent inhibition of biomass in the treated algal culture as compared to the control ranged from -4.6 to 99.5% after 120 hours exposure. The NOE and prC₅₀ values were 180 and 19.4 Dµg a L, respectively. The percent inhibition of growth tate in the treated algar culture as compared to the control ranged from -0.8 to 100% after 120 hours exposure

#### Assessment and conclusion by applicant.

Validity criteria according to the OECD 201 gyrdeline (2011) have been re-assessed and the outcome S presented below.  $\bigcirc$ 

- 1) The cell density increase in the control culture to be at least a factor of 16 (actual 281 after 72 hours and 1638 after 20 hours);
- 2) The mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2, and  $2\beta$ ) in the control culture to be  $\leq 35\%$  (actual 23.9% after 72 hours and 48.3% after 120) hours):
- 3) The coefficient of variation of the average specific growth rates over the whole test period should be  $\leq 7\%$  (actual 1.54% after 2 hours and 0.72% after 120 hours).

Using the 72-hour data, the valuatity cheria according to the current test guideline have all been achieved. However, when the 20-hour data are considered, not all of the criteria are achieved with the mean coefficient of variation being >35%.

On this basis the study is considered to be supporting information only.

The  $E_r C_{\omega}$  value was determined to be 19.43 µg a.s./L.

The data have been subjected to statistical re-evaluation and the results have been presented in the following study summary.



Data Point:	KCA 8.2.6.1/13
Report Author:	
Report Year:	2020
Report Title:	Calculation of EC10, EC20 and EC50 values for Selenastrum capricornutum with
	KWG 4168 in an algal growth inhibition test
Report No:	0471836-ECO25
Document No:	<u>M-761402-01-1</u>
Guideline(s) followed in	None
study:	C L L L
Deviations from current	None 🕅 🖉 🖉 🖉
test guideline:	
Previous evaluation:	No, not previously submitted $\mathcal{O}$ $\mathcal{O}$ $\mathcal{O}$ $\mathcal{O}$
GLP/Officially	No, not conducted under GLP/Officially recognised testing facilities
recognised testing	No, not conducted under GEP/Officially recognised testing facilities
facilities:	
Acceptability/Reliability:	Yes O' A A A

#### **Executive Summary**

The report <u>M-006518-01-1</u> on the effect of exposure to KWG 4168 on the growth of algae (*Selendstrum capricornutum*) did not provide estimates of  $EC_{10}$  of  $EC_{20}$  values. Therefore, these values as well as  $EC_{50}$  values have been calculated in accordance with the Annex to Com. Rep 283/2013 for yield and growth rate.

The resulting  $EC_{10}$ ,  $EC_{20}$  and  $EC_{50}$  values for yield at 120 h were 3,60, 4.73 and 7.99 µg a.s./L, respectively. For growth rate after 120 h, the  $EC_{10}$   $EC_{20}$  and  $EC_{50}$  values were 9.20, 10.94 and 15.24 µg a.s./L, respectively.

#### I. Methods

The statistical evaluation was performed with statistical software ToxRatPro v3.3.0.

Effect concentrations with 10, 20 and 50% from the test item treatment when compared to the control were determined for yield and growth rate after 120 hours exposure. A Probit regression was performed for both parameters with confidence limits estimated according to Fielder's theorem.

#### II. Results and Discussion

#### Yield at 120 hours

Regarding the calculation of EC5, EC5, and EC50 values for yield at 120 h, a statistically significant concentration/response was found (p(b) < 0.001) for this parameter.

The resulting  $C_{10}$ ,  $C_{20}$  and  $C_{50}$  values and the respective confidence intervals are represented in the following table below.

# Table CA 8.2.6.1/13-1 Results of the Probit analysic of yield at 120 h: Selected effective concentrations (EC) of the rest item and their 95%-confidence limits

'¥			
		Yield	
L. A	∖ <i>©</i> E€ Q	EC20	EC50
Parameter	95 % confidence	(95 % confidence	(95 % confidence
	interval)	interval)	interval)
	C Aug a.s./L	[µg a.s./L]	[µg a.s./L]
Effect on yield at 120	~ 3.60	4.73	7.99
h h	(3.03 - 4.09)	(4.17 - 5.22)	(7.43 - 8.59)
0 60 10	a V		

The resulting EC₁₀, EC₂₀ and EC₅₀ values of 3.60 (95%CL: 3.03 - 4.09), 4.73 (95%CL: 4.17 - 5.22) and 7.99 (95%CL: 7.43 - 8.59) µg a.s./L, respectively, meet the goodness of fit criteria and therefore the estimated ECx values are considered reliable.



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#### Growth rate at 120 hours

Regarding the calculation of EC₁₀, EC₂₀ and EC₅₀ values for growth rate at 120 h, a statistically significant concentration/response was found (p(F) < 0.001) for this parameter.

The resulting EC10, EC20 and EC50 values and the respective confidence intervals are represented in the following table below.

#### Table CA 8.2.6.1/13-2 Results of the Probit analysis of growth rate at 120 h Selected effective concentrations (EC_x) of the test item and their 95% confidence limits

		Growth rate	
	EC ₁₀	EC20	
Parameter	(95 % confidence	(95 % confidence 🖉	(95 % confidence
	interval)	🧳 interval) 🗸	mteryally
	[µg a.s./L] 🧳	(μg æs./L] 🖉 🦼	⁰ _ [μg a.s. [L] ~ [
Effect on growth	9.20	© \$10.94 \$ ~ @	15.24
rate at 120 h	(8.75-9.62)	(1054 - 1052)	(1460-1559)

The resulting EC₁₀, EC₂₀ and EC₅₀ values of 9,20, 95% CL: 8.75 – 9.62), 10,94 (95% CL: 10.54 – 1.32) and 15.24 (95%CL: 14.90 - 15.59) µg as./L, respectively, meet the goodness of fact riteria and therefore the estimated ECx values are considered refrable.

#### III. Conclusion

The resulting  $EC_{10}$ ,  $EC_{20}$  and  $EO_{50}$  values for yield at 120-hours were determined to be 3.60, 4.73 and 7.99  $\mu$ g a.s./L, respectively. The resulting EQ₁₀, EC₂₀ and EC₅₀ values for growth rate a 2120-hours were Ø determined to be 9.20, 10.94 and  $15.24 \mu s a.s./I_{a}$ 

#### Assessment and conclusion by applicant:

The statistical re-evaluation of the data has determined veliable EC10 EC20 and EC50 values for both growth rate and yield. The ErC ordetermined in this re-evaluation work of 15.2 µg a.s./L is slightly lower than the  $E_{r}C_{50}$  determined in the original study report of 19.4 µg a.s./L therefore the  $E_{r}C_{50}$  of 15.2 µg a.s./L Shall be taken as the stitical endpoint determined from this study.

The values determined in the re avaluation work are considered to be fully valid

the revaluation work are considered to the re



Data Point:	KCA 8.2.6.1/05
Report Author:	ذ 😞
Report Year:	1995
Report Title:	Growth of Selenastrum capriornutum cells in nutrient medium containing logh
	concentrations of KWG 4168
Report No:	AJO/131995
Document No:	<u>M-006206-01-1</u>
Guideline(s) followed in	EEC Directive 79/831/E, Annex V, C.3, Algal Inhibition Test, Revised Version
study:	No. L383 A/179 (1992)
	ISO Guideline 8602: 1080 (E) "Water Quality (Fresh Water Model Growth We I was
	Inhibition Test with Scenedes rus subspicatus and Selenastrum capteornutan"
	OECD Guideline 201 "Alea Growth Inhibition Tost" (1984)
Deviations from current	Yes OECD 201 (2011) Validity criteria could not be assessed
test guideline:	Yes OECD 201 (2011) Validity criteria could not be assessed was evaluated anticlassified
	Validity criteria could not be assested of the two sets of the two sets as a set of the two sets of tw
Previous evaluation:	
	DAR (1997), RAR (2010), RAR (2017)
GLP/Officially	No, not condicted under GLF/Officially recognised testing facilities
recognised testing	
facilities:	
Acceptability/Reliability:	Supportive only a co co m
<b>Executive Summary</b>	

#### **Executive Summary**

This study was conducted in order to assess the growth and recovery of the green alga Selenastrum capricornutum during and after exposure to KWG 4158 for 420 hours. Ľ

The ErC50, NOErC and LOE values for S. capricornutum exposed to KWG 4168 for 120 hours are 19.3, 1.8 and 3.2 µg@.s./L respectively

To determine if the test item had long-term effects on the growth of the algal cells, cultures of S. capricornution that had been exposed to the test item for 120 hours were transferred into fresh test , S Ś media and incubated for 528 hours

In cultures treated with 1.0 and 1.8 for a.s./L, there were no significant differences in cell numbers between treated and control after 96 h. In cultures treated with 3.2, 5.5 or 10 µg a.s./L, significant differences had disappeared after 168 h. On the cultures treated with 18 and 32 µg a.s./L, growth was initially slow, but after 528 h cellonumbors in the treated and control cultures were the same. When cells from 528 h old culture overe moculated into resh, &WG 4768-free nutrient solution, and cells counted after 72 h, there were fewer cells in the 48 and 32 µg a.s./L cultures than in the controls. Differences between treated and control cultures were no longer evident at the time of the second counting (192 h).

ormoon nouroa ana contigi	
I. Materialsanc	l Methods 🕺 🔬
A. Materials	
Test Material	
lest Material	KAWG 4168 _O
Lot/Batch#:	898119002 Q
Purity S	³ 96.40%
Description:	Solourless liquid
Stability of test	Not reported
Resnalysis/Expiry	07 August 1995
date:	07 11ugust 1995
Density:	Not reported



#### Treatments

11 cutilities	
Test rates:	0.18, 0.32, 0.56, 1.0, 1.8, 3.2, 5.6, 10.0, 18.0 and 32.0 μg a.s./L
Solvent/vehicle:	None
Analysis of test concentrations:	0.18, 0.32, 0.56, 1.0, 1.8, 3.2, 5.6, 10.0, 18.0 and 32.0 µg a.s./L None Yes (reported in (1995). <u>M-006518-0151</u> ) Green alga <i>Selenastrum capricornutum</i> (1995). <u>M-006518-0151</u> )
Test organisms	
Species:	Green alga Selenastrum capricornutum from Raphidocelis subcapitata), strain 61.84 Collection of Algal Cultures, Universität Göttingen, Göttingen, Germany Not reported Nutrient solution corresponding to medra described in OECD 201.
Source:	Collection of Algal Cultures, Universität Cottingon, Görfingen, Germany
Test design	
Test vessel:	Not reported A
Test medium:	Nutrient solution corresponding to media described in OEQD 201
<b>Replication:</b>	None reported & for the for the former of th
Initial density:	$3 \times 40^3$ cells/mL $6^{-1}$ $5^{-1}$ $6^{-1}$ $6^{-1}$ $5^{-1}$ $5^{-1}$
<b>Duration of test:</b>	120 / 526 hours & & &
Environmental test conditions	None Yes (reported in (1995). M-006518-051) Green alga Selenastrum capricornutum flow Raphidorellis subcapitata), strain 61.84 Collection of Algal Cutures, Universität Gottingen, Görfingen, Germany Not reported Nutrient sclution corresponding to medra described an OEQD 201 None reported 3 x 40 ⁵ cells/mL 120 / 526 hours Continuous lighting at 8000 lbs Continuous lighting at 8000 l
Temperature: 🔬	$23^3 \pm 2$ °C $3^3 \pm 2$ °C $3^3$
pH:	Noteported and a long the long
Photoperiod.	Continuous lighting at \$000 lox
B. Study Design 🔬	
This study was conducted i capricorputum during and a	n order to assess the growth and recovery of the green alga Selenastrum for exposure to KWG 2168 for 120 pours.
	epared from a stock solution. Test media were inoculated with enough 3- i density of 3 x 10 cells/mL.
Incubation was at 23. 2°C substance was prevented by	and under continuous light at 8000 lux. Sedimentation of the cells or test intermittent during of the pole on which test flasks were suspended.
Cell numbers were determin	econicroscopically using a Thoma counting chamber.

Routine growth inhibition test

The 120-hour  $E_rC_{50}$ , LOP, C and NOE, C values determined after exposure to test concentrations 0.18, 0.32, 0.56, 1.0  $(21.8, 3.2, 5.6, 10.0, 18.0 \text{ and } 32.0 \ \mu\text{g}$  a.s./L, along with a control. Cell counts were conducted after 24, 48, 72, 96 and 120 hours. ~Ć

# Growth repovery test Ô

Growth recovery test To follow the growth of cells on cultures that had been inhibited by KWG 4168 during the routine growth inhibition test, incubation was continued up to 528 hours. Additional cell counts were made after 168, 264m 336 and 456 hours. For control cultures and those treated with 18.0 or 32.0 µg a.s./L, further counts were made after 528 hours.



#### Growth of cells from treated cultures in fresh media

To determine if incubation with high concentrations of KWG 4168 impaired the capacit@of S. capricornutum to grow in fresh media, cells from the 528-h controls and 18.0 and 32.0  $\mu$ g a.s./L groups were inoculated at 3 x 10³ cells/mL into 150 mL portions of fresh, untreated nutrient solution. The number of cells that had formed in each culture were counted after 72 and \$92 hours.

#### II. **Results and Discussion**

*Routine toxicity test* 

The ErC₅₀, NOErC and LOErC values for S. capricornutum exposed KWG 416  $3.2 \,\mu g \, a.s./L$ , respectively.

#### Growth recovery test

After 168 hours of incubation, there was not statistically significant difference between the numbers of cells in cultures treated with 1.0, 1.8, 3.2, 5.6 and 10, Jug a st and the controls. Ar cultures treated with 18 and 32 µg a.s./L, growth recovery was notably slower however once growth statted it progressed rapidly and after 528 hours there were no statistically significant differences from the controls. Ø 47

-				<u> </u>				<u>ģ</u>
Time (h)	Number of	f cells (x10 ³ /"	mL) ¹ by con	centration (	µg a.s.⊮L)		A	Č.
	Control	1.0	YIQ Ø	<b>3</b> .2	5.	10.0	18.0	32.0
24	14.2	18.3 ± 2.9	23.3	16.7 °	£3.3 ~~~	20	015	8.3
	± 5.8	± 2.9%	(± 5.8	± 50.8	± 12.6	\$.5.0 Ø	$\pm 5.0^{\circ}$	± 5.8
48	128.3	90 <i>(</i> )		105	78.3	\$3.3	25	15
	± 9.3	±25.2*	± 17.6*	£¥ 10.0 <b>*</b> ≶∕	±Q?6*	± 16 1	\$5.0*	$\pm 5.0*$
72	844.2	565 S	6 <b>05</b> C	445	. 443 🖇	248.3	245	11.7
	± 7.5	×±39.₽*	31.2*	±26.5* <	$2 \pm 7.6 * 0^{10}$	Ð10.4* 🏑 🎽	± 8.7*	± 7.6*
96	2403 $\pm 6152$	244,67	5222 <i>6</i> .9	1586.7	1400	688.3	38.3	5.0
	$\pm 6152$	±204.3	± 185.8	⊁± 109;⊀*	±180.3	± 4408*	± 7.6*	$\pm 5.0*$
120		04/00	5243.3	413333	3640	2006.7	85	0.0
	±262.2	$\pm 40^{9}$	₽300.2√	±\$71.7*	² ± 3254*	@113.7*	$\pm 5.0*$	$\pm 0.0*$
168	GD 630	6070	58904	5240 0	5510	\$5250	350	7
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	¥ ± 478.0	¥864	± <b>304</b> .1	± 422.6	£255.1~	$\pm 517.6$	$\pm 115.7*$	± 5.2*
264	5490	6370	5930 _v	668 0 °	6470 ^O	5280	3290	70
	±447.80	± 582.3	5930 * 4640 *		± 440.1	± 404.8	$\pm 141.8*$	$\pm 20.0*$
336	5450 2	6030	6000 ⁹	<i>"Ø</i> "/60 ∪	5480	5290	4040	1000
	± 439.3	∂ ⊒ 326.£	± 40 1.6 ~	± 284.7	\$567.4	± 369.8	$\pm 641.5*$	$\pm 172.2*$
456	55240	5670	6250 O	50,90	Ø 5 010	5070	3400	3990
	³ €575.0 ⁽⁾	±-397.2 ~	¥± 442.3∛	₩330.5	± 314.6	± 363.7	$\pm 236.0*$	$\pm 260.1*$
528 🗳	4930	- 3	- 7		-	-	4250	4320
	± 398.5 🔩	Ŷ.Q					± 260.1	± 368.3

Table CA 8.2.6.1/05-1 Cell density during the foxicity phase and the growth recovery phase

Mean of three repticates (six for the control)

Statistically significantly different to the controls (T-test)

Ô Growth of cells from treated outures In freshmedia

°~

. Ŵ

Seventy-two bours after insculation into fresh nutrient media, algal cells from the treated cultures had grown but at an initially Nower rate than that of cells from the untreated control cultures. After 192 hours, there was no statistically significant difference in the number of cells in cultures grown using inoculum from control or treated cultures. Y R

19491e (A & S2 6 1/05-2	Cen density of cells transferred to fresh media

Time (b)	Number of cells (x10 ³ /mL) ¹ by concentration (µg a.s./L)		
	Control	18	32
0	3.3 ± 0.3	2.8 ± 0.2	2.9 ± 0.2
72	1420 ± 285	913 ± 95*	497 ± 49



Time (h)	Number of cells (x10 ³ /mL) ¹ by concentration (µg a.s./L)				
	Control	18	32		
192	4993 ± 434	4840 ± 120	5293 ± 363		
¹ Mean c	of three replicates (six for the con	trol) ±SD (standard deviation)		X R	
* Statisti	cally significantly different to the	e controls (T-test)	ð		
III.	Conclusion		<i>A</i>		
The ErC50.	NOErC and LOErC values fo	r S. capricornutum exposed 1	o KWG 4168 fo	x 920 hears are	

19.3, 1.8 and 3.2 μ g a.s./L, respectively.

To determine if the test item had long-term effects on the growth of the algebra cells cultures of \circ S. capricornutum that had been exposed to the test item for 120 hours were transferred into fresh test media and incubated for 528 hours.

In cultures treated with 1.0 and 1.8 μ g a.s./L, there were no significant differences in cell numbers between treated and controls after 96 h. In cultures treated with 3/2, 5.5 or 10 μ g a.s./L, significant differences had disappeared after 168 h. In the cultures treated with 18 and 32 μ g a S/L, growth was initially slow, but after 528 h cell numbers in the treated and control cultures were the same. When cells from 528 h old cultures were inoculated into fresh, KWG 4168-free putrient solution, and cells counted after 72 h, there were fewer cells in the 18 and 32 μ g a.s./L cultures that in the controls. Differences between treated and control cultures were no longer evident at the time of the second counting (192 h).

Assessment and conclusion by applicant:

The study was conducted to the OECD test guideline 201 (1984), the carrent version of which is the OECD 201 "Freshwater alga and cyanobacteria" growt winhibition test", adopted 28 July 2011.

This study was a recovery test which is not considered to be appropriate for use of the risk assessment. As such the validity criteria has not been re-assessed against the current test guideline.

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The E_rC_{50} value was determined be 19.3 µg a.s./L
```

Data Point 2 , KCA \$2.6.1 4 2
Data Point Q , KCA 82.6.1/96 Q Q
Report Author:
Report Year: $2000 & 7 & 7 & 7$
Report Tear.
with piroxaprine (KWG 4168)
Report No: \mathcal{O} \mathcal{O} \mathcal{O} \mathcal{O} \mathcal{O} \mathcal{O} \mathcal{O}
Document No 0 $028201-01-7$
Guideline (a) followed in OECP Guideline 20 % Alga Growth Inhibition Test" (1984)
study: \mathcal{D}' \mathcal{D}' \mathcal{D}' \mathcal{D}' \mathcal{D}' \mathcal{D}' \mathcal{D}'
Deviations from current Yes v V
test guideline: $\sqrt{2}$ $OECD 201 (204)$
Test was run for 22 @ys
Previous evaluation: yes evaluated and assified
A R (2010), RAR (2017)
GLP/Officiely Ses, conducted under GLP/Officially recognised testing facilities
recognised testing of of
facilities
Acceptability Reliability: Supportive only

The study is considered to be supporting information only?

Executive Summary

In a 22-day toxicity study, cultures of *Scenedesmus subspicatus* were exposed to spiroxamine (KWG 4168) at nominal test concentrations of 1.00, 1.80, 3.20, 5.60, 10.0, 18.0 and 32.0 µg test item/L under static conditions.



Cells from the 18.0 and 32.0 µg test item/L concentrations were then incubated for a further 7 days in freshly prepared, uncontaminated media to study the ability to recover.

There were no significant differences in cell numbers between the treatment concentrations 1.00 and 5.6 μ g/L and the controls during the entire exposure period. In cultures treated with 10.0, μ and 32.0 µg test item/L growth was initially slower and cells showed some visible effects after 5 days. Cultures treated with 18.0 and 32.0 µg test item/L showed some visible effects after 8 days. After 19 days, only cultures treated with 32.0 μ g test item/L were still significantly reduced in growth and the algal cells showed some visible effects.

Twenty-four hours after transfer into fresh, uncontaminated test media, no significant differences between the control and both previously treated current were observed. Algabelis from previou exposure levels up to at least 32.0 µg spiroxamine/L are able to recover after elimination of the compound.

prination of the second I. Materials and Methods A. Materials **Test Material** Spiroxamin Lot/Batch #: **Purity:** ellowish, fiauid **Description:** reporte Stability of test compound: **Reanalysis/Exp** date: **Density:** Treatments 60, 🔞 ominal: 1.00. 80 1840 and 32.0 µg test item/L Test rates ¢ Solvent/vehicle: None reported in the highest two concentrations and the stock solution (mean Analysis of 60 concentrations 63% of nominal) easured Test organisms Species(≥en∕alga edesmus subspicatus, strain 86.61 ESP Cultures, University of Göttingen, Germany Source: Test design Test vessel: 300 mL Evenme ver flasks containing 150 mL test medium Test mediam Media prepared (with slight modifications) to OECD 201 Replication ot reported Initial cell density 10^4 cells/mL Duration of test days Environmental test conditions **Temperature:** $22.4 - 24.4^{\circ}C$ 7.88 - 9.11pH:



Photoperiod:

Continuous illumination at mean 6875 lux

B. Study Design

This study was conducted in order to assess the effects of exposure to spiroxamine (KWG 4168) on the green alga *Scenedesmus subspicatus* in a static test over 22 days.

Test vessels were 300-mL Erlenmeyer flasks containing 150 mL test medium, sealed with cotton wool and/or cellulose plugs. These were suspended on a pole, the intermittent turning of which prevented sedimentation of the cells. The test media were prepared with slight modification to the OECD 201 guideline. Media were then inoculated with approximately 10,000 cells mL, from a pre-culture prepared five days before the start of the test and cultivated under equivalent conditions.

Nominal test concentrations were 1.00, 1.80, 3.20 7.60, 10.0, 18,0 and 2.0 µgvest iten/L.

After 22 days of exposure, the cells from the control and the 58.0 and 32.0 fg/L treatment levels were transferred into new untreated nutrient medium. For this purpose the cells were filtered and incubated for another 24 hours in freshly prepared medium (log-phase). Then approximately 1 x 00^4 cells mL were inoculated into fresh, spiroxamine-free nutrient medium to reach a well comparable start situation for growth. These cultures were counted after 3, 4 and 6 days of growth

Cell numbers were determined microscopically at a magnification of 400 times using a Thoma counting chamber.

Quantitative analysis of spiroxathine in samples of the nutrient medium was conducted at test start.

Analytical method

Samples of water were analysed using the varidated analytical method 00622 report reference <u>M-031628-01-1</u> (see Doc MCA Section 4).

II. Result and Discussion ~

The study was not conducted to any guidelines and validity criteria can therefore not be assessed. The quantities of spiroxamine (KWC 4168) found at the beginning of the test were 60 to 63% (average 62%) of the nominal concentrations. All reported results are based on nominal concentrations.

Nominal concentr (µg test item (a.s.))		Mean measured concentration (µg a. s./L)	3 % of nominal
5.60 (5.48)	A		63
32.0 (31.3) 10000 (9780)		1000 × 5× 5×	60
4		Means a g	61 62
¹ Stock olution			·

Table CA.8.2.6 006-1 Nominal and neasoned concentrations at test start

There were no significant differences in cell numbers between the treatment concentrations 1.0 and 5.6 µg/L and the controls during the entire exposure period. In cultures treated with 10.0, 18.0 and 32.0 µg test item/L growth was initially slower and cells showed some visible effects after 5 days. Cultures treated with 18.0 and 32.0 µg test item/L showed some visible effects after 8 days. After 19 days, only cultures treated with 32.0 µg test item/L were still significantly reduced in growth and the algal cells showed some visible effects.

Twenty four hours after transfer into fresh, uncontaminated test media, no significant differences between the control and both previously treated cultures were observed.

The following table details the effects of exposure on biomass (cell density) during the exposure phase:



Nominal	Number of cells	$(x10^4/mL)^1$ by time \pm SD		<u> </u>
concentration	2 days	5 days	8 days	19 days
(µg/L)				
Control	8.17 ± 2.50	209 ± 15.89	311 ± 18.58	394.67 ± 32.66
1.00	9.17 ± 3.88	219.33 ± 13.01	324.67 ± 9.87	405.33 ± 68.86 •
1.80	10.83 ± 4.73	215.33 ± 15.14	313.33 ± 20.03	376 ± 29.4
3.20	8.00 ± 1.80	200.00 ± 13.11	320.67 ± 27 #4	397.33 ± 38.02
5.60	8.67 ± 3.69	212.00 ± 17.09	$290.00 \pm 36,06$	386,67±4,62
10.0	5.67 ± 1.44	156.00 ± 9.17	284.00 ± 28.00	4 2 8.00 ± 2 0.00
18.0	7.17 ± 2.84	125.67 ± 24.98	238.00 <i>≢</i> 15.62	590.67 34.00 S
32.0	3.33 ± 0.29	100.33 ± 10.41	139.00 ± 12,12	\$ 159.67 ± 13.98

Table CA 8.2.6.1/06-2	2 Cell density during the exposure period to spiroxamine
-----------------------	--

¹ Mean of two counts of three replicates (six replicates for the control) ±@andard reviation

The following table details the effects of exposure on thomas (cell density) during the recovery phase:

		. ~ ~ ~ ~	<u>A.</u>	\bigcirc ,	
Nominal	Number of cells (x10 ⁴ /mL)	¹ by time # SD			
concentration	3 days	4 days		6 dave	S O
(µg/L)					
Control	18.08 ± 3.68	28.83 ± 4.75		30.42 ± 301	8
18.0	20.17 ± 8.02	29.00 A.09	0 5	\$\$4.17 ⊕ 3.7	5, 7
32.0	19.33 ± 7.97 ×	30.110 4.19		30.6ر 6.1	Ľ×

Mean of two counts of three replicates (six replicates for the control) ± standard deviation

III. Conclusion

In a 22-day toxicity study, cultures of *Scenedesmus subspicatus* were exposed to spiroxamine (KWG 4168) at nominal test concentrations of 1 60, 1.86, 3.20, 5.60, 10.0, 18.0 and 92.0 µg test item/L under static conditions.

Cells from the \$.0 and 32.0 ug test trem/Leoncentrations were then incubated for a further 7 days in freshly prepared, uncontaminated media to study the ability to becove?

There were no significant differences in cell numbers between the treatment concentrations 1.0 and 5.6 μ g/k and the controls during the entire exponence period. Incultures treated with 10.0, 18.0 and 32.0 μ g test item/L growth was initially slower and cells showed some visible effects after 5 days. Cultures treated with 18.0 and 32.0 μ g test item/L showed some visible effects after 8 days. After 19 days, only cultures treated with 32.0 μ g test item/L were still significantly reduced in growth and the algal cells showed some visible effects.

Twenty-four hours after mansfer into tresh, uncontaminated test media, no significant differences between the control and both previously treated cultures were observed. Algal cells from previous exposure levels up to at least 32.0 μ g spiroxamine/L are able to recover after elimination of the compound.

Assessment and conclusion by applicants

The study was based on the OECD 201 test guideline but was a non-standard test as it was conducted for a 22-day period for lowed by a recovery period. It is therefore not considered necessary or appropriate to a assess the data against the validity criteria for the current OECD 201 test guideline.

Concentrations of the test item in the solution was determined only for the two highest test concentrations and the stock solution, none of which had recoveries within $\pm 20\%$ of nominal. Analytical verification is therefore insufficient to adequately describe test concentrations.

The study is therefore considered as supporting information only.



Metabolites

victabolites	
Data Point:	KCA 8.2.6.1/07
Report Author:	
Report Year:	2007
Report Title:	Desmodesmus subspicatus growth inhibition test with spice amine - desemble 2
Report No:	EBKWX080
Document No:	<u>M-288232-01-1</u>
Guideline(s) followed in	OECD Guideline 201: Freshwater Alga and Cyanobacteria, Growth Inhibition
study:	Test (March 23, 2006)
Deviations from current	Yes S S S
test guideline:	OECD 201 (2011)
	The inoculum was approximately 1 x 104 cols/mL more than the recommended
	$2-5 \times 103 \text{ cells/mL}$
Previous evaluation:	yes, evaluated and accepted
	RAR (2010), RAR (2017) 0 2 2 2
GLP/Officially	Yes, conducted under GLFOfficially recognised esting facilities
recognised testing	
facilities:	
Acceptability/Reliability:	Yes a the the the second secon
Executive Summary	
	and the second

Executive Summary

In a 72-hour toxicity study, cultures of *Desmodesmus subspicatus* were exposed to spirotamine-desethyl at nominal test concentrations of 0,0763, 0,244, 0,781, 2.50 and 8.00 mg/L under statio conditions. Ò

at nominal test concentrations of 00703, 0277, 0701, 200 and 0.737 mg/L respectively. The percent inhibition of average specific growth rate in the treated algor cultures compared to the controls ranged from 26.2 to 78.6% after 72 hours exposure. I. Materials of the treated algor cultures of the controls ranged A. Materials of the treated algor the treated algor the controls ranged

Tort Motoria	
Test Materia	Spirovamine-Hesetlight
Lot/Batch #:) 92 1403 ELB 02 5 0 0 5
Purity:	Spiro@mine_flesethyl 92 103ELB02
Description:	Clear brown oily fiquid
Stability of test	Not reported
Reanalysis/Expiry	07 December 2009
dateo 🔪 🔬	07 December 2009
Density:	A Not poported
Treatments	F E A S
Test rates:	0.0765, 0.244, 0.781, 2.50 and 8.00 mg/L
Solvent vehicle:	None $\sqrt[6]{}$ N
Analysis of test	$\sqrt{2}$ we s, measured concentrations 88 – 103% of nominal (mean 96.6%)
concentrations;	$\sqrt[3]{}$ on day 0 and 80 – 104% of nominal (mean 90.0%) on day 3
Test organisms	
Species:	Desmodesmus subspicatus
Source:	Collection of Algal Cultures, University of Göttingen, 37077 Göttingen, Germany



8	_
Test vessel:	300-mL Erlenmeyer flasks containing 150 mL test medium
Test medium:	Prepared according to OECD 201 (2006)
Replication:	Three per test vessel, six per control
Initial cell density:	10,000 cells/mL
Duration of test:	72 hours
Environmental test conditions	
Temperature:	21.6 – 22.1°C
рН:	7.9-8.2 & g° 5° 4° 4° 4° 4° 4°
Photoperiod:	Under continuous illimination at $64\% - 78\%$ lux $\%$ $\%$ $\%$
B. Study Design	

This study was conducted in order to assess the effects of exposure to spiroxamine-deserved on the green alga *Desmodesmus subspicatus* in a static test over 72 hours.

Test vessels were 300-mL Erlenmeyer flasks containing 150 mL test medium. These were placed on a tablet rotating at 100 rpm to prevent sedimentation of the cells while preventing further aeration, and were sealed with cellulose plugs. The test media were prepared to the DECD 201 guideline, sterilised by membrane filtration and aerated with sterile air. Media were then inoculated with approximately 10,000 cells/mL, from an exponentially-growing preculture prepared four days before the start of the test and cultivated under equivalent conditions.

Nominal concentrations were 0.0763, 0.244, 0.781, 2.56 and 800 mg/L, along with a control and solvent control, with three replicates per test concentration and six replicates per control. Mean measured concentrations ranged from 88 to 103% of pominakat test start and from 80 to 104% at test end, therefore results are based on pominal concentrations.

Morphological examination of cells were made over the exposure period on each study day by a microscope. Cell numbers per volume were estimated photometrically as a surrogate for biomass per volume.

Temperature was determined by one continuous measurement of an additional glass vessel filled with an equivalent amount of de-ionised water as in the test vessels. The pH was measured daily in all test levels and the control. Samples were analysed for spiroxamine-desethyl concentration at test start and end.

Analytical method

Samples of water were analysed using the validated analytical method 01046, report reference \underline{M} -<u>287479-01-1</u> (see Doc MCA Section 4).

II. Results and Discussion

Validity créteria according to the OECD 201 guideline were met.

- Biomass of control cultures to have increased exponentially by a factor of at least 16 (actual: increased by a factor of 35)
- Mean coefficient of variation for section-by-section specific growth rates in the control cultures to not exceed 35% (actual: 32.8%)
- The coefficient of variation of average specific growth rates over the whole test period between the replicate control cultures should not exceed 7% (actual: 1.0%)



The pH of the control medium to not increase by more than 1.5 units during the test (actual: control pH ranged from 7.9 to 8.2)

The mean recoveries of treated, cell-free test vessels at test start ranged from 88 to 103% of noninal with an overall mean recovery of 96.6%. At test end, mean recoveries ranged from 80 to 94% of nominal, with an overall mean recovery of 90.0%. The results of the study have therefore been presented in terms of nominal test concentrations. elsat day 0 5

Nominal concentration	Mean measured concentration & of nominal 7
(mg/L)	(mg/L) (mg/L) (mg/L) (mg/L) (mg/L)
Control	<0.00508
Solvent control	
0.0763	0.0739
0.244	0.233
0.781	
2.50	
8.00	8.219
-	Mean: & & & 96 & Ø & O

Table CA 8.2.6.1/07-1	Nominal and measured	concentrations in	treated, cell-free	vesselsard
			(a l	e,

Nominal concentration		% of nominal &
(mg/L)	(mg/L)	
Control	×0.00508 ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	
Solvent control		y- ~~ ~~
0.0763	0.0647	85 6
0.244		Strain Strain
0.781	9.62Z ~ ~ ~ ~ ~	80 ~~
2.50	2.409 ~ 2.409	96 <i>Q</i>
8.00	8/294 × 4	104
-	Mean: C C C	20.0

Table CA 8.2.6.1/07-2 Nominal and measured concentrations in treated Cell-free vessels at day 3

The following table details the effects afexposure on biomass (cell ensity):

		•
Nominal	Sumber of cells (x10 ⁴ /mL) ¹ by time⊕ SD	
concentration	$24 h^{4}$	72 h
(mg/L)		
Control 🔊	2.4 ± 0.60 3.7 ± 0.01	35.2 ± 4.32
Solvent control	2.3 ± 0.60 3 4 ± 209	29.2 ± 0.94
0.0763	2.3 ± 0.10 0 7.6 ± 0.41	12.1 ± 0.65
0.244	1,0,≠ 0.21,	8.2 ± 0.20
0.781	1×8 ± 0.69 ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~	5.5 ± 0.55
2.50	1.6 ± 0.44 0^{-7} 3.3 ± 0.53	3.7 ± 0.37
8.00	0.3 ± 0.00 0.2 ± 0.26	2.1 ± 0.21

Table CX 8.2.6.1/07-3 Cell density during exposure to spiroxamine-desethyl

Mean of hree replicates six replicates for the control) \pm standard deviation

Statistically significant inhibition of the 0-48 hour and 0-72 hour growth rates to the solvent control could be observed at all test concentrations.

Table CA 8,26.1/07 Growth rates and inhibition of treated cultures

Nomina	0 - 24 h		0 - 48 h		0 - 72 h	
concentration	Growth	%	Growth	%	Growth	%
(mg/L)	rate	inhibition	rate	inhibition	rate	inhibition
Solvent control	0.802	-	1.119	-	1.125	-



Nominal	0 - 24 h		0 - 48 h		0 - 72 h	
concentration (mg/L)	Growth rate	% inhibition	Growth rate	% inhibition	Growth rate	% inhibitien
0.0763	0.836	-4.3	1.013*	9.4	0.830*	
0.244	0.664	17.1	0.930*	16.8	0.703*	37.5
0.781	0.605	24.6	0.740*	33.9	0,566*	49.9
2.50	0.441	45.0	0.599*	46.4	₄ 0.431*	61 .7
8.00	-1.217	251.8	0.078*	93.0	0.241*	» P78.6 Q x

Statistically significantly different to the control (William Multiple Sequential t-test, $\alpha = 0.05$, or $\alpha = 0.05$, $\alpha = 0.0$ Condesethyl 2 9 2 0 1 smaller)

A summary of the results is presented in the table below

Table CA 8.2.6.1/07-5	Summary of results after	72 hour exposure t	o spiroxamine-desethyl

Nominal concentration (mg/L)	Cell number per mL after 72 h	0-724 average specific growth average specific (days) sate per day growth rate (%)
Solvent control	292,000	1.125 V V D A 0 0 0.616
0.0763	121,000	0.830 26.20 26.20
0.244	82,000	0,003 ~ ~ 37,5 ~ ~ 0.986
0.781	55,000	05566 × × 249.7 × 2× 122 v
2.50	37,000 🖓 🤅	0.4310 0 61.7 0 0 01.61 %
8.00	21,000 🖉 🞺	0.249 2.8%

A summary of the relevant endpoints is presented in the table below:

Table CA 8.2.6.1/07-6 Summary of derived endpoints

		Ô Ó			, O`	
Growth rate	L. V.			O' ×		
E _r C ₅₀ (95% CI):	0.737 mg/L	£2.54340.0.997	mg a.s. (D) (
E _r C ₂₀ (95% CI):	گُ [®] 0,0 0 29 mg/1	2 (0.0196 to 0.9	742 mg a.s./L		Ŷ	
E _r C ₁₀ (95% CI).	🤇 Not determi	nable 🔿			7	
LOE _r C:	€_0.07 6 9 mg		ê ê	2 Qu		
NOE _r C:	^{#0} <0.0763 mg			~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~		
00 V	<u> </u>		r ()	. "0"		

III, Conclusion

In a 72-hour toxicity study sultures of Desnodesnus subspications were exposed to spiroxamine-desethyl at nominal test concentrations of 0.0763, 0.244, 0.781, 2.50 and 8.00 mg/L under static conditions.

The growth rate NOEC and ErCso values were 0.076 and 0.737 mg/L, respectively. The percent inhibition of average specific growth rate in the freated algal cultures compared to the controls ranged from 26.2 th 78.6% after 72 hours expositive.

Assessment and conclusion by applicant

The study was conducted to the DECDQtest guideline 201 (2006), the current version of which is the OECD 201 "Freshwater alga and cyanobacteria, growth inhibition test", adopted 28 July 2011.

Validity criteria according to the OECD 201 guideline (2011) are the same as those in the version in force at the time this study was conducted.

- Biomass of control cotures to have increased exponentially by a factor of at least 16 (actual: increased by a factor of 35)
- Mean coefficient of variation for section-by-section specific growth rates in the control cultures to not exceed 35% (actual: 32.8%)
- The coefficient of variation of average specific growth rates over the whole test period between the replicate control cultures should not exceed 7% (actual: 1.0%)



The pH of the control medium to not increase by more than 1.5 units during the test (actual: control pH ranged from 7.9 to 8.2) Q_{μ}^{*}

It is noted that the study report assessed the validity criteria of the solvent control data only therefore the validity criteria for the control data have also been assessed below.

- Biomass of control cultures to have increased exponentially by a factor of at least 16 (actual increased by a factor of 35)
- Mean coefficient of variation for section-by-section specific prowth rates in the cont cultures to not exceed 35% (actual: 30.4%)
- The coefficient of variation of average specific growth rates over the whole test between the replicate control cultures should not exceed 75 (actual: 3.58%)

The validity criteria have been met therefore the study is considered to be acceptable

The E_rC_{50} value was determined to be 0.737 ftpg/L.

The data have been subjected to statistical re-evaluation and the results have been presented in the following study summary.

Data Point:	KCA 8.2. 6714 5 5 5 6 5 5 6
Report Author:	KCA 8.2.60714 5 5 5 6 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5
Report Year:	
Report Title:	2020 Calculation of EC10, EC20 and EQ50 values for Desmodesmus subspicatus with
	KWG 4168- desethyl in an algal growth inhibition test
Report No:	A471836 ECO32 Q Q Q
Document No:	<u>M-761465-019</u>
Guideline(s) followed in	$\begin{array}{cccccccccccccccccccccccccccccccccccc$
study:	Notes of the second sec
Deviations from current	None β
test guideline:	
Previous evaluation:	No, not previously submitted
GLP/Official	No, not conducted under GLP/QPicially recognised testing facilities
recognised resting	
facilities:	
Acceptability/Reliability:	Y_{0}
Executive Summa	

Executive Summary

The report <u>M-288232-6121</u> on the effects of exposure to KWG 4168-desethyl on the growth of algae (*DesmodesmuGsubspicatus*) and not provide estimates of EC_{10} , EC_{20} or EC_{50} values for yield. Therefore, these values have been capsulated in accordance with the Annex to Com. Reg. 283/2013.

The resulting EC₅₀ value was 30.59 μ ga.s./L EC₁₀ and EC₂₀ values could not be calculated due to values being beyond the tested concentrations.

1. Methods

The statistical evaluation was performed with statistical software ToxRatPro v3.3.0.

Effect concentrations with 10, 20 and 5% effect from the test item treatment when compared to the control wore determined for yield after 72 hours exposure. A linear Probit regression was performed, with confidence limits for the EC_x estimated according to Fieller's theorem

IL Results and Discussion

Yield at 22 hours

Regarding the calculation of the EC₅₀ value for yield at 72 d, since p(F) = <0.001, there is no significant lack of fit.

The resulting EC₅₀ value and confidence intervals are represented in the following table below.



Table CA 8.2.6.1/14-1 Results of the Probit analysis with yield at 72 h: Selected effective concentrations (ECx) of the test item and their 95%-confidence limits

		Yield [µg a.s./L]	
Parameter	EC10 (95 % confidence	EC ₂₀ (95 % confidence	EC50 (95 % confidence)
	interval)	interval)	interval)
Effect on yield at 72	n.d.	n.d.	30,59 5
h		× ×	(24.94 36.54)
n d · not determined since	e value is beyond the tested o	oncentrations	

n.d.: not determined since value is beyond the tested concentrations

The resulting EC₅₀ value of 30.59 (95%CL: 24.94 – 36,54) μ g a.s./L meets the goodness of fit criteria \bigcirc showing a significant concentration/response relationship, and therefore is considered reliable. EC₁₀ and EC₂₀ values could not be calculated.

III. Conclusion

The resulting EC₅₀ value for yield at 72-hours overe determined to be 30.50 µg as L.

Assessment and conclusion by applicant

EC₁₀, EC₂₀ and EC₅₀ values for growth rate were calculated in the study report therefore only yield endpoints have been determined in this statistical report.

The statistical re-evaluation of the data has determined a reliable EC_{10} value for yield. Rehable EC_{10} and EC_{20} values for yield could not be calculated.

The E_rC_{50} determined in the original study report of 737 µg a.s. (F shall remain as the critical endpoint determined from this study.

ſ

(1)

The values determined in the re-evaluation work are considered to be fully value

KWG 4168-desprop

$\frac{3^{2}}{2} O^{2} \frac{3^{2}}{2} \frac{3^{2}}{2$
Data Point: CAX8.2.6(1/15 X 4/ X 4
Report Year $1 2020_{\text{H}}$ $2 220_{\text{H}}$ $2 220_$
Report Title: 6 KWG4168 despropyl: Toxigity to Pseudokirchnella subcapitata in an algal
growth inhibition test
Report No: $\sqrt{3}/43071270$ $\sqrt{3}/57$
Document No: $M - 6 2 695 - 04 2 3$
Guideline(s) followed in Regulation 107/2009 (Europe)
study: , a log by log by so a log by so
Deviations from current None concerned and c
est guidefine:
Previous evaluation: No, aot previously Submitted
GLR/Officially Yes, conducted under GLP/Officially recognised testing facilities
recognised testing
acilities:
Acceptabilit, Reliability: Ses 🖉
Acceptability Reliability:
xecutive Summary

The surpose of this jest was to determine the inhibitory effect of the test item KWG 4168-despropyl on the growth of the freshwater green algae *Pseudokirchneriella subcapitata*.

In a 72-hour algae inhibition test, triplicate cultures of *Pseudokirchneriella subcapitata* were exposed to KWG 4168-despropyl at nominal test concentrations of 0.038, 0.122, 0.391, 1.25 and 4.00 mg/L under static conditions.



The 72-hour E_yC_{50} was calculated to be 0.0425 mg pure metabolite/L and the E_rC_{50} 0.383 mg pure metabolite/L. The 72-hour E_yC_{10} could not be determined and the E_rC_{10} was determined to be 0.0203 mg pure metabolite/L. The 72-hour NOE_yC was determined to be <0.0293 mg pure metabolite/L and the associated 72-hour LOE_yC of 0.0293 mg pure metabolite/L. The 72-hour NOE_rC was determined to be <0.0293 mg pure metabolite/L and the associated 72-hour LOE_yC of 0.0293 mg pure metabolite/L.

Ante/Lo, Ant **Materials and Methods** I. A. Materials **Test Material** KWG 4168-despropyl Lot/Batch #: AE 1344303-PU-01 **Purity:** 99.1% w/w **Description:** Clear colourless oilŷ piration date Stability of test Sufficient based On compound: **Expiry date:** 13 May 20 **Density:** Not report Treatments and 4.00 mg test frem/k **Test rates:** No@ainal: @2038 Initial mean measured: 0.0293, 0.0992 0,293, 0.962 and 9.10 mg pure metabolite/1 Solvent/vehicle None entrations 75 \neq 80.4% of nominal nitial mean mea Analysis of te concentratio Test organism eudokirchneriellasubcapilata, Strain, No. 61.81 SAG formerly Species known as Selenastrum capricornutum and recently renamed as Raphidocelis subcapitata (KORSHIKOV). Cultivated in the laboratories of ibacon; original source: "Sammlung Source: von Algenkulturen Albrecht-von-Haller-Institut für Pflanzenwissenchaften / niversität Göttingen, 37073 Göttingen, Germany. Test design of 50 mL volume with approximately 50 mL of test Test vessel: Erlenmevé medium covered with air permeable glass dishes, stoppers or caps. D medium **∦**¶est medium% Ś Three replicates per test concentration and six replicates in the control **Replication** Initial cell density 3000 čells/mI 72 bours Duration of test: Environmental test conditions Temperature: 22.4 to 23.3°C pH: 8.1 to 9.2 **Photoperiod:** Continuous illumination at 4470 to 5070 lux



B. Study Design

The purpose of this test was to determine the inhibitory effect of the test item KWG 4168-desprop. I on the growth of the freshwater green algae Pseudokirchneriella subcapitata.

A stock solution of 10 mg test item/L was prepared by dissolving 20.2 mg test from into 2020 mL test water by intense stirring for 24 hours. Adequate volumes of this stock solution were diluted with test water to prepare the test media of the desired nominal test concentrations; 02038, 0.122, 0.391, 1223 and 4.00 mg test item/L. The test media were prepared just before introduction of the algae = start of the test).

Pseudokirchneriella subcapitata, strain no. 61.81 SAG formerly known as Selenastwum capricorfutum and recently renamed as Raphidocelis subcapitata. The algae were cultivated in the laboratories of ibacon under standardised conditions according to the test guidelines. The test was storted (hours by inoculation of a biomass of nominal 5000 algal cells per mL test medium. These cells were taken from an exponentially growing pre-culture, which was set up 4 days prior to the test start under the same conditions as in the test.

The test was performed with three replicates per test concentration and six replicates in the control Test units were 50 mL Erlenmeyer flasks with approximately 50 mL volume of test medium covered with a permeable glass dishes, stoppers or caps.

The cell density on each observation time was determined by spectrophotometric measurement. Therefore, defined volumes of the algal suspensions from all repricated and from the blanks were sampled after 24, 48 and 72 hours of exposure, and were not replaced. The algal cell densities were calculated by subtracting the absorption of the blanks, from each of the measured absorption of the test media (with algae).

Based on the counted cell densities and the absorption from an algar suspension and its dilutions, a linear regression was performed for the calculation of the cell densities of the replicates during the test.

Incubation was at \$2.4 to 23.3°C and under continuous illumination at 4476 to 5070 lux. The pH of the test and control media anged from \$\$ to 943

Analytical method

Ś Samples of water were analysed using the validated analytical method 01046 adapted, report reference M-680693-01-2 (see Doc MCA Section 4). SY

II. **Results and Discussion**

Validity criteria according to the OECD 201 guideline (2011) were met:

- Cell density of control cultures to increase by a least 16x (actual: 178.7x)
- Mean coefficient of variation for section-by section specific growth rates in control cultures to be ≤35% (actual: 18.3%) ∧
- Coefficient of variation of average specific growth rates in control cultures over the test period to be $\leq 7\%$ (actual: 2.0%)

At the start of the test, 78% of the nominal test concentrations were found (average of all test concentrations). After 72 bours test duration, 76% of the nominal value was determined (average of all test concentrations). During the test, the algae were exposed to a mean of 77% of nominal. Initial mean measured concentrations ranged from 75.4 to 80.4% of nominal.



Nominal c	oncentrations	Fresh (0 h	iours) ¹	Aged (72 h	ours) ¹	Initial mean concentration	n measured O
[mg test item/L]	[mg pure metabolite/L]	% of nominal	RSD [%]	% of nominal	RSD [%]	[mg_test item L]	[mg puốye metabolite/Iĝ
Control	0	-	-	-	-	- 🖓	- ~~~
0.038	0.0377	78	0	69	2	<u>_</u> 0.0296	Ø293 S
0.122	0.121	80	1	77	0 🕺	≠0.0981 <u>`</u>	0.097 <u>2</u> *
0.391	0.387	76	4	78	1 67	0.295	0.223
1.25	1.24	78	2	78	0 2	0.971 🖉	0,962 🖉 🔗
4.00	3.96	78	1	78	0 2	3.13	\$210 O \$
¹ Mean valu	e of all measured	samples per	treatment g		Q' b	° & 4	

Table CA 8.2.6.1/15-1 Summary of analytical results

²Tabulated results represented results rounded to three Significant digits Ċ

RSD: relative standard deviation per treatment group, number of analysed samples 2 per group

Table CA 8.2.6.1/15-2 Mean algal cell densities during the test period of 72 bours

Initial measured	
concentration [mg pure	24 hours 72 hours 77 hours
metabolite/L]	
Control	2.7480 \$ \$ 215408 0 5 \$ \$89.338
0.0293	2.788 17.398 55.502 4
0.0972	2.947 2 11.520 22918
0.293	Q.470 0 6.20 Q 6044 V
0.962 🐇	1.835 3.820 2.3.820 3.344
3.10	0.644 5 2.232 5 1.498
A 5	
Table CA 8.2.6.1/15-3 Mean al	gal yield during the test period of 72 hours

Table CA 8.2.6.1/15-3 Mean algar yield during the test period of 7 hours

Initial measured	Mean yield (ells/ml and	inhibition a	ufter O	\$	
	24 hours 🔊		48 hours of		72 hours	
pure metabolite(L]	Xield 🔗	‰ inhib. У́	Yteld 📎	%nhib.	Yield	% inhib.
Control 🔊 ,	2.248 0	Θ $\langle \langle O \rangle$	20.908		88.838	-
0.0293	2.288 è	-1.8	16.898	9.2*	55.012	38.1*
0.0972	2,4497 🔊	-8,8-	11.023	47,30	22.218	75.0*
0.293	} 970 ₹	12.4*	5,502	.707*	5.544	93.8*
0.962 **	∭.335°∕∕ ×	A0.6*	≫.320 ₆	84 .1*	2.844	96.8*
3.10	0.144	93.6*	1.73	91.7*	0.938	98.9*

*Mean value significantly different from the control Õ

Table CA 8.2.01/15-4 Growth rate during the test period of 72 hours

Initial measured	Mean growt	Mean growth rates per day				
concentration [mg	🔊 - 24 hours		🖉 48 hours		0 - 72 hours	
pure metabolite/L]~	Growth	% inhib. 🔬	Growth	% inhib.	Growth	% inhib.
	rate of	~~~ _O*	rate		rate	
Control	1.700		1.874	-	1.727	-
0.0293	1.71	-1.0	1.775	5.3*	1.570	9.1*
0.0972	1972	-4.2	1.568	16.3*	1.271	26.4*
0.0972 0 0 0.293 0 0 0.295 0 0 0.295 0 0 0.295 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	A.598	6.0	1.259	32.8*	0.831	51.9*
04962	1.298	23.6*	1.015	45.8*	0.631	63.5*
3.10 0	0.239	85.9*	0.746	60.2*	0.351	79.7*

*Mean value significantly different from the control



The determined endpoints have been summarised in the table below:

Table CA 8.2.0.1/13-5 EI	rupoints deter inned in the 72-nour test	<u> </u>
Parameter	Yield	Growth rate
	[mg pure metabolite/L]	[mg pure metabolite/L]
72 hour EC ₅₀	0.0425(0.0400 - 0.0451)	0.383(0.923 - 0.456)
72 hour EC_{20}	0.0148* (0.0131* - 0.0165*)	0.05572(0.0402 - 0.0725)
72 hour EC_{10}	n.d.	0.0203* (0.0129* - 0.0291*)
72 hour NOEC	< 0.0293	
72 hour LOEC	0.0293	

Table CA 8.2.6.1/15-5 Endpoints determined in the 72-hour test

95 % confidence intervals reported in parentheses

* Values are extrapolated

III. Conclusion

The influence of KWG 4168-despropyl on the growth of the treshwater grown algo Pseudokirchneriella subcapitata was assessed in a static concentration response test subcapitata was assessed in a static concentration response test.

The 72-hour E_yC_{50} was calculated to be 0.042.5 mg pure metabolite/K and the 72-hour E_xC_{50} value was calculated to be 0.383 mg pure metabolite/I& The 72-hour NOE C was determined to be <0.0293 mg pure metabolite/L and the associated 72-hour LOE C was 0.0293 mg pure metabolite/L. The 72-hour NOErC was determined to be <0.6293 mg pure metabolite/L and the associated 72 hour EOErC was 0.0293 mg pure metabolite/L

Assessment and conclusion by applicants

This is a new study that has not been previously submitted or evaluated

Validity criteria according to the OECD 200 guideline (2011) were met:

- Cell densite of control caltures to increase by at least 16x (actual: 178.7x)
- Mean coefficient of variation for section-by-section specific growth rates in control cultures
- Coefficient of variation of average specific growth dates in control cultures over the test

moralue was determined to be 0.383 mg pure metabolite/L.

the tribule to be the tribule

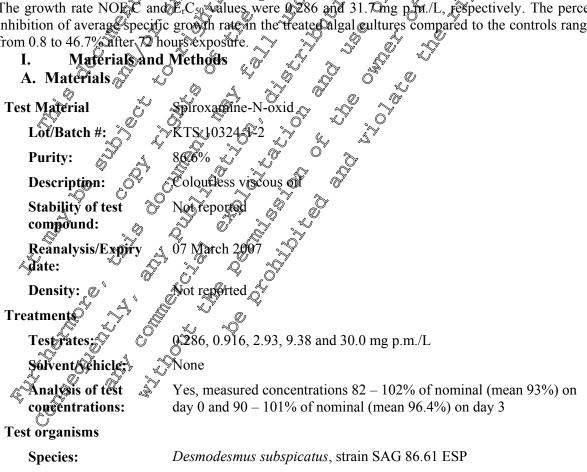


KWG 4168-N-oxide (M03)

Data Point:	KCA 8.2.6.1/08
Report Author:	
Report Year:	
Report Title:	Desmodesmus subspicatus growth inhibition test with spiroxamine - N - oxid
Report No:	EBKWX081
Document No:	<u>M-288235-01-1</u>
Guideline(s) followed in	OECD Guideline 201: Freshwater Alga and Cyatobacteria, Growth Inhibition 2
study:	Test (March 23, 2006)
Deviations from current	Yes of the of the
test guideline:	OECD 201 (2011)
	The inoculum was approximately 1 x 104 cells/1002, more than the recommended
	Yes OECD 201 (2011) The inoculum was approximately 1 x 104 cells/nd, more than the recommended 2-5 x103 cells/mL The concentration series did not cover the proterable range of growth inhibition
	I ne concentration serves did not cover the preserve range or growth inhibition
	of 5 - 75%
Previous evaluation:	yes, evaluated and accepted
	RAR (2010), RAR (2017)
GLP/Officially	Yes, conducted under GLP Officially recognised testing facilities and the second secon
recognised testing	Yes, conducted under GLP/Officially recognised testing fa@ities &
facilities:	
Acceptability/Reliability:	Yes y g g g g g g g g g g g g g g g g g g
Executive Summary	

In a 72-hour toxicity study oulture of Desmodesmus subspicatus were exposed to spiroxamine-N-oxid at nominal test concentrations of 0.286, 0.916 2.93, 38 and 30.0 mg p.m. L under static conditions.

The growth rate NOE and E_rC_{50} values, were 0.286 and 31.70 mg p m./L, respectively. The percent inhibition of average specific growth rate in the treated algal cultures compared to the controls ranged from 0.8 to 46.7% after 72 hours exposure





Source:	Collection of Algal Cultures, University of Göttingen, 37077
	Göttingen, Germany
Test design	Göttingen, Germany
Test vessel:	300-mL Erlenmeyer flasks containing 150 mL test medium
Test medium:	300-mL Erlenmeyer flasks containing 150 mL test medium Prepared according to OECD 201 (2006) Three per test vessel, six per control
Replication:	Three per test vessel, six per control
Initial cell density:	10,000 cells/mL
Duration of test:	72 hours
Environmental test conditions	10,000 cells/mL 72 hours $21.5 - 22.2^{\circ}C$
Temperature:	21.5 – 22.2°C O C C C C C C C C C C C C C C C C C C
pH:	8.0 - 8.2 4 7 7 7 4 6 4 6
Photoperiod:	Under continuous illumination at 6130 – 7850 lux 5
B. Study Design	

This study was conducted in order to assess the effects of exposure to spiroxamine-N-oxid on the green alga *Desmodesmus subspicatus* in a static test over 72 hours.

Test vessels were 300-mL Erlenneyer flasks containing 150 mL test medium. These were placed on a tablet rotating at 100 rpm to prevent sedimentation of the cells while preventing further aeration, and were sealed with cellulose plugs. The test media were prepared to the OECD 201 guideline, sterilised by membrane filtration and aerated with sterile air. Media were then inoculated with approximately 10,000 cells/mL, from an exponentially growing pre-oulture prepared four days before the start of the test and cultivated under equivalent conditions.

Nominal concentrations were 0.286, 0.916, 2.93, 9.38 and 30.00ng put metabolite (p.m.)/L, along with a control and solvent control, with three replicates per test concentration and six replicates per control. Mean measured concentrations ranges from 82 to 102% of nominal at test start and from 90 to 101% at test end, therefore results are based on nominal concentrations.

Morphological examination of cells were made over the exposure period on each study day by a microscope. Cell numbers per volume were estimated photometrically as a surrogate for biomass per volume.

Temperature was determined by one continuous measurement of an additional glass vessel filled with an equivalent amount of de-ionised water as in the test vessels. The pH was measured daily in all test levels and the control. Samples were analysed for spiroxamine-N-oxid concentration at test start and end

Analytical method

Samples of water were analysed using the validated analytical method 01046, report reference \underline{M} -287479-010 (see Poc MOA Section 4)

II. Results and Discussion

Validity criteria according to the OECD 201 guideline (2011) were met.

- Bromass of control cultures to have increased exponentially by a factor of at least 16 (actual: Increased by a factor of 25 and 23 in the control and solvent control, respectively)
- Mean coefficient of variation for section-by-section specific growth rates in the control cultures to not exceed 35% (actual: 34.8%)



- The coefficient of variation of average specific growth rates over the whole test period between • the replicate control cultures should not exceed 7% (actual: 3.1%)
- The pH of the control medium to not increase by more than 1.5 units during the test (actual: • control pH ranged from 8.0 to 8.2)

The mean recoveries of treated, cell-free test vessels at test start ranged from \$2 to 102% of nonpiral, with an overall mean recovery of 93.0%. At test end, mean recoveries ranged from 95 to 100% of nominal, with an overall mean recovery of 96.4%. The results of the study have therefore been based and nominal test concentrations. N.

Nominal concentration (mg p.m./L)	(mg p.m./L) \sim \mathcal{O}' \mathcal{O}' \mathcal{O}' \mathcal{O}'
Control	<0.0051800 [°] 2
Solvent control	<0.005t80 ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
0.286	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$
0.916	0755 4 . T . T . T . T . T . T . T . T . T .
2.93	\$2.982 & & S DI02 & & ~
9.38	
30.0	
-	<u> </u>

	Nominal and measured		~~···	. W. O.
Table CA 8 2 6 1/08_1	Nominal and measured	concentertions in	treated cell_	ree vessels at hav 0
1 abit CA 0.2.0.1/00-1	Trommai and measured	concenter actions in	u causu, ccm-	u = u = v = u = u = v = u

Table CA 8.2.6.1/08-2 Nominal and measured concentrations in treated cell-free vessels at day 3

Nominal concentration O	Mean measured concentration	% of nominal
(mg p.m./L)	$(\operatorname{ng} p.m_{A}\mathbb{Z})$	\mathcal{A}_{λ}
Control & Q	Q0.005480 2 2	(T)
Solvent control) <0.0 0 5180 ~ ~ ~ ~	9-
0.286	0.203 ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	95
0.916		90
2.93	2.853 0 × × ×	97
9.38	9.510° °	101
30.0	29766 2 27	99
- Q Q	Mean: O . O O	96.4

The following table details the effects of exposure on fromass (cell density):

Table CA 8.2.6.1/08-3 Cell den	nsity during exposure to spiroxamine-N-oxid
--------------------------------	---

Nominal	Number of cells (x1040nL)	¹ by time ± SD	
concentration		() ⁴ 8 h	72 h
(mg p.m./L) 🦼		2	
Control 🔘	27±0.2	9.7 ± 0.77	24.5 ± 1.54
Solvent control	2.3 ± 0.64 $\sqrt{2}$	9.5 ± 0.69	23.3 ± 2.69
Pooled controls	2.3 ± 0.47	9.6 ± 0.71	23.9 ± 2.19
0.286	2.2 ± 0.10	9.7 ± 0.30	23.2 ± 0.32
0.916/	22 ± 0.42	9.9 ± 0.30	17.0 ± 1.19
2. 4 3	1.9 ± 427	7.9 ± 0.34	10.4 ± 0.64
9.38	2.1 ± 0.10	7.5 ± 0.56	7.7 ± 0.19
30.0 0	2.1 ± 0.27	5.7 ± 0.00	5.4 ± 0.11

Mean of three replicates (six replicates for the control) \pm standard deviation



Statistically significant inhibition of growth rates to the pooled controls could be observed at test concentrations 2.93, 9.38 and 30.0 mg p.m./L after 48 hours exposure, and at test concentrations 0.916, 2.93, 9.38 and 30.0 mg p.m./L after 72 hours exposure.

					Š	
Nominal	0 - 24 h		0 - 48 h		0 [©] 72 h	
concentration	Growth	%	Growth	%	Growth	6% <u>8</u> % (2
(mg p.m./L)	rate	inhibition	rate 🔊	inhibition	rate	inhibition
Pooled controls	0.813	-	1.129	- 0	1.057	
0.286	0.809	0.5	1.135	-0.5	1.049 🥒	
0.916	0.800	1.6	1.145	-1.4	0.943	10.8 O V
2.93	0.630	22.5	1.030%	8.8 🖓 🙆	0.779*	26.3
9.38	0.755	7.2	1-009*	107 .	0.679*	3528
30.0	0.721	11.3	¢0.873*@°	\$2.7 5	9 .563*°	¥46.7 V

Statistically significantly different to the coorol (Walliams Multiple Sequential t-test, smaller)

A summary of the results is presented in the table

Table CA 8.2.6.1/08-5	C	- f	- (n × 7)	10×		. <u>.</u>	Ô		
1 able CA 8.2.0.1/08-5	Summary	or results	aner /2	-nour	exposur	engo spi	roşamı	ne≂rv-o:	X MC

	&			
Nominal	Cell number ger	0-72 h average	Inhibition of	Doubling time
concentration	mL after 72 h 🎣	spectfic growth	Paverage specific	(days)
(mg p.m./L)		rate per day 🗸	growth rate (%)	
Pooled controls	239,000 🐇	£057 ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	~ ~ Q	0.656
0.286	232,000 0	P1.049	0.8	0.661
0.916	170,000	0.948	10,8 🗸 🐒	0.735
2.93	104,000	0.979 0 %	26,3 &	0.890
9.38	77,000	Ø.679 S	35.8 0 4	1.02
30.0	54, 000	0.563	46.7 2	1.23

A summary of the derived endpoints is presented in the table below:

Table CA 82,6.1/08-6 Summary of derived endpoints ô

		<u></u>		<u>0</u> _ 0	
Growth cate					
$E_r C_{50} (95\% \text{ CI})$: 31.7 mg p.m.	L (15.3 🕼 169	mg p.m./L)	\sim	
ErC ₂₀ (95% CI)	2.50 mg p.mQ	Ľ (0:541 to 53	03 mg n./I		
$E_r C_{10} (95\% C)$	0.458 mg m	./L.10.041 to/1	.81 mg p.m.	D)	
LQErC	: 19916 mg p.m	./£	Ô Â		
-XODErC	: 0.286 mg p.m	M. ~~			
		<u> </u>			

III. Conclusion III. Conclusio at nominal test concentrations of 0.286, 0.916, 2.93, 9.38 and 30.0 mg p.m./L under static conditions.

The growth rate NOErC and ErC₅₀ values were 0.286 and 31.7 mg p.m./L, respectively. The percent inhibition of average specific growth rate in the treated algal cultures compared to the controls ranged from 0.8 to 45.7% after 72 hours exposure.

Assessment and conclusion by applicant:

The study was conducted to the OECD test guideline 201 (2006), the current version of which is the OFCD 200 "Freshwater alga and cyanobacteria, growth inhibition test", adopted 28 July 2011.

Validit Criteria according to the OECD 201 guideline (2011) are the same as those in the guideline in force at the time this study was conducted. It is noted that the report has assessed the validity criteria on the pooled control and solvent control data therefore the control data only has been reassessed against the validity criteria and the results presented below:



- Biomass of control cultures to have increased exponentially by a factor of at least 16 (actual; increased by a factor of 25)
- Mean coefficient of variation for section-by-section specific growth rates in the control cultures to not exceed 35% (actual: 33.3%)
- The coefficient of variation of average specific growth rates over the whole test period between the replicate control cultures should not exceed 7% (actual: 1.96%)

The validity criteria have all been met therefore the study is considered acceptable

The E_rC_{50} value was determined to be 31.7 mg p.m./L.

The data have been subjected to statistical re-evaluation and the sesults have been presented in the following study summary.

Data Point:	$KCA 8.2.0.1/10$ 0^{*} 0^{*} 0^{*} 0^{*}
Report Author:	
Report Year:	
Report Title:	Calculation of EC10, EC20 and EC50 Values for Desmodespurs subspicatus with
	KWG 4168 Soxidexin an algal growth inhibition test 🖉 🖉 🔍
Report No:	Calculation of 4C10, EC20 and EC50 Values for Desmodesmus subspicatus with KWG 4168 boxidean an algal growth inhibition test 0 5 0 0471836-EQ033 5 5 5 5 <u>M-761467-01-1</u>
Document No:	<u>M-761407-01-1</u>
Guideline(s) followed in	None is a a a a a a a
study:	
Deviations from current	None & by fy or by in a
test guideline:	
Previous evaluation:	No, not previously submitted
1.	
GLP/Officially	No, not conducted under GLP/Officially recognised testing facilities
recognised testing	
GLP/Officially recognised testing facilities:	

Executive Summary

The report M-288235-6-1 on the effects of exposure to KWG 4768-N-oxide on the growth of algae (*Desmodesmus subspicatus*) and not provide estimates of EC₁₀, EC₂₀ or EC₅₀ values. Therefore, these values have been calculated in accordance with the Appex to Com. Reg. 283/2013.

The resulting EC², EC² and E²₅₀ values for yield at 72 h were 217.92, 525.75 and 2834.80 μg p.m./L, respectively.

I. <u>A</u>Methods

The statistical evaluation was performed with statistical software ToxRatPro v3.3.0.

Effect concentrations with 10, 20 and 50% from the test item treatment when compared to the control were determined for yield after 72 hours exposure. A linear Probit regression was performed, with confidence limits for the EC values stimated according to Fieller's theorem.

II. Results and Discussion

Yield at 72 hours

Regarding the calculation of EC_{10} , EC_{20} and EC_{50} values for yield at 72 h, a statistically significant concentration/response was found (p(F) <0.001) for this parameter.

The resulting EC_{10} , EC_{20} and EC_{50} values and the respective confidence intervals are represented in the following table below.



Table CA 8.2.6.1/16-1 Results of the Probit analysis with yield at 72 h: Selected effective concentrations (ECx) of the test item and their 95%-confidence limits

		Yield [µg p.m./L]	
Parameter	EC10	EC20	EC50
	(95 % confidence	(95 % confidence	(95 % confidence
	interval)	interval)	interval)
Effect on yield at 72	217.92	525.75	283 80 (2136.28 3759 4) (2136.28 3759 4)
h	(99.94 – 368.02)	(299.54 – 780.08)	

The resulting EC_{10} , EC_{20} and EC_{50} values of 217.92 (95% CL: 99.94 – 368.02), 525.75 (95% CL: 299.54 – 780.08) and 2834.80 (95% CL: 2136.28 – 3759.14) µg p.m./L, respectively, meet the goodness of fit criteria showing a significant concentration/response relationship and therefore the estimated EC_{80} values are considered reliable.

III. Conclusion

The resulting EC₁₀, EC₂₀ and EC₅₀ value for yield at $\frac{12}{3}$ -hours were determined to be 217.92, 525.75 and 2834.80 µg p.m./L, respectively.

Assessment and conclusion by applicant:

 EC_{10} , EC_{20} and EC_{50} values for growth rate were calculated in the study report therefore only yield endpoints have been determined in this statistical report.

The statistical re-evaluation of the data has determined a reliable E_{10}° , E_{20}° and E_{50}° value for yield.

The E_rC_{50} determined in the original study report of 31,700 µg p.m/L shall remain as the critical endpoint determined from this study.

The values determined in the re-evaluation fork are considered to be fully values

	2		"O"	- 6
KWG 4168-carbo	xx	acid	(M0)	6À
	10 C	~	, ,	V,

Data Point: KCA'8.2.6 1/09
Report Author Star Contraction of the second s
Report Yeat 2008ζ 2008ζ 2008ζ 2008ζ
Report Title: Destrodes subspicatus prowth whibition test with spiroxamine - carbocylic
Report No: NO ABBKW6018 NO NO NO NO
Document No: $M-30618-041$
Guideline(s) followed in OECD Guideline 201: Freshwater Alga and Cyanobacteria, Growth Inhibition study:
Deviations from current Ves of a g
test guideline: OEGD 201 (2011) The inoculum was approximately 1 x10 ⁴ cells/mL, more than the
The inoculum was approximately 1×10^4 cells/mL, more than the recommended 2-5 $\times 0^3$ cells/mL
• The concentration series did not cover the preferable range of growth anhibition of 5 \$275%
Previous evaluation: Ses, evaluated and accepted
\$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$
GLP/Officially Veo conducted under GLP/Officially recognised testing facilities
recognised testing
facilities:
Acceptability/Reliability: Yes
\bigcirc ^{ν}



In a 72-hour toxicity study, cultures of Desmodesmus subspicatus were exposed to spiroxan@necarboxylic acid at nominal test concentrations of 9.54, 30.5, 97.7, 313, 1000 and 3200 µg p.m./L mider static conditions.

The growth rate NOErC and ErC50 values were 1000 and >3200 µg p.m./L, respectively. The persent inhibition of average specific growth rate in the treated algal cultures compared to the controls ranged from -2.8 to 7.1% after 72 hours exposure.

- I. **Materials and Methods**
- h the second sec A. Materials **Test Material** Spiroxamine-carbocytic acid Lot/Batch #: SES 10277-2-1 90.6% w/w **Purity: Description:** Light yellow Stability of test compound: y 2009 **Reanalysis/Expiry** 18 Janua date: **Density:** Treatments and 3200 µg p.m./l **Test rates:** O Solvent/vehicle - 163% of nominal (mean 100%) on Yes, measured concentrations 98 Analysis of test and 98 A110% of nominal (mean 103%) on day 3 concentrations: dåy Ø Test organisms pesmodesmus subspicatus, strain SNG 86.61 ESP Species: Source: ollection of Algal Cultures, University of Göttingen, 37077 Götningen, Germany Test design denmeyer flastes containing 150 mL test medium Test vessel: pare@according to ØECD 201 (2006) Test medium: **Replication:** , six per control Three ber te Initial cell density 000 cell **Duration** of IOU Environmental **Kes**i conditions emperature; 4 -22.2°C 7.9 - 8.1Photoperiod: Under continuous illumination at 7220 – 7580 lux



B. Study Design

This study was conducted in order to assess the effects of exposure to spiroxamine-carbocylic acie on the green alga *Desmodesmus subspicatus* in a static test over 72 hours.

Test vessels were 300-mL Erlenmeyer flasks containing 150 mL test medium. These were placed on a tablet rotating at 100 rpm to prevent sedimentation of the cells while preventing further aeration, and were sealed with cellulose plugs. The test media were prepared to the OECD 201 guideline, steplised by membrane filtration and aerated with sterile air. Media were then inoculated with approximately 10,000 cells/mL, from an exponentially-growing pre-culture prepared four days before the start of the test and cultivated under equivalent conditions.

Nominal test concentrations were 9.54, 30.5, 97.7, 513, 1000 and 3200 µg pure metabolite (p.m.)/12, along with a control, with three replicates per test concentration and and replicates per control. Mean measured concentrations ranged from 82 to 102% of nominal at test start and from 90 to 101% at test end, therefore results are based on nominal concentrations.

Morphological examination of cells were made over the exposure period on each study day by a microscope. Cell numbers per volume were estimated photometrically as a surrogate for biomass per volume.

Temperature was determined by one continuous measurement of an additional glass sessel falled with an equivalent amount of de-ionised water as in the test vessels. The DH was measured daily in all test levels and the control. Samples were analysed for spiroxamine carborylic and concentration at test start and end.

Analytical method

Samples of water were analysed using the alidated analytical method 01122 report reference <u>M-308346-01-1</u> (see Doc MCA Section 4).

II. Results and Discussion

Validity criteria according to the OECD 201 guideline were met

- Biomass of control cultures to have increased exponentially by a factor of at least 16 (actual: increased by a factor of 21)
- Mean coefficient of variation for section-by-section specific growth rates in the control cultures to not exceed 35% (actual 26.12)
- The coefficient of variation of average specific growth rates over the whole test period between the reparate ontroloultures should not exceed 7% (actual: 5.6%)
- The pH of the control medium is not increase by more than 1.5 units during the test (actual: control pH ranged from 7.9 to 8.0)

The mean recoveries of treated, coll-free dest vessels at test start ranged from 98 to 103% of nominal, with an overall mean recovery of 100%. At dest end, mean recoveries ranged from 98 to 110% of nominal, with ap overall mean recovery of 103%.

Nominal conceptration	Mean measured concentration	% of nominal
$(\mu g p_{\mu} m_{\mu} L) \gtrsim A$	(μg p.m./L)	
Control of the second	<1.32	-
957 2	9.44	99
30.5	30.5	100
97.7 ^C	101	103
313	313	100
1000	995	100

Table CA 8.2.6.1/09-1/ Nonomal and measured concentrations in treated, cell-free vessels at day 0



Nominal concentration (µg p.m./L)	Mean measured concentration (µg p.m./L)	% of nominal	°
3200	3144	98	
-	Mean:	100	X Ø

Table CA 8.2.6.1/09-2 Nominal and measured concentrations in treated, cell-free vessels at day

Fable CA 8.2.6.1/09-2 Nomin Nominal concentration	al and measured concentrations in treat	ed, cell-free vessels at day 3
(µg p.m./L)	(µg p.m./L)	
Control	<1.32	
9.54	9.35	
30.5	33.5	
97.7	98.5	104° L L
313	331 🔊	
1000	1042 (104 0 0 20 20
3200	3166 0 0 4	99 0 5
-	Mean:	

The results of the study have been presented based on nominal concentrations The following table details the effects of exposure on biomass (cell/density

Table CA 8 2 6 1/00 3	Coll donsity Quaring	"U	no to conin	vomite	a state a set	ia Ĉaj	S	
Table CA 8.2.6.1/09-3	Cen density during	gerhose	e to spire	oxanane-	cargocyn	Gaciu	<u> </u>	

Nominal	Number of cells $(x_10^{\circ})^{1}$ by time $E SD_{1}$
concentration	24 h 2 48 h 2 72 h
(µg p.m./L)	
Control	2.5 ± 0.55 (7.5 ± 200) (21.3 ± 3.53)
9.54	2.7 ± 1.15 6.8 ± 2.08 0° 22.00×3.97
30.5	$1.7 \neq 0.76 \%$ 3.69% 23.2 ± 4.75
97.7	297 ± 0.76 9.2 ± 4.49 0.78 ± 0.29
313	51.3 ± 0.78 6.7 ± 2.52 717.0 ± 2.18
1000	2.0 ± 1.32 9.7 ± 3.21 19.0 ± 1.80
3200	$1.3 \pm 1.26^{\circ}$ $6.0 \pm 2.18^{\circ}$ $17.2 \pm 1.76^{\circ}$

Mean of two counts of three replicates (six replicates for the control # standard deviation

Statistically significant inhibition of growth rates to the control was observed only at the test concentrations 3200 pg p.m. after 72 hours exposure. No significant inhibition was observed after 48 hours exposure.

Nominal	0 - 24 b	Nº Q.	(4) - 48 h		0 - 72 h	
concentration	Growth 🕺	7% / ×	Growth	%	Growth	%
(µg p.mcL)	rate Q	inhibition	ratey	inhibition	rate	inhibition
Control	~~0.8981	× - *	s≫0.992	-	1.015	-
9.54	0.920 V	-2,0	0.943	4.9	1.026	-1.1
30.5	0.441	َ ^ل َّ 50.9 ا	1.062	-7.0	1.043	-2.8
97.7 🖉	0.954	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	1.076	-8.5	0.960	5.4
313	0.231	≪ 74.3 ♥	0.922	7.1	0.942	7.1
1000	0.44	50	1.112	-12.1	0.980	3.4
3200	» -2 . 63* _^	393	0.876	11.8	0.946*	6.7

Table CA 8.2.64/09-4 Browth ates and inhibition of treated cultures

The sticall significantly different to the control (Williams Multiple Sequential t-test, α =0.05, one-sided smaller or Weight test for inhomogeneous variances with Bonferroni adjustment)

A summary of the results is presented in the table below:



Nominal concentration (µg p.m./L)	Cell number per mL after 72 h	0-72 h average specific growth rate per day	Inhibition of average specific growth rate (%) 🔈	Doubling time (days)
Control	213,000	1.015	- 2	0.683
9.54	220,000	1.026	-1.1 🔊	0.676
30.5	232,000	1.043	-2.8	0.665 2 2
97.7	178,000	0.960	5.4 🔊	0.728
313	170,000	0.942	7.1	0,736 2 0
1000	190,000	0.980	3.4	@ 707 3 2 6
3200	172,000	0.946	6.7	0.733

Table CA 8.2.6.1/09-5 Summary of results after 72-hour exposure to spiroxamine-carbocylic acid

A summary of the endpoints determined in the study are presented in the table below.

Table CA 8.2.6.1/09-6 Summary of derived endpoints

Growth rate	
E _r C ₅₀ : >3200 μg p.m./L	
E_rC_{20} : >3200 µg p.m./L 2^{10}	
$E_r C_{10}$: >3200 µg p.m./LQ	
LOE _r C: 3200 µg p.m./k ^O [×] [×] [×] [×]	
NOE _r C: 1000 μg p.m. Δ	

III. Conclusion

In a 72-hour toxicity study, cultures of Desmodesmus subspication were exposed to spiroxaminecarboxylic acid at nominal test concentrations of 9.54/30.5 of 7, 313, 1000 and 3200 µg p.m./L under static conditions.

The growth rate NQPrC and E_rC_{0} values were 2000 and >3200 µg p.m./L, respectively. The percent inhibition of average specific growth rate in the treated algabelium compared to the controls ranged from -2.8 to 7.1% after 72 hours exposure. L.

Ø

Assessment and conclusion by applicant:

S. The study was conducted to the OECD test guideline 201 2006, the current version of which is the OECI 201 "Freshwater algo and cyanobacteria, growth "inhibition test", adopted 28 July 2011.

Validity criteria according to the current 201, version of the OECD 201 guideline are the same as that used in the guide line in force at the time of study cooduct and therefore remain the same. The validity criteria have been met:

- Biomass of control cultures to have increased exponentially by a factor of at least 16 (actual: increased by a factor (P21)
- Mean coefficient of variation for section-by-section specific growth rates in the control Cultures to tot exceed 35% (actual: 26.1%)
- The coefficient of vagation of average specific growth rates over the whole test period between the replicate control cultures should not exceed 7% (actual: 5.56%)

The study is therefore considered acceptable.

The $E_r Q_r^{\alpha}$ value was determined to be >3200 µg p.m./L.

The data have been subjected to statistical re-evaluation and the results have been presented in the following study summary



Data Point:	KCA 8.2.6.1/17
Report Author:	
Report Year:	2020
Report Title:	Calculation of EC10, EC20 and EC50 values for Desmodesmus subspicatus with
	KWG 4168- carbocylic acid in an algal growth inhibition test
Report No:	0471836-ECO34
Document No:	<u>M-761469-01-1</u>
Guideline(s) followed in	None
study:	
Deviations from current	None $\nabla $ \mathcal{O} \mathcal{O} \mathcal{O} \mathcal{O} \mathcal{O}
test guideline:	
Previous evaluation:	No, not previously submitted of a start of the start of t
GLP/Officially	No, not conducted under GLP/Officially recognised testing factuities
recognised testing	
facilities:	
Acceptability/Reliability:	Yes Δ ∂ ∂ Q ∂ ∂ ∂' ∂'

The report <u>M-309818-01-1</u> on the effects of exposure to KWG 4168-catbocylic acid on the growth of algae (*Desmodesmus subspicatus*) and not provide estimates of EC_{10} , EC_{20} and EC_{50} values for yield.

For the determination of EC_x values, no reliable fit could be achieved, therefore \mathbb{C}_x values could not be calculated.

I. Methods

The statistical evaluation was performed with statistical software Tox Rat Pro v3 20

Due to a lack of statistically significant concernation/response, EC values could not be reliably calculated.

II. Results an Discussion

Yield at 72 hours

Regarding the calculation of EC₁ \approx EC₂₀ and EC₃₀ values for yield at 72 h, the Probit analysis could not produce reliable EC_x values due to lace of a statistically significant concentration/response.

III Conclusion

Regarding the calculation of EC₀, EC₂, and E $_{50}$ values for yield at 72 h, the Probit analysis could not produce reliable EC_x values due to lack of a statistically significant concentration/response.

Assessment and conclusion by applicant:

 EC_{10} , EC_{50} and EC_{50} values for growth rate were calculated in the study report therefore only yield endpoints have been determined in this statistical report.

The statistical re-evaluation of the data could not determine reliable EC_{10} , EC_{20} and EC_{50} values for yield due to a lack of a dose-response. These values are therefore considered to be >3,200 µg p.m./L.

The $E_r C_{50}$ determined in the original study report of >3,200 µg p.m./L shall remain as the critical endpoint determined from this study.

The values determined in the e-evaluation work are considered to be fully valid.

For procedural reasons studies listed in the Table 8.2.6.1-1 below are included in the current dossier as available data or information previously submitted but not necessarily evaluated. However, these reports have been fully superseded by newer studies. Consequently, no summaries of the reports have been included in the dossier.



Table CA 8.2.6.1-1: Studies	nreviously submitted	and not relied upon	for the risk assessment
Table CA 0.2.0.1-1. Studies	previously submitted	i anu not i cheu upon	for the fisk assessment

Data	Document	Date	Title	a,°	
Point	No.				, Cor
KCA	M-309818-	2008	Desmodesmus subspicatus growth inhibition test with spiroxamine	- 67	°
8.2.6.1/09	01-1		carbocylic acid		

Effects on growth of an additional algal specie CA 8.2.6.2

	ets on growth of an additional algal species
CA 8.2.6.2 Effec	ets on growth of an additional algal species 🖉 🖉 🖉
Data Point:	KCA 8.2.6.2/01
Report Author:	
Report Year:	
Report Title:	Toxicity of 14C-KWG 4168 to the matche diatom Skeletoneme costatum
Report No:	107927 0 0 5 5 5
Document No:	<u>M-006512-01-1</u>
Guideline(s) followed in	No EU Guideline followed
study:	
Deviations from current	None of the transformed to the t
test guideline:	
Previous evaluation:	yes, evaluated and accepted a strain of a strain of the st
	RAR (2010), (SAR (2017), (SAR (2017))
GLP/Officially	Yes conducted under GLP/Officially recognised testing facilities
recognised testing	
facilities:	
Acceptability/Reliability:	
0	

Spiroxamine is not an herbicide or plant growth regulator (PGR), nor does it have herbicidal activity therefore studies will additional agal species from a different taxonomic group to green algae are not specifically required. However, data are available with several other algal species which have been summarised below.

Executive Summary

Ì In a 96-boar toxicity study, and ures of Skeletonena costatum vere exposed to ¹⁴C-KWG 4168 at nominal first concentrations of 0.63, 1.25, 2,5, 5.0 and 10 µg a.s. P under static conditions.

The 96-hour growth data was malyses as sell density, growth rate and cumulative biomass (as represented by the area under the growth eurve). For each endpoint the data were analysed using ANOVA folloged by the Dynnett's test to determine the towest observed effect concentration (LOEC) and the no-observed effect Concentration (NOEC). The 96-hour LOEC was 1.29 µg a.s./L and the 96hour NOEC was 0.63 µg a.s./L for all epopoints.

The ECs based on cette density was determined to be 1.3 µg a.s./L. The EC50 based on growth rate was determined to be 6.3 μ g a s. L. The EC₅₀ Based on biomass was determined to be 1.3 μ g a.s./L.

Ĩ. Materials and Methods A. Materials **Test Mate** '-KWG∰168 98.2% a.s. Description Not reported Stability of test Not reported compound:



Reanalysis/Expiry date:	Not reported
Density:	Not reported
Treatments	
Test rates:	Nominal: 0.63, 1.25, 2.5, 5.0 and 10 µg a.s./L
Solvent/vehicle:	Acetonitrile
Analysis of test concentrations:	Not reported Not reported Nominal: 0.63, 1.25, 2.5, 5.0 and 10 µg a.s./L Acetonitrile Yes, mean measured concentrations 97 5107% of nominal Marine diatom <i>Skeletonema costatum</i> The Starr Collection, University of Jexas, Austin, Jexas, Sterile, 250-mb borosilicate glass culture flasks filled with approximately 100 mb of text solution and capped with sterile glass closures Emriched saltware media was filter sterilized (0.450 m). The batch of
Test organisms	
Species:	Marine diatom Skeletonema costatum
Source:	The Starr Collection, University of Texas, Gustin, Fexas,
Test design	
Test vessel:	Sterile, 250-mb borgsilicate glass culture fissks filed with
	approximately 100 not of test solution and capped with sterile glass
Test medium:	Epriched saltwarer media was riter sterilized (0.450pm) The batch of
Test meutum;	within media used to prepare the test solutions was prepared at pH 8.0
l'a	and did not require pH adjostment 27 29
Replication:	Three replicate vessels were prepared for each concentration and used
	for determine daily cell density. The highest test concentration had five replicates: & replicates for cell density determinations and two
L. A	additional replicates that were only used to provide a sufficient volume
	of solution for Day 4 measured concentration analysis
Initial cel density: "	96 hours 1 x 10 cells mL 96 hours 19.8 - 20.8°C 250/20 19.5 - 20.8°C 19.5 - 20.8°C
Duration of test: 🔬	96 hours of O' O O
Environmental test	
conditions	
Temperature:	19.8 - 20.8°C
Salinitxo o ^{ox}	$\tilde{\mathcal{C}}^{25\%}$
pH: A	8.0 - 9.0 - 9.0 - 2 - 2
Photoperiod:	P6 hour light 8 hours darkness, light intensity at 4300 lux
B. Study Design 🕉	
	norder to asses the toxicity of the marine diatom Skeletonema costatum
when expose to ¹⁴ C-KWG	#168 $\delta \forall er 72$ hours.

Test concentrations were prepared from a stock solution, and 100 mL of the solution was used in 250mL borosilicate glass culture flasks filled during the test. Nominal test concentrations were 0.63, 1.29, 2.46, 5.55 and 10.36 ng a s.L.

Test media were inoculated with enough 3-day-old pre-culture to give a density of 1×10^4 cells/mL.

Incubation was at 19.8 to 20.8°C under a photoperiod of 16 hours light to 8 hours dark at 4300 lux. Sedimentation of the cells and test substance was avoided by housing the vessels on a shaker table, which was operated at 100 revolutions per minute.



Each day, density was determined in three replicates at each test concentration using a light microscope and an Improved Neubauer haemocytometer.

II. **Results and Discussion**

The study was conducted to the American Society for Testing and Materials (ASTM). 1990, Standard Guide for Conducting Static 96-h Toxicity Tests with Microalgae. ASTM Standard El 2 Philadelphia, PA. No specific validity criteria were included in the report.

The initial measured concentrations of ¹⁴C-KWG 4168 were 0.63, 1.29, 2.46, 5.35 and 10.36 Jg a.s. which represents 98 to 107% of the nominal test concentrations. The SC-KWG 416 was not stable in the test system as determined by the 72-hour radio-TLC analysis. Due to this breakdown of the parent compound in the test system, the statistics were based upon the initial measured concentration of the test solutions (EPA, 1994). No undissolved test substance was visually observed in the test vessels throughout the test period.

Table CA 8.2.6.2/01-1	Measured test concentr	rations of ¹⁴ C-K	WG 416 8 baced	uponiquid	scintillation 2°
	counting during the ex	posure of Skelet	onema costatum		
	· · · · · · · · · · · · · · · · · · ·		A	\cap^{v}	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

Nominal concentration (µg/L)	Day 0 ^a (μg/L) Percent of Day 4 ^b Percent of nominal Physics Percent of (μg/L) Percent of nominal Physics Percent of (μg/L) Percent of (μg
Control ¹	<0.08 Q' a - a - a - a - a - a - a - a - a - a
Solvent control ¹	
0.63	
1.25	1.29 (103) (103) (1.26) (101)
2.5	2.46 0 98 2.46 99
5	5 .35 A 105
10	× 10.36 × 104 × 104 × 104 × 104 × 104
Lab recoverv ²	1.94 Q 97 X 1.93 V 97

These values opresent the total amount of radioactivity in the test solutions@Percent parent analysis on

- Day 0 determined that 92% of the total radioactivity was present & WG \$168 technical These values represent the total mount Pradioactivity in the tot solutions. Percent parent analysis on Day 4 determined that 20% of the total radioscrivity was present as KWG 4168 technical. The compound was not stable under test conditions after 4 days \sim Not retected at or above the validated limit of detection (6.08 µgA)
- 1
- 2 Lab recovery for Pay 0 and Day 4 based apon a nominal lab concentration of 2.0 µg/L

The growth curves Pearly show becreased growth in the 1.29, 2.46, 5.35 and 10.36 µg a.s./L test levels as compared to the control. (II n

Table CA 8.2.8.2/01-2	Cell density during the	toxicity phase and the gro	wth recovery phase
-----------------------	-------------------------	----------------------------	--------------------

Initial measured	Mean cell demity (cells/mL) x 104		
concentration (mg/L)	Day 🏹 🔪 🦼	Day 2	Day 3	Day 4
Control	3,95, 6" 0"	21 27	72.6	167.2
Solvent control		222	72.4	173.4
0.63	3.89 &	\$1.8	72.2	168.4
1.29		¥17.3	43.2	72.5
2.46	284	4.39	13.8	33.3
5.35	r 7 .82	3.02	4.04	29.3
10.36	2.16 O	0.96	0.84	1.49

A surfarrary of the endpoints derived from the data is presented in the table below:

Table CAD8.2.6.2/01-3 Summary of derived endpoints

Cell density	
EC ₂₅ (95% CI)	0.7 μg a.s./L (95% C.I. 0.4 - 1.2 μg a.s./L)
EC ₅₀ (95% CI)	1.3 μg a.s./L (95% C.I. 0.9 - 2.0 μg a.s./L)



Growth rate			
E _r C ₂₅ (95% CI) E _r C ₅₀ (95% CI)	5.3 μg a.s./L (95% C.I. 3.4 – 8.2 μg a.s./L) 6.3 μg a.s./L (95% C.I. 4.4 – 8.9 μg a.s./L)		
Area under the growth			<u> </u>
E _b C ₂₅ (95% CI) E _b C ₅₀ (95% CI)	0.7 μg a.s./L (95% C.I. 0.5 -1.2 μg a.s./L) 1.3 μg a.s./L (95% C.I. 1.0 - 1.9 μg a.s./L)	ja S	

III. Conclusion

In a 96-hour toxicity study, cultures of *Skeletonema costatum* were exposed to 14 nominal test concentrations of 0.63, 1.25, 2.5, 5.0 and 10 µg a.s./L under static conditions

The 96-hour growth data was analysed as cell deposity, growth rate and cupulative bioppess (a represented by the area under the growth curve) For each endpoint the data were analysed using ANOVA followed by the Dunnett's test to determine the lowest observed effect concentration (LQEC) and the no-observed-effect-concentration (NOEC). The 96-hour LOPC was 1.29 by a.s./Iz and the 96hour NOEC was 0.63 μ g a.s./L for all endpoints.

The EC50 based on cell density was determined to be 1, 3 µg as /L. The EC50 based on growth rate was determined to be 6.3 µg a.s./L. The EC based on biomass was determined to be 13 µg a.s./L

Assessment and conclusion by applicant

The study was conducted to the American Society for Festing and Materials (ASTA). 1999. Standard Guide for Conducting Static @6-h, Toxicit Tests with Microadgae. ASTM Standard El 2 1 8, Philadelphia, PA. The control data have therefore been re-assessed against the Galidity criteria according to the current QECD 201 test guideline (2010).

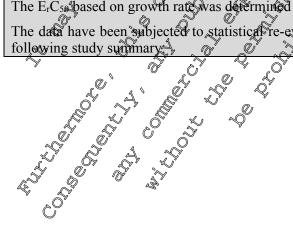
Validity criteria according to QECD 201 (2021) were met

- Cell density of control cultures to increase by at least 10x (actual: 170)
- Mean coefficient of variation for section-by-section specific growth rates in control cultures to be $\leq 35\%$ (actual: 29,4%)
- Coefficient d'variation of average specific growth rates in control cultures over the test S. period to be 10% (actual: 0.96%)

The validity criteria according to the DECD 201 test guideline have been met therefore the study is considered acceptable. It should be noted that the results have been based on initial measured concentrations when they would preferably have been based on mean measured concentrations which took the Day 4 measurements of to account, fowever, based on total radioactivity the total residues at Day 4 were consistent with the noninal concentrations therefore it is believed to be acceptable to use the initial measured concentrations here.

The $E_r C_{50}$ based on growth rate was determined to be 6.3 µg a.s./L.

The data have been subjected to statistical re-evaluation and the results have been presented in the





Data Point:	KCA 8.2.6.2/05
Report Author:	
Report Year:	
Report Title:	Calculation of EC10, EC20 and EC50 values for Skeletonema costatum with
	14C-KWG 4168 in an algal growth inhibition test
Report No:	0471836-ECO26
Document No:	<u>M-761414-01-1</u>
Guideline(s) followed in	None
study:	
Deviations from current	None of A None
test guideline:	
Previous evaluation:	No, not previously submitted
GLP/Officially	No, not conducted under GLO Officially recognised testing facilities
recognised testing	
facilities:	
Acceptability/Reliability:	Yes A Y Y Y Y Y Y

The report <u>M-006512-01-1</u> on the effects of exposure to 14 C-KWC 416C on the growth of algae (*Skeletonema costatum*) did not provide estimates of EC₁₀ of EC₂ values. Therefore, these values as well as EC₅₀ values have been calculated in accordance with the Annex to Com. Reg. 283/2013.

The resulting EC₅₀ value for yield at 96 h was 10^{9} µg as./L. The resulting EC₅₀ value for growth rate at 96 h was 6.33 µg a.s./L.

I. Methods

The statistical evaluation was performed with statistical software ToxRatPro v3.3.0.

Effect concentrations with 10, 20 and 50% from the test item treatment when compared to the pooled control were colculated for yield and growth rate after 96 hours of posure but due to the steep curve EC_{10} and EC_{20} values could not be considered reliable for yield and growth rate. A Probit analysis was conducted on the yield data with confidence limits based on Fieller's theorem, while a non-linear regression 3-parameter logistic (weighted) with confident limits estimated by Monte-Carlo simulation.

II. Results and Discussion

Yield at 96 hour

Regarding the EC_{50} calculation for yield at 96 h, a statistically significant concentration/response was found (p(F) = 0.001) for this parameter Q

The resulting EC_{50} value and the respective confidence interval is presented in the following table below.

Table CA 8.2.6.2/05-1 Results of the Probit analysis of yield at 96 h: Selected effective concentrations (ECx) of the test jtem and their 95%-confidence limits

	Yield	
Parameter 5	EC50 (95 % confidence interval) [µg a.s./L]	
Effect on yord at 95	1.29 (0.99 – 1.66)	

The resulting EC₅₀ value of 1.29 (95%CL: 0.99 – 1.66) μ g a.s./L, met the goodness of fit criteria and therefore the estimated EC₅₀ value is considered reliable. Reliable EC₁₀ and EC₂₀ values could not be determined.



Growth rate at 96 hours

Regarding the EC₅₀ calculation for yield at 96 h, a statistically significant concentration/response@ras found (p(F) < 0.001) for this parameter. Ô

The resulting EC_{50} and the respective confidence interval is presented in the following table below

Table CA 8.2.6.2/05-2 Results of the 3-param. Logistic analysis of growth rate at 96 h: Selected effective concentrations (EC_x) of the test item and their 95%-confidence limits

	Growth rate	
Parameter	(95% confidence aterval)	
	ြို႔g a.s.(L) တို	
Effect on growth		
rate at 96 h		

The resulting EC50 value of 6.33 (95%CL: 4.51 - 20) us a.s./ Respectively, met the goodness of fit criteria and therefore the estimated EC_{50} value is considered reliable. Reliable EC_{10} and EC_{20} values could not be determined. Õ

III. Conclusion

The resulting EC_{50} value for yield at 96 pours was determined to be 9.29 uga.s./LOThe resulting EC50 value for growth rate at 96-hours was determined to be 6.32 ug a sQL. Reliable C10 and EC20 values could not be determined. Ľ

Assessment and conclusion by applicant:

The statistical re-evaluation of the data could not determine reliable EC10 and EC20 values for yield

The ErC50 determined in this re-evaluation work of 6.33 µg a.s./L is considered to be the same as the growth rate EG₅₀ value of 6.3 µg a.s./L from the original study report. The ErC₅₀ of 6.3 µg a.s./L shall

aluntion work are considered to be fully valid

the second of the original study is a second of the original secon



Data Point:	KCA 8.2.6.2/02
Report Author:	
Report Year:	1997
Report Title:	Toxicity of KWG 4168 technical to the blue-green alga Anabaena flos-aqua
Report No:	107706
Document No:	<u>M-006537-01-1</u>
Guideline(s) followed in study:	
Deviations from current test guideline:	Yes OECD 201 (2011) Mean coefficient of variation for section-by section specific prowth ates in control cultures to be $\leq 35\%$ (actual: 44.9%)
Previous evaluation:	yes, evaluated and accepted RAR (2010), RAR (2017) Inspite of the high coefficient of variation the mean of the sectional growth rates (37.19 %,day to day) after three days the test is acceptable.
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Supportive may be a set of the se

In a 96-hour toxicity study, triplicate cultures of Anabaena floss aquae were exposed to KWG 4168 Technical at a mean measured test concentration of 0.99 mg a.s./L under static conditions. Since there was no adverse effect on the *Inab ena flos-aquae* at the limit test concentration of 0.99 mg a.s./L, a 4-Day EC₅₀ was established as >0.95 mg a SL.

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Materials and Methods Materials I. Δ

A. Materials		
Test Materia	KWG 4168 technical	¹² C-KycG 4168
Lot/Batch #: 🔗	199216 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	Vial Rumber. C-681A
Purity:	96.2% a.s.	29.1% as (specific activity 47.9 μ Ci/mL)
Description:	96 8% a.s. 9 Not reported 5 Not reported 5 Not reported 5 Not reported 5	Not reported
Stability of test	Not coported	Not reported
compound:		Ê.
Reanalysis/Expiry	Not reported y by	Not reported
date:		
Density:	Not reported	Not reported
Tréatments	Novreported A A	
Test rates	Nominal: 4.00 mg/a.s./L	
	Nean measured: 0.99 mg a.s.	/L
Solvent/vehicle:	Methanol 🔗	
Analysis of test	Xes, measured concentrations	s 98 – 99% of nominal
concentrations.		
Test organisms		
Species:	Blue-green alga, Anabaena fl	os-aquae
Source:	The Starr Collection, Univ. of	f Texas at Austin



Test design

1000 000-8-	
Test vessel:	250-mL borosilicate glass culture flasks filled with approximately 100 mL of test solution and capped with sterile glass closures
Test medium:	Freshwater Nutrient Media (ASTM, 1990)
Replication:	Three replicate vessels
Initial cell density:	Freshwater Nutrient Media (ASTM, 1990) Three replicate vessels 1 x 10 ⁴ cells/mL 96 hours
Duration of test:	96 hours
Environmental test conditions	
Temperature:	23.9 – 24.2°C 91 – 97 μmhos/om 75 – 9.2
Conductivity:	91 – 97 μmhos/Δm ,
pH:	7.5 - 9.2
Photoperiod:	Continuous lighting at 2200 har 5 5 5
B. Study Design	91 – 97 μ mhos/2m 7.5 – 9.2 Continuons lighting at 2200 for 2
The objective of the study	was to data mine the growth offering of KW/C 1169 took field to the blue groop

The objective of the study was to determine the growth effects of KWG 4168 technical to the blue-green alga, Anabaena flos-aquae.

Test concentrations were prepared from a stock solution and 100 mL of the solution was added to each 250 mL Erlenmeyer flask at the start of the test. The nominal test concentration was 1.0 mg a.s./L. The corresponding measured test concentration was 0.99 mg a.s./L.

The Anabaena flos aquae used for this study was obtained from an in-house culture (AF-28). The algae culture was maintained in the laboratory since July 26, 1996. The culture was originally obtained from The Starr Collection, Univ. of Texas at Austin. The algae used in the definitive test was taken from a three day old batch culture of Anabaena flos-aguae (in log phase growth). Test media were inoculated with enough 3-day old pre-culture to gore a density of 1 x10^o cells plL.

The exposure of *Anabagena flos-aquae* to KWG 4168 was conducted under static conditions. Test vessels were sterile, 250 mb borostricate class culture flasks filled with approximately 100 mL of test solution and capped with sterile glass closures. Testing was conducted in an environmental chamber. The position of the test vessels was re-randomized daily. The haker table was operated at 100 revolutions per minute. An array of cool white fluorescent lights produced a 24-hour light photoperiod and a light intensity of approximately 2200 flax.

Each das density was determined in three replicates at each test concentration using a light microscope and an Improved Nerbauer haemosytom der. Samples of KWG 4168 test solutions, including controls, were taken on day zero and day four to measure actual exposure concentrations.

Incubation was at 23.9 to 24 2°C and under Continuous light at ~2200 lux.

II. Results and Discussion

The study was conducted to an older EPA test guideline and no specific validity criteria were included in the peport. S

The recoveries of treated sest vessels during the study was 98 to 99% of nominal. The study result has been based on the mean measured test concentration.



Table CA 8.2.6.2/02-1	Measured test concentrations based upon LSC during the exposure of Anabaa	ena	
	flos-aquae	_	0

	J			
Nominal	Measured con	ncentration ¹ (mg a	.s./L)	
(mg a.s./L)	Day 0	Day 4	Mean ± SD	Percent of 6
		-		, nominal , o
Control	ND	ND	- 01	-
Solvent control	ND	ND	- 1	- 5 5
1.0	0.98	0.99	0.99 ± 0.01	99 ~ ~ ~
Lab recovery ²	0.52	0.52	0.52 ± 0.00	
ND Not detected	d at or above the	validated limit of de	etection (0.1 mg/L) \mathcal{R}	

¹ Measured concentration based upon liquid scintillation counting

² Lab recovery based upon a lab spike of 0.5 mg/L Δ

The growth curves clearly show similar growth through Day 4. Since there was no adverse effect on the *Anabaena flos-aquae* at the limit test concentration 000.99 mg a.s./L, a 4 day EC was established as >0.99 mg a.s./L.

Table CA 8.2.6.2/02-2	Day 4 cell growth	h darin	g the c	expostere	ofAnd	ibaena	flos-aqu	<i>ae</i> to	KWG	, 168 _	Ć
	Technical	A.			A Or	~~"·			L	A.	n"

Mean measured	Rep.	
concentration		Day 4 2 Day 4 mean 2 control growth
(mg a.s./L)		
Control	А	134.50 2 137.50 2 1090 4
	В	
	С	
Solvent control	A 🗞	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$
	В	
	C∜	
Pooled control	<i>Ŵ</i>	
0.99	A c	$\begin{array}{cccccccccccccccccccccccccccccccccccc$
õ	B _∞ ∖	
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	GO.	12450 (130.50 (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120) (120
	B C V.	

III. Conclusion

In a 96 hour toxicity study, opplicate cultures of *mabaena flogaquae* were exposed to KWG 4168 Technical at a mean measured test concentration of 0.99 mg as *IL* under static conditions.

Since there was no adverse effection the Anabagena flos-aquice at the limit test concentration of 0.99 mg a.s./L, a 4-Day  $EC_{50}$  was established as >0.29 mg as./L.

## Assessment and conclusion by applicant:

The study was conducted to the FIFRA Guideline 123-2 Growth and Reproduction of Aquatic Plants (Tier 2). The study has therefore been re-assessed against the validity criteria according to the current OECD 201 test guideline (2011)

The below validity criteria according to OECD 201 (2011) were met:

- Celbiensity of control cultures to increase by at least 16x (actual: 138)
- Coefficient of ariation of average specific growth rates in control cultures over the test period  $\infty$  be  $\leq 10\%$  (actual: 1.13)

The criterion relating to section-by-section specific growth rates was not met:

• Mean coefficient of variation for section-by-section specific growth rates in control cultures 235% (actual: 44.9%)



Not all of the validity criteria have been met and the study results clearly show that this was an insensitive species to spiroxamine. The study is therefore considered to be supporting information only.

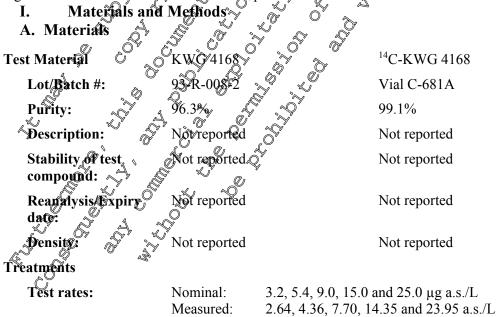
As this was a limit test, the data are not suitable for statistical re-evaluation for Cx values. The f and  $E_vC_{50}$  can both be considered to be >0.99 mg a.s./L.

Data Point:	KCA 8.2.6.2/03
Report Author:	
Report Year:	
Report Title:	Toxicity of 14C-KWG 4968 to the freshwater diatom Navicula pelliculosa
Report No:	
Document No:	<u>M-006542-01-1</u>
Guideline(s) followed in	ASTM (1990) guideline Brandard Guide for Conductine Static 96 h Testicity
study:	Tests with Microalgae V V C
Deviations from current	Yes OECD 201 2011 Mean coefficient of variation for section by-section specific growth rates in the
test guideline:	OECD 20102011)
	Mean coefficient of variation for section by-section specific growth rates in the
	control cultures to not exceed $\bigcirc$ % (as trais 34 $\square$ %) $\bigcirc$ $\bigcirc$ $\checkmark$
Previous evaluation:	yes, graluated and accepted accepted and acc
	RAR (2010), RAR (2017)
GLP/Officially	Yes, conducted under GDP/Officially recognised testing facilities
recognised testing	
facilities:	
Acceptability/Reliability.	Supportive only

#### Executive Summary

In a 96-hour toxicity study, cultures of *Navicula, pelliculosa*, were exposed to ¹⁴C-KWG 4168 at measured test concentration of 2.60, 4.36, 7.70, 34.35 and 23.95 a.s./L under static conditions.

The growth rate NOE₁C and E₂₅₀ values were 7.70 and 11.85 µs a.s./L, respectively. The percent inhibition of average specific prowth rate in the treated algal cultures compared to the pooled controls ranged from -3.9 to 9,4% after 96 hours exposure.





Solvent/vehicle: Methanol Yes, mean measured concentrations 81 – 96% of nominal Analysis of test concentrations: **Test organisms Species:** Freshwater diatom Navicula pelliculosa Source: In-house culture 250 mL borosilicate glass flasks containing 50 mL test solution, capped with sterile 150 mL beakers Sterile freshwater media (ASTM 1990) Four replicates 10,000 cells mL 96 hours  $4200 - 247^{\circ}C$ Test design **Test vessel: Test medium: Replication:** Initial cell density: **Duration of test: Environmental test** conditions **Temperature:** pH: ontinuous illummation approx 4300 **Photoperiod:** B. Study Desig  $\bigcirc$ This study was conducted in order to assess the effect of exposure 40 ¹⁴C-KWG 4168 to the freshwater diatom Navicula pellici Nosa in a 96-hour static test. Test concentrations were selected based on the results of preliminary testing Test concentrations were prepared using volutions of a Stock polution Nominal vest concentrations were 3.2, 5.4, 9.0, 150 and 25.0 µ Da.s./L, with corresponding measured concentrations of 2, 64, 4.36, 7.70, 44.35 and 23,93 a.s. 4. A control and solvent control were also used. The freshwater media was prepared according to ASTM (0990), and was sterilised using a 0.45 µm filter, and was adjusted to pH 7.5 with 0.1 N HCl. Media were inoculated with 10,000 cellson L, from a 2 day old batch culture in log phase growth. Four replicates were used for each test concentration, and three additional replicates were prepared for the 25 µg/L@est concentrations to onsure an adequate volume of test solution for analytical measurements. Replicate test vessels were randomly positioned in an environmental chamber on a 100 rpm shaker table. Vessels were held under continuous illumination at approximately 4300 lux. Test vessels were sterile, 250 mL boosilicoe glass culture flasks filled with approximately 50 mL test solution and copped with storile 150-mL beakers. Cell densify was determined daily using a light microscope and a haemocytometer. The temperature of the test system was automatically determined hourly, and measured manually daily. Measurements of per were taken at test start and test end in the control and 3.2, 9.0 and 25.0 µg a.s./L test concentrations.

# II.O Results and Discussion

The study was conducted to an older EPA test guideline and no specific validity criteria were included in the report.



The mean recoveries of ¹⁴C-KWG 4168 in the test solutions after 96 hours exposure were 81 to 96% of nominal. The results of the study have been presented based on mean measured test concentrations  $\mathcal{R}_{p}^{\circ}$ 

Nominal	Measured concentr	ation (μg a.s./L)	Å.	
concentration	Day 0	Day 4	Mean ± SD	% of nominal 🔊
(µg a.s./L)			4	
Control	<lod< td=""><td><lod td="" 🔊<=""><td>-</td><td></td></lod></td></lod<>	<lod td="" 🔊<=""><td>-</td><td></td></lod>	-	
Solvent control	<lod< td=""><td><lod any<="" td=""><td>- 0</td><td></td></lod></td></lod<>	<lod any<="" td=""><td>- 0</td><td></td></lod>	- 0	
3.2	3.11	2.17	2.64 ± \$7	
5.4	4.23	4.48	4.36 £ 0.13	81 9 0 9
9.0	8.32	7.08	7.70≇0.62° √	85 4
15.0	14.2	14.5 Q	14/35 ± 0/215	96° (\$ Q"
25.0	24.2	23.7 (	23.95 ± 0.25	0°6 × ×
LOD Limit of D	etection: 0.5 µg/I			× · · · · · · · · · · · · · · · · · · ·

Table CA 8.2.6.2/03-1 Nominal and measured concentrations of ¹⁴C-KWG 4168 over the exposure period

LOD Limit of Detection:  $0.5 \ \mu g/L$ 

Significant differences in growth were observed at the 4.35 and  $23.95 \ \mu g$  as ./L test concentrations compared to the pooled controls after 96 bours exposure.

Mean measured concentration	24-hour mean A cell density (x10 ⁴ )	248-hour mean cell density (x10 ⁴ )	cell density	<b>76-hour mean</b> cell densitx (x10 ⁴ )	96-hour inhibition (%)
(µg a.s./L)		<u>k 0° s</u>		×* 0 \$17.01× .9	
Control	2.78 🔵 🔇	20.26	1,18.94	,∞∠∠/.QA \ \ \	-
Solvent control	1.96 🦘 🔬	18.73	31.56	249.56	-
Pooled controls	- 2 5		ž- 🔧 🕺	228.69	-
2.64	189	G16.15 0 S	105,63	Ø13.75 🖑	10.4
4.36	£25 × ·	20.73	AQI.13 6 2	, 248.06	-3.9
7.70	2.34	12.38	<b>19</b> 8.56 N Q	225,00	5.7
14.35	1.42	698° 60 ×	5.4	63.41*	73.4
23.95	1/00 ^a 🗸 🔊	1.04 ^a	1.60ª Õ	<b>Ø</b> .15*	97.4

		0				
Table CA 8.2.6.2/03-2	Mean algal d	ensity	and grow	the inhibition	of treated cu	tures
	intern ungur e				ongreated	

Ø

^a Means are estimates due to some replicates < DOD (1 x10⁴ cols/mL)

* Statistically significantly different to the pooled controls (p\$0.05)

A summary of the endpoints derived in the study is presented below:

# Table CA 8.2.6.2/03-3 Summary of derived endpoints,

#### Table CA 8.2.0.2/05-5 Shummary of depicted endpoints

•	Growth rate of the state of the
	$E_r C_{50}$ (95% CI): 11.85 µg a /L (1092 to 14.03 µg/g.s./L)
	/wNOErC: ] 7/270 μg g /L / 2/2 / 2/2

## III. Conclusion

In a 96-hour toxicity study, cultures of *Novicula pelliculosa* were exposed to  14 C-KWG 4168 at measured test concentrations of 2.64 4.36, 570, 14.35 and 23.95 a.s./L under static conditions.

The growth rate  $NOE_rC$  and  $E_rC_{50}$  values were 7.70 and 11.85 µg a.s./L, respectively. The percent inhibition of average specific growth rate in the treated algal cultures compared to the pooled controls ranged from -3. To 97.4% after 96 hours exposure.

## Assessment and conclusion by applicant:

The study was conducted to the ASTM (1990) guideline "Standard Guide for Conducting Static 96hour Toxicity Tests with Microalgae". Validity criteria have therefore been re- assessed according to the OECD 201 guideline (2011):

After 96 hours exposure, the following criteria were met:



- Biomass of control cultures to have increased exponentially by a factor of at least 16 (actual; increased by a factor of 239)
- The coefficient of variation of average specific growth rates over the whole test period between the replicate control cultures should not exceed 10% (actual: 284%)

After 96 hours exposure, the following criteria were not met:

• Mean coefficient of variation for section-by-section specific growth rates in the control cultures to not exceed 35% (actual: 54.1%)

Not all of the validity criteria have been met therefore the study is considered to be supporting information only.

The  $E_rC_{50}$  value was determined to be 11.85  $\mu g_{s}$ 

A supplemental statistical re-analysis report ( $\underline{W}_{1}$ -280532-01-5) was submitted in order to calculate 72hour endpoints. This has been summarised below  $\underline{W}_{1}$  and  $\underline{W}_{2}$  a

Data Point:	
Report Author:	KCA 8.2.6.2/
Report Year:	
Report Title:	Non-GLP recapulation report avicual pellighosa gowth inhibition test with 14
_	C-KWG4168 0 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2
Report No:	DQM 26021 ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
Document No:	M-280552-01-1 0 0 0 0 0
Guideline(s) followed in S	DECD 201 (March 23 2006)
study:	OECD 201 (March 23, 2006)
Deviations from current	None Ves, evaluated and accepted RAR (2010), RAR (2017) NO, not conducted under CLP/ODicially recognised testing facilities
test guideline:	
test guideline:	yes, evaluated and accepted y
	RAR (2010), RAR (2017) 4 ~ ~ ~ ~
GLP/Official	No, not conducted under CLP/ODicially recognised testing facilities
recognised testing	
facilities:	$\begin{array}{cccccccccccccccccccccccccccccccccccc$
Acceptability/Reliability	
Executive Summary	

#### Executive Summary

This non-GLP recalculation report was conducted to provide a 0-72 hour growth rate  $EC_{50}$  for the freshwater diatom *Naocula pelliculosa* exposed to C-K WG 4168 over 96 hours under static conditions in the study <u>N-006542-01</u>.

The growth rate NOE and  $E_{r}C_{50}$  values were 7.70 and 11.9 µg a.s./L, respectively. The percent inhibition of average specific growth rate in the treated algal cultures compared to the pooled controls ranged from 0.5 to 91.5% after 72/nours exposure.

- I. Materials and Methods
- A. Materials

Refer to study , 2006 (<u>M-0)6542</u>-01-1).

B. Study Design

This pon-GCP recolculation report was conducted to provide a 0-72 hour growth rate  $EC_{50}$  for the freshwater diatom *Navicula pelliculosa* exposed to ¹⁴C-KWG 4168 over 96 hours under static conditions in study 2006 (M-006542-01-1).

Recalculation was done using the program ToxRat Professional v.2.09.



#### II. **Results and Discussion**

Nominal concentration (µg a.s./L)	Cell number per mL after 72 h	Cell number per mL after 72 h	0-72 h average growth rate	Inhibition (C) average specific (C) growth rate (%)			
Pooled controls	10,000	1,056,000	1.605	- 6 ⁴ 5 ⁴			
2.64	10,000	1,211,000	1.546	3.7, 7 ~			
4.36	10,000	1,986,000	1.597	05 5 0			
7.70	10,000	986,000	1.528	Q8 3			
14.35	10,000	54,000	0.386	76.0 %			
23.95	10,000	16,000	0.1 <b>36</b> @°	91.5			
III. Concl A summary of the r Fable CA 8.2.6.2/04	usion recalculated endpoint -2 Summary of deriv	s aner /2-nour expo	0.136				
Growth rate	Q.						
ErC ₅₀ (95% CI)	: 11.9 μg a.s./L.(§.73	to 73.6 ug 2.5./L)	<u>~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~</u>				
$E_r C_{20} (95\% \text{ CI})$			X N N				
E _r C ₁₀ (95% CI)							
LOErC			Û ^V Ø .	Ő			
NOE _r C: 7.70 μg a.s./Ιζ							
				Ý.			
Assessment and correlusion by applicant:							
The study is a m	CI Arroad Watio	Secondary for Audu	Ph06542 01 & and	is considered to be			
The study is a non-GLP/recalculation report for widy <u>1006542-01-6</u> and is considered to be acceptable. Further statistical se-evaluation has been conducted and has been presented below.							
	Or of the	K S S	cied and has been pre	sented below.			
ð			Ő.				
Data Point:	KCA & 2.6.2/	K ST P					
Report Author:		, A					
Report Year:	2020 2		~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~				
Report Year: 7420 Report Year: 7420 Report Title: 8 Calculation of C10, C20 and EC50 values for Navicula pelliculosa with 14C-							
KWG 4168 fr an alsal growth inhibition test							
Report No:	0421836-ECO						
Document No. $\bigcirc$							
Guideline(s) followed in None 2 4 6 0							
study:	Deviations from current None						
study: 5 Deviations from cur	rent None		test, guideline:				
Deviations from cur	rent None						
Deviations from cur	· No por previo	usly somitted					
Deviations from cur test guideline: Previous evaluation GLP/Officially @	· No por previo	usly somitted cted moder GLP/Offici	ally recognised testing	facilities			
Deviations from cur test guideline: Previous evaluation GLP/Officially @ recognised testing	· No por previo	usly somitted cted under GLP/Offici	ally recognised testing	facilities			
Deviations from cur test guideline: Previous evaluation GLP/Officially @	No not condu	usly submitted cted prider GLP/Offici	ally recognised testing	facilities			

# Executive Summary

The peptre M-006 2-0 M on the effects of exposure to ¹⁴C-KWG 4168 on the growth of algae (*Navicule pelliculosa*) did not provide estimates of 72-h EC₁₀, EC₂₀ or EC₅₀ values based on yield. Therefore, these values have been calculated in accordance with the Annex to Com. Reg. 283/2013.

The resulting EC10, EC20 and EC50 values for yield at 72 hours were 6.83, 7.60 and 9.32 µg a.s./L, respectively.



#### I. Methods

The statistical evaluation was performed with statistical software ToxRatPro v3.3.0.

Effect concentrations with 10, 20 and 50% from the test item treatment when compared to the pooled control were determined for yield after 72 hours exposure. A Probit regression was performed to determine  $EC_x$  values, with confidence limits determined according to Fieller's theorem.

II. Results and Discussion

#### Yield at 72 hours

Regarding the calculation of EC₁₀, EC₂₀ and EC₅₀ values for yield 72 h, a statistically concentration/response was found (p(F) <0.002) for this parameter 5

The resulting  $EC_{10}$ ,  $EC_{20}$  and  $EC_{50}$  values and the respective confidence intervals are represented in the following table below.

# Table CA 8.2.6.2/06-1 Results of the Probit analysis of yield at 72 b Selected effective concentrations (ECx) of the test item and their 95% confidence limits

	a strend of the
	$EC_{10}$ $(1)$ $(1)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$ $(2)$
Parameter	(95 % confidence) (95% confidence) (5% confidence)
	interval) interval) ( interval)
	[pg a.s./k] [™] [™] [™] [µgʒǎːś./L] [™] [™] [™] [™] [µgʒǎːs./L]
Effect on yield at 72	\$ 6.83 \$ ac   \$ 7.60 \$ ac   \$ 32
ĥ	$(4.73 & 7.64)$ $(5^{\circ})$ $(4.73 & 7.64)$ $(5^{\circ})$ $(4.73 & 7.64)$ $(5^{\circ})$ $(5^{\circ}$

The resulting  $EC_{10}$ ,  $EC_{20}$  and  $EC_{50}$  values of 6  $\times$  (95% CL: 473 – 7.64), 7.69 (95% CL: 6.20 – 8.50) and 9.32 (95% CL: 8.35 – 13.01) or a.s. L. respectively, meet the goodness of fit criteria by showing a significant concentration/response relationship, and therefore the estimated ECx values are considered reliable.

#### III. Conclusion

The resulting  $\Phi C_{10}$ ,  $B C_{20}$  and  $E C_{50}$  values for yield at 72 hours were determined to be 6.83, 7.60 and 9.32 µg a.s. A, respectively.

## Assessment and consusion by applicant:

The statistical re-evaluation of the data was conducted for yield only because growth rate values have already been determined in the re-assessment report M-289532-01-1.

The  $E_rC_{50}$  determined in the re-assessment report M-280532-01-1 of 11.9 µg a.s./L shall be taken as the critical endpoint determined from this algae study.

The values determined in the re-evaluation work are considered to be fully valid.

## CA 8.2.7 Effects on aquatic macrophytes

Spiroxamine is not an herbicide or plant growth regulator (PGR), nor does it have herbicidal activity therefore studies with aquatic macrophytes are not specifically required. However, studies with *Lemna* 

- mave beensum are available and have been summarised below.



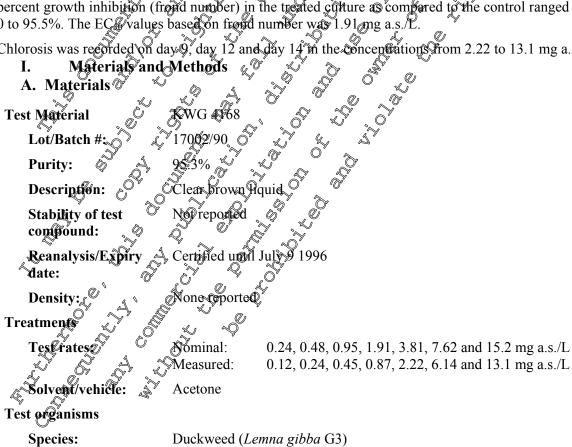
Data Point:	KCA 8.2.7/01
Report Author:	Q° (*
Report Year:	1996
Report Title:	KWG 4168 - toxicity (14 days) to Lemna gibba G3
Report No:	DOM 96013
Document No:	<u>M-006497-01-1</u>
Guideline(s) followed in	FIFRA Guideline 123-2 Growth and Reproduction of Aquatic Plants Tier 2
study:	
Deviations from current	None
test guideline:	
Previous evaluation:	yes, evaluated and accepted a
	RAR (2010), RAR (2017) A Q & A A
GLP/Officially	Yes, conducted under Go Officially recognise desting Pacilities
recognised testing	
facilities:	
Acceptability/Reliability:	Yes a contraction of the state

This study was conducted in order to evaluate the toxicity of KWG 4169 to Dockweed (Lemna gibba G3). The test objective was to determine the 14-day EC25 and EC50 values as well as the NOAEC and the LOAEC values of KWG 4168 for the pest species. He 25 and EC 50 were calculated in two ways: based on the number of fronds on day 4 and based on growth rate  $(\mu)$  from day  $\theta$  to day 14.

In a 14-day toxicity study, Duckweed (Lenua gibba G3) were exposed to KW@ 4168 at mean measured test concentrations of 0.12, 0.24, 0.45, 0.87, 2, 27, 6.14 and 13.1 mg 3.s./Londer static conditions. The 14-day NOEC and EC₅₀ values based on growth rate were 0.24 and 2.65 mg as L, respectively. The percent growth inhibition (frond number) in the treated culture as compared to the control ranged from 0 to 95.5%. The ECE values based on frond number way 1.91 mg a.s./L.

Chlorosis was recorded on day 9, day 12 and day 14 m the concentrations from 2.22 to 13.1 mg a.s./L.

Materials and Methods I.





_	
Source:	Dr. Janet Slovin, Horticulture Crops Quality Laboratory, U.S.
	Department of Agriculture, Beltsville, MD, U.S.A
Acclimatisation	Lemna gibba G3 taken from <14 day old stock culture at test initiation
period:	
Test design	
Test vessel:	400 mL glass dishes: 10 cm diameter x 6 cm height
Test medium:	400 mL glass dishes: 10 cm diameter x 6 cm height Hoaglands E Three test vessels Four plants per test vessel
<b>Replication:</b>	Three test vessels
No. animals/vessel:	Four plants per test vessel
<b>Duration of test:</b>	Three test vessels
Environmental test	
conditions	
<b>Temperature:</b>	Test vessels incubated at $25 \pm 2$ C Test initiation: 4.6 to 4.9 Test termination: 4.8 to 5.2
pH:	Test initiation 4.6 to 4.9 5 a a a a a a a a a a a a a a a a a a
	Test termination: $4.8$ to $5.2$ $5$ $5$
<b>Photoperiod:</b>	Continuous illumination of 4842 lux provider by overhead cool white
	Aghts A O O
B. Study Design 🧳	
Duckweed was exposed for	14 days onder static condition to mean measured concentrations of 0 12

Duckweed was exposed for 14 days ander static conditions to mean measured concentrations of 0.12, 0.24, 0.45, 0.87, 2.22 (6.14 and 13.1) ing a spL. Test vessels were incubated at  $25 \pm 2^{\circ}$ C and continuously illuminated at 4842 lux by overhead cool-white lights for the duration of the study. Four plants, consisting of four fronds each, were aseptically added to each test cessel using a sterile inoculum hook. Hoaglands E medium was used in the test.

Frond counts were made using a lighted magnifying ions on study days 2, 5, 7, 9, 12 and 14 (fronds visible projecting beyond the edge of the parent frond were counted, to determine growth. Samples were analysed on day 0 and 14 for the actual concentration of test substance present in the test medium at each treatment level and in the no treatment controls. The fronts were removed from the vessels at test termination, the contents of all ceplicate vessels were combined and the pH was measured. All test solutions were then submitted for day 14 analysis.

Growth data were used to conduct statistical analysis; stest to determine if controls can be pooled, chisquare test to determine the normality of the data set and Bartlett's test for homogeneity of variances. The non-parametric Kraskal Wallis' and Dunn's Multiple Comparison test were used to determine significant differences between the control and treatment groups for data which did not fit a normal distribution.

#### Analytical method

Samples of vater were analysed using the validated analytical method 00252 M001, report reference <u>M-008490.02-2</u> (see Doc MCA Section 4).

# II. Results and Discussion

The study was conducted to an older EPA test guideline and no specific validity criteria were included in the report.

All results of this study are based upon the mean measured test concentrations of KWG 4168.



Nominal concentration	Measured concentr	ations in mg/L	l l l l l l l l l l l l l l l l l l l
(mg a.s./L)	Day 0 average	Day 14 average	Mean day 0 to day 1
Control	< 0.025	< 0.025	≪Q.025 0° °°
Solvent control	< 0.025	<0.025	0.025
0.24	0.17	0.0642 *)	00.12
0.48	0.27	0.21	A 0.24 5 5 6
0.95	0.43	0.47 🔊	0.45
1.91	0.90	0.85	0.87
3.81	2.70	1.73 *	2.22
7.62	6.14	674 A	6.1
15.2	13.8	2.4	
Limit of quantification for KV	VG 4168: 0.025 mg/L		

Table CA 8.2.7/01-1	Nominal and measured concentrations of KWG 4168
---------------------	-------------------------------------------------

*): Probably microbiological contamination on day 14, visible by a slight (wobidity of median.

Percent inhibition of growth relative to the solvent control, was calculated for each concentration based upon the mean frond counts of each concentration on day 14. Ou day 14, mean frond countwas 244, 240, 236, 202, 222, 114, 19 and 11 at solvent control, 0.12, 0.24, 0.45, 0.80, 2.22, 6.14 and 13,0 mg a.s./L, respectively.

Table CA 8.2.7/01-2	Percent inhibition	of growth (fro	nds) on	day 14	Ĉ
---------------------	--------------------	----------------	---------	--------	---

Mean measured concentration	Mean frond counts on day 14	Percent inhibition on day 14
(mg a.s./L)	NY O' L' OY	
Solvent control	244 d 4	
0.12	240 5	SI.6 S
0.24		3.0 4 2
0.45		1 <u>7</u> ,0 ,0
0.87		Øð Ý
2.22		, 53.2 _Q
6.14	19 ~ ~ ~ ~	92,1
13.1	A C S & S	95.5

The percent, inhibition of growth rate relative to the solvent control was calculated, for each concentration, based upon the growth rate of each concentration from day 0 until day 14. The percent inhibition of growth rate from day 0 until day 14 was 0.61, 1.22, 6.93, 3.47, 27.93, 93.69 and 113.75% at 0.12, 0.24, 0.45, 6.87, 2.22, 6.14 and 101 mg a.s./L respectively.

## Table CA 8.2.7/01-3 Growth sate in whition of day of until Gay 14

Mean measured concentration Growthe rate (pr) (mg a.s./L)	Percent inhibition of growth rate (day 0 – day 14 (Iµ)
Solvent control $\mathcal{O}$ $\mathcal{O}^{\mathcal{V}}$ $0.\mathcal{O}^{\mathcal{V}}$ $\mathcal{O}^{\mathcal{V}}$	
	0.61
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	1.22
0.45 0 0.18 0	6.93
	3.47
2.22	27.93
<u>6.14</u> <u>6</u>	93.69
13.1	113.75

The 14 day EC₅₀ and  $C_{50}$  values for frond count were 0.91 and 1.91 mg a.s./L, respectively.

## Table CA \$2.7/01-4 Stimmary of frond counts at day 14

Endpoint	Effect concentration (mg a.s./L)
EC ₂₅	0.91
EC ₅₀	1.91



Ô

The 14-day EC₂₅ and EC₅₀ values for growth rate were 0.47 and 2.65 mg a.s./L, respectively.

	Table CA 8.2.7/01-5	Summary of growth rate µ at day 14
--	---------------------	------------------------------------

Table CIT 0.2.7701-5 Summary of	growth rate µ at day 14		
Endpoint	Effect concentrat	tion (mg a.s/L)	
EC ₂₅	1.47	Å.	
EC ₅₀	2.65	O,	
		4	

The resulting NOEC and LOEC values after 14-days were 0.24 and 0.5 mg/a.s./L, respectively.

#### NOEC AND LOEC values Table CA 8.2.7/01-6

	.f	$\bigcirc$	1, ~	
Endpoint	Effect	concentration (mg a.s	s./b) ~~	
NOEC	0.24	N O C	S &	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
LOEC	Ø 0.45		§ N	Ô, Ô,
	4. <i>6</i> °		ja v	

#### III. Conclusion

In a 14-day toxicity study, Duckweed (Lemba gibba G3) were exposed to KWG 4168 a mean mean mean field test concentrations of 0.12, 0.24, 0.45, 0,87, 2.22, 6.14 and 130 mg at Lunder static condition. The 14-day NOEC and EC₅₀ values based on growth rate were 0.24 and 2.65 mg a. L, respectively. The percent growth inhibition (frond number) in the treated cather as compared to the control ranged from 0 to 95.5%. The EC₅₀ values based on front number was 1.91 mg a.s. (b).

Chlorosis was recorded on day 9, day 12 and day 14 in the concentrations from 2,02 to 13.1 mg a.s./L.

#### Assessment and conclusion by applicants

Validity criteria according to the OECD 221 (2006) guideline have been assessed as part of the non-GLP recalculation report presented in the subsequent study. This study was 14-days in duration therefore the data have been re-assessed in order to determine 7 day EC vo values based on growth rate

The study is considered acceptable. The EG, values based on frond number was 1.91 mg a.s./L.

A supplemental statistical p-analysis report (Moto342 001-1) was submitted in order to calculate 7-

The EGs values basedon fi analysis report (<u>Medual 201-1</u>) we been summarised below.



Data Point:	KCA 8.2.7/02
Report Author:	
Report Year:	2008
Report Title:	Non-GLP recalculation report: KWG 4168 - toxicity (14 days) to Lemna gobba
-	G3
Report No:	DOM 28002
Document No:	<u>M-303421-01-1</u>
Guideline(s) followed in	Originally reported under US-EPA FIFRA § 123-2, Tier 2 Non-target Aquatic
study:	Plant Toxicity Recent recalculation is based on OFCD 221 Lemia sp. Growth Inhibition Test (March 23, 2006)
	Inhibition Test (March 23, 2006) $\checkmark$ $\checkmark$ $\checkmark$
Deviations from current	None $\sqrt{2}$ $\sqrt{2}$ $\sqrt{2}$
test guideline:	
Previous evaluation:	yes, evaluated and accepted $\sqrt{2}$
	RAR (2010), RAR (2017)
GLP/Officially	No, not conducted under GLP Officially recognised testing facilities
recognised testing	
facilities:	
Acceptability/Reliability:	Yes $\mathcal{A}$ $\mathcal{Y}$ $\mathcal{Y}$ $\mathcal{O}$ $\mathcal{A}$ $\mathcal{Y}$ $\mathcal{O}$

The aim of this non-GLP recalculation report was to fulfil the OECIO 221 requirements at least partly, which ask for the (0-7 day)-ECG for growth fate of frond number. An additional EC₃ calculation for the second endpoint (total frond area of plants or dry weight of plants) according to the new OECD 221 (2006) was not possible, because po such aw data exist for this study.

The 7-day  $E_rC_{50}$  of spiroxamine to Lemna subba 63 is 678 mg a.s./E, based on mean measured concentrations.

- I. Materials and Method
- A. Materials

Refer to M-00097-00 for methods of the biological tes

B. Study Design

The EC for growth rate of frord number was calculated by the applicant. Recalculation was done using the commercial program Tox Rat Professional.

(I)

#### II. Results and Discussion

Validity criteria according to the OECD 22 (2006) guide me met.

• The doubling time in the control must be less than 2.5 days (60 hours) corresponding to approximately a seven fold increase in seven days and an average specific growth rate of 0.275 d (the doubling time in this test for Day 07 was 2.4 days).

 $\bigcirc$ 

At day 7, the final frond counts were 88, f7, 83, f2, 75, 66, 67, 60, 41 and 17 at control, solvent control, pooled controls, 0, 12, 0.24, 0.46, 0.87, 2.22, 614 and 13.1 mg a.s./L, respectively. From day 0 to 7, the average growth rates for frond number were 0.285, 0.265, 0.275, 0.255, 0.261, 0.243, 0.245, 0.231, 0.174 and 0.045 at control, solvent control, pooled controls, 0.12, 0.24, 0.45, 0.87, 2.22, 6.14 and 13.1 mg a.s./L, respectively. Pocent inhibition of average growth rate for frond number from day 0-7 values were 7.1 (5.0, 11.8, 10.8, 16.2, 36.7 and 83.5% at 0.12, 0.24, 0.45, 0.87, 2.22, 6.14 and 13.1 mg a.s./L, respectively.

Table CA 82.7/02-10° Frond numbers, average growth rates and % inhibition

Mean*) measured concentration (mg a.s./L)	Final frond no. (replicate means, day 7)	Average growth rates for frond no. (day 0-7) [1/day]	% inhibition of average (day 0-7) growth rate for frond no.
Control	88	0.285	



Mean*) measured concentration	Final frond no. (replicate means, day 7)	Average growth rates for frond no. (day 0-7)	% inhibition of average (day 0-7) growth rate °
(mg a.s./L)		[1/day]	for frond no.
Solvent control	77	0.265	
Pooled controls	83	0.275	
0.12	72	0.255	\$.1 × ~
0.24	75	0.261	5.0
0.45	66	0.243	11.8
0.87	67	0.2450	10.8 2 2 2
2.22	60	0.23	$\begin{array}{c} 10.0 \\ 16.2 \\ 36 \\ 7 \\ \end{array}$
6.14	41	0.0.74	36.7
13.1	17	«Ø.045	83.9

*): Because of missing analytical data at day 7, mean measured data (day 0-7) were calculated based on initial (day 0) and a (day 14) data from the study, which is also supported by relative stable recoveries of the testmem over the elapsed time of 14 days

The resulting NOEC and LOEC values at day 7 were 0.24 and 045 mg a.s./L, respectively The E  $C_{50}$  value was 6.78 mg a.s./L, respectively with a corresponding 95% confidence interval of 3.57 to 12.54 mg a.s./L.

#### Table CA 8.2.7/02-2 Results for the endpoint frond number based on growth rate

$\begin{array}{c c c c c c c c c c c c c c c c c c c $		Q*	
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	Endpoint (0-7 day)		Effect on frond no. [mg.a.s./L]
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	E _r C ₁₀ (Cl 95%)		2006 (0,06-3.79)Q
LOEC Y A Q AS C Y Y	ErC ₂₀ (Cl 95%)		
LOEC 'N A C Q45 C C C C	ErC ₅₀ (Cl 95%)	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	
	LOEC		
INOLC Y Y Y Y Y	NOEC	the test of te	

#### III. Conclusion

The 7-day  $E_rC_5$  of sphoxamitie to Lemna gibba 33 is 578 new a.s. 15, based on mean measured concentrations

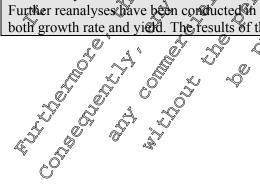
Ľ

## Assessment and conclusion by applicant:

This recalculation report was produced in order to determine a 5 day EC₅₀ based on growth rate. Validity criteria according to the OECD 21 (2006) guideline were met.

• The doubling time in the control must be less than 2.5 days (60 hours) corresponding to approximately a seren-fold increase in given days and an average specific growth rate of 0.275 d⁻¹ (the doubling time in this test for loav 0-7 was 2.4 days and the growth rate was 0.285 d⁻¹).

The validity criterion was met therefore the endpoint derived here is considered to be acceptable. Further reanalyses have been conducted in order to determine  $EC_{10}$ ,  $EC_{20}$  and  $EC_{50}$  values in terms of both growth rate and yield. The results of these reanalyses have been summarised below.





Data Point:	KCA 8.2.7/05
Report Author:	
Report Year:	2020
Report Title:	Calculation of EC10, EC20 and EC50 values for Lemna gibba with KWG 4068 in
	a Lemna sp. growth inhibition test
Report No:	0471836-ECO35
Document No:	<u>M-760417-01-1</u>
Guideline(s) followed in	None V V V
study:	
Deviations from current	None $\nabla $
test guideline:	
Previous evaluation:	No, not previously submitted
GLP/Officially	No, not conducted under GLP/Officially recognised testing facilities
recognised testing	
facilities:	
Acceptability/Reliability:	Yes $\underline{A}$ $\underline{\partial}$ $\underline{\partial}$ $\underline{\partial}$ $\underline{Q}$ $\underline{Q}$ $\underline{\partial}$ $\underline{\partial}$ $\underline{\partial}$ $\underline{\partial}$ $\underline{\partial}$ $\underline{\partial}$ $\underline{\partial}$

The report <u>M-006497-01-1</u> on the effects of exposure to KWG 4168 on the growth of *Lemna gibba* did not provide estimates of  $EC_{10}$  or  $EC_{20}$  values. Therefore, these values as well as EC solutes have been calculated in accordance with the Annex to Comp. Reg 283/2013.

The resulting EC₁₀, EC₂₀ and EC₅₀ values for yield at 7 d were 0 22' (95% CL: 0.08 – 640), 0.62 (95% CL: 0.31 – 0.95) and 3.02 (95% CL 2.21 – 4.06) for a s. L. respectively.

The resulting  $EC_{10}$ ,  $EC_{20}$  and  $EC_{50}$  values for yield at 14 d were  $0.56 \pm 95\%$  CE: 0.33 - 0.76), 0.93 (95%CL: 0.67 - 1.15) and 1.99% (95%CL: 1.02 - 2.34) mg as./L respectively. For growth rate after 14 d, the  $EC_{10}$ ,  $EC_{20}$  and  $EC_{50}$  values were 1.26 (95%CL: 1.05 - 1.44), 1.82 (95%CL: 1.61 - 2.01) and 3.17 (95%CL: 2.94 - 3.45) mg as./L, respectively.

#### I. Methods

The statistical valuation was performed with statistical software ToxRatPro v3.3.0.

Effect concentrations with 10, 20 and 50% from the test itera treatment when compared to the controls were determined for yield after 7 and 14 days exposure and growth rate of frond number after 14 days exposure. A Weibull regression was performed, with confidence limits for the  $EC_x$  values estimated according to Fieller's theorem. Effect that for this study C

#### II. Results and Discussion

## Yield (frond number) at days

Regarding the calculation of  $C_{10}$ ,  $EC_{20}$  and  $EC_{30}$  values for yield at 7 d, a statistically significant concentration/response was found  $p(F) \neq 0.001$ ) for this parameter.

The resulting  $EC_{10}$ ,  $EC_{20}$  and  $EC_{50}$  values and the respective confidence intervals are represented in the following table below.

# Table CA 8,27/05-1Results of the Webull analysis of yield at 7 d: Selected effective concentrations $\sqrt[3]{2}$ $\sqrt[3]{2$

	, <u>, , , , , , , , , , , , , , , , , , </u>	Yield [mg a.s./L]	
A Parameter	(95 % confidence interval)	EC ₂₀ (95 % confidence interval)	EC ₅₀ (95 % confidence interval)
Effect on yield at 7 d	0.22 (0.08 - 0.40)	0.62 (0.31 - 0.95)	3.02 (2.21 – 4.06)



The resulting  $EC_{10}$ ,  $EC_{20}$  and  $EC_{50}$  values of 0.22 (95% CL: 0.08 – 0.40), 0.62 (95% CL: 0.31 – 0.95) and 3.02 (95% CL: 2.21 – 4.06) mg a.s./L, respectively, meet the goodness of fit criteria showing a significant concentration/response relationship, and therefore the estimated ECx values are considered reliable.

#### Yield (frond number) at 14 days

Regarding the calculation of  $EC_{10}$ ,  $EC_{20}$  and  $EC_{50}$  values for yield at 14 d, a statistically significant concentration/response was found (p(F) <0.001) for this parameter.

The resulting  $EC_{10}$ ,  $EC_{20}$  and  $EC_{50}$  values and the respective confidence intervals are represented in the following table below.

Table CA 8.2.7/05-2	Results of the Weibull ana	lysis of yield at 14 d	Selected	effective	ncentrations
	Results of the Weibull ana (EC _x ) of the test item and t	heir 95%-confidenc	e limits	~ ^ ~	

	[™] [™] Yield [™] [™] [™]
Parameter	$EC_{10}$ $C$ $CC_{20}$ $C$ $CC_{50}$ $EC_{50}$ $C$
rarameter	(95 % confidence (95 % confidence (95 % confidence)
	interval) interval) 🖉 🖓 interval)
Effect on yield at 14	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$
d	(0.33 - 0.76) $(1.37 - 1075)$ $(1.37 - 2.24)$

The resulting EC₁₀, EC₂₀ and EC₅ values of 0.56 (95% CL: 0.33 - 0.76), 0.93 (95% CL: 0.67 - 1.15) and 1.99 (95% CL: 1.71 - 2.349 mg at the period of the store of the store of the relation of the relation of the store of the

#### Growth rate (frond number) at 14 days

Regarding the calculation of  $\mathbb{DC}_{10}$ ,  $\mathbb{EC}_{20}$  and  $\mathbb{EC}_{50}$  values for growth rate at 14 d, a statistically significant concentration/response was found  $\mathbb{P}(F) = 0.001$ ) for this parameter.

The resulting EC( $\frac{1}{6}$ , EC) and EC  $\frac{1}{50}$  values and the respective confidence intervals are represented in the following table below  $\frac{1}{2}$ 

# Table CA 8,2.7/05-3 Results of the Weibull analysis of growth rate at 14 d: Selected effective pricent ations (ECx) of the test item; and their 95%-confidence limits

* 1/					
	$\sim$	4		Growth rate mg a.s./L]	
	ST .	∆ <b>£</b> Č	10 1 2	<b>EC</b> 20	EC ₅₀
Parameter 🧔	Í	) (95 🐝 con	fidence 📎	(95 % confidence	(95 % confidence
		Öinter		interval)	interval)
Effect on growth	<b>V</b>	2 J2	6 Q 4	1.82	3.17
rate at 14 d		(1.05-	1.44	(1.61 – 2.01)	(2.94 - 3.43)
_^())	0/			0, -	

The resulting EC₁₀, EC₂₀ and EC₅₀ values of 1.26 (95%CL: 1.05 - 1.44), 1.82 (95%CL: 1.61 - 2.01) and 3.17 (95%CL: 2.94 - 3.43) and a.s. L, respectively, meet the goodness of fit criteria showing a significant concentration/response relationship, and therefore the estimated ECx values are considered reliable.

#### III. Conclusion

The resulting  $EC_{10}$   $EC_{20}$  and  $EC_{50}$  values for yield (frond number) at 7 days were determined to be 0.22, 0.62 and 2.02 mg a.s./L. The resulting  $EC_{10}$ ,  $EC_{20}$  and  $EC_{50}$  values for yield (frond number) at 14 days were determined to be 0.56, 0.93 and 1.99 mg a.s./L. The resulting  $EC_{10}$ ,  $EC_{20}$  and  $EC_{50}$  values for yield (frond number) at 14 days for growth rate (frond number) at 14 days were determined to be 1.26, 1.82 and 3.17 mg a.s./L.

## Assessment and conclusion by applicant:

The statistical re-evaluation of the data was conducted in order to complete the data set for  $EC_x$  values for yield and growth rate. 7-day  $EC_x$  values based on growth rate have already been calculated in the 2008 re-calculation report (M-303421-01-1) therefore calculation of these values was not repeated



here. 14-day EC_x values for growth rate and 7-day and 14-day EC_x values based on yield have been determined.  $Q_n^{\circ}$ 

The lowest  $EC_{50}$  remains the 14-day value determined in the original study report of 1,910 µg as /L. The 7-day  $E_rC_{50}$  value was determined to be 6,780 µg a.s./L. In order to maintain a conservative risk assessment, the original  $EC_{50}$  of 1,910 µg a.s./L shall be taken as the critical endpoint determined from this algal study.

The values determined in the re-evaluation work are considered to be fully valid

Data Point:	KCA 8.2.7/03
Report Author:	
Report Year:	
Report Title:	14C-KWG 4168- tox(city (14)) to Eemist gibba $43$
Report No:	DOM 97017 O C C C C C C C C C C C C C C C C C C
Document No:	<u>M-006540-01-1</u> A & & Q & & O & O & & A
Guideline(s) followed in	FIFRA Guideline 123,2 Growth and Reproduction of Aquatic Plants (Fier 2)
study:	
Deviations from current	M-006540-01-1 FIFRA Guideline 123-2 Growth and Reproduction of Aquatic Plants (Fier 2) None yes, evaluated and accepted
test guideline:	
Previous evaluation:	
	$RAR_{4/2}2010)$ $x$ $RAR(2017)$ $x$ $RAR(2017)$ $x$ $RAR(2017)$
GLP/Officially	Yes conducted under GLP Officially recognised sesting facilities
recognised testing	
facilities:	
Acceptability/Reliability:	Yes A S OF SY A
&	

#### Executive Summar

This study was conducted in order to evaluate the toxicity of ¹⁴ KWG 4168 to Duckweed (*Lemna gibba* G3). The est objective was to determine the 14-day  $EC_{25}$  and  $EC_{50}$  values as well as the NOAEC and LOAEC values of ¹⁴C-KWG 4168 for the test species.

In a 14-day toxicity study, Duckweed (Lemma gibba G3) were exposed to ¹⁴C-KWG 4168 at mean measured test concentrations of 0.37, 0.70, 1.38, 2.73, 5.28 or 10.42 mg a.s./L under static conditions. Growth was determined by counting from numbers on day 2.57, 7, 9, 12 and 14 and by determination of the biomass, based on the dry weights of the whole plant on day 14. The 14-day NOEAC and EC₅₀ values based on from number were 0.60 and 2.76 mg a.s./L respectively. The 14-day NOEAC and EC₅₀ values based of biomass were 2.73 and 9.38 mg as./L respectively.

Frond count values at concentrations  $\geq 138$  mg/L on day 14 were significantly different from the pooled controls. Chlorosis was accorded from day 510 day 14 in the two highest test levels and also from Day

12 to Day 14 at 2.73 mg/L.
 Materials and Methods
 A. Materials
 Test Material
 Lot/Batch #
 Description:
 Density:
 Not reported



#### Treatments

11 catilicities	
Test rates:	Measured: 0.37, 0.70, 1.38, 2.73, 5.28 and 10.42 mg a.s./L Nominal: 0.3, 0.6, 1.2, 2.4, 4.8 and 9.6 mg a.s./L
Solvent/vehicle:	Acetone
Analysis of test concentrations:	Acetone Yes, mean measured concentrations represent 109-123% of nominal Duckweed ( <i>Lemna gibba</i> G3)
Test organisms	
Species:	Duckweed ( <i>Lemna gibba</i> $G3$ )
Source:	Yes, mean measured concentrations represent 109-123% of nominal Duckweed ( <i>Lemna gibba</i> G3) Dr. Janet Slovin, Hortfeulture CropsQuality Laboratory, U.S. Department of Agriculture, Beltswille, MD, U.S.
Acclimatisation period:	Lemna gibba G3 taken from <12 day old stock culture at test initiation
Test design	
Test vessel:	400 mL glass distres: 10 cm diameter @ 6 cm height #
Test medium:	Hoaglands En S S S S S
<b>Replication:</b>	Three test@essels?
No. animals/vessel:	Five plants of three fronds per vessel (16 fronds per vessel)
Duration of test:	Three test coessels for the plants of three fronds per vessel (16 fronds per vessel) 14 days 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5
Environmental test	
conditions	Test vessels incubated ap $25 \pm 2^{\circ}C$
pH:	Test initiation; 3.0 to 5.3; Test termination 5.2 to 5.7
Photoperiod: 🔗 🔸	Continuous illumination of 51229ux provided by overhead cool white
Temperature pH: Photoperiod: 7 B. Study Design	Y L G X & SY
Duckweed was exposed for	14 days under static conditions to mean measured concentrations of 0.37,

Duckweed was exposed for 14 gays under static conditions to mean measured concentrations of 0.37, 0.70, 1.38, 2.73, 3.28 and 10 42 mg as./L. Test vessels were incubated at  $25 \pm 2^{\circ}$ C and continuously illuminated at 122 fax by coverhead coo-whit lights for the duration of the study. Five plants, consisting of three fronds each, were aseptically added to each test vessel using a sterile inoculum hook. Hoagland is medium was used in the test. Frond counts were made using a highted magnifying lens on study days 2, 5, 7, 9, 12 and 14 (fronds visible projecting beyond the edg of the parent frond were counted) to determine growth.

Growth data expressed as frond courts and biomass (based on dry weights of plants) on day 14 were used to conduct statistical analysis, chi-square test to determine the normality of the data set and Bartlett's test for homogeneity of variances. The non-parametric Kruskal-Wallis' and Dunn's Multiple Comparison test were used to determine significant differences between the control and treatment groups for data which did not fit a normal distribution. In case of frond number, statistical analyses were performed in comparison to the pooled controls (solvent control and pure medium control). In case of biomass, statistical analyses were compared to the solvent control.

#### **Results and Discussion** II.

The study was conducted to an older EPA test guideline and no specific validity criteria were included in the report.



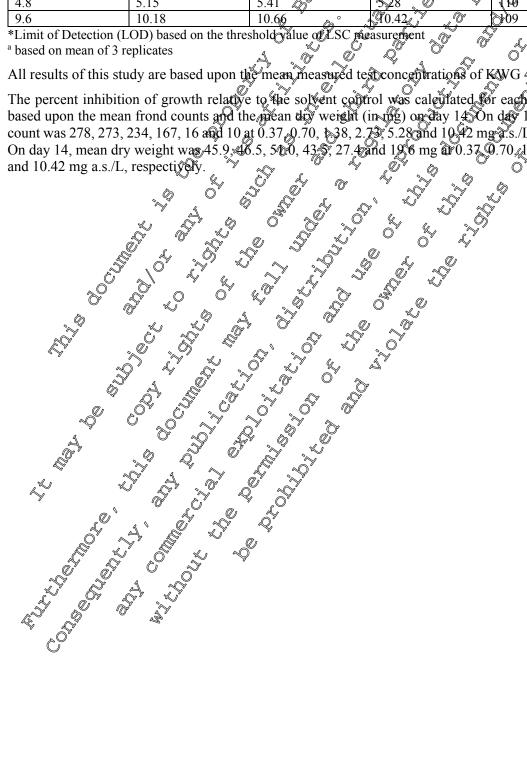
The measured concentrations determined during the test are summarised in the table below.

Nominal	Measured con	centration (mg a.s./L)	Mean measured	% of nominal
concentration	Day 0 ^a	Day 14 ^a	concentration (mg a.s./L)	× 2 2
(mg a.s./L)			(mg a.s./L) 🔊	
Control	<0.03*	<0.03*	<0.03*	- 5 5
Solvent control	<0.03*	<0.03*	<0.03*	
0.3	0.37	0.37	0.37	123
0.6	0.69	0.70	0.70	QU7 3 2
1.2	1.37	1.38	1.38	0 ¹¹⁵ Q 0 [*]
2.4	2.70	2.75	2.75 ° °	5 114 J
4.8	5.15	5.41	5,28 0 ~	
9.6	10.18	10.66 °	910.42 × 0	0109 200

Limit of Detection (LOD) based on the threshold Ŷ

All results of this study are based upon the mean measured texpconcentrations of KWG 4168

The percent inhibition of growth relative to the solvent control was calculated for each concentration based upon the mean frond counts and the prean div weight (in mg) on day 14. On day 14, recan frond count was 278, 273, 234, 167, 16 and 10 at 0.37, 0.70, 138, 2.73, 5.28 and 10 42 mg a.s./L, respectively. On day 14, mean dry weight was 45.9, 46.5, 560, 43 5, 27.4 and 19 6 mg at 0.37, 0.70, 1.38, 2.73, 5.28 and 10.42 mg a.s./L, respectively.





Mean frond	Percent inhibition	Mean dry weight	Percent inhibition
counts on day 14	(frond number)	in mg on day 14 🗋	(dry weight) on
	on day 14	Ď	day 14 🦉 🏠
277		&	* , \$
		39.4	\$7 ~~~
278	-0.3→0*)	45.9	-17 <del>~0*</del> ) ~ ~ ~
273	1 Ö	46.5	-18++0*)~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
234	16	51.0 Q	$29 \rightarrow 0$
.67	40 🖌	43.5 ⁰	
6	94	27.	30 J. C. C
0	96	19.6 Q	
	<b>ounts on day 14</b> 77 78 73 34 67 6	ounts on day 14     (frond number) on day 14       77        78     -0.3 $\rightarrow$ 0*)       73     1       34     16       67     40       6     94       0     96	ounts on day 14       (frond number) on day 14       in mg on day 14 $77$ $77$ 39.4 $78$ $-0.3 \rightarrow 0^*$ )       45.9 $73$ 1       46.5 $34$ 16       51.0 $67$ 40       43.5 $6$ 94 $27.4$

Table CA 8.2.7/03-2	Percent inhibition	of growth on day 14

*): Negative values for percent inhibition indicates stimulation. Stimulation of growth as observed very often near the effect threshold is going unnoticed. Therefore such values are settled as zero before further calculations.

The 14-day EC₂₅ and EC₅₀ values for frond count were 1.82 and 2.76 mg a.s./Le respectively, the NOEC and LOEC values for frond count were 0.00 and 3.38 mg a.s./19 respectively. The 14 day EC25 and EC50 values for biomass were 5.92 and 9.38 mg a. 42, respectively, the NOEC and LOBC values for Giomass were 2.73 and 5.28 mg a.s./L, respectively 

#### Summary of endpoints 2 Table CA 8.2.7/03-3

Endpoint	Effect concentration (mg. 8.5./L)
	Evend number @ Siomass @
EC ₂₅	3.82 A 1 5.92 A
EC ₅₀	2.76 2.76
NOEC	
LOEC	

#### III. Conclusion

In a 14-day toxicity study, Puckweed (Lemna abba (2)) were exposed to 14C-KWG 4168 at mean measured test concentrations of \$37, 0.70, 1.38, 2.73, 5.28 or 10.42 mg a.s./L under static conditions. Growth was determined by counting frond numbers on day 2, 5, 5, 9, 12 and 14 and by determination of the bomass, based on the dry weights of the woole plant on day 14. The 14-day NOEAC and EC₅₀ values based on frond number were 0.70 and 2.76 mg ass./L respectively. The 14-day NOEAC and EC50 values based on biomass were 203 and 9.38 mg a.s./ respectively.

#### Assessment and conclusion by applicant

Validity criteria according to the OECP 221 (2006) Buildeline have been assessed as part of the non-GLP regulation report presented in the subsequent study. This study was 14-days in duration therefore the data have been re-assessed in order of determine 7-day EC₅₀ values based on growth rate and sield. Q

The study is considered acceptable. The 14-day EC₅₀ value based on frond number was 2.76 mg a.s./L.

A supplemental statistical re-analysis report (M-303443-01-1) was submitted in order to calculate 7day endpoints, where possible. This has been summarised below.



KCA 8.2.7/04
2008
Non-GLP recalculation report: 14C-KWG 4168- toxicity (14 days) to Lemba
gibba G3
DOM 28003
<u>M-303443-01-1</u>
Originally reported under US-EPA FIFRA § 123-2, Tier 2 Non-target Aquatic
Plant Toxicity Recent recalculation is based on QCD 221 Lemna sp. Growth Inhibition Test (March 23, 2006)
Inhibition Test (March 23, 2006) 🖉 🧟 🖉 🖉
None
yes, evaluated and accepted $\sqrt{2}$ $\sqrt{2}$ $\sqrt{2}$
RAR (2010), RAR (2017)
No, not conducted under GLF Officially recognised testing facilities
Yes i y y y y y

The aim of this non-GLP recalculation report was to forfil the OECID221, requirements, which ask for the (0-7 day)  $EC_{50}$  for growth rate of frond number and an additional  $EC_{50}$  growth rate calculation for the second endpoint (dry weight of plants), which was done for the total elapsed test period of 14 days.

Based on mean measured concentrations, the 7-day ErC 50 (frond number) of spiroxamine to Lemna gibba G3 is 5.60 mg a.s./L and the 14 day  $E_r C_{50}$  (dry weight) is 212 mg a.s./L

#### Materials and Methods I.

#### A. Materials

Ô <u>p-01-1</u> for methods of the biological test Refer to M-0065 Ś

## B. Study Design

The EC₅₀ for growth rate of from number and dry weight was calculated by the applicant. Recalculation was done dising the commercial program ToxRat Professional.

#### II.[°] Results and Discussion

Validity criteria & ording to the OECD 221 (2006) guideline were met.

The doubling time in the control must be less than 2.5 days (60 hours) corresponding to approximately a seven-fold increase in seven days and an average specific growth rate of 0.275 d the doubling time in this test for Day 0-Z was 2.5 days).

At day  $\hat{\mathcal{P}}$ , the percent mhibition of average grow rate for frond number for each concentration was -1.6, 6, 1, -1.6, 7.8, 42.1 and 98.8, at 0.3, 0.70, 1.38, 2.73, 5.28 and 10.42 mg a.s./L, respectively.

Mean*) measured	Final frond n@	Average growth rates	% inhibition of average
concentration [mg	(replicate means, day 7)	for frond no. (day 0-7)	(day 0-7) growth rate
	Ő	[1/day]	for frond no.**)
Control &	\$	0.278	
Solvent control	\$2	0.273	
Popled controls	83	0.276	
0.37 _B O ^v	85	0.280	-1.6
0.70	83	0.275	0.1
1.38	85	0.280	-1.6
2.73	71	0.254	7.8

#### First endpoint Frond numbers, average growth rates and % inhibition Table CA 8.2.7/09-1



Mean*) measured concentration [mg a.s./L]	Final frond no. (replicate means, day 7)	Average growth rates for frond no. (day 0-7) [1/day]	% inhibition of average (day 0-7) growth rate ° for frond no.**)
5.28	37	0.160	42.1
10.42	12	0.003	988

*): Because of missing analytical data at day 7, mean measured data (day 0-7) were valculated bases on instal (day 0) and final (day 14) data from the study, which is also supported by relative stable recoveries of the test item over the elapsed time of 14 days.

**): Negative values mean stimulation of growth relative to the controls

At day 7, the percent inhibition of average growth rate for dry weight of plants for cach concentration was -1.3, -1.3, -4.9, 0.4, 15.2 and 25.9% at 0.37, 0.70, 1/38, 2.73, 5.28 and 10.42 mg a.s./L@respectivel

Table CA 8.2.7/04-2	Second endpoint: Dry	weight of plants,	average growth	rátes and%	inhipition Ø
	Second enapointe Dij	weiger of planes,		i aces and - / o	

	Ŷ	
Mean measured (day 0-	Final dry weight of 🎉	Average growth rates % inhibition of average
14) concentration [mg	plants (replicate means, _s	for dry weight of plants (day 0-14) growth rate
a.s./L]	day 14) 🔬 🦿	
		a plants*)
Control	50	$(02.32 \times 0) \xrightarrow{p} (02.32 \times 0) \xrightarrow{p} (02.$
Solvent control	39 & &	
Pooled controls	45 K O ^N N	0.224 ~ ~ ~ ~ ~ ~
0.37	46 8 6 6	0227 2 0 1.36 ~
0.70	47_0 _ 🗸 🖉	\$227 @ _ ~ ~ ~ ~ + ~ & &
1.38	51	0.235 2 2 4.9 0
2.73	44 4 0 4	0.223 0.4
5.28	27 6 2	Q.190 V V 15 2
10.42		@.166 ~ (c) 25,9
*): Negative values mean sti	imulation of growth relative	

The 7-day ErC₅₀ value for average growth rates for frond number was 5.60 mg a.s./L. The NOEC and LOEC values were 1.38 and 2.73 mg(a.s./L respectively. Ŵ

## Table CA 8,2,7/04-3 Results for the first endpoint (average growth rates for frond number)

Endpoint (0-7 day)	Affect of frond no. [mg a.s./L]
$E_r C_{10}$ (GV 95%)	3.51 (2.44-4,08)
$E_rC_{20}$ (CI 95%) $\sqrt[3]{4}$ $\sqrt[3]{4}$	4.13 (3.23-4.60)
ErC ₅₀ (CI 95%)	5.60 (5.15-6.19)
LOEC	Q.73 S
NOEC NO C NY NY	1.38

The 14-day ErC₅₀ value for average growth rates for dry weight of plants was 21.2 mg a.s./L. The NOEC and LOFC values were 2.73 and 5.28 mg a L, respectively.

#### Results for the second endpoint (average growth rates for dry weight of plants) Table CA 8.2.7/04

Endpoint (0-14@day)	Effect on dry weights of plants [mg a.s./L]
ErC ₁₀ (CI 95%	4.76 (2.85-6.03)
ErC ₂₀ (CI 95%)	7.96 (6.36-10.0)
$E_rC_{50}(CL \mathfrak{S})$	21.2 (14.9-51.8)
LOEC	5.28
NOEC OF A	2.73

HII. & Conclusion

Based on mean measured concentrations, the 7-day ErC₅₀ (frond number) of spiroxamine to Lemna gibba G3 is 5.60 mg a.s./L and the 14-day  $E_rC_{50}$  (dry weight) is 21.2 mg a.s./L.



#### Assessment and conclusion by applicant:

This recalculation report was produced in order to determine a 7-day  $EC_{50}$  for frond number based on growth rate.

Validity criteria according to the OECD 221 (2006) guideline were met.

• The doubling time in the control must be less than 2.5 days (60 hours) corresponding to approximately a seven-fold increase in seven days and an average specific growth rate of 0.275 d⁻¹ (the doubling time in this test for Day 0-7 was 2.5 days and the growth rate was 0.278 d⁻¹).

The validity criterion was met therefore the endpoints derived hore are considered to be acceptable? Further reanalyses have been conducted in order to determine  $EC_{10}$ ,  $EC_{20}$  and  $C_{50}$  values in terms of both growth rate and yield. The results of these reanalyses have been summarised below.

Data Point:	
Report Author:	
Report Year:	
Report Title:	Calculation of ECT0, EC20 and £050 values for benna bba with 140KWG 4168 in Lemna sp. growth inhibition test
Report No:	0471836-ECO36
Document No:	<u>M.760416-80-1</u> 0 2 2 2
Guideline(s) followed in	Norie & State of the second se
study:	
Deviations from current	None S S S S S S
test guideline:	
Previous evaluation:	No, not previously submatted 2 0 4
GLP/Officially	No, not conducted under GLP Officially recognised resting facilities
recognised testing	
facilities: 🔊 🖇	
recognised testing facilities:	Yes A A A A A A A A A A A A A A A A A A A

#### Executive Summary

The report M-006540-01-16 in the effects of exposure for  14 C-KWG 4168 on the growth of Lemna gibba did not provide estimates of EC or EC 20 values. Therefore, these values as well as EC 50 values have been calculated in accordance with the Annex to Com. Ref. 283/2013.

The resulting  $\mathbb{P}C_{10}$ ,  $\mathbb{E}C_{20}$  and  $\mathbb{E}C_{50}$  values for yield at 7 (were 2.34, 2.86 and 4.23 mg a.s./L, respectively. The resulting  $\mathbb{E}C_{10}$ ,  $\mathbb{E}C_{20}$  and  $\mathbb{E}C_{50}$  values for yield at 14 d were 1.29, 1.77 and 2.86 mg a.s./L, respectively. For growth rate after 14 d, the  $\mathbb{E}C_{10}$ ,  $\mathbb{E}C_{20}$  and  $\mathbb{E}C_{50}$  values were 2.53, 2.79 and 3.67 mg a.s./L respectively.

### I. Methods 🕅

The statistical evaluation was performed with statistical software ToxRatPro v3.3.0.

Effect concentrations with 10, 20 and 50% from the test item treatment when compared to the control were determined for yield after 7 and 14 days exposure, and growth rate after 14 days. A Probit regression was performed, with confidence limits for the  $EC_x$  values estimated according to Fieller's theorem for growth rate at 7 and 14 days, while a Weibull regression was performed, with confidence limits for the  $EC_x$  values estimated according to Fieller's theorem, for yield at 14 days.

s J



#### II. Results and Discussion

#### Yield (frond number) at 7 days

Regarding the calculation of  $EC_{10}$ ,  $EC_{20}$  and  $EC_{50}$  values for yield at 7 d, a statistically significant concentration/response was found (p(F) <0.001) for this parameter.

The resulting  $EC_{10}$ ,  $EC_{20}$  and  $EC_{50}$  values and the respective confidence intervals are represented in the following table below.

 Table CA 8.2.7/06-1
 Results of the Probit analysis of yield at 7 d: Selected effective concentrations (ECx) of the test item and their 95%-confidence@mits

		a'	~	$\bigcirc$	<u>~~</u> .0* _*
		A Yield [1	mg a&/L] 👝 °	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	
Parameter	EC10	~~// //	Cxy Ű		EC50 O
rarameter	(95 % confidence		onfidence /	or ( <b>95</b> %)	confidence
	interval) 💍	iati	erval) 🏹 💦	in 🖉 in	terval)
Effect on yield at 7 d	2.34	<u>v</u> 02	2.86 0	ġ Ø	4.23 💭 🛴 °
	(2.02 - 2.60)	× ~ 2.57	- 3.11)	\$ (3.9	8 – 49) 0

The resulting  $EC_{10}$ ,  $EC_{20}$  and  $EC_{50}$  values of 2.34 (95% CL 2.02 – 2.60), 2.86 (95% CL 2.57 3.11) and 4.23 (95% CL: 3.98 – 4.49) mg as /L respectively, meet the goodness of fit criteria showing a significant concentration/response relationship, and therefore the estimated EOx values are considered reliable.

#### Yield (frond number) at 14 day

Regarding the calculation of EC p EC₂₀ and EC₅₀ values for yield at 14 d a statistically significant concentration/response was found (p(F) < 0.00 for this parameter.

The resulting EC₁₀, EC₂₀ and  $C_{50}$  values and the respective confidence intervals are represented in the following table.

# Table CA 8.2.7/0622 Results of the Weibull analysis of yield at 14 d. Selected effective concentrations

, Q		ng a.s, E
Pasameter	EC C (95% confidence)	C20 EC50 onfidence (95 % confidence
~	A interval) O' K K int	interval)
Effect on yield at		.77 2.86
d 🖉	0.91 - 1057) ~ (1C41	-2.03) (2.59 $-3.17$ )

The resulting  $C_{10}$ ,  $C_{20}$  and  $C_{50}$  values of 1.29 (95%, CL: 0.91 – 1.57), 1.77 (95% CL: 1.41 – 2.03) and 2.86 (95% CL: 2.59 – 3.17) mg as L, respectively, meet the goodness of fit criteria showing a significant concentration/response relationship, and therefore the estimated ECx values are considered reliable.

## Growth rate (frond number) at 14 days

Regarding the calculation of  $\vec{b}C_{10}$ ,  $\vec{E}C_{20}$  and  $\vec{C}C_{50}$  values for growth rate at 14 d, a statistically significant concentration espense was found ( $\vec{p}(F) \neq 0.001$ ) for this parameter.

The resulting ECG, EC₂₀ and EC₅₀ values and the respective confidence intervals are represented in the following tables  $\frac{1}{2}$ 



Table CA 8.2.7/06-3	Results of the Probit analysis of growth rate at 14 d: Selected effective
	concentrations (EC _x ) of the test item and their 95%-confidence limits

concentrations (ECx) of the	e test item and then 5570-co	
	Growth rate [mg a.s./L]	
<b>EC</b> ₁₀	EC ₂₀	EC50 0
(95 % confidence	(95 % confidence	95 % confidence interval
interval)	interval)	🔗 interval)
2.53	2.79	367 5
(2.39 - 2.64)	(2,69-2.90)	
	EC10 (95 % confidence interval) 2.53	EC10EC20(95 % confidence interval)(95 % confidence interval)2.532.79

The resulting EC₁₀, EC₂₀ and EC₅₀ values of 2.53 (95% CL: 2.39 – 2.64), 2.79 (95% CL: 2.69 – 2.90) and 3.67 (95% CL: 3.20 – 3.67) mg a.s./L, respectively, meet the goodness of fit criteral showing a significant concentration/response relationship, and therefore the estimated ECx values are considered reliable.

#### III. Conclusion

The resulting EC₁₀, EC₂₀ and EC₅₀ values for field (frond number) at 7 days were determined to be 2.34, 2.86 and 4.23 mg a.s./L. The resulting EC₁₀, EC₂₀ and EC₂₀ and EC₂₀ and EC₂₀ and EC₂₀ and EC₅₀ values for yield (frond number) at 14 days were determined to be 1.29, 1.77 and 2.86 mg a.s./L. The resulting EC₁₀,  $EC_{20}$  and EC₅₀ values for growth rate (frond number) at 14 days were determined to be 2.53, 0.79 and 3.67 mg a.s./L.

### Assessment and conclusion by applicants

The statistical re-evaluation of the data was conducted in order to complete the data set for  $EC_x$  values for yield and growth rate. 7-day EC, values based on growth rate (frond number) and 14-day  $EC_x$ values based on growth rate (biomass) have already been calculated in the 2008 re-calculation report (<u>M-303443-01-1</u>) therefore calculation of these values was not repeated here.

The lowest  $E_rC_{50}$  determined was 3,670 µg  $\otimes$  5./L which is based on frond number after 14-days. The 7-day  $E_rC_{50}$  value based on frond number was determined to be 5,600 µg a.s./L. In order to maintain a conservative risk assessment the 14 day  $E_rC_{50}$  of 3,670 µg a.s./L shall be taken as the critical endpoint determined from this algal study.

The values determined in the re-evaluation work are considered to be fully valid.

# CA 8.2.8 Further testing on aquatic organisms

No further data with spiroxaphine technical are available. The studies presented above are considered sufficient to address the data requirements therefore no additional studies are considered to be necessary.

## Relevant literature on aquatic organisms

No relevant soientifically peer-reviewed open literature could be found on spiroxamine or its major metabolites, from an ecotoxicological perspective, on equatic organisms. Details of the literature search undertaken can be found in the M-CA Section 9 of the current submission.

# CA.8.3 Effect on arthropods

# CA 8.3.1 Effects of bees

Studies have been conducted using spiro amine technical which have been submitted and summarised here. Other studies are also available with the representative formulations which have been presented and summarised in M-CP Section 10.

The spidpoints are spinmarised below.

Table CA

× · · ·	G 01		• • • •
8.3.1	Summary of bee	toxicity studies	with spiroxamine

Organism	Test item	Test type	Endpoints	Reference		
Adult honey bee ( <i>Apis mellifera</i> )	Spiroxamine	Acute oral	48 h LD ₅₀ >100 μg a.s./bee	EU	<u>M-008208-01-1</u>	



Organism	Test item	Test type	Endpoints	Reference	
Adult bumble bee ( <i>Bombus terrestris</i> )	Spiroxamine	Acute oral	48 h LD ₅₀ >50.9 $\mu$ g a.s./bumblebee	NEW	<u>M-688128-01</u>
Adult honey bee ( <i>Apis mellifera</i> )	Spiroxamine	Acute contact	48 h LD ₅₀ 4.2 μg a.s./bee	EU	<u>M-008208691-1</u>
Adult bumble bee ( <i>Bombus terrestris</i> )	Spiroxamine	Acute contact	48 h LD ₅₀ >100 $\mu$ g a.s./bumblebee	<b>W</b> EW	<u>M-510841-04-</u>
Honey bee larva ( <i>Apis mellifera</i> )	Spiroxamine	Chronic larva (22 day emergence)	LD ₅₀ >33 µg (ass./larva NOED 33 µg (a.s./larva	NEW	<u>54-623362-01-6</u> 7

EU: previously evaluated as part of the original EU review and listed in EPSA conclusion and DAR NEW: new study or data generated since the previous Ro reviously fot submitted

#### Acute toxicity to bees CA 8.3.1.1

#### Acute oral toxicity CA 8.3.1.1.1

Data Point:	KCA 8.3.1 81/01 KCA 8.3.1 81/0
Report Author:	
Report Year:	1994 KWG 4168 Acute toxicity to honey bees Apis melliferal
Report Title:	KWG 4168-Acute toxicity to honey bees Apis mellifera
Report No:	BAY169(A)932259 L & & &
Document No:	Appl-008208-01-15         Appl-008208-01-15           YEPPO 170         Appl-002208-01-15
Guideline(s) followed in	YEPPO 170
study:	
Deviations from current	None of the of t
test guideline:	
Previous evaluation:	yes, evaluated and accepted DAR (1997), RAP (2010), RAR (2017) Wes, conducted under GDP/Officially recognised testing facilities
<u> </u>	DAR(1927), RAB(2010), RAR(2017)
GLP/Officially	Tes, conducted under GDP/Officially recognised testing facilities
recognised opsting	
facilities:	
Acceptability/Reliability/	Y
"Ó"	

#### Executive Summar

Honey bees (Apis methyera), Vere exposed of KW 416 in a 48-hour acute oral and contact toxicity test.

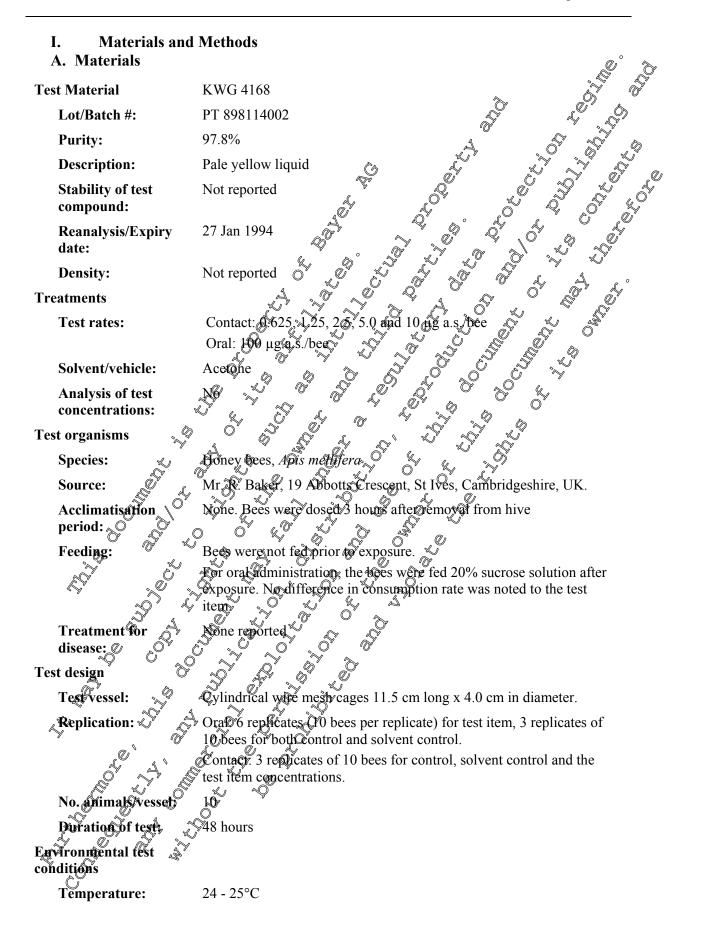
In the oral Toxicity study, KWCA168 Fas administered in the feeding solution at an application rate of 100 µg as./bee. An untreated control group and a solvent control group was also tested. The oral LD₅₀ values with 95% confidence, was gl'00 µg/a.s./bee.

In the contact toxicity study, KWG 4168 was administered to bees at concentrations of 0.625, 1.25, 2.5, 5.0 and 10 µg a.s./bee. The LO 50 value after 48-hour contact with KWG 4168 was 4.2 µg a.s./bee, with 95% confidence limits of 32 to 5.4 µg a s/bee.

The cumplitude prortative prortative for oral administration after 48 hours was 10 bees out of the original population of 60 bees. The total out of the control group was one bee out of the original 30 bees.

The semulative mortality for contact administration after 48 hours was 26 bees out of the original population of 30 bees when exposed to maximum 10 µg a.s./bee of KWG 4168. The total out of the control group was one bee out of the original 30 bees.







#### Photoperiod:

Darkness, except for during procedures where subdued lighting was used.

#### B. Study Design

This study was conducted in order to assess the acute toxicity of KWG 4168 on honey bees (*mellifera*) in oral and contact tests over 48 hours.

Sterile female honey bees (Apis mellifera) were collected from hives and dosed within 3 hours

Ten bees were placed in each cylindrical wire mesh cages 11.5 cm long x 4.0 cm in diameter. For the contact administration the bees were anaesthetised with CO₂ one vessel at a time  $\stackrel{\checkmark}{\xrightarrow{}}$  1.0 all droplet of the diluted test item was applied to the ventral thorax of each bee using a micrometer syringe.

For the oral administration a 50  $\mu$ l aliquot of the stock solution was added to 950  $\mu$ P of 20% success solution. 0.2 mL of this solution was administered per test vessel. It was assumed that the bees in the test vessel received similar doses of 20  $\mu$ L/beo of the test item.

For the oral test the test item was administered in the feeding solution at an application rate of  $100 \mu g$  a.s./bee. Control concentrations containing 20% sucress solution only were administered, as were solvent control concentrations of 1.0  $\mu g$  acetone/bes

Following oral administration of the test item the bees were fed a 20% sperose polution and no deviations between feeding patterns of this solution and the test item solution were noted.

For the contact test the concentrations of KWG 4168 applied were 625, 4.25, 2.5, 5.0 and  $10 \mu g$  a.s./bee to the ventral thorax at an application rate of 1.0 µL droptets (test substance dispersed in acetone). Toxic standard concentrations of 0.025, 0.050, 0.10, 0.20 and  $0.40 \mu g$  a.s./bee of the twee applied as were solvent control concentrations of  $1.0 \mu L$  acetore/bee.

The test vessels were kept in darkness (except for during procedures) and at 24-25°C with a relative humidity of 61-66%.

Observations on mortality were made at 24 and 48 hours and were defined as the absence of response to physical stimulation.

#### II. Results and Discussion A

Specific assessment of guideline validity criteria were not reported in the study report.

Cumulative mortality data are given below for the oral and contact routes of administration. All results are expressed in terms of the notatinal concentration.

Table CA 8.3.4.1.101 Cumulative mortality data for honey bees exposed to KWG 4168 for 48 hours (oral administration)

Cum	alativ	mo	rtality	by ret	blicate	(%)							
24 1	Dours A A A A												
¢,	2	3	4	<b>5</b> ,	<u> </u>	Total	1	2	3	4	5	6	Total
0	Ø	0°	-	¥- "(	) <u>*</u>	0	0	10	0	-	-	-	3.3
°0 ,	10	ő,Ø		- Ó ^Y	-	3.3	10	10	0	-	-	-	6.7
20	0 &			10	10	13.3	20	10	20	20	20	10	16.7
	24 h	24 bours 1 2 3 0 0 0 10	<b>24 bours</b> <b>1 2 3 3</b> 0 <b>0 0</b> 0 10 <b>0</b>	<b>24 Pours</b> <b>1 2 3 4</b> 0 <b>0 0 -</b> 0 10 <b>9</b>	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	<b>4 2 3 4 5 6</b> Total 0 <b>0 0 - - 0</b> 0 10 <b>9 - - 3</b> .3 <b>20 0 20 20 20 10 10 13 3</b>	24 bours         48 h           4         5         6         Total         1           0         0         0         -         -         0         0         0         0         0         0         0         0         0         0         0         0         10         10         13.3         10         20         20         20         10         10         13.3         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20         20	24 bours         48 hours           4         5         6         Total         1         2           0         0         0         -         -         0         0         10         10           0         10         9         -         -         -         0         0         10         10	Cumplative mortality by replicate (%)           24 bours         48 hours           4         2         3         4         5         6         Total         1         2         3           4         2         3         4         5         6         Total         1         2         3           0         0         0         -         -         0         0         10         0           0         10         2         -         -         0         0         10         0           0         10         2         -         -         0         0         10         0           0         10         2         -         -         -         3.3         10         10         0           20         20         20         10         10         13         20         10         20	24 bours       48 hours         4       5       Total       1       2       3       4         0       0       0       -       -       0       0       10       -         0       10       2       -       -       0       0       10       0       -         0       10       -       -       0       0       10       0       -         2       2       3       4       5       6       Total       1       2       3       4         0       0       -       -       0       0       10       0       -         0       10       20       -       -       3.3       10       10       0       -         2       0       20       20       10       13.3       20       10       20       20	Cumplative mortality by replicate %)           24 bours         48 hours           4         5         6         Total         1         2         3         4         5           4         2         3         4         5         6         Total         1         2         3         4         5           0         0         0         -         -         0         0         0         -         -           0         10         2         -         -         0         0         -         -           0         10         2         -         -         0         0         -         -           0         10         2         -         -         3.3         10         10         0         -           20         20         20         10         13         20         10         20         20         20	24 bours       48 hours         40       2       3       4       5       6         1       2       3       4       5       6         0       0       -       -       0       0       10       0       -       -         0       10       -       -       -       0       10       10       -       -       -         0       10       20       20       20       10       13       20       10       20       20       20       10

Initial population: 10° per replicate

Table CV 8.3.1.1.1/01-2 Cumulative mortality data for honey bees exposed to KWG 4168 for 48 hours

Nominal	Cumulati	umthative mortality (%)								
concentration	24 hours				48 hours					
(µg a.s./bee)	1	2	3	Total	1	2	3	Total		
Control	0	0	0	0	0	10	0	3.3		
Solvent control	0	0	0	0	10	0	0	3.3		



Nominal	Cumu	Cumulative mortality (%)										
concentration	24 hou	irs			48 hours							
(µg a.s./bee)	1	2	3	Total	1	2	3	Totat				
0.625	0	10	0	3.3	0	10	0	3.3 7 0				
1.25	20	10	0	10.0	20	10 🐧	0	£9.0 A				
2.5	40	20	60	40.0	40	30	60	43.3				
5.0	40	30	40	36.7	50	50 🔬	40 🔊	× 46,7,×				
10	80	70	60	70.0	90	9.0	80 0	860 4				
Initial population	• 10 per r	enlicate		Ô	`&	- L	st i					

Initial population: 10 per replicate

No marked reactions to exposure (other than death) were noted in any of the confol or jest any hal throughout the duration of the study.

The 24-hour oral and contact LD50 values for honey bees after exposure to KWG 4168 were >10 a.s./bee and 5.5 µg a.s./bee, respectively.

The 48-hour oral and contact LD50 values for honey Bees after exposure to KWS 4168 were 2100 µg a.s./bee and 4.2 µg a.s./bee, respectively. Ù

The 48-hour LD₅₀ values with 95% confidence limits for the reference substance, dimethorte, were 0.11  $\mu$ g/bee (0.085 – 0.13  $\mu$ g/bee) for the oral test and 0.12  $\mu$ g/ber (0.02) J¥ μg/bee) for the contact test, respectively.

#### III. Conclusion

Honey bees (Apis mellifera) were exposed to KWG4168 in a 48 hour acute oral and contact toxicity Ç, test. W  $\bigcirc$ 

The 48-hour oral and contact LD50 values for stoney bees after exposure to KWG 4168 were >100 µg a.s./bee and 4.2 µg a.s./bee, respectively.

The 24-hour LD₅₀ values with 95% confidence limits foothe reference substance, dimethoate, were 0.12  $\mu g/bee (0.095 - 0.14 \mu g/bee)$  for the oral test and  $0.14 \mu g/bee (0.11 - 0.14 \mu g/bee)$  for the contact test, respectively. The 48-hour LD50 values with 95% confidence fimits for the reference substance, dimethoate, were  $0.19 \mu g/bee (0.085 - 0.13 \mu g/bee)$  for the oral test and 0.12  $\mu g/bee (0.094 - 0.14)$ µg/bee) for the contact test, respectively

#### Assessment and conclusion by applicant:

Validity criteria according to the current OECD 213 (1998) and OECD 214 (1998) test guidelines has been assessed.

- Average mortality for the total number of controls must not exceed 10% at the end of the test (actual: max. 6.7% In the oral test and 3.3% in the contact test, respectively)
- The 24-hour LD₅₀ of the toxic standards to be 0.10 to 0.35 µg/bee for oral toxicity and 0.10 to (0.30 µg/bee for contact toxicity (actial: 24 hour LD₅₀ 0.12 µg/bee and 0.14 µg/bee in the oral and contact tests, respectively)

Validity criteria were met therefore the study is considered acceptable.

The 48-hour frai and contact LD values for honey bees after exposure to KWG 4168 were >100 µg a.s./bee and 4.2 µg a.s./bee, respectivel

THE STREET



Data Point:	KCA 8.3.1.1.1/02
Report Author:	;
Report Year:	2020
Report Title:	Spiroxamine tech.: Effects (acute oral) on bumblebees (Bombus terrestris b) in
	the laboratory
Report No:	143051105
Document No:	<u>M-688128-01-1</u>
Guideline(s) followed in	Regulation (EC) No. 1107/2009
study:	OECD (2017), Test No. 247: Bundhebees, Acutogral Toxicity Test, OECD Guidelines for the Testing of Chemicals
Deviations from current	Yes OECD 247 guideline (2017) The stock solution was potentallysed due to human error.
test guideline:	OECD 247 guideline (2017) $Q$ $Q$ $A$ $A$
	The stock solution was no analysed due to human error. This deviation did not have any detrimental impact on the study
	This deviation did not have any detrimental impact on the study
Previous evaluation:	No, not previously submitted 2 2 2
GLP/Officially	Yes, conducted under GLP/Officially recognised testing facilities
recognised testing	
facilities:	
Acceptability/Reliability:	Yes of y y y y y

°°° Executive Summary Bumblebees (Bombus terrestrie L) were exposed to spiroxamine technical in a 48-hour oral toxicity study. The purpose of the study was to determine the effects of spirovamine technical on the behaviour and survival of the test organisms. L. C. S.

and survival of the test organisms. Bumblebees were expressed to spirox amine technic at concentrations of 50.9, \$3.2, 19.9, 11.4 and 5.8  $\mu$ g a.s./bumblebee (nominally 100, 50, 25, 12.5 and 6,25  $\mu$ g a.s./bumblebee, respectively), a water control and solvent control and dimethorate as a reference item.

The NOED and OED values were  $50.9 \mu g$  a.s./bumblebee, respectively and the LD₅₀ value was >50.9  $\mu$ g a.s./bumblebee.

µg a.s./bumblebee.		, O
I. Materials and	Methods ~ ~ ~ ~	\$ }
A. Materials	Methods	
Test Material	Spiroxamine technical &	
Lot/Batch 🗱 🔔	AE 134 293-01-07	
Purity:	©97.0% w/w ~ ~ ~ ~ ~	
Description:	Light-yellow liquid	
Reanalysis/Expiry		
Aate: 🗸		
Density:	Not reported	
Treatments		
Test kates:	Nominal: 6.25, 12.5, 25, 50 and	100 µg a.s./bumblebee
	Measured: 5.8, 11.4, 19.9, 33.2 at	nd 50.9 µg a.s./bumblebee
Solvent vehicle:	Tween80	
Anatysis of test	Yes, $93 - 97\%$ of the nominal	
concentrations:		



Test organisms

**Species:** Bumblebees, *Bombus terrestris* L. (Insecta, Hymenoptera) Source: Koppert Deutschland GmbH, D-47638 Straelen 45.5 hours Acclimatisation period: 50% w/v sucrose solution ad libitum **Feeding: Test design** Cylindrical, latticed plastic cages (Nicot **Test vessel:** approx. 7.3 cm and a drameter of 2.2 cm ap the large and 1.7 small opening. **Replication:** 30 per treatment goup/og No. animals/vessel: Individually house **Duration of test:** 48 hours **Environmental test** conditions **Temperature:** ot during treatment procedures and observation) **Photoperiod:** 0 **B.** Study Design Bumblebees were exposed to spiro admine technical in an acute oral test over 48 hours. The design of the study was based on OECD 240 (2019) and SANCO 3029 99. The test organisms were adult female worker Bombus terrestris L. Acute oral to vierty of spiroxamine Ochnical to adult buildblebees was assessed by exposing 30 worker

Acute oral toxicity of spiroxamine achieved to adult buildblebees was assessed by exposing 30 worker bumblebees to nominal concentrations of 6.25, 42.5, 25, 50 and 100 ug a.s./bumblebee.

The test units consisted of cyludrical latticed plastic cages with a length of 7.3 cm and a diameter of 2.2 cm at the large and 1.7 cm at the small opening; the test organisms were kept in the test units throughout the exposure period. Temperature and relative humidity were kept at 25.0 to 25.4 °C and 58.7 to 62.6%, respectively during exposure. The bees were kept in darkness except for during observation. All test conditions were measured with suitable instruments.

The test item was applied to the bees as a diffation series of spiroxamine tech. in acetone and was transferred to 50 % w/v sucrose solution containing  $k^{\circ}_{0}$  v/v Tween80. The final treated feeding solutions contained 50 % w/v sucrose, maximum 5 % acetone w/w and 1 % v/v Tween80. For the solvent control, 50 % w/v sucrose solution containing 5 % w/w acetone and 1 % v/v Tween80 was used. For the water control, 50 % w/v sucrose solution was used. For the reference treatment, dimethoate was diluted in 50 % w/v sucrose solution.

Approximate 40 at food solution per bomblebee was provided in syringes which were weighed before and after introduction into the cages in order to determine the exact consumption.

The becomer observed after  $\Re$  (± 0.5 hours), 24 and 48 (± 2 hours) hours for mortality and behavioural abnormalities. Sub-tothal affects were defined by the categories of moribund (unable to walk, weak response to stimulus) and affected (reduced coordination).

Statistical analysis was performed on the data using ToxRat Professional, Version 3.2.1, ToxRat Solutions GmbH.



#### Analytical method

Samples of feeding diet were analysed using the validated analytical method M-688128-01reference M-688128-01-1 (see Doc MCA Section 4).

#### **Results and Discussion** II.

Validity criteria according to the OECD 247 guideline (2017) were met.

- Mortality in the water control should be  $\leq 10\%$  at the end of the test. If include control mortality should be  $\leq 10\%$  at the end of the test (actual 10% in both the water and solvent controls)
- Mortality in the toxic reference substance group should be 50% at the odd of the test actua 0° 100%) Ø)

Analytical verification of the feeding solutions at the highest and lowest test concentrations (nominally 100 and 6.25 µg a.s./bumblebee) confirmed the correct dosing of the bees with analytical recoveries of 97% and 93%, respectively.

At test termination, no mortality was observed for the treated bundblebee at any test concentration applied. Similarly, no mortality was observed in either the water or solvent control. There were no behavioural abnormalities observed many test concentration or ineither of the control.

For the reference item, at test termination there was 100% moreality to the organism's tested Ő n

Treatment group (µg	4 hours o	N D	24 hours	2	≫48 hours	
a.s./bumblebee)	Mortality	Abnorma	Mortality	Abnormad	Mortality	Abnormal
		gbehav@ur 🗌	🖋 mean)	Behaviour	( 🎾 mean)	behaviour
Water control		' (% mean) 🔬		(% mean)	L'Y	(% mean)
Water control	QC S			0	Ő.	0
Solvent control	Q X	ŏ ∕∕			0	0
Spiroxamine technical:	0		NO A		0	0
5.8 8						
5.8 Spiroxamine technical:		04 8	0 %	0 0	0	0
11.4		<i>.</i>		* ¥		
Spirox mine technical	0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,		Dð 🔊 🦏	Ģ.	0	0
19.9			k A	V		
Spiroxamine technical:	40 O	Q7 JO	00' 🔊	0	0	0
33.2						
Spiroxamine technical		0_0′ 、	Do O	0	0	0
50.9			ð			
Reference item: 4.5	0	96.4	100	-	100	-

Table CA 8.3.1.1.1/02-1 Summary of mortality and behavioural data observed

In the of a test the target dose levels of 6.25, 12.5525, 50 and 100 µg a.s./bumblebee would have been achieved if exactly 20 mg theated reeding solution were consumed by each exposed bumblebee. The mean food uptake was carculated considering all replicates per treatment group. However, actual food uptake in the treatment groups ranged between 5 and 51 mg per bumblebee. Bumblebees which did not consume at least 80 % of the mean food uptake per treatment group were excluded from the derivation of the end points, as well as from the calculation of the actual mean oral doses in the test and reference item treatment groups. This was done to avoid potentially overestimating the final endpoints. The actual mean oral doses following the adjustment for non-feeding bumblebees were 5.8, 11.4, 19.9, 33.2 and 50.9 pg a.s./bumblebee.

For the 5 11.4, 19.9, 33.2 and 50.9 µg a.s./bumblebee test item treatment group 27, 22, 18, 17 and 14 bumblebees were considered for the evaluation. For the water control (50 % w/v sucrose solution) and solvent control (50 % w/v sucrose solution containing 5 % w/w acetone and 1 % v/v Tween80) treatment groups 29 and 25 bumblebees were considered for the evaluation. At the end of the oral toxicity test (48 hours after application) 5.8, 11.4, 19.9, 33.2 and 50.9 µg a.s./bumblebee led to no mortality. No mortality



occurred also in the water control (50 % w/v sucrose solution) and in the solvent control (50 % w/v sucrose solution containing 5 % w/w acetone and 1 % v/v Tween80) treatment groups. No test item induced behavioural effects were observed at any time during the oral test.

The reference item target dose level of 4 µg dimethoate/bumblebee would have been achieved if active the second se 40 mg treated feeding solution was consumed per bumblebee. Considering bimblebees with a food uptake of at least 80 % of the mean food uptake the measured consumption corresponded to an avial oral dose of 4.5 µg dimethoate/bumblebee. For the reference item treatment group 28 burblebees were considered for the evaluation. The mortality in the reference item treatment group was 100 % (24 hours after application).

As the mortality in the test item treatment groups did not reach or exceed 10% abtest termination, LD₅₀, LD₂₀ and LD₁₀ were not statistically calculated. Thus, the LD₅₀, LD₂₀ and LD₁₀ for Spiroxamine technical were all considered to be  $>50.9 \ \mu g$  a.s. An imblebee.

#### III. Conclusion

Bumblebees (Bombus terrestris L) were exposed to spiro aming technical in a 48-hour oral toxicity study. The purpose of the study was to determine the effects of spiroxamine technical on the behaviour and survival of the test organisms.

As the mortality in the test item treatment groups did not reach of exceed 10% at test dermination, the LD₅₀, LD₂₀ and LD₁₀ were not statistically calculated. Thus, the DD₅₀, LD₂₀ and LD₁₀ were all considered to be  $>50.9 \ \mu g$  a.s./bumblebee. 1

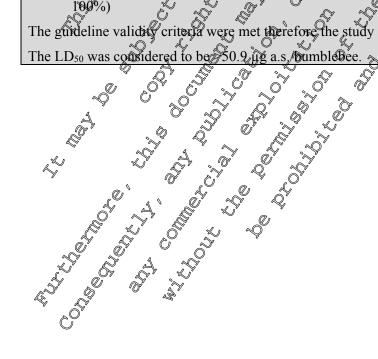
The oral NOED and LOED were calculated to be >50.9 µg a.s./b@mblebee

#### Assessment and conclusion by applicant

guideline (2017) were mor Validity criteria according to the current OBCD 247 This is the version of test guideline to which the study was conducted.

- Mortality of the stater control abould be  $\leq 1.0\%$  at the end of the test. If included, also solvent control mortality should be \$10% at the end of the test (actual: 0% in both the water and the solvent controls)
- Mortality if the toxic reference substance group should be 30% at the end of the test (actual: 100%)

The guideline validity criteria were met therefore the study is considered to be acceptable.





Data Point:	KCA 8.3.1.1.2/01
Report Author:	
Report Year:	2015
Report Title:	Effects of spiroxamine tech. (acute contact) on bumblebees (Bombus terrestris 1)
	in the laboratory
Report No:	88621105
Document No:	<u>M-510841-01-1</u>
Guideline(s) followed in	No specific guidelines available; study design based on OECD 214 (1298) Vat
study:	der Steen (2001) and ICPPR pon-apis group (2014)
Deviations from current	Yes OECD 246 (2017) It is noted that the study the not include analytical verification of the dosing solution used to treat the bees which is a requirement of the current OECD 246 test guideline, however this study was conducted pror to the issue of this formal
test guideline:	OECD 246 (2017)
	It is noted that the study did not include analytical verification of the dosing
	solution used to treat the bees which is a requirement of the current OECD 246
	test guideline, however this study was conducted proor to the issue of this formal
	OECD test guideline and & therefore considered to be valid and based of the
	accepted test methodology at the time of 0 2
Previous evaluation:	No, not previously submitted
GLP/Officially	Yes, conducted under GLP/Officially recognised testing facilities
recognised testing	
facilities:	
Acceptability/Reliability:	Yess y w y w y o y

#### CA 8.3.1.1.2 Acute contact toxicity

#### **Executive Summary**

Executive Summary Bumblebees (*Bombus terrestric*).) were exposed to spiroxamine technical in a 48-hour contact toxicity study. Exposure was at 100 µg as /bumblebee along with a water control and a solvent control. Dimethoate at 12 µg a.s./bee as a reference item was also tested.

Dimethoate at 12  $\mu$ g a.s./bee as a deference item was doo tested. Mortality in the reatment concentration of 100  $\mu$ g/s.s./bumblebee was 8% after 48 hours therefore the NOED and LO₂₀ values have been considered to be  $\geq$ 100 and 700  $\mu$ g a.s/bumblebee, respectively.

VOLD and DReso values have	
I. Materials and	Methods & V
I. Materials and A. Materials Test Material Lot/Batch #	Methods Spiroxamin@technical
Test Material	Spiroxamino technical &
Lot/Batch # A	FOTH008883
Purity; O	98.7% Copourless fiquit Not reported 19/06/2016
Description:	Coourless liquito
Stability of test	Not reported to a start a star
<b>co</b> mpound:	
Reanalysis/Expiry	1\$406/2016 L ^O ^v
date:	
Reanalysis/Expiry date: Density: Treatments	0.93 g/cm ² (20°C)
Treatments 0	õ
Treatments C Test rates:	Spiroxamin@ technical EDTH008883 98.7% CoPourlese fiquid Not reported 19/06/2016 0.93 g/cm (20°C) 100 µg a.s./L Acetone No
Solvent/vehicle:	Acetone
	No
concentrations:	



Test organisms	
Species:	Bumblebee, Bombus terrestris L.
Source:	Bumblebee colonies, healthy and queen-right, obtained from Biobest Belgium N.V., Ilse Velden 18, 2260 Westerlo, Belgium
Acclimatisation period:	19 hours 40 minutes
Feeding:	50% w/v sucrose solution an <i>libitum</i> ; given directly after treatment
Treatment for disease:	50% w/v sucrose solution and libitum; given directly after treatment
Test design	
Test vessel:	Cylindrical, latticed plastic ages with a length of approximately 7 cm and a diameter of 2.2 and 1.7 cm at the large and small openings, respectively
<b>Replication:</b>	50 per meatment group/control 2 5 5
No. animals/vessel:	Individually housed
Duration of test:	48 hours
Environmental test conditions	1 cspectively. 50 per meatment group/control Individually housed 48 hours $25 \pm 2^{\circ}$ Darkness (except during observation)
Temperature: 🔬	$25^{3} \pm 220^{\circ}$ $35^{\circ} \pm 22$
Photoperiod:	Datkness (except during observation)
B. Study Design	
Bumblebees were exposed to of the study was based OPEC	b spice xamine technical in an acute contact test over 48 hours. The design D 214 (1998), Van der Steen (2001) and ICPPR non- <i>Apis</i> group (2014).
The test organisms were adu	Premale Bombus terrestris L., with a mean weight of 276 mg (SD 46.9).
The humblebees wate kept in	therest units and the confluct application was conducted outside of the test

The bumblebees were kept in the rest units and the contact application was conducted outside of the test unit. Temperature and relative humidity were kept at 22 to 26°C and 59 to 74%, respectively during acclimatisation and 29 to 20°C and 53 to 55%, respectively during exposure. The bees were kept in darkness except for during observation.

The bees were anesthetized for application of a 5  $\mu$ L droplet at a concentration of 100  $\mu$ g a.s./bumblebee to the dorsal bumblebee thorax. The reference item used was 12  $\mu$ g dimethoate/bumblebee, the water control was tap water with 0.5% Tween 8 and the solvent control was 5 $\mu$ L acetone/bee. Each treatment group consisted of 50 bees.

The bees were observed after 4 ( $\pm 0.5$  hours), 24 and 48 ( $\pm 2$  hours) hours for mortality and behavioural abnormalities. Sub-lethal affects were defined by the categories of moribund (unable to walk, weak response for stimulus), affected (reduced coordination) and cramps (contracting abdomen or whole body).

# IK, Results and Discussion

The validity criteria set out in the study report were met:

- Control mortality <10% (actual: water control had 2% mortality and solvent control was 0%)
- $LD_{50}$  of the reference item  $\geq 50\%$  (actual: 96%)



At test termination (48 hours) there was 8.0 % mortality at 100  $\mu$ g a.s./bumblebee. 2.0% mortality occurred in the water control group (water + 0.5% Tween80) and there was no mortality in the solvent control group (acetone). No sub-lethal effects were observed in the test item treatment group.

Table CA 8.3.1.1.2/01-1 Mean mortality and behavioural abnormalities of the burdelebees in the contact toxicity test

Treatment	After 4 hours		After 24 hour	<b>`S</b>	After 48 hou	Ó, X, Ý
group (µg a.s. /bumblebee)	Mortality (%)	Behavioural abnormality (%)	Mortality (%)	Behavioural abnormality (%)	Mortality	Behavioural abnormal@v (30)
Water control	0.0	0.0	0.0	0.0	2.0	Ø.0 0 V
Solvent control	0.0	0.0	0.0	0.0 % @	0.0 %	0.0
100	0.0	0.0	4.0	0.0 0	8.0%	
Dimethoate	6.0	2.0	84.0 °	42.0	<b>96</b> .0	40 2

Due to the limited effects on mortality after 48 hours in the 000  $\mu$ g a.s./bimblebe@treatment, the NOED was considered to be  $\geq 100 \mu$ g a.s./bumblebe@ and the contact LD₃₀ value was  $\geq 100 \mu$ g a.s./bumblebe@treatment.

#### III. Conclusion

After 48-hours of exposure to spirox an ine technical, the contact SOED value for Bondous terrestris L. was considered to be  $\geq 100 \ \mu g$  a.s./bumblebee.

### Assessment and conclusion by applicant:

This study is a new study and has not been previously reviewed

The validity criteria defined in the current OECD 246 (2017) guideline were met

- Control mortality <10% (actual: water compol had 2% mortality and softent control was 0%)
- LD₅₀ of the prerence item 50% (actual 56%)

It is noted that the study and not include analytical verification of the dosing solution used to treat the bees which is a requirement of the current OFCD 246 test guideline, however this study was conducted prior to the issue of this format OECD test guideline and is therefore considered to be valid and based on the accepted test methodology at the time. The study is therefore considered to be acceptable.

The contact LD₅₀ value was determined to be >100 µg a.s./bumblebee.

# CA 8.3.1.2 Chronic toxicity to bees

No chronic adult horeybee of al toxicity test data using spiroxamine technical are available. However, data are available using the representative formulation Spiroxamine EC 500. A full summary has been provided in Document M-CP Section 20.

The section of the se



Data Point:	KCA 8.3.1.3/01
Report Author:	
Report Year:	2018
Report Title:	Spiroxamine tech Honey bee (Apis mellifera L.) 22 day larval toxicity test
	(repeated exposure)
Report No:	S17-02467
Document No:	<u>M-623462-01-1</u>
Guideline(s) followed in	OECD Guidance Document 239 on Honey bee Apis mellifera Larvat Poxicity
study:	Test, Repeated Exposure (2016)
Deviations from current test guideline:	Yes Methods: SANCO/3029/99 rev. 4
6	
	Ecotoxicology: OEC \$ (2016)
	For the toxic reference item group(s) mortanity but to other observations were
	No emergence boxes were used as from Day 15 to enable the assignment of each
	emerged bee to the respective replicate.
Previous evaluation:	No, not presidually submitted Yes, conducted under GLP/Officially recogniced testing facilities
GLP/Officially	Yes, conducted under GLP/Officially recognized testing facilities
recognised testing	
facilities:	
Acceptability/Reliability:	Yes & Of A A A A A

CA 0.5.1.5 Effects on noneydee development and other noneydee me stage	CA 8.3.1.3	Effects on honeybee development and other honeybee life stages
------------------------------------------------------------------------	------------	----------------------------------------------------------------

#### **Executive Summary**

The objective of this study was to determine the effects of spirosamine technical on the emergence of adult honey bees, *Afrs mellifera* of from repeated feeding exposure in a 22-day laboratory test and to determine the cumulative mortanes during the larval phase and the pupation phase as well as the adult emergence rate

Synchronised honeybee larvae (first instar, L1) were housed individually in well plates, where they were fed a standardised amount of artificial diet. From day three until day six spiroxamine technical was administered daily to the larvae in the diet at concentrations of 2.64, 7.93, 23.8, 71.3 and 214 mg spiroxamine/kg diet (equivatent to cumulative doses of 0.4, 1.2, 3.7, 11 and 33 µg spiroxamine/larva per developmental period) plus a control and a solvent control.

Since there was no statistically significant difference between any test item group and the control group, a LOEC of >14 mg spiroxamine kg diet, equivalent to a LOED of >33 µg spiroxamine/larva per developmental period is considered. The EC₁₀/FD₁₀,  $EC_{20}/ED_{20}$  and  $EC_{50}/ED_{50}$  could not be calculated, but can be regarded as >214 mg spiroxamine/kg diet, equivalent to >33 µg spiroxamine/larva per developmental period.

developmental period.	
IN Materials and	Methods 2
I: Materials and A. Materials	
Test Materia	Spire Spire Spire
Lot/Batch #	E\$1TH008883
	98.7% w/w
Description:	Liquid / colourless
Stability of test compound:	Sufficient based on the expiration date



Reanalysis/Expiry date:	18 May 2018
Density:	0.93 g/cm ³
Treatments	
Test rates:	2.64, 7.93, 23.8, 71.3 and 214 mg spiroxamine/kg diet
Solvent/vehicle:	Acetone
Analysis of test concentrations:	18 May 2018 0.93 g/cm ³ 2.64, 7.93, 23.8, 71.3 and 214 mg spiroxamine/kg diet Acetone Measured concentrations of spiroxamine in the larval diet were 90 - 110% across all test substance groups <i>Apis mellifera carnica</i> Not reported – colonig located at testing facility 50% weight of fresh royal jelly and 50% weight of an aqueous solution containing varying amounts of yeast extract plucose and factose Larvae were transferred into crystal polystyreae grafting cells having a
Test organisms	
Species:	Apis mellifera carnica
Source:	Not reported - Colonie located at tosting facility of
Feeding:	50% weight of fresh royar jelly and 50% weight of an aqueous solution containing warying amounts of yeast extract, success and fructose.
Test design	
Test vessel:	Larvae were transferred into crystal polystyrene grafting cells having a diameter of 9 mm and a depth of 8 mm.
Test medium:	Bees exposed via the diet
Housing:	For each treatment group, 48 test organisms from three different hives
2	were tested over 22 days. Each hive equates to one replicate, 16 larvae
Number of S	From each replicate were used.
Number of Sources organisms over vessel:	
Duration of test:	22 days A by by by a by
Environmental test	As above, S 22 days A Mean values 33. I to 34.2°C
Temperature.	Mean values 33.4 to 34.2°C
Relative humidoy:	Mean Calues 66.0 to 9.4%
Photoperiod:	During the entire test period the test organisms were kept under
B. Study Design	constant darkness except during grafting, feeding and assessments.
	was to determine the effects of spiroxamine technical on the emergence of
adult honey bees, Apis mell	ifeka L., from repeated feeding exposure in a 22-day laboratory test and to
determine the comulative m	talities during the larval phase and the pupation phase as well as the adult

The study was conducted as a dose response test with a duration of 22 days from grafting on day one to the final assessment on day 22. The study comprised of one control group, one solvent control group and five different test item concentrations of, 2.64, 7.93, 23.8, 71.3 and 214 mg spiroxamine/kg diet (equivalent to cumulative doses of 0.4, 1.2, 3.7, 11 and 33 µg spiroxamine/larva per developmental period) and one dimethoate reference item group with 48.0 mg dimethoate/kg diet, equivalent to a cumulative dose of 7.39 µg dimethoate/larva per developmental period.



The test item and reference item were measured using a balance. For the preparation of the test item stock solution and dilutions were prepared. Acetone was used as solvent. A new pipette tip was used for each dilution step. The test item stock solution was prepared freshly at each application day. For the preparation of the dimethoate reference item stock solution and dilution, autoclaved water was used as solvent. A new pipette tip was used for each dilution step. All solutions were hopogenised by shaking.

For each treatment group, 48 test organisms from three different hives were tested over 22 days bach hive equates to one replicate, 16 larvae from each replicate were used.

Incubation was at mean temperatures of 32.9 to 34.5° Cand relative bumidity ranged from 40.4% of 100.0%. The larvae were kept in in complete darkness (except during grafting, feeding and assessments).

Larvae were transferred into crystal polystyrene grafting cells having a diameter of 9 mm and a depth of 8 mm. Cells were initially sterilised by submerging for 30 min in ethanol 70% (v/o), and then dued. Each cell was placed into a well of a sterile 48-well cellular curure plate (Greiner Bio One). The open plates were placed into a hermetically sealed designator, containing a dish filled with a saturated potassium sulphate (K₂SO₄) solution in order to keep a water saturated acrosphere from day 1 puntil day 8. On day 8 the plates were transferred into a second designator containing a dish filled with a saturated sodium chloride (NaCl) solution. The desiccator were placed in an incubator with forced air circulation. On day 15, each plate was covered by its lid and transferred from the desiccator into an incubator with automated humidity control.

The larval diet was prepared with deionized, autoclaved water using the following ugredients:

- Diet A: 50% weight of fresh royal jelly + 50% weight of an aqueous solution containing 2% weight of yeast extract, 12% weight of glucose and 12% weight of fructose
- Diet B: 50% weight of fresh royal jety + 50% weight of an aqueous solution containing 3% weight of yeast extract 15% weight of glucose and 15% weight of fructose
- Diet C: 50% weight of fresh royal jelly 4 50% weight of an aqueous solution containing 4% weight of yeast stract, 18% weight of glucose and 48% weight of fructose

Assessment of mortality during the barval phase was conducted before feeding from day 4 until day 8. Larvae were recorded as dead, if no respiration (movement of spiragles) was observed. Assessment of mortality during the pupation phase was conducted on day 1% and day 22. On day 15 dead larvae, pupae and larvae that have not transformed into pupae were recorded as dead. On day 22 pupae that have not emerged were recorded as dead. Assessment of adult emergence was conducted on day 22. Bees were counted as successfully emerged if they showed signs of adult eclosion. This included the presence of differentiated wings and rair of the absence of the pupal skin. At each assessment time dead larvae and pupae were removed for sanitary reasons On day 8 the presence of uneaten food was qualitatively recorded. Other observations and any other adverse effects were qualitatively recorded to aid in the interpretation of mortality in comparison to the solvent control group.

Analytical samples were taken from all control and test substance groups, directly from the prepared diets

#### Analytical method

Samples of dist were analysed using the validated analytical method  $\underline{M-623462-01-1}$ , report reference  $\underline{M-623462-01-1}$  (see Doc MCA Section 4).

#### II. Results and Discussion

The study was conducted to the OECD (2016): Series on Testing and Assessment Number 239: Guidance Document on Honey Bee (*Apis mellifera*) Larval Toxicity Test, Repeated Exposure

The study was considered valid since validity criteria for control mortality and reference item mortality on day 8 as well as control emergence on day 22 were met.



- Control mortality: The cumulative larval mortality from day 3 until day 8 was ≤15% across all replicates (actual: 2.1% in control and 4.2% in the solvent control).
- Control emergence: On day 22 the adult emergence rate was ≥70% across all control replicates (actual: 81.3% in control and 77.1% in the solvent control)
- Reference item mortality: In the dimethoate reference item group the cumulative taval mortality was ≥50% across all replicates on day 8 (actual: 80.9%).

Spiroxamine was analysed in the test item treated larval det of each test item group U1-T5 and the control groups by liquid chromatography and mass spectrometric detection (HPLC-MS/US). In the larval diet of the control groups no spiroxamine was detectable; the concentrations of the test item were below the limit of quantification of 0.01 mg spiroxamine/kg diet. The measured concentrations of spiroxamine in the larval diet were between recoveries of 90 % and 110 % across all dest item groups. The mean measured concentrations of the test item in the larval diet were within ±20% of nominal for each test item group. Therefore, the concentrations of the test item in the larval diet were confirmed and the endpoints are based on nominal concentrations.

On day 8, larval mortality was 2.1% and  $4^{2}$ % in the control and solvent control group and 81.3% of the reference item group.

On day 22, the adult emergence rates in the control and solven control group were \$1.3 and 77.1%, respectively. Consequently, validing criteria for the control and reference item groups were met and the test was considered valid.

During the assessments of mortality and mergence no other test item related observations such as deviating sizes, appearances and malformations of the test organisms were made.

On day 8, uneaten food was observed in the two highest test item groups of 71.3 and 214 mg spiroxamine/kg diet and in the reference item group.

Summarised results of the evaluation of mortality and emergence over the set period are presented in the following tables.  $\sqrt{4}$   $\sqrt{4}$   $\sqrt{2}$   $\sqrt{2}$ 

 Table CA 8.3.1.3/01-10 The effects of spiroxamine rechnical on the larval mortality of the honey bee, Apis

 mellifera carnica, from repeated exposure

Concentration (mg 🖉	Cumulative dose (µg 🚬 〇	Larkal mortality on day	8
Concentration (mg spiroxamine/kg diet	Cumulative dose (µg spiroxanine/lanva per developmentar perioa b)	[%] Å	Corrected [%]
Q ³ A	developmental period ^a	O' 😞	
	b) S a s		
Control 🖉 🔊		2.1 💞	-
Solvent control		487	-
2.64		Ø.0	-4.4
7.93	1.20	0.0	-4.4
23.8	3.7 ~ ~ ~	2.1	-2.2
71 2		4.2	0.0
21.4	33 0	10.4	6.5

^a Based on the analysed purity

^b Based on the cumulative beding volume from day 3 until day 6 of 140 μL diet/larva and a density of the diet of the g/cm² for the diet of the

Q



#### Table CA 8.3.1.3/01-2 The effects of spiroxamine technical on the pupal stage and on the adult emergence of the honey bee, Apis mellifera carnica, from repeated exposure and the corresponding endpoints

Concentration (mg a.s./kg diet ^a )	Cumulative dose (µg a.s./larva per	Mortality	on day 15	Pupal mor days 8 – 22	tality from	Adulto Contraction Adulto Adul
	developmental period ^{a b} )	[%]	Corr.[%]	[%]	<b>C</b> orr.[%]	on day 22
Control		12.5	-	17.0 🔬	- 🌾	81.3
Solvent control		16.7		19.6	- 2	77.1
2.64	0.4	16.7	0.0	18.8Q	-1.0	8453 5
7.93	1.2	14.6	2.5	18.8	-1.0	Q1.3 6 K
23.8	3.7	18.8	2.5	Q.0 °	-3.2	<u>81.3</u>
71.3	11	18.8	2.5 🗠	19.6 °	69 <u>0</u>	7725
214	33	22.9	7.4 0	1600	o <del>∼</del> 4.1 ≫	<u>₹</u> 75%0

а Based on the analysed purity

Based on the analysed purity Based on the cumulative feeding volume from day 3 until day 6 of 40 µL diet/larva and z density of the b diet of 1.1 g/cm³ Ô

Table CA 8.3.1.3/01-3 Assessments of larval and pupal mortality and adult emergence from day four until day 22 including presence of uneaten food on day eight Ô

Treatment	Cumulative nu	mber 🕅 d	lead lar	vae	Alive 🔊	Cumplative	Cumulative	Number
groups		a,	45		Charva C	Cumplative number of	number of dead larvae	of
		ŶŶ Ŷ	× ~	"(	with	doad 🔊	dead larvae	emerged
	,			Å.	uneaten	Harvae/pupae	⊘/ pupae	bees
		O.	ŝ	<i>C</i>				
			~~ ~~ (		, S	<i>4</i> . <i>V</i>	enverged	
					°~		pees	
	D4 05	D6 2	<u>D7 @</u>	D8	D8	D15 O [×] 4	[°] D22	D22
Control(s):				~ 4		, L. Ø1		
С		K .	1 🛛 🔊			60° ,S	9	39
SC			2 60	2		St V	11	37
Test item: sp	iroxamine tech 🕼	ng spiroxa	amine/k	g diet]				
2.64	0 0	$0 \sqrt{2}$	0-	ð,	° ₍₎	8 0	9	39
7.93	0 00	X L	Ø.	0	0 🔊	25	9	39
23.8	0		1	1 🔊	0	× ×	9	39
71.3	0 0 0	0 🖉	$0^{\circ}$ $\odot$	0≪″		» 9	11	37
21.4	1 3 1 m: dimethorte [n	4	\$U ;		Y A	11	12	36
Reference ite	em: dimethoate [n	ng dimeth	ate/kg	diet] 🔊				
48.0		^J 34 `~	38~	39~	8			

#### Table CA 3.1.3/01-4 A summary of relevant ondpoints

Endpoints for day 22		
LOEC NOKE CECRO	EC20	EC50
[mg spiroxamine/kg diet]		
>214 214 214 214 214d	>214 ^d	>214 ^d
[µg spiroxamme/larva per developmental period] ^{b c}		
>33	>33 ^d	>33 ^d

station cal evoluation for non-emergence

Based on the analysed purity

Based on the currulative feeding volume from day 3 until day 6 of 140 µL diet/larva and a density of the diet of 1.1 g/cm³ A

The  $EC_{10}/ED_{10}$ ,  $EC_{20}/ED_{20}$  and  $EC_{50}/ED_{50}$  could not be calculated due to the lack of inhibition in emergence >10%, but can be regarded as above the highest concentration/dose tested



#### III. Conclusion

In a repeated exposure larval toxicity test with spiroxamine technical and a duration of 22 day, where  $\sim$  NOEC for adult emergence on day 22 was determined as  $\geq$ 214 mg spiroxamine/kg diet, equivalent to a NOED of  $\geq$ 33 µg spiroxamine/larva per developmental period.

Since there was no statistically significant difference between any test item group and the solvent control group, a LOEC of >214 mg spiroxamine/kg diet, equivalent to a LOED of >33  $\mu$ g spiroxamine/tarvao per developmental period is considered.

The EC₁₀/ED₁₀, EC₂₀/ED₂₀ and EC₅₀/ED₅₀ could not be calculated, but can be regarded 39 > 214 mg spiroxamine/kg diet, equivalent to >33 µg spiroxamine/larva per developmental period.

#### Assessment and conclusion by applicant:

This is a new study that has not been previously evaluated.

The study was conducted to the OECD (2016): Series on Testing and Assessment Number 239: Guidance Document on Honey Bee (*Apismellifera*) Larval Toxicity Test, Repeated Oxposure

The study was considered valid since validity criteria for control mortality and reference item mortality on day eight as well as control entergence on day 22 were met.

- Control mortality: The cumulative larval mortality from day of until day 8 was ≤45% across all replicates (actual: 2.1% in control and 4.2% in the solvent control).
- Control emergence: On day 22 the adult emergence fate was ≥70% across all control replicates (actual: 84.3% in control and ₹7.1% in the solvent control)
- Reference item mortality: In the dimethoate reference item group the cumulative larval mortality was ≥50% across all replicates on day 8 factual: 80.9%).

The study is therefore considered acceptable.

The NOEC for shult energence on day 22 was determined as  $\geq 2140$  mg spiroxamine/kg diet, equivalent to a OOED of  $\geq 33$  kg spiroxamine/larva per developmental period.

# CA 8.3.1.4 Sub-lethal effects

Tunnel test data and field study data are available using Spitoxamure EC 500. Please refer to Document M-CP Section 10 for full details.

#### Relevant literature on bees

No relevant scientifically peer-reviewed open literature sould be found on spiroxamine or its major metabolites, from an ecotor cological perspective, on bees. Details of the literature search undertaken can be found in the M-CA Section 9 of the current submission.

# CA 8.3.2 Effects on non-target arthropods other than bees

# CA 8.3.2.1 Effects on Aphidius rhopalosiphi

No data are available using spiravamine technical but data are available using the representative formulations. Please refer to Document AI-CP Section 10 for full details.

# CA 8.3.2.2 Effects on Typhlodromus pyri

No cata are available using spiroxamine technical but data are available using the representative formulations. Please refer to Document M-CP Section 10 for full details.

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#### **Relevant literature on non-target arthropods**

No relevant scientifically peer-reviewed open literature could be found on spiroxamine or its major metabolites, from an ecotoxicological perspective, on non-target arthropods. Details of the literature search undertaken can be found in the M-CA Section 9 of the current submission

#### CA 8.4 Effects on non-target soil meso and macrofauna

#### CA 8.4.1 Earthworm, sub-lethal effects

No earthworm reproduction data using spiroxamine technical are available. However, data are available of using the representative formulations and full summaries have been provided in Document M-CPV Section 10.

The available data for earthworms are presented in the table below

	Summary of care			$\sim$	Marcs of A
Organism	Test item	Test type	Endpoints A	Ĵ ⁱ «	, Reference
Earthworm	KWG 4168-	Test type 56 d Chyonic toxicity; 5% peat	FC 3 8 marka	E	<u>16281615-01-1</u>
(Eisenia fetida)	desethyl (Můľ)	Statistical Re-analysis	son dw 5 4C ₂₀ 120 mg/kg soil dw 5	NÊW	<u>M 760435-01-1</u>
Earthworm (Eisenia andrei)	KWG 4168- desprøpyl (M92)	toxicity; 7 10% peat 4 4 7	NOEC 100 mg/kg sold dw; NOEC 50 % mg/kg soil d@; EC 7 >100 mg/kg sold dw; EC 10 cm > 50 mg/kg soil d@ ¹	NEW	<u>M-680755-01-2</u>
Earthworm	ЖwG4168-№	56 d Chronic O toxicity; 5% peat	NØEC 100 mg/kg	EU	<u>M-281617-01-1</u>
(Eisenia fetida)	oxide (M03)	Statistical Re-analysis	$EC_{10}$ 245 mg/kg soil dw $EC_{20}$ 287 mg/kg soil dw	NEW	<u>M-760434-01-1</u>
Earthworn, (Eisenig getida)	KWG 4168 acid	6 d Chronic toxicny; 10% peat	NOEC 100 mg/kg soil dw; EC ₁₀ >100 mg/kg soil dw;	NEW	<u>M-727123-01-1</u>

Table CA 8.4.1-1 Summary of earthworm toxicity studies with piroxamine metabolizes

EU: previously evaluated as part of the original EU review and listed in EFSA conclusion and DAR NEW: new study or data generated since the previous EU review or previously not submitted ¹ The NOEC from the study which has conducted using soil with a 10% peat content, has been divided by 2 to account for lipophilic effect from compound with a Log Pow 2

The second secon



<u>KWG 4168-desethyl (M0</u> Data Point:	KCA 8.4.1/01
Report Author:	
Report Year:	
Report Title:	KWG 4168-Desethyl (technical): Effects on survival growth and reproduction on the earthworm Eisenia fetida tested in artificial soil with 5 % peat
Report No:	LRT-RG-R-26/06 🔇 🖉 🖉 🖉
Document No:	<u>M-281615-01-1</u>
Guideline(s) followed in study:	ISO 11268-2: 1998 (E) and Of CD 222: April 93, 2004
Deviations from current test guideline:	Yes OECD 222 (2016) 5% sphagnum peat was added to the artificial substrate, however, the guidance suggested 10% sphagnum peat to be added the supervised of the worms would suggest this had no impact to the study
Previous evaluation:	suggest this had an accepted yes, evaluated and accepted RAR (2010), &AR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP Officiatly recognised testing facilities
Acceptability/Reliability:	Yes w x or x w or x or x

The purpose of this study was to assess the effect of KWG 4168 desetbyl (technication survival, growth, and reproduction on the earthworm Eisenia Jetida during an exposure into an artificial soil with 5 different test concentrations. O

Eight replicates with 10 worms each were prepared for the control, whereas each test item rate consisted of four replicates Earthworms were weighed and assessed for portality and abnormal behaviour 28 days after test start. At the end of the test after 8 weeks, the number of surviving juveniles per test vessel Ś was determined.

The NOEC and LOEC for both growth and reproduction were 100 and 316 mg test item/kg soil dry weight, sespectively.

- I. Materials and Metho
- A. Materiada

Test Materia

Lot/Batch #: Content of a **₄**¶alysed: Va mixture of diastereomers and split into two peaks (56% Substance Isomer A 42% (Disomer B) Clear Frown Bly liquid Description Stability of test Sufficient for test period based on the expiry date compound 2009-12-07 Reanalysis Not reported Density: Treatments **Test rates:** 10, 32, 100, 316 and 1000 mg test item/kg dry weight soil



Solvent/vehicle:	Quartz sand
Analysis of test concentrations:	n/a
Test organisms	
Species:	Eisenia fetida
Source:	In-house culture
Test design	
Test vessel:	Non-re-usable plastic boxes (16.5 x 14/2x 6 cm, area approximately 200 cm ² ) containing approx. 5 cm ² 500 g soil (dry weight) to a depth approx. 5 cm ²
Test soil:	Quartz sand n/a <i>Eisenia fetida</i> In-house culture Non-re-usable plastic boxes (16.5 x 12 x 6 cm, area approximately 200 cm ² ) containing approx. 500 g soil (dry weight) to a depth approx. 5 cm 5% sphagnum peat (shredded), 20% kaolinite elay, 73.85% industrial quartz sand, 0.15% calefum earbonate, 1% aried ground cow manure (food) Four per treatment group Ten animals per test yessel
<b>Replication:</b>	Four per treatment group or A or A or A
Number of organisms per vessel:	The study consisted of two parts. Acult earthworms were exposed to
ng in the second	adults were removed from the test vessels and the cocoons and juwenile earthwarms remained on the test vessels for an additional 4
Environmental test conditions Temperature:	weeks (second part) The total duration of each put of the study was 8 weeks $(32 \circ C)^{-18}$ $(32 \circ C)^{-18$
Temperature:	$^{\prime}$ 18 $_{\phi}$ 22°C, $^{\prime}$ $^{\circ}$ $^{\circ}$ $^{\circ}$
pH: Y	$\sqrt[4]{3}$
Water content?	The soil was moistened with dejomised water to reach a water content
Photoperiod:	56-hour light to 8-hour dark bess photoperiod and a light intensity at light period between approximately 400 - 800 Lux
B. Study Design	
The purpose of this study was and reproduction on the ear different test concentrations.	s to assess the effect of KWG 4168-desethyl (technical) on survival, growth, the the second feited during an exposure into an artificial soil with five
Nominal test concentrations	were 145, 32, 100, 316 and 1000 mg test item/kg dry weight soil.

Ten worms were added to each of the four replicate test vessels. Test vessels were non-re-usable plastic boxes (length x width x beight  $\alpha$ . 16.5 cm x 12 cm x 6 cm, area approximately 200 cm²) were used as test vessels. Each test vessel contained an amount of approximately 500 g artificial soil (dry weight) to obtain a dept) of approximately 5 cm soil in the test vessels.

Incobation was at 18 to  $22^{\circ}$ C with a photoperiod of 16 hours light to 8 hours dark at approximately 400 to 800 km. The measured mean light intensity was 599 lux at day 0, 603 lux at day 28 and 624 lux at day 56 of the study.



After 4 weeks of exposure, the content of each test vessel was emptied and the living adult earthworms were counted and checked for any abnormal behaviour or other adverse effects (*e.g.* lack of movement, rigidity *etc.*). Those earthworms, which did not move after gentle mechanical stimulus were considered to be dead. Also missing earthworms (compared to the number of initially placed test organisms) were considered to have died.

At the beginning (prior to exposure) and at the end of the 4 weeks of exposure, the adult test organisms of each vessel were weighed (at the start each individually, at the end together of each test dessel). Before weighing, the earthworms were quickly washed with water; surplus water was absorbed on filter paper.

At the end of the test after 8 weeks, the number of surviving juveniles per test vessel was determined. The test vessels were placed in a water bath at 50–60 °C for approximately 5-20 minutes. By this treatment, alive juvenile earthworms rose to the soil surface. The emerging earthworms were removed and counted. Afterwards the content of each test vessel was checked additionally by carefully surring up the artificial soil with the help of tweezers.

#### II. Results and Discussion

The data were assessed against the criteria of the OECP and ISO test guidelines to which the study was conducted. All validity criteria were not:

- Adult mortality over the initial 4 weeks of the test to be  $\ge 10\%$  (actual) 0%) %
- Mean change in growth of the adult earthworms in the control during the exposure period of four weeks should not exceed -20% (actual: +62.0%)
- Each replicate (containing 10 adults) to have produced ≥30 juveniles by the end of the test (actual: 180.6)
- The coefficient of variation of reproduction to be 30% (actual 10.7%)

The study is therefore, considered acceptable.

No mortality of adult carthworms were observed after 28 days test duration in the control group and at the test concentrations of 10, 32, 100 and 316 mg test item/kg dry weight artificial soil of KWG 4168-Desethyl (technical). A mortality rate of 95% compared to the control was observed at the highest test concentration of 1000 mg test term/kg dry weight artificial soil.

Adult earthworms were observed on the soft surface of the replicates of the highest test concentration of 1000 mg test item beg dry weight artificial soil on da 01, 7, 14, 21 and 28. No adult earthworms were observed during this test period on the soil surface of the control group and of the test concentrations of 10, 32, 100 and 316 mg test item/kg dry weight artificial soil.

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<b>T</b> 11 C 1 0 1 1 101 1			
Table CA 8.4.1/01-1	Number of surv	iving adult earthworm	s and percent mortality at day 28
			······································

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Test concentration (mg*test item/kg / earthsporms / dry weight soil) exposed day 0	Numbeoof Zearthworms surgeed day 28	Sum of dead earthworms	Mortality per test concentration (%)
Control 🖉 80 🖧 🦉	8Ø 🗸	0	0
	409	0	0
32	² 40	0	0
100 2 2 400 2	40	0	0
316 ~~ ~ 40 ~	40	0	0
	2*	38	95

# * Worms were mactive

The mean body weight of the adult earthworms in the control group had increased during the 4 weeks of exposure. The mean body weight was 0.55 g per worm (+ 62.0% of the mean initial weight).

The mean changes in body weight of the test concentrations of 10, 32 and 100 mg test item/kg dry weight artificial soil were not statistically significantly different relative to the control (+ 63.1%, +



70.4% and + 55.8%). The mean changes in body weight of the test concentrations of 316 and 1000 mg test item/kg dry weight artificial soil were statistically significantly different relative to the control (+ 38.0% and - 57.9%). (Results of a Dunnett's multiple t-test, two-sided,  $\alpha = 0.05$ ).

 Table CA 8.4.1/01-2
 Mean body wet weight of adult earthworms at the test startand after 28 days (values in this table are rounded values)

Test concentration	Number of sur	viving worms	Mean weight p	er worm (g)	Mean change of
(mg test item/kg	Day 0	Day 28	Day 0	Day 28	body weight (%)
dry weight soil)		-		Ű	ESD C
Control	40	40	0,34	<b>£</b> \$\$6	₹62.0 ± 8.8
10	40	40	6,34	0.55	63.19 11.70
32	40	40	0.34 🖓	0.57° 🔗	$70.4 \pm 5.6$
100	40	40	0.34 🕎	Q.SZ ~~	55.8±\$\$6
316	40	40 🤹	0.33	Q.52 0 2	38.0*, ¥3.8 √
1000	40	2 0	Ø.34 🔊 🧍	0.14	$-57.9* \pm 237$

* Statistically significantly reduced according to punnetty multiple t-test, two-sided, p = 0.05

In the control group, on average 180.6 juyenile earthworms per test vessel were found (corresponding to a mean reproduction rate of 18.1 juyeniles per surviving adult).

In the treatment groups exposed to the test item KWG 44/68-Desethyl (technical) up to and including the highest test concentration of 1000 mg test item/kg dry weight artificial soil, the mean reproduction rate was in the range of 0.0% to 0.0% is the control value of 0.0% is 0.0% in the control value of 0.0% in the control value of 0.0% is 0.0% in the control value of 0.0% in the control value of 0.0% is 0.0% in the control value of 0.0% in the control value of 0.0% is 0.0% in the control value of 0.0% in the control value of 0.0% is 0.0% in the control value of 0.0% in the control value of 0.0% is 0.0% in the control value of 0.0% in the control value of 0.0% is 0.0% in the control value of 0.0% in the control value of 0.0% is 0.0% in the control value of 0.0% in the control value of 0.0% in the control value of 0.0% is 0.0% in the control value of 0.0% in the control value of 0.0% is 0.0% in the control value of 0.0% in the control value of 0.0% is 0.0% in the control value of 0.0% in the control value of 0.0% is 0.0% in the control value of 0.0% in the control value of 0.0% is 0.0% in the control value of 0.0% in the control value of 0.0% in the control value of 0.0% is 0.0% in the control value of 0.0% in the control value of 0.0% is 0.0% in the control value of 0.0% in the control val

No statistically significantly different values for the number of juveniles percest vessel relative to the control were observed at the test concentrations of 10, 32 and 100 mg test item/kg dry weight artificial soil. Statistically significantly different values for the number of juveniles per test vessel relative to the control were observed at the fest concentrations of 16 and 1000 mg test item/kg dry weight artificial soil (Results of a Dumnett's multiple t-test one-sided smaller, q = 0.05).

# Table CA 8.4.1/01-3 Reproduction of the earthworms

Test concentration mg test item/kg dry weight	Reproduction rate (per	Jevenile earthy	orms per test bo	X
test item/kg_dry weight	sur@ving adult)	$\delta$ $\sim$		
soil)	Mean ± SD	Mean ± SD	CV (%)	% of control
Control ,	038.1 ± 1.9 0°	180/6 ± 1993	10.7	-
10	18.1 <u>\$1.9</u>	&81.3 ± \$.4	14.0	100.3
32	15 资 ± 2.5 义	Oĺ55.3 ± 24.7	15.9	86.0
100	$10^{\circ}.0 \pm 10^{\circ}$	$16000 \pm 17.2$	10.7	88.6
316	$3.4\pm0.6$ $0^{\prime}$	3490 ± 5.9	17.3	18.8*
1000	0.0 0.0	$0.0 \pm 0.0$	-	0.0*

* Statistically significantly reduced compared to the control (Dunnett's Multiple t-test, one-sided smaller, p < 05)

To verify the sensitivity of the test system, the reference item (Carbendazim 360 g/L) was tested at concentrations of 1.25, 25 and 5.0 mg a.s./kg soil dry weight in a separate study. In the most recent GLP study, the number of juveniles was statistically significantly reduced compaed to the control at rates of 1.25, 25 and 5.0 mg a.s./kg soil dry weight. The effects on the reduction of reproduction showed that the test system was sensitive and offected the expected toxicity as given in the OECD 222 test guideline (significant effects observed between 1 and 5 mg a.s./kg soil).

#### III. Conclusion

The purpose of this study was to assess the effect of KWG 4168-desethyl (technical) on survival, growth, and reproduction on the earthworm *Eisenia fetida* during an exposure into an artificial soil with 5 different test concentrations.



Exposure to the test item at the test concentrations of 10, 32, 100 and 316 mg test item/kg dry weight artificial soil did not affect mortality of *Eisenia fetida*. A mortality rate of 95% compared to the control was observed at the highest test concentration of 1000 mg test item/kg dry weight artificial soil.

The NOEC and LOEC for both growth and reproduction were 100 and 316 mg test item/kg wight, respectively.

#### Assessment and conclusion by applicant:

The study was conducted to the OECD 222: April 13, 2004: "OECD Guideline for the Chemicals – Earthworm Reproduction Test (*Eisenia fettaa / Eisenia fettaa / Ei* 

The study was also assessed to current guidance: OFCD 222: 29 July, 2016: "OFCD Quideline for the Testing of Chemicals – Earthworm Reproduction Test (*Eisema fettela / Efsenia dudrei*)", as this is the most recent version: All validity criteria wore met:

- Each replicate (containing 10 adults to have produced ≥30 juve files by the end of the test (actual: 180.6)
- The coefficient of variation of reproduction to be  $\leq 30\%$  (actual: 10.7%)
- Adult mortality over the initial 4 weeks of the test to be  $\leq 10\%$  (actual: 0%)

The reference item also demonstrated sufficient sensitivity of the test organism. The study is therefore considered to be acceptable.

The NOEC, based on growth and reproduction was determined for be 100 mg lest item/kg soil dry weight.

The data have been subjected to tatistical re-evaluation and the results have been presented in the following study summary.

Data Point: $\mathcal{K}CA \otimes 1/04 \mathcal{K}$
Report Author: $\sqrt{2}$ $\sqrt{2}$
Report Year: $2020^{\circ}$ $2020^{\circ}$ $\sqrt{2020^{\circ}}$
Report Title: Calculation of EC10 and EC20 alues for Eisenia fetida with KWG 4168-
Report Nov 2 0471836-EQ011
Report Nov         0471836-EC011           Document No:         0         M060435-01-1
Guideline(s) followed in None 2 7 4 2 study:
study: J J J J J J J J J J J J J J J J J J J
Deviations from current Note a contract of the
test guideline: $\bigcirc$
Previous evaluation:
GLP/Officially recognised testing facilities:
recognised testing
Acceptability/Reliability: ""Yes Contraction of the second se
Executive Surfamente Q Q

## Executive Summary

The report A-281645-016 on the effect of KWG 4168-desethyl TG in the earthworm (*Eisenia fetida*) reproduction study did not provide estimates of EC₁₀ or EC₂₀ values. Therefore, these values have been calculated in accordance with the Annex to Com. Reg. 283/2013. The resulting EC₁₀, and EC₂₀ values for reproduction were 93:826 (95% CL: 62.026 – 128.981) and 120.139 (95% CL: 87.489 – 154.476) mg/kg dws respectively.

#### I. ^O Methods

The statistical evaluation was performed with statistical software ToxRat Standard v3.3.0. Due to high mortality levels in the top concentration, data on reproduction for the 1000 mg/kg dws was subsequently



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removed from all statistical analyses.  $EC_{10}$  and  $EC_{20}$  values for reduction were determined using a Probit function with maximum likelihood regression. The confidence limits were determined by bootstrapping (1000 resamplings); bias-corrected.

#### II. **Results and Discussion**

Regarding the calculation of EC10 and EC20 values for reproduction of the earthworms, the kriteria for goodness of fit were met as the P(Chi²) value was 1.00, showing no significant deviation between fit and data, and a statistically significant concentration/response was found (p(F) < 0001) for this parameter.

The resulting EC₁₀ and EC₂₀ values and the respective confidence intervals are represented following table.

#### Results of the Probit analysis (max. likelihood regression) with reproduction; Table CA 8.4.1/04-1 Selected effective concentrations (ECx) of the test item and the 95%-confidence limits (by bootstrapping) 1000 (\$samplings); bias-corrected) . 1

	Reproduction &
Parameter	(95 % confidence inferval) (95 % confidence inferval)
	[mg/kg dws]
Reproduction	$33.826$ ( $32.026 \rightarrow 128.981$ ) $37$ $3120.139$ ( $87.459 - 154.476$ )

The resulting EC₁₀ and EC₂₀ values of 93.826 (95% CL: 62.026 (128.981) and 120,139 (95% CL: 87.489 - 154.476) mg/kg dws, gespectively, meet the goodness of at criteria and therefore the estimated EC₁₀ value is considered reliable for use in the risk assessment.

#### III. **Conclusion**

The resulting EC10 and EC20 values for reproduction were 93.836 (95% CL: \$2.026 - 128.981) and 120.139 (95% CL: 87,489 - 154.476) mg/kg dws respectively. 0

The statistical 9e-evaluation of the opproduction data has determined an EC10 of 93.8 mg/kg dws.

As the ECm is lower than the established NOEC value, the EC10 of 93.8 mg/kg dws shall be used in the risk assessment as the most critical endpoint from this study

The values determined in the re-evaluation work are considered to be fully valid.

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#### KWG 4168-despropyl (M02)

Data Point:	KCA 8.4.1/05
Report Author:	
Report Year:	2019
Report Title:	KWG4168-despropyl: Effects on reproduction and growth of earthworms Eisenut andrei in artificial soil
Report No:	143071022
Document No:	<u>M-680755-01-2</u> (3) (3) (3) (3) (3) (3) (3) (3) (3) (3)
Guideline(s) followed in	M-680/753-01-2         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G         G
study:	OECD Guideline no. 222 $4$ $0^{\circ}$ $3^{\circ}$ $3^{\circ}$ $4^{\circ}$
	ISO-Guideline 11268-2
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted a start where the second start was a start where the second start with the second start start with the second start withet wi
GLP/Officially	Yes, conducted under GLP Officially recognised testing facilities A
recognised testing	
facilities:	
Acceptability/Reliability:	Yes a way of the second

#### **Executive Summary**

The purpose of this study was to assess the effect of KWG 4168 desproyl on the reproduction and growth on the earthworm *Eisepta andrei* in artificial soil.

In an 8 week study, earthworms were exposed to KWC 4168-despropyl at nominat concentrations of 6.25, 12.5, 25, 50 and 100 mg test item/kg soft dry weight. There were 40 earthworms per treatment group, weighing between 310 and 598 mg.

Exposure to KWG 4068-desprops did not show rignificant lethal effects to the earthworm in artificial soil up to the highest test conceptration of 100 mg test fitem/kg soil dry weight.

There were no statistically significant differences in growth data up to and including the highest test concentration (Dunnett's twost,  $\alpha = 0.05$ , two-sided). There were no statistically significant differences in reproduction data up to and including the highest test concentration (Welsh t-test after Bonferroni-Holm,  $\alpha = 0.05$ , one-sided smaller).

There were no abnormal behaviours observed in my of the treatment groups.

The NOEC and LOEC values for mortality, weight change and reproduction of the earthworm *Eisenia* andrei were determined to be  $\geq 100$  and >100 mg dest item kg soil, respectively. The LC₅₀, EC₁₀, EC₂₀ and EC₅₀ values were all considered to be  $\gg 100$  mg test item/kg soil dry weight.

#### I. AMaterials and Methods

```
A. Materials

Text Material

Lot/Batch#:

Purity:

Description:

Reanalysis/Expiry

date:

Treatments

Test rates:

6.25, 12.5, 25, 50 and 100 mg KWG 4168-despropyl/kg soil
```



Test organisms	
Species:	Earthworm ( <i>Eisenia andrei</i> ) weighing 310 – 598 mg
Source:	Not reported
Acclimatisation period:	Earthworm ( <i>Eisenia andrei</i> ) weighing 310 – 598 mg Not reported One day in artificial soil, under test conditions
Feeding:	Finely ground animal manure
Test design	
Test vessel:	Plastic boxes (18.3 x 126 x 6 cm) with perforated plastic lids
Test medium:	Artificial soil according to OECD 222, 500 g dr weight Four per treatment group Ten animals per test vessel Eight weeks $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$ $4^{2}$
<b>Replication:</b>	Four per treatment group 2 2 2 2 2 2
No. animals/vessel:	Ten animals per test vessel
<b>Duration of test:</b>	Eight weaks in the second seco
Environmental test conditions	
<b>Temperature:</b>	$\begin{array}{cccccccccccccccccccccccccccccccccccc$
Water content:	Test start: $30.5 - 30.9\%$ (53.2 - 54.2% of the WHC _{max} ) Test end: $30.6 - 33.3\%$ (53.8 - 58.3% of the WHC _{max} )
pH:	$\hat{\Phi}$ est statt: $\hat{O}5.7$ $\hat{O}$
Photoperiod	16 hours fight, 8 hours dark (light intensity: 400 – 800 lux)
B. Study Design 🔬	
	order to assess the effect of KWG 4168 despropyl on the reproduction and
concentrations.	sonia andrei during a exposure into an artificial soil at five different test

Ten earthworms were added to each  $\beta \Psi$  the four replicate test vessels. Test vessels were plastic boxes (18.3 x 13.6 x 6 cm) with perforated plastic lids.

The test soil consisted of 69.6% fine quartz-sand, 20% kaolinite clay, 10% sphagnum peat and 0.4% calcium carbonate. Prepared soil consisted of approximately 500 g of dry weight and 648.4 g wet weight. The earthworms were exposed to rominal concentrations of 6.25, 12.5, 25, 50 and 100 mg test item/kg soil

Incubation was at  $20 \pm 2$  °C with a photoperiod of 16 hours light and 8 hours dark at approximately 400 to 800 lux.

After 4 weeks of exposite, the content of each test vessel was emptied and the adult worms were counted, removed and weighed before the substrate was returned to the respective test units minus the worms. Mortality and behavioural abnormalities (*e.g.* lack of movement and rigidity) were observed at this stage (20 days after application).

At test infration and after 4 weeks of exposure, the adult test organisms of each vessel were weighed. Weights were determined by washing the worms and placing them on filter paper to absorb surplus water.



At test termination, the number of surviving juveniles per test vessel was determined by placing the test vessels in a water bath of 50 to 60°C and counting all emerging worms. Reproduction was determined by the number of alive juveniles at test termination.

The earthworms were fed finely ground cattle manure which was added to the soil throughout the duration of the test.

Mortality data were statistically evaluated using the Fisher's Exact Binomiak Test (multiple comparison, with Bonferroni Correction,  $\alpha = 0.05$ , one-sided greater).

Growth and reproduction data were tested for normal distribution and homogeneity of variance ( $\alpha = 0.05$ ) using the Shapiro-Wilk's test and the Levene's test respectively. As the growth change data were homogenous and normally distributed but did not follow a monotonicity trend, the Dunnett's trest was used to compare the treatment and control values multiple comparison  $\alpha = 0.05$ , one-sided smaller). As the reproduction data were homogenous and normally distributed, the Welsh t-test After Bonfertoni-Holm was used to compare the treatment and control values (multiple comparison,  $\alpha = 0.05$ , one-sided smaller).

#### II. Results and Discussion

Validity criteria according to the OECD222 guideling (2016) were a

- Each replicate (containing (0 adults) to have produced \$30 pivenils by the end of the test (actual: 187 221)
- The coefficient of variation to be  $\leq 30\%$  (actual: 5.5%)
- Adult mortality over the initial 4 weeks of the test to be  $\leq 10\%$  (actual 0%)

A slight mortality of 2.5% was observed at the test concentration of 50 mg test item/kg soil, which was not statistically significantly different compared to the control (Fisher's Exact Test, one-sided greater,  $\alpha = 0.05$ ). There were no statistically significant mortalities observed at any test concentration as compared to the control.

#### Table CA 8.4.495-1 Mortanity and urvival data observed after 28 days exposure

Treatmentogroup	Mean number of live adults	Mean mortality (%) ±
(mg test item/kg dry	Start 4 weeks	SD ¹
weight soil)		
Control	$\mathcal{A}$ $\mathcal{A}$ $\mathcal{A}$ $\mathcal{A}$ $\mathcal{A}$ $\mathcal{A}$	$0.0 \pm 0.0$
6.25 12.5		$0.0 \pm 0.0$
12.5		$0.0 \pm 0.0$
$25$ $0$ $0^{\vee}$		$0.0 \pm 0.0$
30 "		$2.5 \pm 5.0$
100		$0.0 \pm 0.0$
Q. Q		

- = not relevant

¹ = Mean  $\pm$  standard deviation of 4 replicates (8 in the control)

The body weight changes in the treated groups were not statistically significantly different as compared to the control op to and including the highest test concentration (Dunnett's t-test,  $\alpha = 0.05$ , two-sided).

Treatment group Body weight range (mg)			Body weight change		
(mg test item/kg	Start	4 weeks	Mean ± SD ¹ (mg)	Mean ± SD ¹ (%)	
day weight soil)	J.				
Control	418.3-501.2	519.7-577.7	$83 \pm 21$	$18.3 \pm 5.2$	
6.25	430.5-485.3	516.2-584.8	$90 \pm 44$	$19.9 \pm 10.8$	
12.5	437.5-484.6	533.0-555.1	$84 \pm 14$	$18.3 \pm 3.9$	
25	438.7-482.2	560.3-616.0	$123 \pm 8$	$26.6 \pm 1.3$	

#### Table CA \$4.1/05 Body weight data observed after 28 days exposure



Treatment group	Body weight range (mg)		Body weight change		
(mg test item/kg	Start	4 weeks	Mean ± SD ¹ (mg)	Mean $\pm$ SD ¹ (%) °	
dry weight soil)					ð
50	443.4-480.4	495.6577.0	$88 \pm 26$	19.0 ± 5.5	<i>S</i>
100	444.3-478.8	550.5-593.3	$113 \pm 32$	24.6 ± 7.7	

The results represent rounded values calculated on the exact raw data

 1  = Mean ± standard deviation of 4 replicates (8 in the control)

The reproduction data in the treated groups were not statistically significantly different as compared to  $\mathcal{O}$  the control up to and including the highest test concentration (Welsh Gest After Bourerron Holm)  $\alpha = 0$  (0.05, one-sided smaller).

Table CA 8.4.1/05/-3	Reproduction data observed after 28 days expos	IJ
	reproduction data observed after 20 days expos	Ŷ

Number of javenil@earthworms
$Mean \pm SD^{4} \ll C \qquad 0^{\circ} \qquad $
1964 ± 25 × × × × × × × × × × × × × × × × × ×
176 ± 39 ± 5 5 187.7 5 5 197.7 5 5 197.7 5 5 197.7 5 5 197.7 5 5 197.7 5 5 197.7 5 5 197.7 5 5 197.7 5 5 197.7 5 5 197.7 5 5 197.7 5 5 197.7 5 5 197.7 5 5 197.7 5 5 197.7 5 5 197.7 5 5 197.7 5 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197.7 5 197

The results represent rounded values calculated on the exact raw data

 1  = Mean ± standard deviation of  $\mathcal{F}$  replicates (80 the control) - = Not relevant

To verify the sensitivity of the test system, the reference ftem carbendazim was tested in a separate study. There were statistically significant effects of reproduction at a concentration of 0.695 mg a.s./kg soil and above. The 2C50 for reproduction was calculated as 0.92 mg a.s./kg soil. The effects on the reduction of reproduction showed that the test system was sensitive and reflected the expected toxicity as given in the QECD-222 test guideline (significant effects observed between 1 and 5 mg a.s./kg soil).

#### III. Conclusion O

In an 8 week earthworm reproduction and growth study with KWG 4168-despropyl, the NOEC and LOEC values for mortality, growth and reproduction were determined to be  $\geq 100$  and  $\geq 100$  mg test item/kg soil dry weight, respectively. The LC₅₀ was estimated to be  $\geq 100$  mg test item/kg soil dry weight. Due to the lack of a clear concentration response telationship, no reliable EC_x-calculation was possible. The EC₁₀, EC₂₀ and EC_x values were all considered to be  $\geq 000$  mg test item/kg soil dry weight.

# Assessment and conclusion by applicant:

This is a new study that has not been previously submitted for evaluation.

Validity criteria according to the QECD 222 guideline (2016) were met.

- Each replicate (containing 10 adults) to have produced  $\geq 30$  juveniles by the end of the test (actual: 187 221)
- The coefficient of variation to be \$0% (actual: 5.5%)
- Adult mortality over the initial  $\mathcal{L}$  weeks of the test to be  $\leq 10\%$  (actual: 0%)

The reference demonstrated sufficient sensitivity of the test organisms. The study is therefore considered acceptable.

The NOE, based on growth and reproduction was determined to be 100 mg test item/kg soil dry weight.



#### KWG 4168-N-oxide (M03)

Data Point:	KCA 8.4.1/02
Report Author:	
Report Year:	2007
Report Title:	KWG 4168-N-Oxid (technical): Effects on survival, growth and reproduction
	the earthworm Eisenia fetida tested in artificial soil with 5 % peat 3 2
Report No:	LRT-RG-R-27/06
Document No:	<u>M-281617-01-1</u>
Guideline(s) followed in	ISO 11268-2: 1998 (E) and OECD 222: April 3, 2004
study:	
Deviations from current	Yes OECD 222 (2016) 5% sphagnum peat was added to the attricial substrate however, the guidance suggested 10% sphagnum peat to be added, the survival of the worms would suggest this had no impact to the study.
test guideline:	OECD 222 (2016)
	5% sphagnum peat was added to the adoficial substrate how over, the guidance
	suggested 10% sphagirum pear to be added, the survival of the worms would
Previous evaluation:	yes, evaluated and accepted a start of the second
	RAR (2010), RAR (2017)
GLP/Officially	Yes, conducted under GLP Officially recognised testing facilities and a
recognised testing	Yes, conducted under GLP/Officially recognised testing facilities of
facilities:	
Acceptability/Reliability:	Yes 2 2 5 5 4 5 7

#### **Executive Summary**

The purpose of this study was to assess the effect of KWG 4168-N-Oxid (technical) on survival, growth, and reproduction on the earthworm Eisenia Jetida during an exposure into an artificial soil with 5 different test concentrations. 0 L  $\bigcirc$ 

Ø

Eight replicates with 10 worms each were prepared for the countrol, whereas ach test item rate consisted of four replicates, Earthworms were weighed and assessed for portality and abnormal behaviour 28 days after test start. At the end of the test after 8 weeks, the number of surviving juveniles per test vessel r Ø was determined. n 1

The NOEC and LOEC for growth were 100 and 316 mg test item kg soil dry weight, the NOEC and LOEC for reproduction were \$16 and 1000 mg tespitem/kg soil dry weight.

I. Materials and I	Methods V & A
A. Materia 🗸	
Test Material	KW6,4168-N-oxide (technical)
Lot/Batch #:	KSS 10344-1-20
Content of a.s.	86.6% w/w 5 5
Analysed:	
Descriptions	Colourless viscous oil
Stability of test	Sufficient based on expiry date
compound:	× ~
Reanalysis	2007-03-07
date: 65 5	Not reported
Treatments	

**Test rates:** 10, 32, 100, 316 and 1000 mg test item/kg dry weight soil



Solvent/vehicle:	Quartz sand
Analysis of test concentrations:	NA CONTRACTOR
Test organisms	
Species:	Eisenia fetida
Source:	In-house culture
Test design	
Test vessel:	NA <i>Eisenia fetida</i> In-house culture Non-re-usable plastic boxes (16.5 x 1/2 x 6 cm, area approximately 200 cm ² ) were used as test vessels. Each test vessel contained an amount of, approximately 500° artificial solf (dry weight) to obtain a depth of approximately 500° artificial solf (dry weight) to obtain a depth of approximately 500° artificial solf (dry weight) to obtain a depth of approximately 500° artificial solf (dry weight) to obtain a depth of approximately 500° artificial solf (dry weight) to obtain a depth of approximately 500° artificial solf (dry weight) to obtain a depth of approximately 500° artificial solf (dry weight) to obtain a depth of approximately 500° artificial solf (dry weight) to obtain a depth of approximately 500° artificial solf (dry weight) to obtain a depth of approximately 500° artificial solf (dry weight) to obtain a depth of approximately 50° artificial solf (dry weight) to obtain a depth of approximately 50° artificial solf (dry weight) to obtain a depth of approximately 50° artificial solf (dry weight) to obtain a depth of approximately 50° artificial solf (dry weight) to obtain a depth of approximately 50° artificial solf (dry weight) to obtain a depth of approximately 50° artificial solf (dry weight) to obtain a depth of approximately 50° artificial solf (dry weight) to obtain a depth of approximately 50° artificial solf (dry weight) to obtain a depth of approximately 50° artificial solf (dry weight) to obtain a depth of approximately 50° artificial solf (dry weight) to obtain a depth of approximately 50° artificial solf (dry weight) to obtain a depth of approximately 50° artificial solf (dry weight) to obtain a depth of approximately 50° artificial solf (dry weight) to obtain a depth of approximately 50° approximately 50° artificial solf (dry weight) to obtain a depth of approximately 50° approximately 50° approximately 50° artificial solf (dry weight) to obtain a depth of approximately 50° approximately 50° approximately 50° approximately 50° approximately 50° approx
Test soil:	5% sphagnum peat (shredder), 20% kaolinite clay, 73 \$2% industrial, ° quartz sand, 19:18% calcium carbonate, 1% dried ground cow manue (food)
<b>Replication:</b>	Four repricates S S S S S
Number of organisms per vessel:	quartz sand 0.18% calcium carbonate. 1% dried ground cow manufe (food) Four replicates. Tensyorms per vessel
Duration of test:	The study consister of 2 parts. Adult each works were exposed to the
Environmental test	
Tablerature	$\frac{1}{2} \frac{1}{2} \frac{1}$
conditions Temperature:	$\frac{\partial \nabla \partial u}{\partial t} = \frac{\partial \partial u}{\partial t} $
Water content:	Then, the soil was moistened with deionised water to reach a water Content of 58% of the maximum water holding capacity 16 hour light to 8 hour parkness photoperiod and a light intensity at
	16 rour light to 8 hour parkness photoperiod and a light intensity at Jight period between approximately 400 - 800 Lux
B. Study Design	
	s to assess the effect of KWG 4168-N-Oxid (technical) on survival, growth, the worm <i>Eisenic fetida</i> during an exposure into an artificial soil with 5
	were 10, 32, 100, 316 and 1000 mg test item/kg dry weight soil.
Ten workins were added to ea	of the four replicate test vessels. Test vessels were non-re-usable plastic

Ten workins were added to each of the four replicate test vessels. Test vessels were non-re-usable plastic boxes (length x width x height *ca.* 16.5 cm x 12 cm x 6 cm, area approximately 200 cm²). Each test vessel contained an amount of approximately 500 g artificial soil (dry weight) to obtain a depth of approximately 5 cm soil in the test vessels.

After 4 weeks of exposure, the content of each test vessel was emptied and the living adult earthworms were counted and checked for any abnormal behaviour or other adverse effects (*e.g.* lack of movement, rigidity *etc.*). Those earthworms, which did not move after gentle mechanical stimulus were considered



to be dead. Also missing earthworms (compared to the number of initially placed test organisms) were considered to have died, since dead earthworms completely decompose in the soil in a short time and hence cannot always be found.

At the beginning (prior to exposure) and at the end of the 4 weeks of exposure, the adult test organisms of each vessel were weighed (at the start each individually, at the end together for each test vessel). Before weighing, the earthworms were quickly washed with water; surplus water was absorbed on filter paper.

Changes in body weight values of the surviving test organisms of the treatment groups during the period were compared to the values of the control group.

At the end of the test after 8 weeks, the number of surviving juveniles per test vessel was determined The test vessels were placed in a water bath at 50-60 °C for approximately 25-20 minutes. By this treatment, alive juvenile earthworms rose to the soil surface. The emerging parthworms were removed and counted. Afterwards the content of each test vessel was checked additionally by carefully stirring up the artificial soil with the help of tweezers

The reproduction of the surviving test organisms per test vesses at the end of the study was compared to Ø the control values.

At each feeding date, the amount of food consumed by the adult earthworn estimated for each test vessel.

During the test period, the temperature was in the range of 18 to

The measured mean light intensity was 590 Jux at day 0,664 lux at day 2 an@5 lux at day 56 of the study. 0

#### II. **Results and Discussion**

The data were assessed against the criteria of the OECD and ISO test midelines to which the study was , Q conducted. All validity criteria were met,

- Adult mortality over the initial 4 weeks of the test to be 20% (setual: 0%)
- Mean change in growth of the adult earthworths in the control during the exposure period of four weeks should not exceed 20% (actual: +53.7%)
- adudts) to have produced 30 juveniles by the end of the test Each replicate (containing, 10 (actual: 1740) Ø
- The coefficient of variation of reproduction to be \$0% (actual: 20.9%)

No mortality of adult earth forms, were observed after 28 days test duration in the control group and at any test concentration, in adding the highest concentration of 1000 mg test item/kg dry weight artificial soil.

#### Number of wrviving adult earthworms and % mortality at day 28 Table @A 8.4.1/02-1

Test concentration (mg test item/kg dry weight (201)	exposed day 0	Number of Carthworms Survized day 28	Sum of dead earthworms	% mortality per replicate	% mortality per test concentration
Control 2	801 ~~	80	0	0	0
10 5	AP N	40	0	0	0
	40 &	40	0	0	0
100 0	40	40	0	0	0
316	40	40	0	0	0
1000	40	40	0	0	0



The mean body weight of the adult earthworms in the control group had increased during the 4 weeks of exposure. The mean body weight was 0.52 g per worm (+ 53.7% of the mean initial weight).  $\rho_{a}^{\circ}$ 

The mean changes in body weight of the test concentrations of 10, 32 and 100 mg test item/kg sold dry, weight were not statistically significantly different relative to the control (+ 51,8%, + 44.1%) and + 45.9%). The mean changes in body weight of the test concentrations of 316 and 1000 mg test item/kg sold dry weight were statistically significantly different relative to the control (+ 37.2% and + 23.7%). (Results of a Dunnett's multiple t-test, two-sided,  $\alpha = 0.05$ ).

Test concentration	Number of su	rviving worms	Mean weight	er worm (g) 🎸	Mean change of
(mg test item/kg dry weight soil)	Day 0	Day 28	Day 0		body weight (%)
Control	80	80	d 34 🔬 i	QŨ.52 🖉 🦂	\$53.7 ± 7.4
10	40	40	0.34 0	0.52	51.8±8.8
32	40	40	0.35	0,50	44.1 ± 3.6° 0°
100	40	40	¢34 °	(\$49 ×	¥5.9 ± 8.6
320	40		0.34 🚀 🤘	0.46	37.2⊕ 4.6* O
1000	40	40 5 5	0.34	0.42	23 ± 7.0*

 Table CA 8.4.1/02-2
 Mean body wet weight of adult earthworms at the rest start and after 28 days, (values in this table are rounded galues)

s. Mean value statistically significantly different compared to the control (p < 0.05)

In the control group, on average 174, Yuvenile earthwormsper test vessed were found (corresponding to a mean reproduction rate of 17.5 juveniles per surviving adult)

In the treatment groups exposed to the test item KWG 4168-N-Oxid (technical) up to and including the highest test concentration of 1000 mg test item/kg soll dry weight, the mean reproduction rate was in the range of 0.4% to 109.2% of the control value.

No statistically significantly different values for the number of juveniles per test vessel relative to the control were observed at the test concentrations of 10, 32, 100 and 316 mg test item/kg soil dry weight. A statistically ognificantly different value for the number of juveniles per test vessel relative to the control was observed at the test concentration of 1000 mg test item/kg soil dry weight. (Results of a Dunnett's multiple t-test, one-sided smaller,  $\alpha \ge 0.05$ ).

Test concentration mg test	Reproduction rate	<b>b</b> venile earthworms per test box		
item/kg dry weight soil	(per surviving adult)			
a .õ ^v t	Mean 🗘 SD 🍈 🕺 🔿	Mean ± SD	CV (%)	% of control
Control 🖓 🗘 🔊	17.5 ¥ 3.7 🗡 🕺	$154.9 \pm 36.6$	20.9	100.0
10 🔬 🔿	$1/2 \neq 3.7$ $1000 \pm 3.3$	$0.3 \pm 33.2$	20.7	91.6
32	Ĵ9.1±49	$191.0 \pm 46.7$	24.5	109.2
100	18.3×3.7 ~ ~	$183.3 \pm 36.7$	20.0	104.8
316 4 2	12.50± 5.9	$125.3 \pm 59.5$	47.5	71.6
1000 0	$00^{7} \pm 0.1^{10}$	$0.8 \pm 1.0$	127.7	0.4*
*1 1 0 1 1	of a 1 the	1, 1, 1, 1,	0.05	

## Table ( 8.4.1/02-3 , Keproduction of the earthworms

*Mean value statistically significantly ofference compared to the control (p < 0.05)

To verify the sensitivity of the test system, the reference item carbendazim was tested in a separate study. There were statistically significant effects on reproduction at concentrations of  $\geq 1.25$  mg a.s./kg soil. The effects on the reduction of reproduction showed that the test system was sensitive and reflected the expected toxicity as given in the OECD 222 test guideline (significant effects observed between 1 and 5 mg as /kg soil).

#### MII. S Conclusion

No statistically significantly different values for the growth relative to the control were observed at the test concentrations of 10, 32 and 100 mg test item/kg soil dry weight. Statistically significantly different



values for the growth relative to the control were observed at the test concentrations of 316 and 1000 mg test item/kg soil dry weight.  $Q_{\mu}^{\circ}$ 

No statistically significantly different values for the number of juveniles per test vessel relative to the control were observed at the test concentrations of 10, 32, 100 and 316 mg test nem/kg soil dry weight. A statistically significantly different value for the number of juveniles per test vessel relative to the control was observed at the test concentration of 1000 mg test item/kg soil dry weight.

Considering all measured parameters and endpoints of the study, the overall NOEC is determined to be 100 mg test item/kg soil dry weight. Thus, the overall LOEC is determined to be 316 mg test item/kg soil dry weight.

Assessment and conclusion by applicant:

The study was assessed to current guidance: OFCD 222: 29 haly, 2016: "OECD Quideline for the Testing of Chemicals – Earthworm Reproduction Test (Eisenia fetica / Eisenia andrei)"; as this is the most recent version:

- Each replicate (containing 10 adults) to have produced ≥30 juveniles by the end of the lest (actual: 174.9)
- The coefficient of variation of reproduction to be \$30% factual 20.9%
- Adult mortality over the initial 4 weeks of the test to be  $\leq 10\%$  (actual 0%) ( $\approx$

The reference item also demonstrated sufficient sensitivity of the test organisms. The study is therefore considered acceptable.

The NOEC, based on growth, was determined to be 100 mg test item kg soil dry weight.

The data have been subjected to statistical re-evaluation and the results have been presented in the following study summary.

Data Point: KCA 84.1/06 Y Y Y
Report Year: 0 2020
Report Title? Calculation of EC10 and EC20 values for Eisenia fetida with KWG 4168-N-oxid
$\mathcal{O}$ TGM a reproduction study $\mathcal{O}$
Report No: 04/1836-ECO12
Document No: $\sqrt[n]{1-760(34-0)}$
Guideline(s) followed in A None
Deviations tron current None Y Y
Previous valuation: No not previously submitted
GLROfficially not applicable
GLPAOfficially and applicable
Tacilities.
Acceptability/Reliability. Ye
Acceptability/Reliability? Ver S

#### Executive Summary

The report M-24/617-01-1 or the effects of KWG 4168-N-Oxid TG in the earthworm (*Eisenia fetida*) reproduction Grady did not provide estimates of  $EC_{10}$  or  $EC_{20}$  values. Therefore, these values have been calculated in accordance with the Annex to Com. Reg. 283/2013. The resulting  $EC_{10}$  and  $EC_{20}$  values were determined to be 244.53 (95% CL: 236.89 – 250.87) and 286.64 (95% CL: 282.94 - 289.71) mg/kg dws, respectively and are considered reliable as the criteria for goodness of fit were met.



#### I. Methods

The statistical evaluation was performed with statistical software ToxRat Standard v3.3.0. To calculate ECx values, Probit analysis using linear maximum likelihood regression was performed along with 95% EC_x confidence limits based on Fieller's Theorem.

#### II. **Results**

Regarding the calculation of  $EC_{10}$  and  $EC_{20}$  values for reproduction of the arthworms, the criteria for goodness of fit were met as the P(Chi²) value was 1.00, showing no significant deviation between fit and data, and a statistically significant concentration/response was round (p(F) & 0.004) for wis parameter.

The resulting EC10 and EC20 values and the respective confidence intervals are represented in following table below. 

#### Results of the Probit analysis (max. likelihood regression) with reproduction at 56 Table CA 8.4.1/06-1 d: Selected effective concentrations (ICCx) of the test tem and their 95%confidence limits (according to Fieller's theorem) -Q

	Reproduction at sest end (56 days)
Parameter	
	$\langle mg/kg dws \rangle_{\sim} \sim \langle ng/kg dws \rangle_{\sim}$
Effect on	244.53 0 S O S 280.64 k
reproduction	(236.89 - 250.87) $(282.94 - 286.71)$

The resulting EC₁₀ and EC₂₀ values were determined to be  $244.53 \oplus 5\%$  CL: 23689 - 250.87) and 286.64 (95% CL: 282.94 289.71) mg/kg dws respectively and are considered reliable as the criteria for goodness of fit were met.

#### III. Conclusion

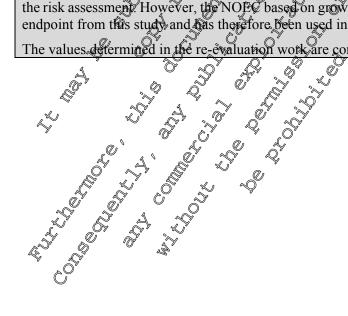
The resulting EC and  $\overrightarrow{\text{BC}}_{20}$  values were determined to  $\overrightarrow{\text{BC}}_{244.5}$  (95% CL: 236.89 – 250.87) and 286.64 (95% CL: 236.89 – 250.87) and 286.64 (95% CL 282 24 - 289.71) mg/kg dws, respectively K.

Assessment and conclusion by applicant:

The statistical re-evaluation of the refroduction data has determined an EC10 of 245 mg/kg dws.

As the EC10 is lower than the established NOE walue, the EC10 of 245 mg/kg dws would be used in the risk assessment. However, the NOEC based on growth of 100 mg/kg dws remains the most critical endpoint from this study and has therefore been used in the risk assessment.

The values determined in the re-exaluation work are considered to be fully valid.





#### KWG 4168-carboxylic acid (M06)

D ( D ) (	
Data Point:	KCA 8.4.1/07
Report Author:	
Report Year:	
Report Title:	KWG 4168-carboxylic acid: Effects on reproduction and growth of earthworms
	Eisenia andrei in artificial soil
Report No:	
Document No:	<u>M-727123-01-1</u> (b) (c) (c) (c) (c) (c) (c) (c) (c) (c) (c
Guideline(s) followed in	Regulation (EC) No 1107/2009 (2009)
study:	OECD 222: Guideline for the testing of chemicals - Earthworm Reproduction
	Test (Eisenia fetida/Eisenia andrei; adopted July 29, 2016)
	ISO-Guideline 11268-2: Soil quality - Effects of collutants on eachworms - Part
	2: Determination of effects on reproduction of Eisenia fetida/Eisenia andrei, 2012
Deviations from current	2: Determination of effects on reproduction of Fisenia fetida/Exenia andrei, 2012 None
test guideline:	
Previous evaluation:	No, not previously submitted Q Q
GLP/Officially	Ves conducted inder GP/Ostically progrised testing facilities
recognised testing	
facilities:	$\begin{array}{cccccccccccccccccccccccccccccccccccc$
Acceptability/Reliability:	

#### **Executive Summary**

The purpose of this study was to assess the effect of KWG 4168-carboxylic acid on the reproduction and growth of the earthworm *Eisedia andrei* in stificial soil.

In an 8-week study, earthworms were exposed to KWG 41@-carboxylic acid at dominal concentrations of 1.63, 2.94, 5.29, 9.53, 17.17 30.9 \$5.6 and 100 mg/kg soil. There were 40 carthworms per treatment group, weighing between 302 and 500 mg.

Exposure to KWG 4168-carboxylic acid showed slight mortality of 2.5% and 7.5% at the test concentrations of 30.9 and 55.6 mg/kg, respectively, which was not statistically significantly different as compared to the pooled control (Welsh t-test after Bonferron Holtz,  $\alpha = 0.05$ , one-sided greater).

There were no statistically significant differences in growth data up to and including the highest test concentration of 100 mg/kg (Dannett's t-test,  $\alpha = 0.95$ , two-sided). There were no statistically significant differences in reproduction data up to and including the highest test concentration of 100 mg/kg (Dunnett's t-test,  $\alpha = 0.05$ , one sided smaller).

There were no abnormal behaviours observed in any of the treatment groups.

The NOEC and LOEC values for mortably, weight change and reproduction of the earthworm *Eisenia* andrei were determined to be 2100 and >100 mg/kg, tespectively. The LC₅₀ and EC₅₀ values were both determined to be >100 mg/kg.

	· · · · · · · · · · · · · · · · · · ·
L. Materials and	Methods ~~~
A. Materials	
Test Material	KWG 4168-carboxylic acid
Lot/Batch #>	AC 1344313-01-03
Parity: A	90.6%
	<i>y</i>
Description:	Turbid brown liquid
Remalysis/Expiry	13 March 2021
date:	



Treatments

Traincins	
Test rates:	1.63, 2.94, 5.29, 9.53, 17.1, 30.9, 55.6 and 100 mg/kg soil
Test organisms	
Species:	Earthworm ( <i>Eisenia andrei</i> ) weighing 302 – 600 mg, approximately 80 months old
Source:	In-house culture
Acclimatisation period:	Earthworm ( <i>Eisenia andrei</i> ) weighing 302 – 600 mg, approximately 8 months old In-house culture 4 days in artificial soil, under test conditions Finely ground cattle manure Plastic boxes (19.3 x 19.6 x 6 cm) with perforated plastic lids
Feeding:	Finely ground cattle manure
Test design	
Test vessel:	Plastic boxes (19.3 x 19.6 x 6 cm) with perforated plastic lids
Test medium:	Artificial soil according to OECD 222,500 g bry weight, 654 g wet weight
<b>Replication:</b>	weight 4 per treatment group 8 per control 10 per replicate 8 weeks
No. animals/vessel:	10 per replicate
<b>Duration of test:</b>	& Weeks to be a first of the second s
Environmental test conditions	
Temperature: 🔬	$48^{2}-22^{2}C$
Water conten	Test vart: $29.5\% - 31.2\% + 50.0 - 53.0\%$ of the WHC _{max} ) Test end. $32.8\% - 35.9\% + 60.9\%$ of the WHC _{max} )
pH: of of a	Test shaft: $6.3 \neq 6.4$ Test end: $6.3 = 6.4$
Photoperiod:	to hours agent, 8 hours dark (400 - 800 lux)
B. Study Design	
The study was conducted in	order to assess the effect of KWO 4168-carboxylic acid on the reproduction

The study was conducted in order to assess the effect of KWO 4168-carboxylic acid on the reproduction and growth of the earth form *Bisenic andrei* during an exposure into artificial soil at eight different test concentrations

Ten earthworms were added to each of the four replicate test vessels in the treatment groups and to each of the eight replicate test vessels in the controls. Test vessels were plastic boxes (18.3 x 13.6 x 6 cm) with perforated plastic lids A

The test soil consisted of 69.6% fine quartz and, 20% kaolinite clay, 10% sphagnum peat and 0.4% calcium carbonate. Prepared foil consisted of approximately 500 g of dry weight and 654 g wet weight.

The earthwarms were exposed to nominal concentrations of 1.63, 2.94, 5.29, 9.53, 17.1, 30.9, 55.6 and 100 mg/kg soil.

Incubation we within 18 and 22°C with a photoperiod of 16 hours light and 8 hours dark at approximately 400 a 800 Jux.

After 4 weeks of exposure, the content of each test vessel was emptied and the adult worms were counted removed and weighed before the substrate was returned to the respective test units minus the worms. Mortality and behavioural abnormalities (e.g. lack of movement and rigidity) were observed at this stage (28 days after application).



At test initiation and after 4 weeks of exposure, the adult test organisms of each vessel were weighed.

At test termination, the number of surviving juveniles per test vessel was determined by placing the est vessels in a water bath of 50 to 60°C and counting all emerging worms. Reproduction was determined by the number of alive juveniles at test termination.

The earthworms were fed finely ground cattle manure which was added to the soil throughout the duration of the test.

Mortality data were tested for normal distribution and homogeneity of variance ( $\alpha \neq 0.01$ ) using the Shapiro-Wilk's test and the Levene's test, respectively. As the data were normally distributed and heterogeneous, Welsh t-test after Bonferroni-Holm was used to compare the treatment and pooled of control values (multiple comparison, one-sided greater).

Growth and reproduction data were tested for normal distribution and homogeneity of variance  $\alpha = 0.01$ ) using the Shapiro-Wilk's test and the Levene's test, respectively. Since both the growth and reproduction data were normally distributed and homogeneous but did not follow a monotonicity trend, the Dunnett's t-test was used to compare the treatment and pooled control (growth; multiple comparison,  $\alpha = 0.05$ , two-sided) and solvent control (reproduction; multiple comparison,  $\alpha = 0.05$ , one sided smaller) values.

#### II. Results and Discussion

The data were assessed against the criteria of the OECD and ISO test guidelines to which the study was conducted. All validity criteria were met.

- Each replicate (containing 10 adults) to have produced  $\geq$  30 inveniles by the end of the test (actual: 162 183)
- The coefficient of variation to be  $\leq 36\%$  (actual: 4.%) &
- Adult mortably over the intrial 4 weeks of the test to be  $\leq 10\%$  (actual 10%)

A mortality of 0% was observed in the control and solvent control. No mortality was observed in the test item treated groups except at the test concentration of 30.9 mg test item/kg soil where 2.5% mortality was observed and at the test concentration of 50.6 mg test new/kg soil where 7.5% mortality was observed. The mortality was not statistically significantly different compared to the pooled control (Welsh t test after Bonferroni Holm one-sided greater, q = 0.05). The Welsh t-test after Bonferroni-Holm showed no significant statistical difference at the two test concentrations where mortality was observed compared to the pooled control (Welsh t test after Bonferroni-Holm showed no significant statistical difference at the two test concentrations where mortality was observed compared to the pooled control. At the other/test concentrations without mortality, the test could not be performed due to mathematical reasons. Nevertheless, as there was no mortality observed, no test item related effect was observed.

Treatment group	Mean number of l	ive adults >>	Mean mortality	Significance
(mg/kg)	Start 2	A weeks	$(\%) \pm SD^1$	
Control		Q 10 X	$0.0 \pm 0.0$	-
Solvent control			$0.0 \pm 0.0$	n.s. ¹⁾
		$Q^{\gamma}$	Pooled control	
Ö ^y A		Ø.	$0.0 \pm 0.0$	
1.63		Q 10	$0.0 \pm 0.0$	n.s. ⁺
2.94		10	$0.0 \pm 0.0$	n.s. ⁺
5.29	A0 ~	10	$0.0 \pm 0.0$	n.s. ⁺
9.53	\$10 .	10	$0.0 \pm 0.0$	n.s. ⁺
1751 2 30.9	10.2	10	$0.0 \pm 0.0$	n.s. ⁺
30.9	10	9.75	$2.5 \pm 5.0$	n.s.
55.6 0	10	9.25	7.5 ± 9.6	n.s.
100	10	10	$0.0 \pm 0.0$	n.s. ⁺

Table CA 84.1/07-1 Summary of adult mortality following 4 weeks of exposure

- not relevant



¹ mean  $\pm$  standard deviation of 4 replicates (8 in the controls)

n.s.¹⁾ not significantly different compared to the control, Fisher's exact test,  $\alpha = 0.05$ , two-sided

n.s.+ test could not be performed due to mathematical reasons, but as no mortality was observed they are determined to be not statistically significantly different compared to the pooled control n.s. not significantly different compared to the pooled control, Welsh t-test after Bonfertoni Holm,  $\alpha =$ onesided greater

The body weight changes in the test item treated groups were not statistically significantly different compared to the pooled control up to and including the highest test concentration of 100 mg (Dunnett's t-test,  $\alpha = 0.05$ , two-sided).

Treatment	Body weight ran	ge (mg)	Body weight cha	ange? Q	Significance &
group (mg/kg)	Start	4 weeks	Mean ± SP	Mean + SD ¹	
		<u>k</u>	(ŵg) 🔊 🏅		$\sim \sim \sim$
Control	359.7 - 424.8	468.0 - 50 8	$89 \pm 26$	23,00 ± 7.3 ⊙	£ A
Solvent control	360.3 - 430.2	461.4 - 493.2 0	97±08 Q	$24.8 \pm 5.4$	
			Peoled control	Fooled@ontrok	
		Ű, N	987 ± 22 🔨 🕐	23.9 + 6.3	
1.63	360.5 - 418.5	4581-490.8	89±15, ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	22@±4.9	Ø <b>1</b> .S.
2.94	362.3 - 416.9	494.8 – <b>S</b> Å3.1 📎	119₽30 ∕∕	\$0.6 ± 8.9	n.s.
5.29	365.0-412.2	₹444. <b>3</b> – 513. <b>3</b>	960≠21 🖉	024.5 + 3.3	n.š.y
9.53	367.6 - 409.0	49456 – 52408	\$02 ± 200	26.4 6.7	⟨n,s.
17.1	370.4 - 408	478.2 - 496.3	[ ⁹ 5±177 ₆ @ [™]	$2456 \pm 5.3$	Qn.s.
30.9	374.7 - 408.0	\$491.9 \$13.5 \$	1147#18	29.4±5,3 0	n.s.
55.6	375.1 → 404.1	455.2 519	$104 \pm 33$ %	26.8 9.1	n.s.
100	375.5 - 402.9	477.2 - 515.4	$@ 100 \pm 6$	25.7 ± 5.1 8	n.s.

Table CA 8.4.1/07-2	Body weight data	observed following 4	weeks exposure
	Doug weight data	observed tong mg	meens sapusure

- not relevant

- not relevant  $\sqrt{3}$   $\sqrt{3}$ 

n.s.¹⁾ not significantly different compared to the control, Student t-test,  $\alpha = 0.05$ , two-sided n.s. not significantly different compared to the posted control, Dunnett's stest,  $\alpha \neq 0.05$ , two-sided The reasonable of the posted control of the posted control of the state o

The reproduction rates were not statistically significantly different compared to the solvent control up to and including the highest test concentration (Dunnet s t-test,  $\alpha = 0.05$ , one-sided smaller).

1 able (3) 8.4.1/0/-3			weeks exposure	
Treatment group	Number of rev	venile earthworms		Signi

Treatment group       Number of invenile carthy         (mg/kg)       Image: Additional statement of the statement of		Significance
	% of solvent control	
Control $\mathcal{O}$ $\mathcal{O}$ $\mathcal{O}$ $\mathcal{O}$ $\mathcal{O}$ $\mathcal{O}$	- 7	-
Solvent control $\bigcirc$ $\bigcirc$ $\bigcirc$ $58 \pm 17$ $\bigcirc$ $\bigcirc$		*
1.63 $152$ $20$ $37$	096.6	n.s.
$2.94  \bigcirc  \lor  \bigcirc  120 \pm 22  \bigcirc  \bigcirc  \bigcirc  \bigcirc  \bigcirc  \bigcirc  \bigcirc  \bigcirc  \bigcirc $	82.0	n.s.
5.29 (148 ± 24)	94.1	n.s.
9.52 $\sqrt{162 \pm 20}$	102.5	n.s.
17.1 [°] 1630 22 ° O	103.3	n.s.
$30.9 \qquad \qquad$	102.1	n.s.
55.6 0 ⁴ 45 ± 10 ⁹	92.0	n.s.
100	101.2	n.s.

- not relevant

¹ mean = Standard deviation of 4 Seplicates (8 in the controls)

* significantly difference compared to the control, Student t-test,  $\alpha = 0.05$ , two-sided

n.s not significantly different compared to the solvent control, Dunnett's t-test,  $\alpha = 0.05$ , one-sided smaller

To verify the sensitivity of the test system, the reference item carbendazim was tested in a separate study. There were statistically significant effects on reproduction at a concentration of 0.695 mg a.s./kg soil and above. The EC50 for reproduction was calculated as 0.92 mg a.s./kg soil. The effects on the



reduction of reproduction showed that the test system was sensitive and reflected the expected toxicity as given in the OECD 222 test guideline (significant effects observed between 1 and 5 mg a.s./kg soil).

#### III. Conclusion

In an earthworm reproduction and growth study with KWG 4168-carboxylic acid, the NOPC for mortality of the earthworm *Eisenia andrei* was determined to be  $\geq 100 \text{ mg/sg}$  soil. The  $\downarrow OEC$  for mortality was estimated to be  $\geq 100 \text{ mg/kg}$  soil. The LC₅₀ was estimated to be  $\geq 100 \text{ mg/kg}$  soil.

The NOEC for growth was determined to be  $\geq 100 \text{ mg/kg}$  soil. The LOEC for growth was estimated to be  $\geq 100 \text{ mg/kg}$  soil.

The NOEC for reproduction was determined to be  $\geq 100 \text{ mg/kg soil}$  The LOEC for reproduction was estimated to be  $\geq 100 \text{ mg/kg soil}$ . Due to the lack of a concentration response relationship, no reliable EC_x-calculation was possible. Therefore no EC₁₀ can be reported, and the EC₈ an

#### Assessment and conclusion by applicant;

This is a new study that has not been previously subparted for evaluation

Validity criteria according to the OECD 222 guideline (2016) were met

- Each replicate (containing 10 adults) to have produced  $\geq$  30 uvenifes by the end of the test (actual: 162 183)
- The coefficient of variation to be ≤30% (actual: 4.%)
- Adult mortality over the initial 4 weeks of the test to be \$10% (actual 0%)

The reference item also demonstrated sufficient sensitivity of the test organisms. The study is therefore considered acceptable.

The NOEC, based on reptoduction and growth, was determined to be  $\geq 100 \text{ mg/kg}$  soil.

#### Acute earthwoom studies

Acute studies using earthworms are no longer a data requirement but for completeness the available acute data with spiroxartine technical have been presented below as supporting information only.

Data Point:	KCA 804.1/03 2 2 2
Report Author:	
Report Year:	1993 $O' O' O' O'$
Report Title:	Doxicity of KWG 4168 technoto earthworms
Report No	HBF2RG 1814 V V
Document No:	<u>M. 20088806-61-1</u> 2 2
Guideline(s) followed in	QECD Guideling No. 209. OECD Guideline for Testing of Chemicals,
study.	Parthworm, Agute Toxicity Tests' (4 April 1984)
	None O
test guideline:	
Previous evaluation:	res, evaluated and accepted
	ĎAR (1997), ĎAR (2010), RAR (2017)
GLP/Officially	Yes Conducted under GLP/Officially recognised testing facilities
recognised testing	
facilities:	
Acceptability/Reliability:	Supportive only

### Executive Summary

The purpose of this study was to assess the acute toxicity of KWG 4168 (tech.) on survival of the earthworm *Eisenia fetida* during an exposure into an artificial soil with five different test concentrations.

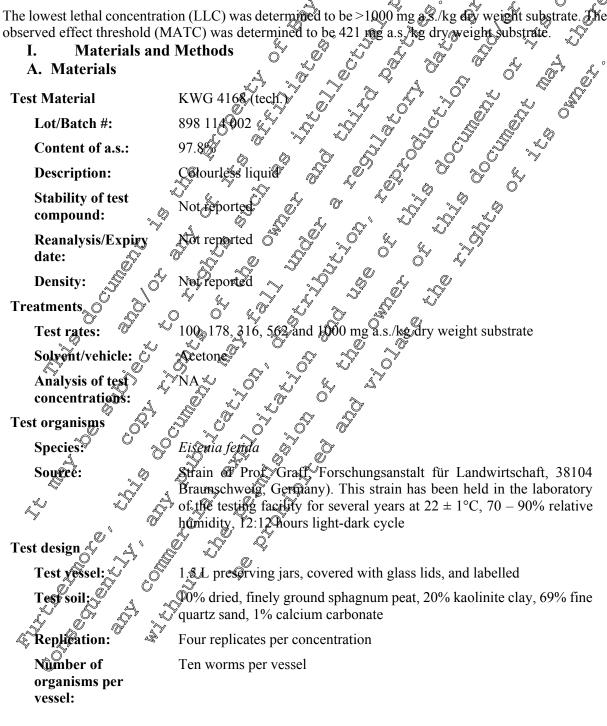


Adult earthworms more than two months old were used for the test. The test substance was applied to the substrate using acetone as a vehicle, and later sprayed onto the artificial soil. Four replicates with 10 worms each were prepared for the control and for the test groups. Earthworms were weighed at test start and at Day 14. Mortality observations were conducted at Days 7 and 14. Moisture content and maximum@ water holding capacity of the test substrate was determined at the start and end I the test.

The 14-day LC₅₀ determined for earthworms exposed to KWG 4168 (tech.) was >1000 ng a weight substrate.

The lowest observed effect concentration (LOEC) was determined to be 562 mg as /kg dw substrate and the no observed effect concentration (NOEC) was determined to be 216 mg/a.s weight substrate.

The lowest lethal concentration (LLC) was determined to be >1000 mg a.S./kg dy weight substrate observed effect threshold (MATC) was determined to be 421 mg a.





<b>Duration of test:</b>	14 days		
Environmental test conditions		1°C	) ,
Temperature:	Range: $20 \pm 2$		
рН:	Test start: Test end:	6.16 - 6.20 6.15 - 6.18 approx. 31% 30.0 - 30.3%	
Water content:	Test stat: Test end:	6.15 - 6.18 approx. 3 km 30.0 - 30.3%	,© 1
Photoperiod:	16-hour light approximatel	t to 8-hout darkness photoperiod and a fight intensity of 19 400 800 lux	
B. Study Design			

## The purpose of this study was to assess the effect of KWG 416Q (tech.) on survival of the carthworm *Eisenia fetida* during an exposure into an artificial soil with five different test concentrations.

Nominal test concentrations were 100 78, 346, 562 and 1090 mg a.s./kg dry worth tsoft.

Ten worms were added to each of the four replicate test vessels. Test containers were 15 Little preserving jars, covered with glass lids. Five handred grants dry weight substrate (725 @ wet veight) was added to each test container.

Incubation was at  $20 \pm 1^{\circ}$ C with a constant photoperiod at approximately 400% 800 Jux.

Seven days after the start of the study, the number of surviving earthworms was counted by emptying the substrate out onto an inert surface and removing the earthworms by hand. The animals were then returned to the test contained with the substrate. After 44 days, the weight us well as the number of surviving earthworms were determined, Farthworms which show no reaction upon being prodded with a blunt probe are considered dead.

The moisture content of the peat and the test substrate as well as the maximum water capacity of the test substrate were determined with the aid of a hydrometer at 105 C, the pH with an electronic measuring instrument.

II. A Results and Distussion

The test was conducted in accordance with the DECD Fuideline No. 207: OECD Guideline for Testing of Chemicals, 'Barthworm, Acute Toxicity Dests' (4 April 1984). The following guidelone validity criterion was met:

• The mortality in the controls should no exceed 10% (actual: 0%)

The study is therefore considered acceptable

No mortality of adult earthworms were observed after 14 days test duration in the control group and at any test concentration, including the highest concentration of 1000 mg a.s./kg dry weight artificial soil.

The  $LC_{50}$  of the reference distance was within the usual range. The test conditions are therefore equivalent to the standard  $\mathcal{Q}$ 

Table CA 8.4.1/03-1	Individual figures obtained in the study with KWG 4168 (tech.)

Concentration	wumber of surviving worms			Mean weigh	nt of worms (g)
Concentration (mg a.s./kg dry weight substrate)	Day	Day 7Day 14Day 0D		Day 14	
weight substrate)					
Contr	40	40	40	0.43	0.41
100	40	40	40	0.43	0.41
178	40	40	40	0.44	0.42
316	40	40	40	0.43	0.38



m

Concentration	Number of surviving worms			Mean weight of worms (g)		
(mg a.s./kg dry	Day 0	Day 7	Day 14	Day 0	Day 14 or	
weight substrate)					DuyII	
562	40	40	40	0.44	0.36	
1000	40	40	40	0.44 🏷	0.30	

#### III. Conclusion

Related to weight alteration and symptoms the no-observed effect concentration NOEC, was \$16 mg/ a.s./kg dry weight substrate, the lowest observed effect concentration (LOEC) was 562 mg a.s./kg and the observed effects threshold 421 mg a.s./kg (MATC, geometric mean of NOEC and LOEC) whe lowest tested concentration with mortality (LLC) was \$1000 mg a.s./kg dry weight substrate.

The values given are nominal concentrations

#### Assessment and conclusion by applicant

The test was conducted in accordance with the QECD Guideline No 207: QECD Guideline for Testing of Chemicals, 'Earthworm Acute Toxicity' Tests' (4 April 1984) which is still the current version. The following validity criterion applies:

• The mortality in the controls should not expeed 10% (actual: 0%)

The study is therefore considered acceptable on the basis that the validity criterion has been met. It is noted that acute earthworm studies are no longer a data requirement therefore this study has been submitted for completeness but considered to be supporting information only.

# CA 8.4.2 Effects on not-target soil meso and macrofauna (other than Searthworms)

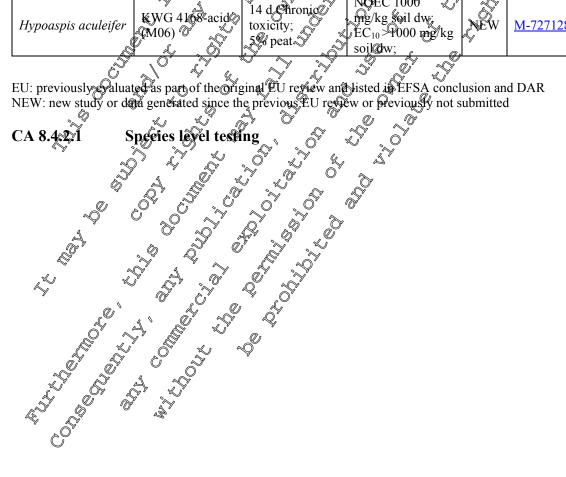
The available cata for meso- and macro-faulta other than arthworms are presented in the table below. Table CA 84,2-01 Summary of soil macro-organism (other than earthworm) toxicity studies with

		,	XX O		
Organism	Test item 🔬	Test type	Endpoints		Reference
Folsomia candida		Vioxieity; 5% peat	NOEC 32 mg As./kg soil dw;	EU	<u>M-289274-01-1</u>
~\$ 1		e-analysis	$EC_{10}/EC_{20}$ not determinable	NEW	<u>M-760433-01-1</u>
		28 d hronic torneity; 26 peat y	NOEC 75 mg a.s./kg soil dw;	NEW	<u>M-405276-01-1</u>
Fotsømia candida	Spinoxamino	<u> </u>	$EC_{10}$ 175 mg a.s./kg soil dw $EC_{20}$ 258 mg a.s./kg soil dw	NEW	<u>M-761559-01-1</u>
Folsomva cantida	KWG 468-	28 d Chronic toxicity; 5% peat	NOEC 316 mg/kg soil dw;	EU	<u>M-289321-01-1</u>
		Statistical Re-analysis	EC ₁₀ /EC ₂₀ not determinable	NEW	<u>M-760431-01-1</u>
Folsomla candida	KWG 4168- despropyl (M02)	28 d Chronic toxicity; 5% peat	NOEC 316 mg/kg soil dw;	EU	<u>M-288905-01-1</u>

spiroxamine and metabolites



Organism	Test item	Test type	Endpoints		Reference
		Statistical Re-analysis	$\begin{array}{c} EC_{10} \ 308 \ mg/kg \\ soil \ dw \\ EC_{20} \ 402 \ mg/kg \\ soil \ dw \end{array}$	NEW	<u>M-760410-051</u>
Folsomia candida	KWG 4168-N- oxide (M03)	28 d Chronic toxicity; 5% peat	EC ₁₀ >100 mg/kg	NEW	M-687854-00-1
Folsomia candida	KWG 4168-acid (M06)	28 d Chronic toxicity; 5% peat	$\sim$ $0^{\circ}$	NEØ	<u>V</u> <u>M-79712691-1</u>
Hypoaspis aculeifer	KWG 4168- desethyl (M01)	14 d Chronic toxicity; 5% peat.	NOLC 50 mg/kg soil dw; Cl ₁₀ 9DI mg/kg soil dw;	NÊŴ	[™]
Hypoaspis aculeifer	KWG 4168- despropyl (M020	Ad Chronic toxicity; 5% peat	NQEC 100 mg/kg soid dw; EC ₁₀ >000 mg/sg soil dw;		₩ <u>680694-01-1</u>
Hypoaspis aculeifer	KWG 4168-N-	14 d Obronic toxicity; 55 peat	NOEC 100 mg/kg aoil dw: EC ₁₀ = 000 mg/kg soil dw;	NEW	<b>Q</b> <u>-680687-01-1</u>
Hypoaspis aculeifer	KWG 4168 ² acid@ GM06)	14 d Shronic toxicity; 5% peat 5	NOEC 1000 mg/kg soil dw; EC ₁₀ P000 mg/kg soil@w;	NEW	<u>M-727128-02-1</u>





 $\bigcirc$ 

Data Point:	KCA 8.4.2.1/01
Report Author:	
Report Year:	2007
Report Title:	KWG 4168 tech.: Influence on the reproduction of the collembola species of Folsomia candida tested in artificial soil with 5 percent p
Report No:	FRM-COLL-52/07
Document No:	<u>M-289274-01-1</u>
Guideline(s) followed in study:	ISO 11267 (1999)
Deviations from current test guideline:	Yes OECD 232 (2016) The reference item used was not boric acid as recommended by the OECD 232 test guideline, however the reference item used was acceptable under the ISO guideline to which the study was conducted and was considered to sufficiently demonstrate the sensitivity of the test organism.
Previous evaluation:	yes, evaluated and accepted and
GLP/Officially	Yes, conducted under GLP/Officially recognized testing facilities
recognised testing facilities:	
Acceptability/Reliability:	Yes A TO A A A A A

#### **Executive Summary**

Collembola (*Folsomia candida*) aged 10 to 12 days were exposed to KWG 4168 technical incorporated into soil in a 4-week study to assess effects on portality and reproduction.

Test organisms were exposed to 10, \$2, 100, 316 and 1000 mg as./kg soil dry weight and to a water control. Betosip (a.s. phenmediphan) was used as a toxic standard in acoordance with ISO 11267 (1999) guidelines.

guidennes. A statistically significant reduction in number of juveniles compared to the control was observed in the treatment groups with 100, 396 and 9000 mg a.s. Ag soil by weight, resulting in reductions of 21.4, 21.9 and 95.6%, gespectively. O ð O

Q,

S.

The NOESe and LOEC for reproduction were 32 and 100 mg a.s. drg soil dry weight, respectively.

Materials and Methods I.

WG@168 with.
ED171004050
Light brow clear pily liquid
Not reported 0
14 August 2007
Not reported
10, 32, 100, 316 and 1000 mg a.s./kg soil dry weight
Because the test item was poorly soluble in water, a test item quartz sand mixture for each concentration was prepared



Analysis of test concentrations:	No
Test organisms	
Species:	Folsomia candida, Collembola, Isotomidae
Source:	<i>Folsomia candida,</i> Collembola, Isotomidae Bred at Bayer CropScience since April 2002. The strain was originally obtained from Ibacon, Institute for Analytic and Consuming, 2 GmbH, D – 64380 Rossdort None reported
Acclimatisation period:	None reported
Feeding:	Approximately 2 mg granulated dry yeas at the start of the study and after 14 days
Treatment for disease:	Approximately 2 mg granulated dry yeast at the start of the study and after 14 days None reported Glass vessels (volume 140 fbL, diameter: 5 cm) covered with glass lids
Test design	
Test vessel:	Glass vossels (volume, 140 mL, diameter: 5cm) covered with glass lids
Test medium:	Artificial soil according to OEOD 207 (1984). With respect to the properties of the test item (log $P_{ow} \geq 2$ ) 5% peat instead of 10% peat were used considering the influence on bioavailability (EPPO 2002).
Replication:	5 (+① without Collembola for measurement of soil morsture during the test and pH and soil morsture or the end of the study
No. animals/vessel:	
Duration of test:	28 Pays S ~ ~ ~ ~ ~
Environmental test	$\begin{array}{cccccccccccccccccccccccccccccccccccc$
Temperature:	$20 \neq 2^{\circ} C \xrightarrow{6^{\circ}} \overset{6^{\circ}}{} \overset{6^{\circ}}{$
B. Study Design	$\frac{1}{28} \text{ ways} = \frac{1}{29} \text{ ways} = \frac{1}$
Collembola (Folsomia candi on mortality and reproduction	da) were exposed to KWG 4 68 technical over 4 weeks to assess the effects n.

The Collembola were 10 to 12 days old at the start of the study. For each replicate, 10 of the juvenile Collembola were placed in the glass test vessels, which had been prepared with the test item quartz sand and artificial soil. The soil was aligned with QECD 207 (1984) standard, but with 5% peat instead of 10% due to considerations on the influence or bioavailability with respect to the test item. The required amount of the test item was mixed thoroughly with 5 g quartz sand. If less than 50 mg test item was required, the est item had to be mixed with quartz sand and a stock mixture was prepared and diluted with quartz sand to teach the demanded set concentrations. Water was added to the soil mix until 50% water holding capacity was achieved.

The artificial soil was kept at 18 to 22°C, with the temperature continuously recorded by a thermohyprograph integrated in the climatic chamber. The test vessels were kept at 450 to 645 lux under a photoperiod of 16 h light : 8 h dark, monitored by an integrated luxmeter of the climatic chamber.

Application rates in this study were 10, 32, 100, 316 and 1000 mg a.s./kg soil dry weight. Five replicates were exposed to control (water) treatment, and five replicated to 10, 32, 100, 316 and 1000 mg a.s./kg soil dry weight. During the study, they were fed with granulated dry yeast.



A reference test with the toxic standard, Betosip, was performed at least once a year to ensure that the laboratory test conditions were adequate and to verify that the response of the test organisms does not change significantly over time.

Water content was checked 14 days after application by reweighing the additional vessels. If the water loss exceeded 2% of the initial water content, the missing amount of water was added to all vessels of the treatment group. At the same time, the food was checked and the Collembola were fed again if necessary. Mortality and reproduction were reported after 28 days and were determined by the mimber of dead adult Collembola and the number of living juvenites detected using digital images.

#### **Results and Discussion** II.

Validity criteria, according to the test guideline that the study was conducted to , V control, the following criteria were met:

- Mean adult mortality <20% at the end of the test (actual: 12%),
- The mean number of juveniles per vessel >100 at the end of the sest (actual: 742 •
- The coefficient of variation calculated for the number of juveniles ≤30% (actual: 21%)

Mortality did not vary from the control greater than 6% at test item Concentrations between 10 and 316 mg a.s./kg soil dry weight. At the highest test item concentration 1000 ng a.s. kg soil dry weight, 86% mortality was observed in adult Collembola.

Table CA 8.4.2.1/01-1	Survival of adult	Collembola aft	er 4 weeks	treatment (1	n=100replicate)
			**		() /

	mg a.s./kg soi	l dry weight 🖓		~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~		
	Control	10 8	X s	100	J 316 V	1000
Mean ¹	00	A.8	9.6 0	<b>9</b> .2 ¢	* <u>9</u> .2	1.4
$SD^1$	1.1	1.6	0.5	0.80	S 1.3 V	0.5
% mortality ²	12.0	120		5 <u>89</u>	8.0	86.0

1 mean and standard destation (SD) of five replicates 2 formula: ((initial placed organisms per cessel - mean of surviving adults per cessel) / 100 × 100 ×

Reproduction in the Collembola was statistically different to the control at 100, 316 and 1000 mg test item/kg artificial solf dry weight geatment groups. At the highest test item concentration, 1000 mg test item/kg artificial soil dry weight reproduction was at 4.4% of the control.

Table CA 8.4.2.1	/01-3 Reproduction of the Collembola after	4 weeks treatm	ent (juveniles/r	replicate)
	ang test item/kg artificial soil dry weight	ð		
0	Control \$10 6 \$32 \$	100	316	1000
Mean ¹	74106 8648 ~ 72672	583.0	579.0	32.4
$SD^1$ $\sqrt[4]{3}$	156.9 7 29.4 2 124.5	112.2	101.1	32.9
$CV^2$		-	-	-
% of control ³	- ~ 116.6 97.95	78.6*	78.1*	4.4*

1 mean and standard deviation (SD) of five replicates

2 Coefficient of Variation

3 formula: mean number of juveniles per  $tail = 100^{10}$  / mean number of juveniles per control group

- = not applicable Q* = significantly afferent compared to the control (Dunnet's Test, one-sided-smaller,  $\alpha = 0.05$ )

To demonstrate the sensitivity of the test system Betosip (phenmedipham 15.4 %) as a toxic standard was regularly tested (once a year) at concentrations of 89, 133, 200 and 300 mg test item/kg artificial soil dry weight in the most cent test the mortality rate of adult Collembola was 8 %, 14 %, 22 % and 32 % at 89, 153, 200 and 300 mg Betosip/kg artificial soil dry weight. In all treatment groups the number of avenues was statistically significant reduced in comparison to the control. The NOEC_{reproduction} was < 89 mg Betosip (13.7 mg a.s)/kg artificial soil dry weight and the LOEC_{reproduction} was 89 mg Betosip (13.7 mg a.s)/kg artificial soil dry weight. The results were considered to demonstrate the sensitivity of the test system.



#### III. Conclusion

In the control group, 12% of the adult Collembola died which is within the tolerated range of  $\langle 00\% \rangle$  mortality recommended by the guideline. The highest mortality rate of 86% was found in the test item concentration of 1000 mg a.s./kg soil dry weight. Concerning the number of juveniles, statistical analysis revealed significant differences between the control and the treatment groups at 100, 316 and 1000 mg a.s./kg soil dry weight.

The NOEC for reproduction: 32 mg a.s./kg soil dry weight

The LOEC for reproduction: 100 mg a.s./kg soil dry weight.

#### Assessment and conclusion by applicant:

Validity criteria according to the current OECD 32 guideline (2016) were met. In the control, the following criteria were met:

- Mean adult mortality <20% at the end of the test (actual: \$2%);
- The mean number of juveniles per yessel @100 a f the end of the test (actual: <math>942); %
- The coefficient of variation calculated for the number of juveniles 90% (setual: 21%).

The study was not specifically conducted to the OPCD 232 test guideline, however the test design and the parameters assessed are consistent with the OECD test guideline. Furthermore the OECD validity criteria have been met.

The reference item used was not boric acid, as acceptable under the ISO guideline to which the study was conducted and was considered to sufficiently demonstrate the sensitivity of the test organism.

The study is therefore considered acceptable

The NOEC for reproduction: 32 rog a.s. Reg soil by weight

The data have been subjected to statistical re-evaluation and the esults have been presented in the following study summary.

(N 1)

Data Points $4$ KCA8.4.2.194 $0$
Report Author:
Report Year: $\sqrt{2}$ $\sqrt{20}$ $\sqrt{2}$ $\sqrt{2}$ $\sqrt{2}$
Report Year:       2020       C       A         Report Title:       Calculation of EC10 and EC20 values for Folsomia candida with spiroxamine TG
a mayepioquetion study a
Report No: $0^{1/2}$ $0^{1/2}$ $1836$ CO14 $0^{1/2}$
Document No:     MI-760/83-01.0       Guidelinets followed in study:     Noncolor       Deviations from current     Noncolor
Guideliness followed in None of the second s
study:
Deviations from current None a star
test guideline:
Previous evaluation: No, not previously submitted
GLP/Officially novapplicable
recognised testing v a a
Previous evaluation: No, not previously submitted GLP/Officially not applicable recognised testing facilities:
Acceptability/Regrability. Yes

### Executive Summary

The report M-289274-09-1 on the effects of spiroxamine TG in a springtail (*Folsomia candida*) reproduction study did not provide estimates of EC₁₀ or EC₂₀ values. Therefore, these values have been calculated in accordance with the Annex to Com. Reg. 283/2013. Due to the wide span of the confidence limits, unfit EC₁₀ and EC₂₀ obtained values and due to the lack of a significant dose response with some



models, when compared to the control, it was not possible to calculate reliable  $EC_{10}$  and  $EC_{20}$  values for reproduction.

#### I. Methods

The statistical evaluation was performed with statistical software ToxRat Standard v3.3.0 For the calculation of  $EC_{10}$  and  $EC_{20}$  values, several statistical models were used. However, due to the write span of the confidence limits, unfit  $EC_{10}$  and  $EC_{20}$  obtained values and due to the lack of a significant dose response with some models, when compared to the control, it was not possible to calculate calculate  $EC_{10}$  and  $EC_{20}$  values for reproduction.

#### II. Results

Due to the wide span of the confidence limits, unfit  $EC_{10}$  and  $EC_{26}$  betained values and due to the lack of a significant dose response with some models, when compared to the control it was not possible to calculate reliable  $EC_{10}$  and  $EC_{20}$  values for reproduction.

#### III. Conclusion

Due to the wide span of the confidence limits,  $unfit EC_{10}$  and  $EC_{0}$  obtained values and due to the lack of a significant dose response with some models, when compared to the confol, it was not possible to calculate reliable  $EC_{10}$  and  $EC_{20}$  values for reproduction.

#### Assessment and conclusion by applicant

The statistical re-evaluation of the production data could not calculate reliable  $EC_{0}$  and  $EC_{20}$  values.

The NOEC of 32 mg a.s./kg dws shall be used in the risk assessment as the most critical endpoint from this study.

The values determined in the re-evaluation work are considered to be full valid

Data Point: $\sqrt{2}$ $\sqrt{2}$ $\sqrt{2}$ $\sqrt{2}$ $\sqrt{2}$
Report Author:
Report Year: $2011^{\circ}$ $2011^{\circ}$ $2011^{\circ}$
Report Title: Spiroxamme a.s. Mnfluence on the reproduction of the collembolan species
Foisoinga cangua tester in angaciai son
Report Nov FRACOLLO1/9/11
Document No: <u>MOD5276-01-1</u> <u>O</u>
Guideline(s) followed in DECD 232 adoped, September 07, 2009: OECD Guidelines for Testing
study: Chemicals - Collemboran Reproduction Test in Soil
Deviations from current Note 2 2 2 2
Previous evaluation: Qo, not previously submitted
GLP/Officially Yeo conducted under GLP/Officially recognised testing facilities
recognised testing and a second secon
facilities:
Acceptability/Reliability: Yes O

### Executive Stonmary

Collembola (*Folsomia condida*) aged 10 to 12 days were exposed to spiroxamine technical incorporated into soil on a 4-week study to assess effects on mortality and reproduction.

Test organisers were exposed to 18.75, 37.5, 75, 150, 300 and 600 mg a.s./kg soil dry weight and to a water control.

The highest mortality rate of 35% was found in the test item concentration of 600 mg a.s./kg soil dry weight. The  $LC_{50}$  for mortality was considered to be >600 mg a.s./kg soil dry weight.



A statistically significant reduction in number of juveniles compared to the control was observed in the treatment groups with 150, 300 and 600 mg a.s./kg soil dry weight, resulting in reductions of 13, 22 and 61%, respectively.

The NOEC and LOEC for reproduction were 75 and 150 mg a.s./kg soil dry weight, respectively EC₅₀ for reproduction was determined to be 501 mg a.s./kg soil dry weight.

y. The **Materials and Methods** I. A. Materials **Test Material** Spiroxamine Lot/Batch #: EDTH008883 98.2% w/w **Purity: Description:** Light yellow liqui Stability of test Not reported compound: **Reanalysis/Expiry** 16 Decem date: **Density:** Treatments 300 and 600 mg a.s. kg weight sochdry **Test rates:** mixture for each concentration was None. A test iten qu Solvent/vehicle: Analysis of tes concentrations Test organism Folsomia candida, Collepibola, Isotomidae Species Bred at Bayer CropScience spice April 2002. The strain was Source: originally obtained from Ibacon, histitute for Analytic and Consulting, GmbH. D Acclimatisati period: granulated dry yeast at the start of the study and oximatel Feeding: 14@avs Treatment for **A**isease: Test design Ś Glass vesses (volume: 140 mL, diameter: 5 cm) covered with glass lids each containing 30 g test soil. Artificial soil according to OECD 232 (74.8% fine quartz sand, 5% sphagnum peat, 20% kaolin clay and 0.2% CaCO₃) Replication: 8 for control and 4 for each test item concentration (one additional replicate also prepared without Collembola for measurement of soil moisture during the test and pH and soil moisture at the end of the study)



No. animals/vessel:	10					0
<b>Duration of test:</b>	28 days					
Environmental test conditions						
Temperature:	$20 \pm 2^{\circ}C$				<i>"0</i> " 1	
Photoperiod:	16 h light	: 8 h dark a	at 545 - 663	3 lux 🖉		
B. Study Design			T A A			
Collembola ( <i>Folsomia cand</i> effects on mortality and repr	<i>dida</i> ) were roduction.	exposed to	spiroxami	ne tronical	over Aweel	to assess the
No. animals/vessel: Duration of test: Environmental test conditions Temperature: Photoperiod: B. Study Design Collembola ( <i>Folsomia cand</i> effects on mortality and repu The Collembola were 10 to Collembola were placed in the and artificial soil. The soil was amount of the test item was artificial soil. The artificial soil was kept chamber. The test vessels were Application rates in this stud replicates were exposed to	dy were 18. control (wa	75, 37.9, 75 iter) (treatmy)	5, <b>3</b> 30, 300 ent, and fo	and @0 mg a	s./kg soil di	weight. Eight
During the study, they were At test start each test vessel water was determined by rev 2-fold amount of the missin After 7, 14 and 21 hays the Mortality and oproduction adult Collembola and the mi	was weight weighing the ng water. The test vessels were report	ed for the d e test vessel test vess were regran ted, after 28	eterminatio S. The vess els were se domised days and	elswere fewe y up randomi were determi	etted with the sed in a clir p ned by the r	e approximately natic test room.
<ul> <li>II. Results and D</li> <li>Validity criteria, according control, the following criteria</li> <li>Mean addit mortalit</li> <li>The mean number of the coefficient of v.</li> </ul>	Discussion to the test g ia were met: ty \$0% aft fjuventies j	uideline the he end of the pervessel	at the study be test (actu	v was conduct al: 5%); end of the tes	ted to, were t (actual: 15	70);
	ght. A0the l d in adult C est item/kg	hghest test ollemDola. dry veight <b>Collembola</b>	item conce A LC ₅₀ co artificial so	entration, 600 ould not be ca	mg a.s./kg alculated an	soil dry weight, d was therefore
	sod dry weig			1 50	200	
		37.5	75	150	300	600
		97		9.8	9.8	6.5
Mean ^y & Control &		9.7 0.6	9.5	9.8	9.8	6.5
		9.7 0.6 3.3		9.8 0.5 2.5	9.8 0.5 2.5	6.5 2.4 35.0



Reproduction in the Collembola was statistically different to the control at 150, 300 and 600 mg a.s./kg artificial soil dry weight treatment groups. At the highest test item concentration, 600 mg a.s./kg artificial soil dry weight, reproduction was at 39.5% of the control.

The No-Observed-Effect-Concentration (NOEC_{reproduction}) was 75 mg a.s./kg artificial soil dry weight. The Lowest-Observed-Effect-Concentration (LOEC_{reproduction}) was 150 mg a.s./kg artificial soil dry weight. The EC₅₀ for reproduction, determined by probit analysis, was 501 mg a.s./kg artificial soil dry weight (95 % confidence limit 422 – 637 mg a.s./kg artificial soil dry weight).

	mg test iter	m/kg artificia	al soil dry wei	ght	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	<u> </u>	
	Control	18.75	37.5	75	Q.50 🔊	<i>3</i> 90 L	600
Mean ¹	1569.6	1587.8	1520.7	1578.0 🥎	13703	[™] ¥220,00″	<b>6</b> 19.8 <b>V</b>
$SD^1$	188.3	207.6	136.9	92.2	96.Y O	12000	74.2
$CV^2$	12.0				4		4
% of control ³	-	101.2	96.9 👋	100,9	87.4*0	<i>1</i> 9.7* 🔗	3 <b>9.</b> 5* ~ °

Table CA 8.4.2.1/05-2	Reproduction of the	Collembola affer 4	weeks treatment	(iuvenilek/replicate)
1 abic CA 0.4.2.1/03-2	Reproduction of the	Concinuoia artea 4	weeks ti gatinent	(juvenines/repaire).

¹ mean and standard deviation (SD) of 8 replicates in the control and 4 replicates per test item concentration

² Coefficient of Variation

³ formula: mean number of juveniles per treatment group * 100/ mean former of savenile for control group * *

* = significantly different compared to the control (Dunnetty Tests one-sided smaller, of 0.05)

To demonstrate the sensitivity of the test system boriglacid as a toxic standard was tested at concentrations of 44, 67, 100, 150 and 225 up test item/kg artificial solid dry weight. In the most recent test boric acid showed an EC₅₀ of 1 mg test item/kg artificial solid dry weight (95 % confidence limits from 80 mg to 104 mg Boric acid/kg artificial solid dry weight) for reproduction. The result is in the recommended range of the guideline (about 100 mg Boric acid/kg artificial solid dry weight). The NOEC_{reproduction} was coculated to be 44 mg Boric acid/kg artificial solid dry weight and accordingly the LOEC_{reproduction} is 67 mg Boric acid/kg artificial solid dry weight according Williams-Test multiple t-test procedure,  $\alpha = 0.05$ , one sided smaller. This demonstrates that the test organisms were sufficiently sensitive.

### III. Conclusion

In the control group, 5% of the adult Collembola died which is within the tolerated range of  $\leq 20\%$  mortality recommended by the guideline. The highest mortality rate of 35% was found in the test item concentration of 600 mg as /kg soil dry weight. Concerning the number of juveniles, a statistically significant reduction in humber of juveniles compared to the control was observed in the treatment groups with 150, 300 and 600 mg as /kg soil dry weight resulting in reductions of 13, 22 and 61%, respectively:

The LC₅₀ for mortality was considered to be  $\approx 600$  mg a.s./kg soil dry weight.

The NOEC and LOEC for reproduction were 75 and 150 mg a.s./kg soil dry weight, respectively. The EC 56 for reproduction was determined to be 500 mg a.s./kg soil dry weight.

### Assessment and conclusion by applicants

Validity criteria according to the current OECD 232 guideline (2016) were met. In the control, the following criteria were wet:

- Mean adult mortality 20% at the end of the test (actual: 5%);
- The mean number of juveniles per vessel  $\geq 100$  at the end of the test (actual: 1570);

The coefficient of variation calculated for the number of juveniles  $\leq 30\%$  (actual: 12%).

The reference item also demonstrated sufficient sensitivity of the test organisms. The study is therefore considered to be acceptable.

The NOEC for reproduction was determined to be 75 mg a.s./kg soil dry weight.



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The data have been subjected to statistical re-evaluation and the results have been presented in the following study summary. V

~ .	
Data Point:	KCA 8.4.2.1/06
Report Author:	
Report Year:	
Report Title:	Calculation of EC10 and EC20 values for Folsomia candida with spiroxamme TG
Report No:	0471836-ECO22
Document No:	<u>M-761559-01-1</u>
Guideline(s) followed in study:	None
Deviations from current test guideline:	None
Previous evaluation:	No, not previously subminded
GLP/Officially recognised testing facilities:	
Acceptability/Reliability:	Yes Q' A A A A A A A A A A A A A A A A A A
Executive Summery	

#### **Executive Summary**

The report M-405276-01-1 on the effects of spiroxamine To in a springrail (Folsomia candida) reproduction study did not provide estimates of EG10 or EC20. Therefore, these values have been calculated in accordance with the Annex to Com. Reg. 283/2013. The resulting EG10 and EC20 values of 175.34 (95% CL: 109 54 - 225.95) and 258.31 (95% CL: 191.18 308.29) mg as /kg dws, respectively, are considered reliable as the criteria for goodness of figwere met. S.

#### I. Methods , O

The statistical evaluation was performed with statistical software ToxRat Professional v3.3.0. To calculate ECx values logit analysis using linear maximum likelihood regression was performed along with 95% EC_X confidence limits based on Fielder's theorem  $\mathbb{Q}$ 

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### II. S Results

For the calculation  $\mathcal{O} EC_{10}$  and  $EC_{10}$  value, the criteria tor goodness of fit were met as the P(Chi²) value was 1.00, showing no significant deviation between fiband data, and a statistically significant concentration/response was found ( $p(\mathbf{F}) = 0.001$ ) for this parameter.

The resulting  $EC_{10}$  and  $EQ_{20}$  values and the aspective confidence intervals are represented in the following table below.

#### Table CA 8.4.2.1/06-1 Results of the Logit analysis (max. likelihood regression) with reproduction at 28 R d: Selected effective concentrations (ECx) of the test item and their 95%confidence limits (according to Fieller's theorem)

	Reproduction at 1	test end (28 days)
Parameter 6	EC ₁₀ (95 % confidence interval)	EC ₂₀ (95 % confidence interval)
	[mg a.s./kg dws]	[mg a.s./kg dws]
Effect on production	175.34	258.31
	(109.54 - 225.95)	(191.18 – 308.29)

The resulting EC10 and EC20 values of 175.34 (95% CL: 109.54 - 225.95) and 258.31 (95% CL: 191.18 - 308.29) mg a.s./kg dws, respectively, for the springtail (Folsomia candida) in a spiroxamine TG reproduction test (28 days period), even if outside the predicted visual estimated range are considered



reliable as the criteria for goodness of fit were met and no better fit was found with any other available statistical model.  $Q_{\mu}^{\circ}$ 

#### III. Conclusion

The resulting  $EC_{10}$  and  $EC_{20}$  values of 175.34 (95% CL: 109.54 – 225.95) and 258.31 (95% CL 291.18 – 308.29) mg a.s./kg dws, respectively, are considered reliable as the criteria for goodness of fit were met.

met.	
Assessment and conclu	sion by applicant:
The statistical re-evaluat	tion of the reproduction data has determined an $EC_{10}$ of 175 mg/kg dws.
As the NOEC is lower	than the EC ₁₀ , the NOEC of 75 mg a.s kg dws shall be used in the risk $\gamma$ critical endpoint from this study.
assessment as the most of	pritical endpoint from this study.
The values determined in	n the re-evaluation work are considered to be fully valid.
Metabolites	
KWG 4168-desethyl (M0	
Data Point:	KCA 8402 1/02
Report Author:	
Report Year:	
Report Title:	
*	of the collembola species Folsomia candida tested in artificial soil with 5 percent peat
Report No:	$\frac{\text{PCd}}{\text{FRM}-\text{COLL}-53/07} \qquad \qquad$
Document No:	(M-289) M-01 k ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~
Guideline(s) followed in (	
study:	
Deviations from current	$y_{\Theta s} \circ \psi \circ $
test guideline:	Y@s 0' 4' ' ' ' ' ' ' ' ' ' ' ' ' ' ' ' ' '
	The reference item used was not borth acid. As recommended by the OECD 232
L L . O	test guideline, however the reference iten orsed was acceptable under the ISO
	gradeline to which the study was conducted and was considered to sufficiently demonstrate the sensitivity of the test organism
Previous evaluation:	yes valuated and accepted
Previous evaluation.	RAR (2010), RAR (2017)
GLP/Official	Ares conducted under (The P/Officially recognised testing facilities
recognised testing	
facilities	Ses, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	$Yes^{\vee} \sim \sqrt{2}$

### **Executive Summary**

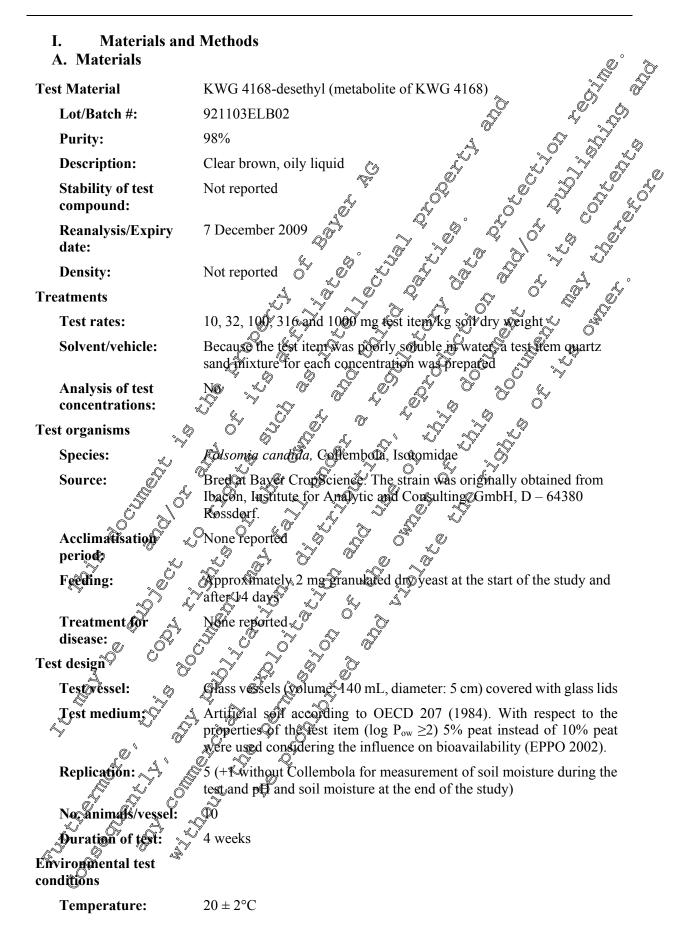
Collembola (*Folsomia candida*) ages 10 to 2 days were exposed to KWG 4168-desethyl incorporated into soil in a week study of assess effects on reproduction.

Test organisms sere exposed \$10, 32, 100, 316 and 1000 mg test item/kg soil dry weight and to a water control. Betosip (a.s. phenmedipham) was used as a toxic standard in accordance with ISO 11267 (1999) guidennes.

A statistically significant reduction in number of juveniles compared to the control was observed in the treatment group at 1000 mg/kg soil dry weight, resulting in a reduction of 76.1%, respectively.

The NOEC and LOEC for reproduction were 316 and 1000 mg/kg soil dry weight, respectively.







#### Photoperiod:

16 h light: 8 h dark at 524-606 lux

#### **B.** Study Design

This study was conducted in order to assess the effects on reproduction of KWG 4168-desethyl on Collembola (*Folsomia candida*) over 4 weeks.

The Collembola were 10 to 12 days old at the start of the study. For each replicate, 10 of the invenile Collembola were placed in the glass test vessels, which had been prepared with the test item quartz sare and artificial soil. The soil was aligned with OECD 207 (1984) standard, but with 5% pear instead of 10% due to considerations on the influence on bioavailability with respect to the test item. The required amount of the test item was mixed thoroughly with 2 & g quartz sand. If less than 2 mg test item had to be mixed with quartz sand a stock mixture was prepared and dilated with quartz sind to reach the demanded test concentrations. Water was added to the soil until 50% water holding capacity was achieved.

The artificial soil was kept at 18 to 22°C, with the temperature continuously recorded by a thermohygrograph integrated in the climatic chamber. The test vessels were exposed to 524-606 fix under a photoperiod of 16 h light: 8 h dark, montrored by an integrated luxmeter in the climatic chamber.

Application rates in this study were 10, 32, 100, 36 and 1000 mg test tem/kg soil dry weight. Five replicates were exposed to control (water) treatment, and five replicates to 10, 32, 100, 346 and 1000 mg test item/kg soil dry weight treatments. During the study the Contembola were red with granulated dry yeast.

A reference test with the toxic standard, Betosip was performed at least once a year to ensure that the laboratory test conditions were adequate and to verify that the response of the test organism does not change significantly over time

Water content was checked 14 days after opplication by reweighing the additional vessels. If the water loss exceeded 2% of the initial water content, the missing appoint of water was added to all vessels of the treatment group. At the same time, the food was checked and the Collembola were fed again if necessary. Mortality and reproduction were reported after 28 days and were determined by the number of dead adult Collembola and the number of living juveniles detected using digital images.

### II. Results and Discussion

Validity criteria, according to the test guideline that the study was conducted to, were assessed. In the control, the following criteria were met:

- Mean addit mortality 20% at the end of the test (actual: 10%);
- The mean number of juvenines per vessel >100 at the end of the test (actual: 826);
- The coefficient of variation calculated for the number of juveniles <30% (actual: 18%).

Mortality did not van, from the control greater that 6% at test item concentrations between 10 and 316 mg test item/kg soil dry weight. At the fighest test item concentration, 1000 mg test item/kg soil dry weight, 54% mortality was observed in adult Collembola.

Table CA 8.4 21/02 T Survival of Adult Collembola after 4 weeks treatment (n=10/replicate)

	mg test item/kg s	soil dry weig	ht			
	Control	10	32	100	316	1000
Mean [®] &	2,0	9.4	9.4	9.4	9.6	4.6
	≫1.4	0.9	0.9	0.9	0.5	1.3
% mortal m ²	10.0	6.0	6.0	6.0	4.0	54.0

¹ mean and standard deviation (SD) of five replicates

² formula: ((initial placed organisms per vessel – mean of surviving adults per vessel) / 10) * 100



Reproduction in the Collembola was statistically different to the control at 1000 mg test item/kg soil dry weight treatment group at which reproduction was at 23.9% of the control.  $Q_{p}^{\circ}$ 

Table CA 8.4.2.1/02-2	Reproduction of the Collembola after 4 weeks treatment	(iuveniles/re	plicate
		<b>U</b>	F

			• • •		<u> </u>	
	mg test item/	/kg soil dry w	eight		-Q [®]	
	Control	10	32	100	<b>316</b>	1000
Mean ¹	825.6	676.8	675.6	700.0	643.8	¥197.6
$SD^1$	149.1	127.1	127.2	166.8	135.6	97:8
$CV^2$	18.1	-		- 0	- 0	
% of control ³	-	82.0	81.8	84.\$	78.0 [©]	23.9*
•	1 = 1 = 1 = 1		- A - A - A - A - A - A - A - A - A - A			

1 mean and standard deviation (SD) of five replicates

² Coefficient of Variation

³ formula: mean number of juveniles per treatment goup * 100 / mean number of juveniles per control group

- = not applicable

* = significantly different compared to the control (Dunnett's Test, one-sided-smaller,  $q \leq 0.05$ )

To demonstrate the sensitivity of the test system Betosip (phenmedipham 15,4%) as a toxic standard was regularly tested (once a year) at concentrations of \$9, 135, 200 and 300 mg test item/kg artificial soil dry weight. In the most recent test the mortality rate of adult Collembola was 8%, 14%, 20% and 32% at 89, 133, 200 and 300 mg Betosip/kg artificial soil dry weight. In all treatment groups the number of juveniles was statistically significant reduced in comparison to the control. The NOEC reproduction was < 89 mg Betosip (13.7 mg a.s)/kg artificial soil dry weight. The results were considered to demonstrate the sensitivity of the test system.

#### III. Conclusion

In the control group, 10% of the adult Collembola died which is within the tolerated range of  $\leq 20\%$  mortality recommended by the test guideline. The highest mortality rate of 54% was found in the test item concentration of 1000 mg test item/kg soil dro weight. Concerning the number of juveniles, statistical analysis revealed significant differences between the control and the highest treatment group at 1000 mg test item/kg soil dry weight.

The NOEC for reproduction: 316 mg test item is soil dry weight.

The LOEC for reproduction: 6000 ms test item/kg soil droweight.

### Assessment and conclusion by applicant:

Validity criteria according to the current QECD 232 guideline (2016) were met. In the control, the following criteria were met

- Mean adult mortality \$20% at the end of the test (actual: 10%);
- The mean number of uveniles pervessel 100 at the end of the test (actual: 826);
- The coefficient of variation calculated for the number of juveniles <30% (actual: 18%).

The study was not specifically conducted to the OECD 232 test guideline, however the test design and the parameters assessed are consistent with the OECD test guideline. Furthermore the OECD validity criteria have been anet.

The reference item used was not borc acid, as recommended by the OECD 232 test guideline, however the reference item used was acceptable under the ISO guideline to which the study was conducted and was considered to sufficiently demonstrate the sensitivity of the test organism.

The study is therefore considered acceptable.

The NOEC for reproduction was determined to be 316 mg test item/kg soil dry weight.

The data have been subjected to statistical re-evaluation and the results have been presented in the following study summary.



Data Point:	KCA 8.4.2.1/07
Report Author:	
Report Year:	2020
Report Title:	Calculation of EC10 and EC20 values for Folsomia candida with KWG 4168-
	desethyl in a reproduction study
Report No:	0471836-ECO15
Document No:	<u>M-760431-01-1</u>
Guideline(s) followed in	None v v v v
study:	
Deviations from current	None $\mathcal{A}^{\mathcal{Y}}$ $\mathcal{A}^{\mathcal{Y}}$ $\mathcal{A}^{\mathcal{Y}}$
test guideline:	
Previous evaluation:	No, not previously submitted
GLP/Officially	not applicable
recognised testing	
facilities:	
Acceptability/Reliability:	Yes A Y Y Y Y Y Y Y Y

#### **Executive Summary**

The report M-289321-01-1 on the effects of KWG 4168-desethyl metabolite of KWG 4168) in a springtail (Folsomia candida) reproduction study did not provide estimates of CI1000r EC20 values. Therefore, these values have been calculated in accordance with the Annex to Com. Reg 283/2013. Due to the lack of a significant dose response, w was not possible to determine reliable EC₁₀ or EC₂₀ values for reproduction. 0

#### I. Methods

The statistical evaluation was performed with statistical software Tox Rat Standard v3.3.0. Due to the lack of a significance dose response on reproduction when compared to the control, it was not possible to calculate reliable EC10 or EC20 values. Ľ.

#### II. Results

II. Results Due to the lack of a significant dose response on the reproduction, when compared to the control, it was not possible to calculate reliable EC1gof EC20 values

#### III. Conclusion

Due to the lack of significant dose response it was not possible to determine reliable EC10 or EC20 values for reproduction

#### Assessmentand conclusion by applicant:

The statistical re-evaluation of the reproduction data could not calculate reliable  $EC_{10}$  and  $EC_{20}$  values. The NOEC of 316 mg/kg dws from the eriginal study report shall be used in the risk assessment as the most critical endpoint from this study.  $\sim$ 

The values determined in there-evaluation work are considered to be fully valid.

- in theore-eve - in t



Data Point:	KCA 8.4.2.1/08
Report Author:	
Report Year:	2019
Report Title:	KWG4168-desethyl: Effects on reproduction of the predatory mite hypoasperior of
	aculeifer in artificial soil
Report No:	143061089
Document No:	<u>M-680684-01-1</u>
Guideline(s) followed in	Regulation (EC) No 1107/2009 (2009)
study:	
Deviations from current	None V O O V V
test guideline:	
Previous evaluation:	No, not previously submitted
GLP/Officially	Yes, conducted under GBP/Officially recognised testing facilities
recognised testing	
facilities:	
Acceptability/Reliability:	Yes A & Q Q A O Q A

#### **Executive Summary**

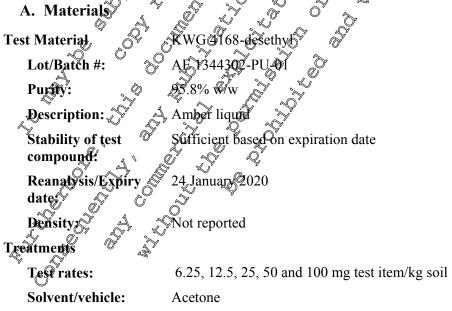
The purpose of the study was to determine the effects of KWG 4168-desethyl on morality and reproduction of the predatory mite *Hypogspis aculeifer*, *S* 

KWG 4168-desethyl was added to soil at concentrations of 6.25, 12.5, 25, 50 and 100 mg test item/kg soil dry weight.

KWG 4168-desethyl caused no statistically significant effects on morality of *Hypeaspis aculeifer* up to and including the concentration of 100 mg test, item/kg soil. Therefore, the No Observed Effect Concentration (NOEC) for morality was determined to be  $\geq 100$  mg test item/kg soil. The Lowest Observed Effect Concentration (LOEC) for morality was estimated to be >100 mg test item/kg soil. The Lowest Observed Effect Concentration (LOEC) for morality was estimated to be >100 mg test item/kg soil. The Lowest Observed Effect Concentration (LOEC) for morality was estimated to be >100 mg test item/kg soil.

The NOEC for reproduction was determined to be 50 mg test item/kg soil and the LOEC for reproduction was determined to be 100 mg test rem/kg soil. The EC₆₀ was determined to be 94.1 mg test item/kg soil, the EC₂₀ was determined to be 102.1 mg test item/kg soil, and the EC₅₀ was determined to be 1170 mg test item/kg soil.

#### I. Materials and Methods





Analysis of test concentrations:	No
Test design	
Test species:	Hypoaspis aculeifer (age: approximately 14 days at test start)
Test vessel:	100 mL glass containers (volume: 100 mL; diameter: 5 cm) with tight screw cap 5% Sphagnum-peat, 20% kaolin clay, 74.8% fine quartz-sand, 0.2% Calcium carbonate Four per treatment group and eight for the control
Test substrate:	5% Sphagnum-peat, 20% baolin clay, 74.8% fine quartz-sand, 0.2% Calcium carbonate
<b>Replication:</b>	Four per treatment group and eight for the control
No. of animals/vessel:	
<b>Duration of test:</b>	14 days
Environmental test conditions	14 days $\begin{pmatrix} 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 $
<b>Temperature:</b>	
рН:	Test start 5 5 to 6 0 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5
Photoperiod:	16 h Hight : Sh dark (400 -800 lux)
Water content:	Test start: Dest end? 22.1% to 23.0% (49.8% to 5).1% of WHC _{max} ) 22.1% to 22.8% (49.2% to 50.6% of WHC _{max} )
B. Study Design	
reproduction of the predator	was to determine the effects of KWG 4168-desethyl on mortality and ry mite Hypoaspos aculation.
Nominal test concentrations	s were 6.25, 12.5, 23, 50 and 100 mg test uem/kg soil.
Ten adult female mites over vessels were glass container evaporation, filled with app in the containers was 1.5 to	e added to each of the four replicate test vessels (eight for the control). Test is volume: 100 mL, duameter: 5 cm), tight screw top closure to avoid water voximately $20 g \pm 10^{\circ} g$ artificial soil dry weight. The height of the soil layer
on days 2, 4, 7, 9 and 11.	
to 800 lux.	

After/14 days exposure the soft was filled into Millipore pots with attached plastic containers for collecting the escaping mites. These extraction units were placed in a heat extractor. The soil including the mites was exposed to a temperature of approximately 25°C and 30°C for approximately 2 days. Escaping mites were collected in a fixing liquid, cooled at a temperature of approximately 16°C. The

fixing liquid contained glycol and a detergent. Adult animals were counted once visually, juvenile animals were counted twice under binocular microscopes. One of the replicates was counted three times because the first two counts deviated more than 10% from their mean value.

#### $II \stackrel{O}{\leftarrow} \stackrel{O}{}$ Results and Discussion

Validity criteria according to the OECD 226 test guideline were met.

Mean mortality in the controls to not exceed 20% (actual: 4%) •



- The mean number of juvenile mites per replicate to be at least 50 (actual: 188 to 222)
- The coefficient of variation for reproduction to be  $\leq 30\%$  (actual: 7.0%)

Mortality of Hypoaspis aculeifer in the test item treated groups ranged from 0 to 8%. The values were not statistically significantly different compared to the control (Fisher's Exact Test,  $\alpha = 0.05$ , the sided greater). Therefore, the NOEC for mortality was determined to be  $\geq 100 \text{ mg}$  test item/kg soil dry weight. The LOEC for mortality was estimated to be >100 mg test item/kg soil dry weight.

No differences in morphology of the mites between the test item treated groups and the control were observed. Table CA 8.4.2.1/08-1 Mortality of adult *Hypoaspis actileifer* after 16 days

Treatment group (mg test item/kg soil dry weight)	Number of surviving (%)     Mean mortality [%)     Standard deviation [%]       females per group     (%)     (%)
Control	77
6.25	$38 \qquad \qquad$
12.5	$39 \qquad \qquad$
25	
50	$39 \qquad \qquad$
100	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$

<b>T 11</b> C 4 0 4 0 1/00 1	Mortality of adult <i>Hypoaspis</i>	and the second		
Table CA 8.4.2.1/08-1	Mortality of adult <i>Hypoaspis</i>	aculeifer after	r <b>l</b> adays	

The results represent rounded values calculated from the exact ray data

There were no statistically significant effects on reproduction of Hypoasfas aculeifer up to and including the concentration of 50 mg test item/kg soil dry weight (Williams t-test, a = 0.05, one-sided smaller). At the concentration of 100 mg test item/kg soil dry weight a statistically significant reduction of reproduction was observed.  $\bigcirc$ 

Therefore, the NOE of reproduction was determined to be 50 mg test item/kg soil dry weight and the LOEC for reproduction was determined to be 100 mg test item item item by soil dry weight. The EC₁₀ for Hypoaspis acule fer in artificial soil was determined to be 94.1 mg test item/kg soil dry weight, the EC₂₀ was determined to be 102.1 mg tes item/kg soil ary weight, and the EC₅₀ was determined to be 117.3 mg test item/kg soil dry weight. @5% confidence limits could not be determined due to mathematical reasons).

Treatment group mg test item/kg soil dry weight	Mean number of juveniles per group	Standard deviation	% of control
Control 🔊 🖒		± 14	-
6.25	214	<u>6</u> 19	106
12.5		J±19	105
25 4 2		± 24	100
50 10 10		± 8	100
100 2	167 8	± 7	83*

Table CA 8.4.2.1/08-2 Reproduction of Hobaspis aculetfer after 14 days

*significantly different from the control

The results represent founded values calculated from the exact raw data

To verify the sensitivity of the test system, the reference item (dimethoate) was tested at concentrations of 1.54, 223, 3.27, 4.69 and 6.80 mg a.s./kg soil dry weight in a separate study. In the most recent GLP study, ar EC₅₀ of 3.34 mg a kg soil dry weight was determined for juvenile production. The effects on the reduction of reproduction showed that the test system was sensitive and reflected the expected to first as given in the OECD 226 test guideline (EC₅₀ for reproduction should be between 3.0 and 7.0 mg a.s. Ag soil dry weight).



### III. Conclusion

Exposure to KWG 4168-desethyl caused no statistically significant effects on mortality of *Hypotepis aculeifer* up to and including the concentration of 100 mg test item/kg soil dry weight. Therefore, the No Observed Effect Concentration (NOEC) for mortality was determined to be  $\geq 100$  mg test item/kg soil dry weight. The Lowest Observed Effect Concentration (LOEC) for mortality was estimated to be >100 mg test item/kg soil dry weight. The LC₅₀ was estimated to be >100 mg test item/kg solv dry weight.

The NOEC for reproduction was determined to be 50 mg test item/kg soil dry weight and the LOECoor reproduction was determined to be 100 mg test item/kg soil dry weight. The EC₁₀ was determined to be 102.1 mg test item/kg soil dry weight, and the EC₅₀ was determined to be 117.3 mg test item/kg soil drg weight.

#### Assessment and conclusion by applicant:

This is a new study that has not been previously expluated

Validity criteria according to the most recent OECD 226 test guideline (2016) were met.

- Mean mortality in the controls to not exceed 20% (actual: 4%)
- The mean number of juverile mittes perpeplicate to be at leasy 50 (actual: 108 to 222)
- The coefficient of variation for reproduction to be \$30% (actual: 7.0)

The reference item also demonstrated sufficient sensitivity of the test organisms. The study is therefore considered acceptable.

The NOEC for reproduction was determined to be 50 mg test item/kg soil dry weigh

KWG 4168-despropylyM02)

Data Point:		KCA 89.2.1 (0) ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~
Report Author:		
Report Year:	Â,	
Report Title:	O,	KWG & 68-despropy (Metabolite of KWG & 68): Influence on the reproduction
	Č,	of the collembola species Folsomia candida tested in artificial soil with 5 percent
Report No:	S	FIRM-CQLL-54497 in a
Document No:	Ŷ	MI-288905-014
Guideline(s) follow	ed in	ISQ 1267 (1999), 4
study:	.Ô¥	
Deviations from cu		Dec DEC 232 (2016) DEC 232 (2016) The eference item used was not boric acid, as recommended by the OECD 232
test guideline:		OECE0232 (2016) 0 0
Q"		The reference item used was not boric acid, as recommended by the OECD 232
	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	test guideline, however the reference item used was acceptable under the ISO
A .	s i	guideling to which the study was conducted and was considered to sufficiently
· »	0	demonstrate the sensitivity of the test organism
Previous evaluation	1:	yes evaluated and accepted
	À.	BAR (2010), RAR (2017)
GLP/Officiaty	, Y	Ses, conducted ander GLP/Officially recognised testing facilities
recognised testing	, O	
facilities		<u></u>
AcceptabilityRelia	bhity:	Х es
	Ô â	$\sum_{n=1}^{\infty}$
Executive Summa	rv	

Collembola (*Folsomia candida*) aged 10 to 12 days were exposed to KWG 4168-despropyl (metabolite of KWG 4168) in a 4-week study in artificial soil with 5% peat to assess effects on reproduction.



CONTRACTION OF STREET

Test organisms were exposed to 10, 32, 100, 316 and 1000 mg test item/kg soil dry weight and to a control. Betosip (a.s. phenmedipham) was used as a toxic standard in accordance with ISO 11267 (1999) guidelines.

A statistically significant reduction in number of juveniles compared to the compol was observed in peetively of pe treatment group at 1000 mg test item/kg soil dry weight, resulting in reduction of 74.6%.

KWG 4168-despropy

921103ELB03

Not reported

reporte

13 Jan@i

97%

The NOEC and LOEC for reproduction were 316 and 1000 mg test item/kg soil dry w

- **Materials and Methods** I.
- A. Materials

Test Material Lot/Batch #: **Purity:**

> Amber oily liqu **Description:**

Stability of test compound:

Reanalysis/Expiry date:

Density:

Treatments

Test rates:

- 34,6, 1000 mg test item kg lominal: Ø0 The test item was poorly soluble in water A test item quartz sand Solvent/vehicle
 - wassprepared for each concentration. ature

Analysis of tes concentrations

Test organisms Species:

Source:

Feeding

somia candida, Collembola, Isofomidae

n-house culture originally from Bacon, Institute for Analytic and Consulting, GmbH, D-@380, Rossdorf.

mg granulated dry yeast at the start of the study and Approximatel 2 ′after ̂%4 da�

Treatment for disease:

Test design

Test vessel:

Test medi

Gass vessels (Colume: 140 mL, diameter: 5 cm) covered with glass lids Artificial soil according to OECD 207 (1984). With respect to the properties of the test item (log $P_{ow} \ge 2$) 5% peat instead of 10% peat were used considering the influence on bioavailability (EPPO 2002). This is in line with OECD 232 (2016)

5 replicates per treatment group. (+1 without Collembola for measurement of soil moisture during the test and pH and soil moisture at the end of the study

No. animals/vessel:

plication:

¹⁰



	4
Duration of test:	4 weeks
Environmental test conditions	
Temperature:	$20 \pm 2^{\circ}C$
pH:	Recorded in each replicate of artificial soil. (5.84 - 6.11)
Photoperiod:	16 h light :8 h dark at 400-800 lux
B. Study Design	
This study was conducted in Collembola in an inhibition of	order to assess the influence on reproduction of KWG 4168-despropyl of of reproduction test over 4 weeks.
The artificial soil was kept hygrograph integrated in the photoperiod of 16 h light. Sh Five replicates were exposed dry weight. During the study A reference test with the oxi laboratory test conditions we change significantly over tim After 14 days, water content Food was also checked at th	4 weeks 20 ± 2°C Recorded in each replicate of artificial soil. (5.84 - 6.11) 16 h light :8 h dark at 400-800 lux order to assess the influence on reproduction of KWG 4168-desptopyl or of reproduction test over 4 weeks. <i>andida</i>) were 10 to 12 days old at the start of the study. For each replicate a were placed in the test essels which had been prepared with the test item 1. The soil was aligned with OECD 207 (1984) standard, but with 5% peat derations on the influence on bioavailability with respect to the test item. est item was mixed thoroughly, with 5 Quartz stand. If less than 50 hig test uartz stard a stock mixture was prepared and diluted with quartz stand to centrations. Water was added until 50% water holding espacity of the soil at 18 to 22°C with the temperature continuously recorded by a thermo climatic chantler. The test ressels were exposed to 559 to 622 lux under a in dark, monitored by an integrated luxmeter of the climatic chamber. to control (water treated), 10, 52, 100, 316 and 1000 mg test item/kg soil the test organisms were feat with granulated dry yeast. ic standard, Betosip, was performed at least office a year to ensure that the tre adecuate and to verty that the response of the test organisms does not i. was checked and replenished itewater toss exceeded 2% of initial content. fy time and the Collembola were fed again if necessary. Mortality and for 28 days and were determined by the number of dead adult Collembola

Validity criteria, according of the test guideline that the study was conducted to, were assessed. In the control, the following criteria were met a second control and the study was conducted to be assessed.

pean adult mortality 20% at the end of the test (actual: 18%); •

The mean number of juveniles per vessel >100 at the end of the test (actual: 417);
The coefficient of variation calculated for the number of juveniles <30% (actual: 10%).

At the highest application rate, 1000 mg test item/kg soil dry weight, 86% mortality was observed. The percent mortality in the control was 18%.

Table CA8	.4.2.070	3-1⊿Su	rvince of	fadult	Collembola	after 4	weeks treatment	: (n=10/replicate)
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	_07	Č ^v	≪J ^v					

		ng test item/kg soil dry weight				
	Control	10	32	100	316	1000
Mean	8.2	6.6	8.4	6.4	7.6	1.4
$SD^1$	1.3	2.8	1.3	1.1	0.9	0.5
% mortality ²	18.0	34.0	16.0	36.0	24.0	86.0

1 mean and standard deviation (SD) of five replicates



	mg test item/kg soil dry weight						
	Control	10	32	100	316	1000	<i>a</i> .°
2 formula: ((initial placed organisms per vessel – mean of surviving adults per vessel) / 10) * 100							

A statistically significant difference in reproduction compared to the control was observed at the highest application rate, 1000 mg test item/kg soil dry weight. Reproduction in this teacher group was 25 4% of reproduction observed in the control.

	Reproduction of the Collembola			
Table CA 0 4 2 1/02 2	Donucduction of the Collombole	often 1 mentre trees	time ( i	anilactonliasta
1 able CA 0.4.2.1/03-2	Reproduction of the Conempoia	i ailes 4 weeks lrea		ennes/reducater

					A.		_ (2
	mg test item/	kg artificial soi	l dry weight	Q,		Ľ,	Ś
	Control	10	∰⁄32	<b>190</b>	316		
Mean ¹	416.8	491.2	628.0	Q491.4_ °	372.0	10508 0	ľ
$SD^1$	41.7	88.4	76.8 🔊	113	Q17.1, O	169.4 Of	
$CV^2$	10.0				≈ .		
% of Control ³	-	115/9	150 T	rm7.9 🔊	8903	25.4*	

 $\bigcirc$ 

1 mean and standard deviation (SD) of five replicates 🗸 🖉

2 Coefficient of Variation

- 3 formula: mean number of juveniles per treatment group * 100 / pean number of fuveniles per control group
- = not applicable
- * = significantly different compared to the control (Durnett's Fest, one sided-smaller 3 = 0.09

To demonstrate the sensitivity of the test system Betosip (phermedionan 15.4 %) as a toxic standard was regularly tested (once a year) at concentrations of 89, 153, 200 and 500 mg est item/kg artificial soil dry weight. In the most resent test the mortality rate of adult collembola was 8 % 14 %, 22 % and 32 % at 89, 133, 200 and 300 mg Betosip/kc artificial soil dry weight. In all treatment groups the number of juveniles was statistically significant/jeduced in comparison to the control. The NOEC_{reproduction} was < 89 mg Betosip (13.7 mg a.s)/kg artificial soil dry weight. The results were considered to demonstrate the sensitivity of the test system.

### III. Conclusion

The highest mortality rate of 86% was found in the test item concentration of 1000 mg test item/kg soil dry weight. Concerning the number of juveniles, statistical analysis revealed significant differences between the control and the treatment group at 1000 mg test item/kg soil dry weight.

The NOEC for reproduction 316 nog test frem/kg soil dry weight.

The LOEC for reproduction: 1000 mg test iten/kg soil dry weight.

### Assessment and conclusion by applicant

Validity criteria according to the current OECD 232 guideline (2016) were met. In the control, the following criteria were met:

- Mean adult prortality <20% at the end of the test (actual: 18%);
- The mean number of juveniles per vessel >100 at the end of the test (actual: 417);
- The coefficient of variation @lculated for the number of juveniles <30% (actual: 10%).

The study was not specifically conducted to the OECD 232 test guideline, however the test design and the parameters associated are consistent with the OECD test guideline. Furthermore the OECD validity criteric have been mot.

The reference item used was not boric acid, as recommended by the OECD 232 test guideline, however the reference item used was acceptable under the ISO guideline to which the study was conducted and was considered to sufficiently demonstrate the sensitivity of the test organism.

The study is therefore considered acceptable.

The NOEC for reproduction was determined to be 316 mg test item/kg soil dry weight.



The data have been subjected to statistical re-evaluation and the results have been presented in the following study summary.

Data Point:	KCA 8.4.2.1/09
Report Author:	
Report Year:	
Report Title:	2020 Calculation of EC10 and EC20 values for Folsevia candida with KWG4168
	despropyl in a reproduction study
Report No:	0471836-ECO13
Document No:	$\underline{M}_{-760410-01-1} \qquad \qquad$
Guideline(s) followed in study:	None y o y y y y y
Deviations from current test guideline:	None $(0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0$
Previous evaluation:	I No not previously subhatted y 20 day so y 20 a
GLP/Officially	not applicable 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4
recognised testing	
facilities:	
Acceptability/Reliability:	Yes y g g g g g g g
Executive Summary	

#### **Executive Summary**

The report M-288905-01-1 on the effects of KWG 4168-despropylymetabolite of KWG 4168) in a springtail (*Folsomia canàdida*) reproduction study did not provide estimates of  $C_{10}$  or  $EC_{20}$  values. Therefore, these values have been calculated in accordance with the Annex to Coni. Reg. 283/2013. The resulting  $EC_{10}$  and  $EC_{20}$  values of 0.8.18695% L: 306.07 – 310.27) and 402.24 (95% CL: 400.09-404.38) mg/kg dws respectively are considered reliable as the criteria for goodness of fit were met.

#### I. Methods

The statistical evaluation was performed with statistical software Tox Rat Standard v3.3.0. To calculate  $EC_X$  values, probit analysis using linear maximum likelihood regression was performed along with 95%  $EC_X$  considence limits based of Fieller's Theorem  $\sqrt{2}$ 

Å

#### II. Results

The criteria for goodness of fit were metras the  $P(Chi^2)$  value was 1.00, showing no significant deviation between fit and data, and a statistically significant concentration/response was found (p(F) < 0.001) for this parameter

X.

The resulting  $EC_{10}$  and  $EC_{20}$  values and the respective confidence intervals are represented in the following table.

 Table CA 8.4.2.1/09-1
 Results of the Probit analysis (max. likelihood regression) with reproduction at 28

 d: Selected effective concentrations (ECx) of the test item and their 95% 

 confidence limits (according to Fieller's theorem)

	Reproduction at t	test end (28 days)
Parameter 0	(95 % confidence interval) [mg/kg dws]	EC ₂₀ (95 % confidence interval) [mg/kg dws]
Effect on Teproduction	308.18 (306.07 - 310.27)	$\frac{402.24}{(400.09 - 404.38)}$

The resulting  $EC_{10}$  and  $EC_{20}$  values of 308.18 (95% CL: 306.07 – 310.27) and 402.24 (95% CL: 400.09-404.38) mg/kg dws, respectively, for springtail (*Folsomia candida*) in a KWG 4168-despropyl



reproduction test (28 days period) are therefore considered reliable as the criteria for goodness of fit were met.  $\mathbb{R}^{\circ}$ 

#### III. Conclusion

The resulting EC₁₀ and EC₂₀ values of 308.18 (95% CL: 306.07 – 310.27) and 408-24 (95% CL:  $\frac{100.09}{404.38}$ ) mg/kg dws, respectively, are considered reliable as the criteria for goodness of fit were met.

#### Assessment and conclusion by applicant:

As the  $EC_{10}$  is lower than the NOEC value, the  $EC_{10}$  of 308 mg/kg dws shall be used assessment as the most critical endpoint from this study.

The values determined in the re-evaluation work are considered to be fully valid.

	KCA 84 21/10 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
Data Point:	KCA 8.4.2.1/10 O V V V V V V V V V V V V V V V V V V
Report Author:	
Report Year:	
Report Title:	KWG4168-desproped. Effects on reproduction of the predatory mit hypotaspis
	aculeifer in artificial soil S S S S S
Report No:	aculeifer pointificial soil $\sqrt{2}$ $\sqrt{2}$ $\sqrt{2}$ $\sqrt{2}$ $\sqrt{2}$ $\sqrt{2}$ $\sqrt{2}$ $\sqrt{2}$ $\sqrt{2}$
Document No:	M-680694-019
Guideline(s) followed in	Regulation (FC) No 1107/2009 (2009)
study:	
Deviations from current test guideline:	None of Star and Star
Previous evaluation .	No pot pregiously Cubmitten . O & . O
- S	
GLP/Officially	Yes, conducteeQinder GLP/Qfficially @cognised testing facilities
recognised testing	
GLP/Officially recognised testing facilities:	
Acceptability Reliability:	

#### Executive Summary

The purpose of the study was to determine the effects of KWG 4168-despropyl on mortality and reproduction of the predatory mits Hypospis aculeifor

KWG 4168-despropyl was added to soll at concentrations of 6.25, 12.5, 25, 50 and 100 mg test item/kg soil dry weight.

KWG 4168-despropyl caused no statistically significant effects on mortality or reproduction of *Hypoaspis aculeifer* up to an Dincluding the concentration of 100 mg test item/kg soil. Therefore, the No Observed Effect Concentration (NOEC) for portality was determined to be  $\geq 100$  mg test item/kg soil dry weight and the Lowest Observed Effect Concentration (LOEC) was estimated to be >100 mg test item/kg soil dry weight.

The NOEC for reproduction was determined to be  $\geq 100$  mg test item/kg soil dry weight and the LOEC for reproduction was estimated to be  $\geq 000$  mg test item/kg soil dry weight. Due to the absence of a concentration-response celationship, the EC₁₀, EC₂₀ and EC₅₀ values have been estimated to be >100 mg test item/kg soil dry weight.

IS Materials and Methods A. Materials

Test Material

KWG 4168-despropyl

Lot/Batch #: AE 1344303-PU-01



Purity:	99.1% w/w
Description:	Colourless liquid
Stability of test compound:	Sufficient based on expiration date
Reanalysis/Expiry date:	13 May 2022
Density:	Not reported
Treatments	
Test rates:	6.25, 12.5, 25, 50 and 100 mg test item/k@soil do weight
Solvent/vehicle:	Acetone
Analysis of test concentrations:	No O C C C C C C C C C C C C C C C C C C
Test design	
Test species:	Hypoators aculeifer & S & S &
Test vessel:	100 mL glass containers (volume) 100 mL; diameter 3 cm), with tight
Test substrate:	99.1% w/w Colourless liquid Sufficient based on expiration date 13 May 2022 Not reported 6.25, 12.5, 25, 50 and 100 mg test item/ke soil dry weight Acetone No <i>Hypoaeprs aculeifer</i> 100 mL glass containers (volume 100 mL; diameters cm), with tight serve cap Four pet treatment group and right for the control 10 14 days
Replication:	Four per treatment group and eight for the control
No. of animals/vesses. Duration of test Environmental test	Four per treatment group and eight for the control 10 14 days $50 \pm 2^{\circ}C$ Test start 58 to 60 Test end 5.9 to 6.0
Duration of test	It days of it is it
Environmental test condition	
Temperature: V	$\tilde{Q} = 2 \tilde{C}$
conditions Temperature:	Test start $5$ to $60$ Test end $5$ 9 to $6.0$
Photoperiod:	16 h% ght : 8 h dark 400 – 800 lux)
Water content:	Test start 22.7% to 23.2% (50.5% to 51.5% of the WHC _{max} ) best end. 22.7% to 23.5% (50.4% to 52.2% of the WHC _{max} )
B. Study Design	
The nurnose of the study v	vacto determine the effects of KWG 4168-despropyl on mortality and

The purpose of the study was to determine the effects of KWG 4168-despropyl on mortality and reproduction of the predatory nite *Hypoaspis aculeifer*.

Nominal test concentrations were 6.25, 12.5, 25, 50 and 100 mg test item/kg soil dry weight.

Ten adult demale mites were added to each of the four replicate test vessels (eight for the control). Test vessels vere gass containers volume: 100 mL, diameter: 5 cm), tight screw top closure to avoid water evaporation willed with approximately 20 g  $\pm$  1.0 g artificial soil dry weight. The height of the soil layer in the containers was 1.5 to 2 cm.

One sparula of cheese mites (*Tyrophagus putrescentiae*) was provided as food at experimental start and on days 2, 4, 7, 9 and 11.



Incubation was at 18 to 22°C with a photoperiod of 16 hours light to 8 hours dark at approximately 400 to 800 lux.  $Q_{\mu}^{\circ}$ 

After 14 days exposure the soil was filled into Millipore pots with attached plastic containers for collecting the escaping mites. These extraction units were placed in a heat extractor. The soil including the mites was exposed to a temperature of approximately 25°C and 30°C for approximately 2 days. Escaping mites were collected in a fixing liquid, cooled at a temperature of approximately 16°C. The fixing liquid contained glycol and a detergent.

Adult animals were counted once visually, juvenile animals were counted twice under binocodar microscopes. None of the replicate counts deviated more than 10% from their mean value

#### II. Results and Discussion

Validity criteria according to the OECD 226 guideline were me

- Mean mortality in the controls to not exceed 20% (actual: 2.5%)
- The mean number of juvenile mites per replicate to be at tast 50 (actual. 169 for 187)
- The coefficient of variation for reproduction to be  $\leq 30\%$  (actual: 3.3%

Mortality of *Hypoaspis aculeifer* in the test item treated groups ranged from 0% to 2%. The values were not statistically significantly different compared to the control where 2.5% of the soil mites were dead (Fisher's Exact Test,  $\alpha = 0.05$ , one-sided greater). Therefore, the NOEC for mortality was determined to be  $\geq 100$  mg test item/kg soil dry weight.

No differences in morphology of the mites between the test item treated groups and the control were observed.

Treatment group mg	Number of surviving	Mean mortality [%]	Standard deviation [%]
test item/kg soil dry 🔊	females per group		
weight)			
Control	78 0 1 24		± 5
6.25		0.0	$\pm 0$
12.5	3965 ~~ 6	[∞] 2.5 √ 0″	± 5
25	AV ST T	0.0	$\pm 0$
50	40 2 2 2	Ø 🐁	$\pm 0$
100	39 8	2.5	± 5
		Y AY I	

### Table CA 8.4.2.1/10-1 Mortality of adult Hypoaspis aculater after 14 days

The results represent ronded alues colculated from the exaction data

There were no statistically significant effects on reproduction of *Hypoaspis aculeifer* up to and including the concentration of 100 mg test item/kg soil dry weight (Dunnett's t-test,  $\alpha = 0.05$ , one-sided smaller). Therefore, the NOEC for reproduction was determined to be  $\geq 100$  mg test item/kg soil dry weight and the LOEC for reproduction was estimated to be  $\geq 100$  mg test item/kg soil dry weight.

Due to the lack of a concentration response relationship no reliable ECx-calculation is possible. Therefore, no  $EC_{10}/EC_{20}$ -value can be reported. The  $EC_{10}$ ,  $EC_{20}$  and  $EC_{50}$  were estimated to be >100 mg test item/kg foil dry weight

Table CA 8.4.2.100-2	Reproduction	of Hypoaspis aculeifer after 14 days
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Treatment group (ng test item/kg soil dry weight)	Mean number of Juveniles per group	Standard deviation	% of control
Control	180	± 6	-
6.25	185	± 23	103
12.5	190	± 16	106
25	184	± 10	102



Treatment group (mg test item/kg soil dry weight)	Mean number of juveniles per group	Standard deviation	% of control	Ø
50	186	± 19	103	
100	174	± 12	9D	
The results represent roun	ded values calculated from	the exact raw data	S	

To verify the sensitivity of the test system, the reference item (dimethoate) was tested at concentrations of 1.54, 2.23, 3.23, 4.69 and 6.80 mg a.s./kg soil dry weight in a separate study. In the most recent GLP study, an EC₅₀ of 3.31 mg a.s./kg soil dry weight was determined for jovenile production. The effects on the reduction of reproduction showed that the test system was sensitive and reflected the expected  $\bigcirc$ toxicity as given in the OECD 226 test guideline (EQC) for reproduction should be between 3.0 and 7 3 mg a.s./kg soil dry weight).

#### Conclusion III.

KWG 4168-despropyl caused no statistically significant effects on mortality or reproduction of Hypoaspis aculeifer up to and including the concentration of 500 mg test item/kg soil dry weight. Therefore, the No Observed Effect Concentration (NOES) for mortality was determined to be ≥100 mg test item/kg soil dry weight and the Lowest Observed Effect Concentration (LOEQ) was estimated to be >100 mg test item/kg soil dry weight Q Ľ.

The NOEC for reproduction was determined to be  $\geq 100$  mg test item/kg soil dry weight and the LOEC for reproduction was estimated to be \$400 mg/test item/kg/spil dry weight. Due to the absence of a concentration-response relationship, the  $EC_{10}$ ,  $EC_{20}$  and  $EC_{50}$  values have been estimated to be >100 mg test item/kg soil dry weight. ^K

 $\bigcirc$ 

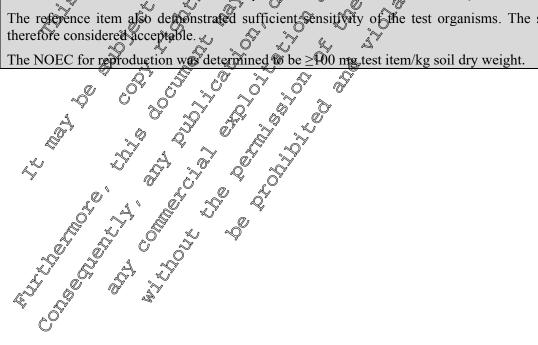
#### Assessment and conclusion by applicant:

This is a new study that has not been previously saluated

Validity criteria according, to the surrent OECD 226 guideline (2016) were met.

- Mean mortality in the controls to not exceed 20% Pactual 2.5%
- The mean number of juvefule mites per replicate to be at least 50 (actual: 169 to 187)
- The coefficient of variation for reproduction  $n^{2}$  be  $\leq 30\%$  (actual: 3.3%)

The reference item also demonstrated sufficient sensitivity of the test organisms. The study was





#### KWG 4168-N-oxide (M03)

Data Point:	KCA 8.4.2.1/11
Report Author:	N N N
Report Year:	2020
Report Title:	KWG4168-N-oxide: Effects on reproduction of the Collembola Folsomia candiga
	in artificial soil 1st final report amendment
Report No:	
Document No:	<u>M-687854-01-1</u>
Guideline(s) followed in	Regulation (EC) No 1107/2009 (2009)
study:	US EPA OCSPP Not Applicable
	OECD-Guideline for testing chemicals No 332 "Collembolan Reproduction Test
	in Soil" (adopted July 29.
	ISO 1126 / Soli Quality – innosition outreproduction of Collegnoola (noisomatic
	2016) ISO 11267 Soil Quality – Inhibition obteproduction of Collectional (Bolsonia candida) by soil contaminants, 2014
Deviations from current	None to the total and the second seco
test guideline:	
Previous evaluation:	No, not presedually submitted
GLP/Officially	Yes, conducted under GLP/Officially recognized testing facilities
recognised testing	
facilities:	
Acceptability/Reliability:	$\begin{array}{cccccccccccccccccccccccccccccccccccc$

#### **Executive Summary**

Collembola (*Folsomiti candida*) aged 9 to 12 days were exposed to KWG 4468-N-oxide incorporated into soil in a 28-day study to assess effect on survival and reproduction.

Test organisms were exposed to 6.25, 12.5, 25, 50, and 100 mg test item/kg soil dry weight and to a water control.

Exposure to KWG 4168-N-oxide did not cause any statistically significant effects on the mortality and reproduction of *Folsonfua cardida* up to and including a concentration of 100 mg test item/kg soil dry weight.

The NOEC and LOEC for both portality and reproduction are therefore  $\geq 100$  and >100 mg test item/kg soil dry weight, respectively. The LC of or mortality and EC₅₀ for reproduction were both determined to be >100 mg test item/kg soil. Due to a lack of a dose-response relationship, no reliable EC₁₀ or EC₂₀ could be calculated therefore these values are considered to be >100 mg test item/kg soil dry weight.

I. Materials and	Methods , L
A. Materials	
Test Material	KårG 4168-N-osride
Lot/Batch#:	AE 1344305 00 1C74 0001 (origin batch no.: M26999)
Purity S	72,9% w/x
Description:	Bight yellow liquid
Stabilit of test	Not reported
Compound:	
Reanalysis/Expiry	28 November 2022
date:	
Density:	Not reported



#### Treatments

11 cutilitentis	0
Test rates:	6.25, 12.5, 25, 50, and 100 mg test item/kg soil dw
Solvent/vehicle:	Deionised water
Analysis of test concentrations:	No significant deviations to the target concentration (<5%)
Test organisms	
Species:	Collembola Folsomia candida, 9 – 12 days old
Source:	In-house cultures
Acclimatisation period:	
Feeding:	Fed 2 mg dry yeast at lest start and our day of a final for the start and our day of the start a
Test design	
Test vessel:	100-mL gaass versels with a drameter of 5 cm, closed to avoid sater
Test medium:	Artificial soil according to OFCD 232
<b>Replication:</b>	Four replicates per concentration, eight in the control
No. animals/vessek	
Duration of test	$\begin{array}{cccccccccccccccccccccccccccccccccccc$
Environmental test conditions	
Temperature:	Within the range $18422^{\circ}$
pri:	Test end $6.2 - 6.3$
Moisture content:	$\sqrt{1}$ fest start: $\sqrt{26}$ $26\% - 27.6\%$ ( $4\%$ ? $-52.1\%$ of WHC _{max} )
Photoperiod:	Test end: $24.6 - 26.2\% (46.4 - 49.4\% \text{ of WHC}_{max})$ So h light : 8 h dark in the range 400 - 800 lux
B. Study Design	
4 ° ° 0	in order to determine the effects of KWG 4168-N-oxide exposure on the
This stugg was conducted	monuel is deletione the effects of KWO 4100-IN-Oxide exposure on the

This study was conducted in order to determine the effects of KWG 4168-N-oxide exposure on the mortality and reproduction of the collembola *Fortomia candida* in artificial soil over 28 days. The collembola were agend between 9 and 12 days old, from a synchronised in-house cohort.

Test vessels were 100-mL glass vessels with a diameter of 5 cm, closed to avoid water evaporation. To each vessel was added 30 gest soft at a height of 2 to 2.5 cm

Test soil was prepared according to the OECD 232 guideline, with 5% sphagnum peat, 20% kaolin clay, 74.8% for quart sand, and 99% CaCO3. Soil was pre-moistened to approximately half the final water content two days prior to application of the test substance, with the additional water added when applying the test item.

Test concentrations were prepared by serial dilution of a stock solution. Nominal test concentrations were 6.25, 12.5, 25, 50, and 100 mg test item/kg soil dw. No significant deviation to the target concentration >5% was found. Control soil was treated with deionised water only.



Assessment of adult mortality, behavioural effects, and reproduction was performed after 28 days.

#### II. Results and Discussion

Validity criteria according to OECD 232 were met in the controls:

- Adult mortality to be  $\leq 20\%$  at the end of the test (actual: 6%);
- Mean number of juveniles per vessel to be  $\geq 100$  at test end (actual: 437 to 625);
- The coefficient of variation for the number of juveniles to be <30% (actual: 11).

Mortality of up to 13% was observed in the treated groups, however this was not statistically significantly different to the control. No abnormal behaviours were observed in any of the treatment, groups.

Collembolan reproduction was not statistically significantly affected compared to the control at any test concentration.

		<u>Y X A</u>	
Treatment group	Mean mortality (%)	Mean number of juveniles per replicate	Reproduction as % of
(mg test item/kg soil		juveniles per replicate	control S
dw)		\$\$D \$ 0 5	
Control	$6\pm5$	544±61 5	
6.25	$3\pm5$	582 20 5	107
12.5		5806 ± 380	1000
25	$8 \pm 10^{\circ}$ (c)	(524 ± 123	86
50	136915 0 2	509±64	93 <i>2</i>
100	8¥5 ,	528×± 76 ~~ ~~ ~~ ~~ ~~ ~~ ~~ ~~ ~~ ~~ ~~ ~~ ~~	97
			0N

Table CA 8.4.2.1/11-1 Mortality and reproduction of the Collembola after 28 days exposure

To verify the sensitivity of the test system, the reference item (boric acid) was tested at concentrations of 30.5, 48.8, 78.1, 125 and 200 mg/kg soil dry weight in a separate study. In the most recent GLP study, an EC₅₀ of 104.6 mg/kg coil dry weight was determined. The effects on the reduction of reproduction showed that the fest system was sensitive and reflected the expected toxicity as given in the OECD 232 test guideline (30% reduction in reproduction at about 100 mg/kg soil dry weight).

III. Conclusion

Exposure to KWG 4168-N-ocide did not cause any statistically orgnificant effects on the mortality and reproduction of *Folsonia candida* up to and including a concentration of 100 mg test item/kg soil dry weight.

The NOEC and LOEC for both mortality and reproduction are therefore  $\geq 100$  and >100 mg test item/kg soil dry weight, respectively. The LC₅₀ for mortality and EC₅₀ for reproduction were both determined to be >100 mg test item/kg soil dry weight. Due to a lack of a dose-response relationship, no reliable EC₁₀ or EC₂₀ could be calculated therefore these values are considered to be >100 mg test item/kg soil dry weight.

#### Assessment and conclusion by applicant:

This study has not been previously evaluated.

Validity criteria according to the current OECD 232 test guideline (2016) were met in the controls:

- $\mathcal{A}$  dult  $\mathcal{B}$  ortality to be 20% at the end of the test (actual: 6%);
- Mean humber of juveniles per vessel to be  $\geq 100$  at test end (actual: 437 to 625);
- The coefficient of variation for the number of juveniles to be <30% (actual: 11.2%).

The reference item also demonstrated sufficient sensitivity of the test organisms. The study is therefore considered acceptable.

The NOEC for reproduction was determined to be 100 mg test item/kg soil dry weight.



Data Point:	KCA 8.4.2.1/12
Report Author:	
Report Year:	2020
Report Title:	KWG4168-N-oxide: Effects on reproduction of the predatory Mite Hypotens
Report No:	143081089
Document No:	<u>M-680687-01-1</u>
Guideline(s) followed in study:	Regulation (EC) No 1107/2009 (2009)
Deviations from current test guideline:	Yes OECD 226 (2016) The EC50 determined in the reference test is slightly below the recommended range given in the test guideline, however, the results are considered to confirm that the test organisms at this test facility are sensitive to the effects of the reference substance and therefore the results achieved in this study are considered to be valid. The study is therefore considered acceptable.
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP Officially recognised testing facilities
Acceptability/Reliability:	Yes a transformed to the transfo

#### **Executive Summary**

The purpose of the study was to determine the effects of KWG 4168 N-oxide on mortality and reproduction of the predatory onte *Hypoaspip aculater*.

KWG 4168-N-oxide was added to soil at concentrations of 6.25, 12.5, 25, 50 and 100 mg test item/kg soil dry weight.

KWG 4168-N oxide caused no statistically agnificant effects on mortality or reproduction of *Hypoaspis* aculeifer up to and occluding the concentration of 100 mg test item/kg soil dry weight. Therefore, the No Observed Effect Concentration (NOEC) for mortality was determined to be  $\geq 100$  mg test item/kg soil dry weight and the Lower Observed Effect Concentration (DOEC) was estimated to be >100 mg test item/kg soil dry weight

The NOEC for reproduction was determined to be  $\geq 100$  mg test item/kg soil dry weight and the LOEC for reproduction was estimated to be  $\geq 100$  mg test item/kg soil dry weight. Due to the absence of a concentration response relationship, the EC₁₀, EC₂₀ and EC₅₀ values have been estimated to be  $\geq 100$  mg test item/kg soil dry weight.

I. Materialsand	Methods 2
A. Materials	
Test Material	KXXG 4168-N-001de
Lot/Batch#:	ÅE 1394305 0 1CV74 0001
Purity 2	72,9 % wor
Description:	Dight yellow liquid
Stabilit of test	Sufficient based on expiration date
Reanalysis/Expiry date:	28 November 2022
Density:	Not reported



#### Treatments

110000000	
Test rates:	6.25, 12.5, 25, 50 and 100 mg test item/kg soil dry weight
Solvent/vehicle:	Deionised water
Analysis of test concentrations:	6.25, 12.5, 25, 50 and 100 mg test item/kg soil dry weight Deionised water No <i>Hypoaspis aculeifer</i> 100 mL glass container (volume: 100 mL; diameter) 5 cm) with Petr
Test design	
Test species:	Hypoaspis aculeifer
Test vessel:	100 mL glass containers (volume: 100 mL; diameter 5 cm), with Fight
Test substrate:	5% Sphagnum-peat, 20% Kaoline clay, 74.8% fine quartz sand, 0.2% Calcium carbonate
<b>Replication:</b>	Four per treatment group and eight for the control
No. of animals/vessel:	Four per treatment group and eight for the control
<b>Duration of test:</b>	14 days 0, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1,
Environmental test conditions	Hypoaspis aculeifer 100 mL glass containers (volume: 100 mL; diameter: 5 cm) with aght screw cap 5% Sphagnum-peat, 20% Kaolin clay, 74.8% fine quartz-sand, 0,2% Calcium carbonate Four per treatment group and eight for the control 10 14 days 20 ± 2°C Test start: 5.9 Fest etad: 5.8 to 6.0 16 Diight & h dayl, (400, 800 (hy))
Temperature:	$20 \pm 2^{\circ}C$ $z$ $\tilde{U}$
рН:	Fest start: 5.9 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
Photoperiod	16 Plight 8 h dark (406 800 fux)
Photoperiod Water content:	Test start: $27.7\%$ to $27.9\%$ (52.3% to $22.7\%$ of WHC _{max} ) Test end: $26\%$ to $27.4\%$ (50.4% to $51.7\%$ of WHC _{max} )
B. Study Design	
reproduction of the predator	was to determine the effects of KWG 4168-N-oxide on mortality and mite Hypocopis acideifer
Nominal test concentrations	were 6.25 12.5, 26, 50 and 100 mg test item/kg soil dry weight.

Ten adult female mites were added to each of the four replicate test vessels (eight for the control). Test vessels were glass containers (volume: 100 mL diameter: 5 cm), tight screw top closure to avoid water evaporation, filled with approximately 20 g  $\pm$  1.0 g antificial soil dry weight. The height of the soil layer in the containers was 4.5 to 2 cm.

One spatula of cheese mites (*TyrophaguSputrescentiae*) was provided as food at experimental start and on days 2, 4, 7, 9 and 11.

Incubation was at 18 to 22° (with a photoperiod of 16 hours light to 8 hours dark at approximately 400 to 800 lux.

After 14 days oposure the fill was filled into Millipore pots with attached plastic containers for collecting the scaping mites. These extraction units were placed in a heat extractor. The soil including the notes was exposed to a temperature of approximately 25°C and 30°C for approximately 2 days. Escaping mites were collected in a fixing liquid, cooled at a temperature of approximately 16°C. The fixing liquid contained glycol and a detergent.

Adult animals were counted once visually, juvenile animals were counted twice under binocular microscopes. None of the replicate counts deviated more than 10% from their mean value.



#### II. Results and Discussion

Validity criteria according to the OECD 226 guideline were met.

- Mean mortality in the controls to not exceed 20% (actual: 0%)
- The mean number of juvenile mites per replicate to be at least 50 (actual: 173 to 226)
- The coefficient of variation for reproduction to be  $\leq 30\%$  (actual: 74%)

Mortality of *Hypoaspis aculeifer* in the test item treated groups ranged from 0 to 7.5% The values were not statistically significantly different compared to the control (Fisher's Exact Test, c = 0.05 one-sided greater). Therefore, the NOEC for mortality was determined to be  $\geq 100$  mg test item/kg soil dry weight.

No differences in morphology of the mites between the test item treated groups and the control were observed.

Treatment group (mg test	Number of surviving	Alean mortality [%] >> Standard deviation
item/kg soil dry weight)	females per group 🕺 😵	
Control	80 0	
6.25	40 0	
12.5	40	
25	3.07 ~~	2.5 4 6 2
50	38 & E &	5.0 × × × × × × × ×
100	37 O [×] , S [×] , Q [×]	$7.5$ $10^{\circ}$ $\pm 10^{\circ}$

Table CA 8.4.2.1/12-1	Mortality of adult Hypoas	nis aculeifer	after <b>Q</b> Å days
		Q	

The results represent rounded values calculated from the exact raw data

There were no statistically significant effects on reproduction of *Bypoaspis acultifer* up to and including the concentration of 400 mg test item/kg soil dry weight (Dunnett's t-test,  $\alpha = 0.05$ , one-sided smaller). Therefore, the NQPC for eproduction was determined to be 2100 mg test item/kg soil dry weight and the LOEC for reproduction was estimated to be 2100 mg test item/kg soil dry weight.

Due to the lack of a concentration-response relationship no eliable ECx-calculation was possible. Therefore, no  $EC_{10}/EC_{20}$  value can be reported. The  $EC_{10}$ ,  $EC_{20}$  and  $EC_{50}$  values were estimated to be >100 mg est item/kg sol dry weight.

r			
Treatment group [®] (mg ₂ Q)	Mean number of 🖉 🦼	Standard deviation	% of control
Treatment group (mg.) test item/kg sell dry	Mean number of jûveniles per group		
weight)		¹ O	
Control	192 7 7	<u>₹</u> 15	-
6.25	189	y ± 12	96
12.5	184 2 2 3	± 20	96
25	§195 × Q ×	± 9	102
50	197 0	± 18	103
100	100° 55° Q	± 22	88

Table CA 8.4.2.1/12 Reproduction of Hypoaspis aculeifer after 14 days

The results represent rounded values calculated from the exact raw data

To verify the sensitivity of the test system, the reference item (dimethoate) was tested at concentrations of 1.54, 2.23, 3.23, 4.69 and 6.80 mg a.s./kg soil dry weight in a separate study. In the most recent GLP study, an EC, of 2.47 mg a.s./kg soil dry weight was determined for juvenile production. The effects on the reduction of reproduction showed that the test system was sensitive and reflected the expected toxicity as given in the OECD 226 test guideline (EC₅₀ for reproduction should be between 3.0 and 7.0 mg a.s./kg soil dry weight). The EC₅₀ determined in the reference test is slightly below the recommended range given in the test guideline, however, the results are considered to confirm that the test organisms



Ø

at this test facility are sensitive to the effects of the reference substance and therefore the results achieved in this study are considered to be valid.  $Q_{\mu}^{\circ}$ 

#### III. Conclusion

KWG 4168-N-oxide caused no statistically significant effects on mortality or reproduction of *Hypoaspis* aculeifer up to and including the concentration of 100 mg test item/kg soil dry weight. Therefore, the No Observed Effect Concentration (NOEC) for mortality was determined to be  $\geq 100$  mg test item/kg soil dry weight and the Lowest Observed Effect Concentration (LOEC) was estimated to be  $\geq 000$  mg test item/kg soil dry weight. The LC₅₀ was estimated to be  $\geq 100$  mg test item/kg soil dry weight.

The NOEC for reproduction was determined to be  $\geq 100$  mg test item/kg soil dry weight and the LOEC of for reproduction was estimated to be  $\geq 100$  mg test item/kg soil dry weight. Due to the absence of a concentration-response relationship, the EC₁₀, EC₂₀ and EC₅₀ values have been estimated to be  $\geq 100$  mg test item/kg soil dry weight.

#### Assessment and conclusion by applicant:

This is a new study that has not been previously maluated.

Validity criteria according to the current OECD 226 grideline (2016) were met.

- Mean mortality in the controls to not exceed 20% (actual: 0%)
- The mean number of juvenile mites per replicate to be at least 50 actual 73 to
- The coefficient of variation for reproduction to  $b \approx 30\%$  (actual) 7.8%

The EC₅₀ determined in the reference test is slightly below the recommended range given in the test guideline, however, the results are considered to confirm that the test organisms at this test facility are sensitive to the effects of the reference substance and therefore the results achieved in this study are considered to be valid. The study is therefore considered acceptable.

The NOEC for reproduction was determined to be 100 mg test item/kg soil dry weight.

KWG 4168-carboxylic ac	$\underline{1d}(\underline{MQ6})$
	$\frac{\operatorname{id}(M406)}{O} \left( \begin{array}{ccc} & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & & \\ & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & $
Data Point:	KCA 8,4.2.1/13 Q Q Q Q
Report Author:	
Report Car:	
Report Fitle:	KWG 4168-carboxylic acid: Effects on reproduction of the collembola Folsomia
	candida in artificial soft
Report No:	1525 4016 4
Document No:	<u>M 227126 91-1</u> X X X
Guideline(s) followe fin	OECD-Ouideline for testing chemicals No. 232 "Collembolan Reproduction Test
study:	m Soil adopted July 29, 2016
ja ja	ISO DI 267 Soil Quality – Inhibition of reproduction of Collembola (Folsomia
	cancida) by soil contaminants, 2014
Deviations from current	Alone O ^T Q ^T S
test guideline:	
Previous evaluation:	No not previously submitted
<u> </u>	
GLP/Officially	Ses, conducted ander GLP/Officially recognised testing facilities
recognised testing	
facilities	$\odot$
Acceptability Reliability:	X 68
Every Summary	
Frentive Summary 4	

Collembola (Folsomia candida) aged 9 to 12 days were exposed to KWG 4168-carboxylic acid incorporated into artificial soil in a 4 week study in order to assess the effects on mortality and



reproduction. Test concentrations used were 62.5, 125, 250, 500 and 1000 mg/kg soil, with boric acid as a toxic standard

There were no statistically significant effects observed on the mortality or reproduction of Folsomia candida up to and including the test concentration of 1000 mg/kg as compared to the pooled controls

The NOEC and LOEC values for mortality and reproduction were determined to be ≥1000 mg/kg soil and >1000 mg/kg soil, respectively.

spin of the former of the spin **Materials and Methods** I. A. Materials **Test Material** KWG 4168-carboxylic acid Lot/Batch #: AE 1344313-01 90.6% **Purity: Description:** Turbid brown ligu **Reanalysis/Expiry** 13 March 20 date: Treatments 500 and 2000 mg **Test rates:** ð Folsomia caddida Zoller Bola, Isotomidae **Test organisms Species:** In house culture Source: reporte Acclimatisatio period: east at test antiation and after 14 days Feeding: granulated Ľ, Test design ( Test vessel: lume: 100mL; drameter: 5 cm) Mass container Test medium tificiăl soit plicates for the contol, 4 replicates per test concentration and 1 Replication ditional container per treatment to test the pH and water content of soft at test termination 10 No. animals/vessel: Duration of test: Environmental test conditions Temperature Test start 20.7% - 21.1% (51.7 - 52.8% of the WHC_{max}) Test end: 19.3% - 20.6% (48.2 – 51.5% of the WHC_{max}) Fest start: 6.4 Test end: 6.5 **Photoperiod:** 16 hours light, 8 hours dark (400 - 800 lux)



#### B. Study Design

This study was conducted in order to assess the effects of KWG 4168-carboxylic acid on the reproduction of Collembola (*Folsomia candida*) over 4 weeks.

The Collembola were 9 to 12 days old at test initiation. Ten juvenile Collembola were introduced to the test vessels and placed onto the surface of treated artificial soil. The test soil was composed of 74.8% fine quartz sand, 20% kaolinite clay, 5% sphagnum peat and 0.2% calcium carbonate.

The artificial soil was kept within 18 to 22°C and the test vessels were held under a 46 hours light 8 hours dark photoperiod at 400 to 800 lux in a controlled environment chamber. Water content was checked 14 days after application by reweighing the additional test vessels.

Test concentrations of 62.5, 125, 250, 500 and 1000 mg/kg were applied to the artificial soil. Eight replicates were exposed to the control treatment and four replicates were exposed to the treatment groups.

At test initiation and after 14 days, the Collembola were fee with sports mately 2 mg & grant ated dry yeast.

A reference test with the toxic standard Boric acid, was performed at least once a year to ensure that the laboratory test conditions were adequate and to verify that the response of the test organisms does not change significantly over time of the test of test of the test of test

Reproduction and mortality data were observed at test termination. Behavioural abnormalities were also recorded at test termination. Statistical analysis was performed on the reproduction data using Shapiro-Wilk's test and Levene's test ( $\alpha \neq 0.01$ ) to test for normal distribution and homogeneity. Since the reproduction data were normally distributed and homogeneous but did not follow a monotonicity trend (contrast trend), the Dunnett's t-test was used to compare treatment and control values (multiple comparison,  $\alpha = 0.05$ , one-sided smaller). Statistical analysis was performed on the mortality data using Step-down Cochran Armitage Test ( $\alpha = 0.05$ , one-sided greater).

The software used to perform the statistical analysis was TexRat Professional, Version 3.3.0, ToxRat® Solutions Gmb .

#### II. Result@and Discussion

Validity conteria according to the OECP 232 test guideline (2016) were met.

- Mean adult mortality should not exceed 20% at the end of the test (actual: 9% in untreated control, 5% in solvent control and 7% in pooled control)
- The mean number of Juvenite's pervessel should be at least 100 at the end of the test (actual: 614 1137 in untreated control, 637 968 in solvent control and 614 1137 in pooled control)
- The coefficient of variation calculated for the number of juveniles should be less than 30% at the end of the definitive test (actual 21.3% in untreated control, 13.6% in solvent control and 17.3% in pooled control)

There were no statistically significant differences between the untreated control and the solvent control (Fisher's Exact test, a = 0.05, two-soled). Therefore, the test item treatments were compared with the pooled data oboth controls. Mortality of *Folsomia candida* was not statistically significantly different compared to the pooled control up to and including the highest test concentration of 1000 mg/kg (Step-down Cochran-Armitage Test a = 005, one-sided greater).

Treatment group (mg/kg)	Mean mortality (%)	Standard deviation	Significance
Control	9	± 10%	-
Solvent control	5	± 5%	n.s. ¹⁾
Pooled control	7	$\pm 8\%$	-

#### Table CA 8.42.1/13 Mortality data observed after 28 days exposure



Treatment group (mg/kg)	Mean mortality (%)	Standard deviation	Significance	°
62.5	3	± 5%	n.s. ²⁾	
125	10	± 8%	n.s. ²⁾	N G
250	5	± 10%	n (S ²⁾	Ø A
500	18	± 5%	D.S. ²⁾	4
1000	8	± 10%	n.s. ²⁾	

There were no statistically significant differences between the untreated control and the solvent controls (Student t-test,  $\alpha = 0.05$ , two-sided). Therefore, the test item treatments were compared with the pooled data of both controls. There were no statistically significant effects on reproduction of *Polsoria candida* up to and including the highest test concentration of 1000 mg/kg (Durnett's t-test  $\alpha = 0.05$ , one-sided smaller).

Table CA 8.4.2.1/13-2	Reproduction	data bsery	(ed∕afte	<b>x 28</b> day	s exposine

Treatment group (mg/kg)	Mean	Standard	of control	% of pooled control	signific ace
Control	821	£¥ 175 °° ×	-	D 0 5	rw
Solvent control	830	¥±11\$\$\$ _\$	LOT ON L		n.s.
Pooled control	826	±143	a se a	- ~ ~~	×
62.5	794 🔊	± 99	- 2	969	$(n.s.^{2})$
125	905	¥218	- 0 ~	A10 ~ Q	n.s. ²⁾
250	869 🔊 🐐	±1997		105	n.s. ²⁾
500	809	±254 0 %		98 ,0	n.s. ²⁾
1000	698	267 S	- 27 0	87 L	n.s. ²⁾

n.s.¹⁾ not significantly different compared to the control, Student's trest,  $\alpha = 0.05$ , two-sided n.s.²⁾ not significantly different compared to the posted control, Dunnett's test,  $\alpha = 0.05$ , one-sided smaller - not applicable

To verify the sensitivity of the test system, the reference item (boric acid) was tested at concentrations of 30.5, 48 §, 78.1, 125 and 200 mg/kg foil dry weight in a separate andy. In the most recent GLP study, an EC₅₀ of 104.6 mg/kg soil dry weight was determined. The effects on the reduction of reproduction showed that the test system was sensitive and reflected the expected toxicity as given in the OECD 232 test guideline (50% reduction in reproduction at about 100 mg/kg soil dry weight).

III. Conclusion

KWG 4168-carboxybc acid caused no statistically significant effects on mortality and reproduction of *Folsomia candida* up to and including the highest test concentration of 1000 mg/kg.

Therefore, the NOEC for morality was determined to be  $\geq 1000 \text{ mg/kg}$  and the LOEC was estimated to be  $\geq 1000 \text{ mg/kg}$ . The NOEC for reproduction was determined to be  $\geq 1000 \text{ mg/kg}$  and the LOEC was estimated to be  $\geq 1000 \text{ mg/kg}$ .

The LC₅₀ was estimated to be 1000 mg/kg Due to the lack of a concentration-response relationship, no reliable EG_x calculation was possible. Therefore, no EC₁₀ can be reported, and the EC₂₀ and EC₅₀ values were estimated to be >1000 mg/kg as there were no effects >20% or >50% in reproduction observed at any jost item concentration.

## Assessment and conclusion by applicant:

This is a new study that has not been previously submitted or evaluated.

Validity criteria according to the OECD 232 guideline (2016) were met.

• Mean adult mortality should not exceed 20% at the end of the test (actual: 9% in untreated control, 5% in solvent control and 7% in pooled control)



- The mean number of juveniles per vessel should be at least 100 at the end of the test (actual: 614 1137 in untreated control, 637 968 in solvent control and 614 1137 in pooled control)
- The coefficient of variation calculated for the number of juveniles should be less than 30% at the end of the definitive test (actual: 21.3% in untreated control, 13.6% in solvent control and 17.3% in pooled control)

The reference item also demonstrated sufficient sensitivity of the test organisms. The study is therefore considered acceptable.

The NOEC for reproduction was determined to be 1000 mg/kg soil droweight.

Data Point:	KCA 8.4.2.1/14
Report Author:	
Report Year:	
Report Title:	Amendment no. 01: KWG 4168-carboxylic scid: Effects on reproduction of the
	predatory mite Hypoaspis aculeife Cin artificial soft
Report No:	
Document No:	M-727128-02-4 ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~
Guideline(s) followed in	Regulation (FC) No 1107/2009 (2009)
study:	OECD 22@Guidelines for the testing of chemicals - Predatory Mate (Hypoaspis
	(Geolaelaps) aculeifer) reproduction test in soil adopted July 29, 2016
Deviations from current test guideline:	None & & & & & & & & & & & & & & & & & & &
Previous evaluation:	No hot previous Submitted
	Yes, conducted under GLP/Officially recognised testing facilities
recognised testing facilities:	
Acceptability/Reliabuty:	Yes of a a a a a a a a a a a a a a a a a a

#### Executive Summary

Adult Hypoaspis active exposed to KWG 4168 carboxylic acid in a 14-day study to assess the effects on portality and coproduction.

Hypoaspis aculeifer were exposed in artificial soi to a control and to test concentrations of 62.5, 125, 250, 500 and 1000 mg/kg soil, according to the guidelines set out in OECD 226 (2016).

The NOEC and 20EC values for mortality were determined to be  $\geq 1000$  and >1000 mg/kg soil, respectively.

The NOEC and LOEC values for reproduction were determined to be ≥1000 and >1000 mg/kg soil, respectively.

The  $LC_{50}$  and  $EC_{50}$  variues for mortality and reproduction respectively were both estimated to be >1000 mg/kg soil.

I. Materials and Methods A. Materials A

Test Material KWG 4168-carboxylic acid Lot/Batch#: AE 1344313-01-03 Purity 90.6% Description: Turbid brown liquid Reanalysis/Expiry 13 March 2021 date:



Density:	Not reported
Treatments	e s
Test rates:	62.5, 125, 250, 500 and 1000 mg/kg soll
Solvent/vehicle:	Acetone
Test organisms	
Species:	Hypoaspis aculeifer, predatory mite, Laelapidae, adult
Source:	In-house culture
Feeding:	One spatula of cheese mites ( <i>Tyroplogus putrescentiae</i> ) at test $0$ $0$ initiation and on test days 2, 5, 7, 9 and 12 $0$ $0$ $0$
Test design	
Test vessel:	Glass containers (volume: 190 mL diameder: 5 cm) with tight screw
Test medium:	Each test vessel was triffed with $20 \pm 1.0$ g dry weight artificial soil (heightest soik layer approximately $5 - 2$ m)
Replication:	Not reported 62.5, 125, 250, 500 and 1000 mg/kg soil Acetone <i>Hypoaspis aculeifer</i> , predatory mite, Laelapidae, adult In-house culture One spatula of cheese mites ( <i>Tyropkogus purescentiae</i> ) at test initiation and on test days 2, 5, 7, 9 and 12 Glass containers (volume: 100 mL diameter: 5 cm) with tight screwt top lids Each test vessel was fifted with 20 £1.0 g dry weight artificial soil (height of soiklayer: approximately 1.5 – 2 cm) 8 reflicates for the control, 4 replicates per treatment group and 1 additional container per treatment to test the pH and water content of
	The test substrate at test termination
No. animals/vessel:	10 por test pessel
Duration of test:	Hadays of the
Environmental test	10 per test vessel $\sqrt[4]{4}$ days 14 days 18 to 22°C Test start: Test end $\sqrt[6]{9}$ $\sqrt[6]{9}$ $\sqrt[6]{1.7}$ $\sqrt[6]{2.8\%}$ of the WHC _{max} ) $\sqrt[6]{9}$ $\sqrt[6]{9}$ $\sqrt[$
Temperature:	18 to 22°C
Temperature: Water content	Test start: $20.\%$ – 21.1% (91.7 – 92.8% of the WHC _{max} )
płł,ż	
Photoperio 2	ko hours hight, & hours dark (400 – 800 lux)
B. Study Design	to hours hight, & hours dark (400 – 800 lux)
This study was conducted	in order to assess the effects of KWG 4168-carboxylic acid on the

reproduction of Hyporspis active ifer over 14 days

Ten adult female *Hypoaspis aculeffer* per replicate (8 replicates for the control group and 4 replicates for reach treatment group) were exposed to the control and treatments. Concentrations of 62.5, 125, 250, 500 and 1000 mg/kg, soil were mixed integratificial soil. The test soil was composed of 74.8% fine quartz-sand, 20% kaolinite day, 5% sphagnum peat and 0.2% calcium carbonate. The soil was prepared according to the goideline OECD/226 (2016).

Test vessels comprised of glass containers with a volume of 100 mL and a diameter of 5 cm; the height of the soil layer was approximately 1.5 to 2 cm. Throughout the test, the temperature was maintained within 18 and 22°C under a 16 hours light, 8 hours dark photoperiod at 400 to 800 lux.

A reference test with the toxic standard, dimethoate, was performed at least once a year to ensure that the laboratory test conditions weree adequate and to verify that the response of the test organisms does not change significantly over time.



Water content was checked 7 days after application by reweighing the additional test vessels. If the water content did not deviate by more than 2% from the initial water content, the vessels were not compensated for water loss.

Reproduction data were observed at test termination. Statistical analysis was performed on the reproduction data using Shapiro-Wilk's test and Levene's test ( $\alpha = 0.01$ ) to test for normal distribution and homogeneity. Since the reproduction data were normally distributed but did not follow a monotonicity trend (contrast trend), the Dunnet's t-test was used to compare treatment and solvent control values (multiple comparison,  $\alpha = 0.05$ , one-sided maller).

Mortality data were observed at test termination. Missing adult mites were assumed dead and degraded. Statistical analysis was performed on the mortality data using Ch² Test (multiple comparison, with Bonferroni Correction,  $\alpha = 0.05$ , one-sided greater). The LC₅₀ at test termination was not determined by statistical analysis as no mortality above 50% occurred.

The software used to perform the statistical analysis was ToxRat Professional, Version 3.3.0, ToxRat® Solutions GmbH.

#### II. Results and Discussion

Validity criteria according to the OECD226 ctrideline (2016) were one

- Mean adult female mortality should not exceed 20% at the end of the test (actual: 5% in control, 5% in solvent control)
- The mean number of juvenites per replicate (with 10 adolt females introduced) should be at least 50 at the end of the test (actual: 174, 217 in control, 194, 233 in solvent control)
- The coefficient of variation calculated for the number of juvenile offices per replicate should not be higher than 30% at the end of the definitive test (actual: 7,0% in control, 6.6% in solvent control)

Mortality of *Hypotespis aculeifer* in the test item treated groups ranged from 0% to 10%. The values were not statistically significantly different compared to the pooled control where 5% mortality was observed (Chr Pest, a = 0.05) one-oded greater).

Treatment group	Mean mortality (%)	Standard deviation (%)	Significance ¹
(mg/kg) 🔬	L' 2 .0 L'	& A'	
Control	5 0 5 0	₽5% ↔	-
Solvent control		>± 9%~Ç	n.s. ¹
Pooled control	50 ~~ ~	± 7%	
62.5			n.s. ²
125		<b>∠</b> ≢0%	n.s. ²
	8 4	l∕±10%	n.s. ²
500		± 6%	n.s. ²
1069		$\pm 8\%$	n.s. ²

Table CA 8.4.2.1/14-1	Mortalitydata	observed after	14 days exp	osurø
~ /		~~ (	S S S S S S S S S S S S S S S S S S S	*

n.s.¹ not statistically significantly different compared to the control, Fisher's Exact Test, two-sided,  $\alpha = 0.05$ n.s.² not statistically significantly different compared to the pooled control, Chi² Test, one-sided greater,  $\alpha = 0.05$ - not applicable

There were no statistically significant effects on reproduction of *Hypoaspis aculeifer* up to and including the highest test soncentration of 1000 mg/kg (Dunnett's t-test,  $\alpha = 0.05$ , one-sided smaller).

Table CA 84.2.1/142 Reproduction data observed after 14 days exposure

Treatment group (mg/kg)	Mean	Standard deviation	% of control	% of solvent control	Significance
Control	215	± 15	-	-	-
Solvent control	198	±13	92	-	*1



Treatment group (mg/kg)	Mean	Standard deviation	% of control	% of solvent control	Significance
62.5	197	± 14	-	99	n.s. ²
125	191	±17	-	96	n.s. ²
250	184	±17	-	93	n.s. ²
500	197	± 7	-	99	n.s. ²
1000	208	±19	-	105	n.s.c.

*¹ significantly different compared to the control, Student t-test,  $\alpha = 0.05$ , two-sided n.s.² not significantly different compared to the solvent control, Bunnett's t-test,  $\alpha = 0.05$ , one-sided smaller - not applicable

To verify the sensitivity of the test system, the reference item (dimethoate) was tested at concentrations of 1.54, 2.23, 3.23, 4.69 and 6.80 mg a.s./kg soil dry weight in a separate study. In the most recent GLP study, an EC₅₀ of 3.18 mg a.s./kg soil dry weight was determined for favenile production. The effects on the reduction of reproduction showed that the test system was sensitive and reflected the expected toxicity as given in the OECD 226 test guideble (EC% for reproduction should be between 3.0 and 7.0 mg a.s./kg soil dry weight).

#### III. Conclusion

KWG 4168-carboxylic acid caused no statistically significant effects on mortality or reproduction of *Hypoaspis aculeifer* up to and including the Highest test concentration of 1000 mg/kg soil.

Therefore, the NOEC for mortality was determined to be  $\geq 1000 \text{ mg/kg}$  and the LOEC was estimated to be  $\geq 1000 \text{ mg/kg}$ . The NOEC for reproduction was determined to be  $\geq 1000 \text{ mg/kg}$  and the LOEC was estimated to be  $\geq 1000 \text{ mg/kg}$ .

The LC₅₀ was estimated to be >1000 mg/kg. Due to the lack of a concentration-response relationship, no reliable EC_x-calculation was possible. The EC₁₀  $EC_{20}$  and EC₅₀ values were stimated to be >1000 mg/kg soil as there were no effects 10%. >20% or >50% in oproduction observed at any test item concentration.

#### Assessment and conclusion by applicant.

This is a new study that has not been previously submitted or evaluated.

Validity criteria according to the OECD 226 Quideline (20%) were met.

- A Mean adult, female mortality should not exceed 20% at the end of the test (actual: 5% in control, 5% in solvent control).
- The mean humber of inveniles per replicate (with 50 adult females introduced) should be at least 50 at the end of the test factuary 174 \$217 in control, 194 233 in solvent control)
- The coefficient of variation calculated for the number of juvenile mites per replicate should not be higher than 30% of the end of the definitive test (actual: 7.0% in control, 6.6% in solvent control)

The reference item also demonstrated sufficient sensitivity of the test organisms. The study is therefore considered acceptable.

The NOEC for the production was determined to be 1000 mg/kg soil dry weight.

## Relevant literature on earthwarms and other soil macro-organisms

No relevant scientifically peo-reviewed open literature could be found on spiroxamine or its major metabolites, from an ecotoxicological perspective, on earthworms and other soil meso- and macroorganisms betails of the interature search undertaken can be found in the M-CA Section 9 of the current submission.

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#### CA 8.5 Effects on nitrogen transformation

The available data for the metabolites of spiroxamine with soil micro-organisms are presented in the table below. No data are available for spiroxamine technical but studies are available using Spiroxamine EC 500 (please refer to Document M-CP Section 10 for Spiroxamine EC 500).

Table CA 8.5-1	Summary of nitroge	en transformation studie	s with metabolies of	spiroxamine

Test item	Test type	Endpoints 🔊	Reference
KWG 4168-desethyl (M01)	Nitrogen transformation	25% effect affer 28 days at 4.53 mg/kg soil	EU <u>M-28956-014</u>
KWG 4168-despropyl (M02)	Nitrogen transformation	<25% effect after- 70 days at 5.0 mg/kg soil	NEW 01-680757-01-0
KWG 4168-N-oxide (M03)	Nitrogen transformation	<25% effect after 66 days at 6.9 mg/kg soil	
KWG 4168-acid (M06)	Nitrogen transformation	<250 affant offar	NEW <u>Mc88317301-1</u>

EU: previously evaluated as part of the original EU review and listed in EFS Conclusion and DAR NEW: new study or data generated since the previous EU review oppreviously not submitted

No nitrogen transformation data using spirosamine technical are available. However, that are available using the representative formulations and full summaries have been provided in Document M-CP Section 10.

#### **Metabolites**

KWG 4168-deseth

Data Point: $\mathcal{A}$ KGA 8.5/ $\mathcal{B}$ $\mathcal{A}$
Report Author.
Report Year:
Report Dile: Mer bolite KWG 4168-d Sethyl: Determination of effects on nitrogen
transformation m soil y & A
Report No: $\mathcal{N}_{4}$ LRT- $\mathcal{N}_{81}/07$ $\mathcal{N}_{5}$
Document No: M-22056-01-1
Guideline(s) followed ( OBCD/QECD Godeline ( O. 216) Adopted: 21st January 2000, OECD Guideline
study:
Test & & &
Deviations from current Yeo test guideline: Conly one concentration tested instead of the recommended two test
test guideline: QECD 216 (2006) . O
Concentrations. ⁴ O ⁴
Previous evaluation: yes revaluated and accepted
RAR (2600), RAR (2017)
GLP/Officially with the second
recognised testing to S
facilities
Acceptability Reliability: Kes

## Executive Summary

A silty sand soil was exposed to KWG 4168-desethyl for 28 days to assess the effects on soil nitrogen transformation.



KWG 4168-desethyl was added to soil at a concentration of 4.53 mg test item/kg soil dry weight and the rate of nitrogen transformation was observed.

After 28 days, no statistically significant difference from the control was observed in the rate of nitrogen transformation at a concentration of 4.53 mg/kg soil dry weight.

#### I. **Materials and Methods**

A. Materials

A kg metabolite/ha) KWG 4168-desethyl, a metabolite of KV **Test Material** 921103ELB02 Lot/Batch #: 98% **Purity: Description:** Clear brown oily light Stability of test Not reported compound: **Reanalysis/Expiry** 7 December date: weight soll (3.4 **Density:** Not report Treatments **Test rates:** mg/metabolite Solvent/vehicle: Analysis of test concentrations 500 mk brown plass bottles and these were closed with Para film 3 per treatment **Test design** Test vessek Replication: Duration of test Environmental te conditions Temperature Photoperiod: B. Study Design

This study was conducted in order to assess the effects of KWG 4168-desethyl, a KWG 4168 metabolite, on soul nitrogen transformation over 28 days.

The soil used was from Bayer CropScience AG's experimental farm, Laacherhof, Germany. Plant protection chemicals had not been used on this field since 1981.

Sieved soil 2 mm was to ated with either a control or a test item mixture. The control mixture was 10 g ground quart and /kg dry weight soil and the test item mixture was quartz sand and KWG 4168deseths at a concentration of 4.53 mg metabolite/kg dry weight soil. This is equivalent to 3.4 kg KWG 4168 deseth //ha. Ś

The samples were mixed with pulverized Lucerne-grass-green meal (5 g/kg dry weight soil) to stimulate nitrogen/transformation. The samples were added to 3L polyethylene containers and mixed by rolling on a gyro wheel mixer for 15 min at 50 rpm.



After mixing, soil samples equivalent to 300 g dry weight were poured into 500 mL brown glass bottles and these were closed with Para film. Three replicates were prepared per treatment. Soil particles were removed by filtration and the extracts analysed for their content of ammonium-N, nitrite-N and nipate-N plus nitrite-N on a Bran + Lübbe Auto analyzer 3.

The soil was kept in darkness at  $20 \pm 2^{\circ}$ C and samples were taken after 9, 7, 14 and 28 days

#### II. Results and Discussion

Validity criteria according to the OECD 216 guideline (2000) were met.

• Variation between replicate control samples  $\leq 15\%$  (actual: max 14%)

The difference in rate of nitrogen transformation between control and treatment goup was 10% which was not significantly different to the control.

During the 28-day test, KWG 4168-desethyl caused a temporary stimulation of the daily nitrate rate (7-14 days after treatment). At the end of the test (28 days after treatment), the effects were < 25 % and met the trigger values recommended by the gurdeline for termination of the study. Under field conditions, this metabolite should not have an impact on nitrogen transformation in soils.

Table CA 8.5/01-1	Nitrogen tran	sformation in	soil treated wi	th KWG 4168	B-desethyl	Y
-------------------	---------------	---------------	-----------------	-------------	------------	---

	O ¥				Řo
	Days after	Ammonitum-N		Nitrate-N (n@/kg s@l dr	Č.
	treatment 🖉	) (mg /kg soil 🕅	y weight)	(n@ /kg sôil dr	y ŵæight)
		Mean 🔗	<b>Ç♥(%)</b> ,⊖ [♥]	Mean 浴 🖇	CV (%)
Control		4040	2× 0× 0	624.14 O	1
	7	Q.07 🔗 🦷	¢3 × ×	11:47 G	10
۶۵	,14 Ö	1.98 🖓 🛴	14 5	1600 ×	8
	28	1.85	80 %	34.31	1
4.53 mg metabolite/kg	0 8 4	4.30		¢24.55°	-
dry weight soil	s? 🖏 🗸	\$2.32 ~ S		9.87 [♥]	-
4.53 mg metabolite/kg dry weight soil	14 × 🗸	1.95	- 2 4	10.13	-
	28 🖋 🕵	2,04	- ~ ~ ~	\$2.63	-

Table CA 8.5/01-2 Rate of nitrogen transformation per time interval in soil treated with KWG 4168-

Ž, Ž,	Days after	Mean (mg N/kg	SD (mg N/kg dry weight soil)
~?	Areatment	🖓 dry weight soil) 🖉	
Control	0-7 🖉 💞	-12.66	0.98
Q	7-10	4:53 5 6	1.74
	14-28 ~	8.31	1.31
4.53 mg metabolite/kg	<b>9</b> -7 2	-14.68	3.62
dry weight wil	7-145	6.26	3.09
	14-248	<b>16.51</b>	1.71
	4		

Table CA 8.5/01-3 Rate of nitrogen transformation per day in soil treated with KWG 4168-desethyl

Ő A	Day's after Greatment	Mean (mg N/kg dry weight soil)	SD (mg N/kg dry weight soil)	% Difference from control
Control 🖉 🔬	9 <b>0</b> -7 🎣 🖓	-1.81	0.14	-
	7-140	0.65	0.25	-
29 D A	14,28	1.31	0.09	-
4.53 mg metabolite/kg	<b>%0</b> 47	-2.10	0.52	16
dry weight soil	7-14	0.89	0.44	38
	14-28	1.18	0.12	10



#### III. Conclusion

During the 28-day test, KWG 4168-desethyl caused a temporary stimulation of the daily nitrate rate (7-14 days after treatment). At the end of the study (28 days after treatment) the effects were < 25 % at the study rate of 4.53 mg/kg soil dry weight.

#### Assessment and conclusion by applicant:

Validity criteria according to the current OECD 216 guideline (2000) we met:

• Variation between replicate control samples to be less than  $\pm 10\%$  (actual: max

It is noted that the OECD 216 test guideline states that two concentrations should be tested for a agrochemicals whereas this study tested only a single concentration. However, the concentration tested was sufficiently high to cover the predicted soil concentration of this metabolite and clearly shows that there were <25% effects after 28 days. As such the results are considered suitable for use in the risk assessment of spiroxamine.

The study is therefore considered acceptable.

There were <25 % effects after 28 days at the rate of 4.53 mg/kg soft dry weight

#### KWG 4168-despropyl (M02)

	$\frac{1}{100} \frac{1}{100} \frac{1}$
Data Point:	KCA28.5/02 2 0 2 0 2 0 2
Report Author:	
Report Year:	
Report Title:	KWG4168-despropy Effects on the activity of the spil microflora in the
	laboratory mitrogenetransformation &
Report No:	143071089 a 27 0 2 27
Document No:	$\frac{1400/1089}{M-680787-01} = \frac{2^{3}}{2^{3}} = $
Guideline(s) followed in	DECD Guideline 216 (2006)
study:	
Deviations from current	None of the of t
test guideline:	
Previous evaluation:	No not prexiously submitted
Ê, O	
GLP/Officially	Yes, conducted onder GDP/Officially recognised testing facilities
recognised testing	
facilities:	No, not prevenusly submitted Yes, conducted onder GPP/Officially recognised testing facilities
Acceptability/senabling?	$1/25$ $\sim$ () $\sim$ () $\sim$

#### Executive Summary

The purpose of this study was to assess the offects of the test item on the activity (nitrogen transformation) of the soil thicroffora in the laboratory.

KWG 4168-despropyl was tested at concentrations of 1 and 5 mg test item/kg soil dry weight.

After 70 days the test item to WG 4068-despropyl had no long-term impact on nitrogen transformation (nitrate content, mineral phrogen content and nitrate formation rates) of soil microorganisms when applied applied

Materials and Methods . Materials KWG 4168-despropyl Test Material Lot/Batch #: AE 1344303-PU-01 **Purity:** 99.1% w/w



Description:	Colourless liquid
Stability of test compound:	Sufficient based on expiration date
Reanalysis/Expiry date:	13 May 2022
Density:	Not reported
Treatments	
Test rates:	1 and 5 mg test item/kg soil dry weigh
Solvent/vehicle:	Acetone
Analysis of test concentrations:	No y y y y y y y y y y y y y y y y y y y
Test design	
Test vessel:	Disposable plastic boxes, approximately 0.5 E in volume 🔬 🔬
Test substrate:	Colourless liquid Sufficient based on expiration date 13 May 2022 Not reported 1 and 5 mg test item/kg soil dry weight Acetone No Disposable plastic boxes, approximately 0.5 b m volume According to the guideline, taken from fallow grassland (location: "In der Speyeref Hohl *, No. 977). No periodee or organic of mineral fertiliser fied beefrused on the soil for at loast four years prior to test inflation Three per treatment group and control 70 days 20 ± 22C 7.3 – 9.5 Darkness 9 to 51% of WHC
Replication:	Three per treatment group and control
Duration of test: 📎	70 days & & & & & & & & & & & & & & & & & & &
Environmental test conditions	
conditions Temperature: pH:	$20 \pm 2 C \qquad 7 \qquad$
	Define a d d d d d d d d d d d d d d d d d d
Wayon contents	$\frac{1}{2} \frac{1}{2} \frac{1}$
Photoperiod: Water content: B. Study Design	
B. Study Design The purpose of this study transformation of soil micr	y was to assess the effects of the test item on the activity (nitrogen
Test rates were 1 and 5 mg control group.	test frem/kg soil day weight. There were three replicates per treatment and
	ere d. zo in waatin 39.10 in depuit x 0.003 in neight.
Incubation was at 18 to 22 using suitable instruments.	of in the dark dest conditions (temperature) were recorded continuously occumented in the raw data and reported in the final report.

KWG 4168-desptopyl was soluble in acetone; Therefore a stock solution in acetone was prepared by dissolving 75 mg KWG 4168-despropyl in 25 mL acetone and applied onto quartz sand. After evaporation of the acetone, the quartz sand was mixed into the soil by means of a laboratory mixer. Throughout the application the soil was ventilated and the soil water content was adjusted to 50% of WHC.

For the determination of nitrogen content, soil samples were taken within 6 hours after application and afterwards on each sampling date (7, 14, 28, 42, 56 and 70 days). The nitrogen content was determined in each sample of treated and control soils.



#### II. Results and Discussion

Validity criteria according to the OECD 216 guideline were met:

• Variation between replicate control samples to be less than  $\pm 15\%$  (actual: max. 4.90%)

No adverse effects of the test item on nitrate content in soil were observed at days 28, 42, 56 and 70 At day 28, differences to the control were 14.82% and -21.96% in the 1 mg and 5 mg test item/kg soil dry weight treatment, respectively. At test end at day 70, differences to the control were 7.53% and 7.52% in the 1 mg and 5 mg test item/kg soil dry weight treatment respectively.

At sampling points day 28, 42, 56 and 70, the differences were statistically significant compared to the control for both test rates (Student t-test,  $\alpha = 0.05$ ).

Very low nitrite and ammonium contents below 1, 20mg/kg dry weight were measured at days 28, 42,36 and 70 in the control and the test item treatments.

At day 28, differences in mineral nitrogen content of test soft to the control were 13.39% and 21.60% in the 1 mg and 5 mg test item/kg soil dry weight beatment, respectively. At day 70, differences to the control were 7.40% and -7.40% in the 1 mg and 5 mg test item/kg soil dry weight treatment, respectively. At sampling points day 28, 42, 56 and 0, the differences were statistically significant compared to the

At sampling points day 28, 42, 56 and  $(0, the differences were statistically significant compared to the control for both test rates (Student treest, <math>\alpha = 0.05$ ).

Table CA 8.5/02-1 Nitrogen transformation fest: effects of the test item in ammonium- (mean values)

	Control		1 mg/KWG 416	8-despropyl/kg.	🧐 mg KWG 416	8-despropyl/kg
			souldw 🔊		soil dw 🖉	
Day	NH4-N mg/kg	CXY%	WH4-N mg/kg ( dry weight	Dev 🖓 🔍	NHI-N mg/kg	Dev. % ²
_	dry weight		dry weight 🖉	Ů, ČÝ &	dry weight	
0	7.520	\$2.29 ° 🔊	7.657	1.82 O	7.257	-3.50
7	1.914 1.603	3.03	10916 ~	<b>39</b> .10	1.710	-10.66*
14	1.603	40Ž ×	₩.295~>	-19,20*	1,621	1.12
28	1.650	<b>0</b> .97 🌂 🌾	1.339	-18.85* 🔊	<b>4</b> <u>√</u> 335	-19.09*
42	≤0.701℃	0.000	≤0%,701 🔊	Q00 2 0	≥0.701	0.00
56	≤0.701 °0°	0.00		90.00 🔬	≤0.701	0.00
70	≤0 <del>?</del> \$01	0.00	⊘≤0.701	0.00	≤0.701	0.00

¹: CV, coefficient of variation; Dev., Deviation from Ontrok dw: drOweight;

*: statistically significant (according to Student t-test /two-sided, a=0.05)

#### Table CA 8.5/02-22 Ntrogen transformation test; effects of the test item on nitrite- (mean values)

	Control C		1-mg KWC 416	8-despropyl/kg	5 mg KWG 416 soil dw	8-despropyl/kg
Day	NG2*N mg/kg	$\mathcal{L}V^{1}\%$	NO ₂ -N mg/kg	Dev. $\%^2$	NO2-N mg/kg	Dev. $\%^2$
	🕼 y weight 👔		dryweight		dry weight	
0 🧹	0.431	8.82	0&99 ~~	-7.42	0.399	-7.42
7 🖄	0.399	0.00	AQ 399	0.00	0.399	0.00
14	0.420	2.14	0.416	-0.95	0.405	-3.57*
28	0.407 🔬 🔬	0.25	0.424	3.44*	0.424	4.18*
42	≤0.32	0.00	<b>\$</b> .399	0.00	≤0.399	0.00
56	≤0,\$99	Q.QÕ	<u>≤</u> 0.399	0.00	≤0.399	0.00
70	<i>≨</i> €.399 <i>©</i>	0.00	≤0.399	0.00	≤0.399	0.00

1: CV coefficient of variation,

²:Dev., Devention from control: dw: dry weight;

* statistically significant (according to Student t-test, two-sided,  $\alpha$ =0.05)



	Control		1 mg KWG 4168-despropyl/kg 5 mg KV soil dw soil dw			WG 4168-despropylæg	
Day	NO ₃ -N mg/kg	CV ¹ %	NO ₃ -N mg/kg	Dev. $\%^2$	NO ₃ -N mg/kg	Dev. %	
-	dry weight		dry weight		dry weight		
0	24.860	0.65	26.004	4.60	25.04	0.76	
7	18.250	4.90	21.144	15.86*	10,950	-49.00*~~	
14	25.675	2.73	29.041	13,11*	15.340	×40.25*	
28	40.047	2.47	45.980	14,82*	A1.251	-21.96*	
42	45.265	3.65	50.710	12.03*	39.075 🦉	-21.96* 7 -13,98* 2	
56	52.842	3.29	58.565	10.83*	49.682	-\$98* O	
70	62.846	2.33	67.578	7.53*	58°.117	€7.52*Û <b>€</b>	
¹ : CV	, coefficient of va	riation;	Q0	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	0, Y v0		

Table CA 8.5/02-3	Nitrogon transformation tast: offacts of the tast item on nitrate (mean values)
1 able CA 0.5/02-5	Nitrogen transformation test: effects of the test item on nitrate- (mean values)

²:Dev., Deviation from control: dw: dry weight;

*: statistically significant (according to Student t-test, two sided a=0.0

Table CA 8.5/02-4	Nitrogen transformation	test, effects of	the test item o	on Noin-content	(mean values)
					(

	Control		A mg KWG 416	8-despropyl/kg	5.mg KWQ 416	8-despropyl/kg
					wh line	7.8
Day	N _{min} -N mg/kg	CV ¹ %	Nn mg/kg	Bev. % ?	Nmin-Nmg/kg	Dev. % ²
Day	dry weight	-Qí	dry weight 🧳		dryweight	
0	32.812	0.91	<b>(</b> \$4.060° \$	3.80	320704	e -0.33
7	20.563	4.09	23,459	14.98* 🖉	∂3.059 (	Ĵ [*] -36.49*
14	27.699	2.60	30,051	102* ♥	vì7.366 à	-37.30*
28	42.104	2,38 U	67.740 S	13.39*\	33.009 🗸	-21.60*
42	46.365	3.56	51.810	11,74 (c)	40.175	-13.35*
56	53.942	3.23	59.665	10%1* 0	<b>%</b> 50.782∕∽∕	-5.86*
70	63.946	2.29	59.665 68,678 S	3.40* (	59.217	-7.40*

¹: CV, coefficient of variation;

²:Dev., Deviation from control: dw: dry weight,

*: statistically significant (according to Studen 4-test two-sided,  $\alpha$ =0.05)

L,

The cumulative soil nitrate formation rates didesceed the trigger range of  $\pm 25\%$  set by OECD guideline 216 at the 0 - 28 day determination for both test rates. Differences to the control were 31.73% and - 59.04% in the 1 mg and 5 mg test item/kg soil dry weight treatment, respectively. Therefore the study was prolonged for 40 days.

The cumulative soll nitrate formation rates did exceed the trigger range of  $\pm 25\%$  set by OECD guideline 216 at the 0 - 42 day determination for the digh test rate. Differences to the control were 20.99% and - 31.28% in the 1 mg and 9 mg test item/kg soil dry weight treatment, respectively. The study was therefore prolonged for a further 14 days.

The cumulative soil prirate formation rates did 400 exceed the trigger range of  $\pm 25\%$  set by OECD guideline 216 at the 0 - 56 day determination and the 0 - 70 day determination for both test rates. At test end at the 0 - 70 day determination, differences to the control were 9.93% and -13.08% in the 1 mg and 5 mg test item/kg soil dry weight treatment despectively.

The incremental solution rates did not exceed the trigger range of  $\pm 25\%$  set by OECD guideline 216 at the 14 28 day determination. Differences to the control were 17.82% and 10.71% in the 1 mg and 5 mg test item/kg soil dry weight treatment, respectively.

The incremental soil nitrate formation rates did exceed the trigger range of  $\pm 25\%$  set by OECD guideline 216 at the 28 - 42 day determination and the 42 - 56 day determination for the high test rate. Therefore the study was prolonged to day 70. In the last incremental nitrate formation rate interval day 56 - 70, the differences to the control were -9.93% and -15.80% in the 1 mg and 5 mg test item/kg soil dry weight treatment, respectively.



Table CA 8.5/02-5	Nitrogen transformation test: effects of the test item on nitrate formation rate	es
	(mean values)	

	Control		1 mg KWG 4168-		5 mg KWG 41	
			despropyl/kg s	oil dw	despropyl/kg s	oil dw S ^r
Day	Mean mg NO ₃ -	N/kg soil dry we	eight per day ³			
	mg/day	CV % ¹	mg/day	Dev. % ²	mg/day	Dev. % ²
0 - 7	-0.944	-12.08	-0.694	-26.48	-2.014	13.35
0 - 14	0.058	79.31	0.217	274.14*	¥9.694	¥1296,53* ×
0 - 28	0.542	6.09	0.714	<b>3</b> 1.73* (	0.222	-590** 0
0 - 42	0.486	7.61	0.588	20.99*	0.334 🖉	-3428*
0 - 56	0.500	6.20	0.581	16.20*	0.440	42.00 ° ×
0 - 70	0.543	3.87	0.594	9.39 🖓	0°.472 🔬 🔍	, -13.08 ⁶
	Control		1 mg KN G 41	68- 🥎 🤇 🖉	5 mg KWG 4	68- @
			despropyl/kg s	oil dw 🖉 🕺 🥎	despropy kg s	oil dw
Day	Mean mg NO ₃ -	N/kg soil dry we	eight per day	Kĩ Kĩ		, 4
	mg/day	CV % ¹	mg/day 📈	Bev. %20		Dev do ²
0 - 7	-0.944	-12.08	<b>√0</b> ?694 ∾	-26.48 🦨	-2.01	11\$\$5*
7 - 14	1.061	3.77	1.128	6.3 t ^O	0.627	<u>40.90*</u>
14 - 28	1.027	2.24	12/40 8	17\$2*	1.137 Q	\$10.71* [©]
28 - 42	0.373	12.87 ₆ 0♥	0.338	2-9.38 O	0.559	49.85*
42 - 56	0.541	9.06	0.561	3.70 3.70	0.758	40.41*
56 - 70	0.715	4.20	0.64	-9.93	0	-15.80

¹: CV, coefficient of variation;

²:Dev., Deviation from control. dw: dry weight;

3: Calculated from the mean galues ONO3-S contend between the sampling date and day

4: Calculated from the mean values of NO3-N content between each sampling date,

*: statistically significant (according to Student  $\oplus$  test,  $\oplus$  -sided,  $\alpha$ =0.05)

#### III. Conclusion .

Ĵ, After 70 days, exposure to the test item KWG 4168 desptopyl had no fong-term impact on nitrogen transformation Quitrate content, mineral mitrogen content and nitrate formation rates) of soil microorganisms when applied at 1 and 5 mg test ftem/kg soil day weight.

 $\bigcirc$ 

#### Assessment and conclusion by applicant:

This is a new study that has not been prestously evaluated.

Validity criteria according to the current OECD 216 guideline (2000) were met:

Variation beoveen replicate control samples to be less than ±15% (actual: max. 4.90%)

The study is therefore considered acceptable

There were <25 % effects after 70 days at cates up to 5.0 mg/kg soil dry weight.



#### KWG 4168-N-oxide (M03)

Data Point:	KCA 8.5/03
Report Author:	
Report Year:	
Report Title:	KWG4168-N-oxide: Effects on the activity of the soil nucroflora in the
	laboratory (nitrogen transformation)
Report No:	143081080
Document No:	<u>M-680759-01-1</u>
Guideline(s) followed in	OECD Guideline 216 (2000)
study:	
Deviations from current	None
test guideline:	
Previous evaluation:	No, not previously submitted
GLP/Officially	Yes, conducted under GLP Officially recognised losting facilities
recognised testing	Yes, conducted under GLP Officially recognised asting facilities
facilities:	
Acceptability/Reliability:	Yes a ky ky ky ky a construction of the second seco
Executive Summary	

The purpose of this study was to assess the effects of the test item on the activity (nitrogen transformation) of the soil microflora in the aboratory. Ŵ

KWG 4168-N-oxide was tested a Conceptrations of 1,4 and 6.9 mg test item kg seif dry weight.

≪ , After 56 days, the test item had no impact or nitrogen transformation (nitrate content, mineral nitrogen content and nitrate formation rates, of soil microorganisms when applied at 1.4 mg and 6.9 mg test item/kg soil dry weight. Ô

Materials and Methods I. A. Materials Test Matérial 4168 -N-oxid Lot Batch #: 13444305 .00 Š **Content:** uid 5 5 onexpiration date Description Sight yollow liquid fficient based Stabilitgof test compound: Reanalysis/Ex date: Density Treatments 1.4 and 69 mg test item/kg soil dry weight Acetone Søł No A∕nalvøiš ŝ concentrations: Test design Disposable plastic boxes, approximately 0.5 L in volume

**Test vessel:** 



Test substrate:	According to the guideline, taken from fallow grassland (location: "In der Speyerer Hohl ", No. 977). No pesticides or organic or mineral" fertiliser had been used on the soil for at least four yers prior to the soil initiation
<b>Replication:</b>	Three per treatment group and control
<b>Duration of test:</b>	56 days
Environmental test conditions	
Temperature:	$20 \pm 2^{\circ}C$
рН:	7.3 – 7.5 $\mathcal{A}$
Photoperiod:	Darkness $\langle \varphi \rangle \langle \varphi \rangle \langle$
Water content:	$49 \text{ to } 52\% \text{ WHC} \qquad \qquad$
B. Study Design	
The purpose of this study transformation) of the soil m	was to ossess the effects of the test item on the activity (filtrogen nicroflore in the taboratory.

Test rates were 1.4 and 6.9 mg test item/kg soil dry weight. There were three replicates per treatment and control group.

Test units were disposable plastic boxes with 300  $\pm$  soil (dev weight), the box volume was approximately 0.5 L and the dimensions were 0.10 x 0.00 x 0.005 m₄.

Incubation was at 18 to  $22^{\circ}$ C in the dark. Test condition (temperature) were recorded continuously using suitable instruments, documented in the raw data and reported in the final report. (Short-term deviations (<2 hours) from the recommended temperature range do normally not result in major disturbances of the test performance and were not reported.

KWG 4168-desprop was, Soluble in actione; Therefore a stock solution in acetone was prepared by dissolving 90 mg KWG 4168-N-spide in 25 mL acetone and applied solution quartz sand. After evaporation of the acetone, the quartz sand was mixed into the soil by means of a laboratory mixer.

Throughout the application the soil was ventilated and the soil water content was adjusted to 50% of WHC.

For the determination of nitrogen content, soil samples were taken within 6 hours after application and afterwards on each sampling date (4, 14, 28, 42 and 56 days). The nitrogen content was determined in each sample of treated and control soils 2

#### II. Results and Discussion

Validity criteria according to the QECD 216 guideline were met:

• Variation between replicate control samples to be less than  $\pm 15\%$  (actual: max. 4.90%)

No adverse effects of the test item on niffate content in soil were observed at day 28. At day 28, differences to the control were -16.43% and 9.73% in the 1.4 mg and 6.9 mg test item/kg soil dry weight treatment. As pechaetic of the sector of the s

No adverse effects of the test item on nitrate content in soil were observed at day 42. At day 42, differences to the control were -9.84% and 8.57% in the 1.4 mg and 6.9 mg test item/kg soil dry weight treatment, respectively.

No adverse effects of the test item on nitrate content in soil were observed at day 56. At day 56, differences to the control were -7.30% and 9.83% in the 1.4 mg and 6.9 mg test item/kg soil dry weight treatment, respectively.



At days 28, 42 and 56, all differences were statistically significant compared to the control for both test rates (Student t-test,  $\alpha = 0.05$ ).

Very low nitrite and ammonium contents below 1.0 mg/kg dry weight were measured at days 28, 27 and 56 in control and the test item treatments.

The mineral nitrogen contents in soil were within the trigger range of  $\pm 25\%$  at day 28. At day 28, differences to the control were -16.31% and 8.52% in the 1.4 mg and 6.9 mg test item/kg soil dry weight treatment, respectively. The mineral nitrogen contents in soil were within the trigger range of  $\pm 25\%$  at day 42. At day 42, differences to the control were -9.61% and 8.36% in the 1.4 mg and 6.9 mg test item/kg soil dry weight treatment, respectively.

The mineral nitrogen contents in soil were within the trigger range of  $\pm 25\%$  at day 56. At day 56 differences to the control were -6.77% and 9.63% for the 1.4 mg and 6.9 mg test term/kg soil dry weight treatment, respectively.

At days 28, 42 and 56, all differences were statistically significant compared to the control for both test rates (Student t-test,  $\alpha = 0.05$ ), except the low test rate at the total of  $\alpha$  and  $\beta$  and  $\beta$ 

Table CA 8.5/03-1 Nitrogen transformation vest: effects of the testitem on ammonium-transalues)

	Control	0 1.4 despror	mg KWG 4168- wl/kg seil dw	to.9 Jing despropyl/kg so	KWG© 4168- bil dw
Day	NH4-N mg/kg dry weight	CV ¹ % C NH4-N CV ¹ % C NH4-N CV ¹ % C NH4-N	mg/kg Dey 2	NH4-N mg/kg	<b>\$Dev. %</b> ²
0	7.520	2.29 0 7.361	0.55	7.829	-3.07
7	1.914	3.03	-3.00 [×] «	1.736	-9.30*
14	1.603	4.12 1.00 007 2 4.347	5 <b>4</b> .18	©1.627√y	1.50
28	1.603 1.650	007 20 4.347	-18.36*	1,299	-20.06*
42	0.701	0.00 0 0.200	x 00 \$	0.701	0.00
56	0.701	0.00 0.905	× \$29.10 ×	0.701	0.00

1: CV, coefficient of variation; 2

²:Dev., Deviation from control, dw: dry weight,

*: statistically significant (according to Student t-test, two sided,  $\alpha = 0.05$ )

Table	Table CA 6.3 02-2 - Settingen in any of macion is effects of the test nem on intrine- (mean values)							
	Control		4.4 mig despropyl/kg so	KWG 4168- bil dw	6.9 mg KWG 4168- despropyl/kg soil dw			
Day _s	NH4-N mg/kg dry weight		NH4-N ing/kg Ary weight	Dev. % ²	NH4-N mg/kg dry weight	<b>Dev.</b> % ²		
0	0.431	8.82	0.576	32.25*	0.434	0.70		
7	0.3990	0.00	07399	0.00	0.399	0.00		
14	0.420	Q14 2	0.412	-1.90	0.414	-1.43		
28	<b>0</b> .407	0.25	0.425	4.42*	0.428	5.16*		
42~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	0.399	0.00	0.399	0.00	0.399	0.00		
56	( <b>Q</b> 399	0.00	0.399	0.00	0.399	0.00		

 Table CA 8.5/03/2
 Nitrogen transformation test? effect? of the test item on nitrite- (mean values)

¹: CV, coefficient of variation;

²:Dev., Deviation from control: dw: dry weight;



	Control		1.4 despi	mg copyl/kş	KWG g soil dw	4168-	6.9 despr	mg opyl/kş	KWG g soil dw	4168-	ð
*: sta	tistically signifi	icant (according to S	tudent	t-test, tv	wo-sided, α	=0.05)				2	F
Table	CA 8.5/03-3	Nitrogen transfo	ormatio	on test:	effects of t	he test ite	em on n	iitçate-	(mean va	()))	
	Control		1.4	mg	KWG	4168-	6.9	mg	К₩Ğ	<b>4168-</b>	Ò

	Control		despropyl/kg so	bil dw	despropyl/kg son dw		
Day	NH4-N mg/kg dry weight	CV ¹ %	NH4-N mg/kg dry weight	Dev. % ²	NH4-N mg/kg dry weight		
0	24.860	0.65	25.042	0.73	26:247	5.58* Č	
7	18.280	4.90	12.950	-29.04*	21.980	20.44*	
14	25.675	2.73	18.349	-28.53*	28,815	12.23*	
28	40.047	2.47	33,466	~16.43* Q	43.945	9.73	
42	45.265	3.65	49.810	29.84* ⁰	49:49:	\$ 57*	
56	52.842	3.29	48.987	-7.60*	59.035 S	9.83*	
1 01			- 102 - 191			4	

¹: CV, coefficient of variation;

²:Dev., Deviation from control: dw; dry weight; @

*: statistically significant (according to Studenty-test, two-sided, a

Table CA 8.5/03-4	Nitrogen transformation	test: effects of the test item	on N _{min} content (mean values)
-------------------	-------------------------	--------------------------------	-------------------------------------------

	Control		1.4 mg KWC 4		6.9 mg WG 4	168-
	01		despropylikg se		despropyl/kg so	
Day	NH4-N mg/kg		MH4-N mg/kg dry weight	Dev. 982	NHa-N mg/kg	Dev. % ²
	dry weight,	$\mathcal{N} \mathcal{A} $	dry weight 🍼	~~~	dry weight	
0	32.812	Q0.91	33,104	\$10 \$°	33.970	3.53
7	20.563	4.0%	15.191	Q26.12°O Q	24.115	17.27*
14	27.699	2,60	20.431	-16.24*	30.855	11.39*
28	42,104	Q.38 🛠 🖉	35.238	-176371*	45.692	8.52*
42	46.365	3.56	41.040	, -9.61* 🔊	50.242	8.36*
56	53.942 🔊	3.25	<u>√5</u> 9.291 ∞	\$¥6.77 [™]	59.135	9.63*

1: CV, coefficient variation;

²:Dev., Deviation from control: Tw: dry weight

*: statistically significant (according to Student t-test, two-sided,  $\alpha$ =0.05)

The cumulative soil nitrate formation rates did exceed the trigger range of  $\pm 25\%$  set by OECD guideline 216 at the 0 - 28 day determination for the low test rate. Therefore, the study was prolonged for 14 days. Differences to the control were -44/46% and 16/91% in the 1.4 mg and 6.9 mg test item/kg soil dry weight treatment, respectively, for the 0 - 2 day determination. The differences were statistically significant for the 0 - 28 day determination for both test rates (Student t-test,  $\alpha = 0.05$ ).

The cumulative soil hitrate formation rates did not exceed the trigger range of  $\pm 25\%$  set by OECD guideline 245 at the 0 - 43 day determination. Differences to the control were -22.84% and 12.14% in the 1.4 mg and 6.9 mg test them/kg soil dry weight treatment, respectively, for the 0 - 42 day determination. The difference was statistically significant for the 0 - 42 day determination for the low test rate (Student ttest,  $\alpha = 0.05$ ).

The cum dative soil nitrate formation rates did not exceed the trigger range of  $\pm 25\%$  set by OECD guideline 216 at the 0 - 56 day determination. Differences to the control were -14.40% and 13.60% in the 1.4 mg and 6.9 mg test item/kg soil dry weight treatment, respectively, for the 0 - 56 day determination. The differences were not statistically significant for the 0-56 day determination for both test rates (Student t-test,  $\alpha = 0.05$ ).



The incremental soil nitrate formation rates did not exceed the trigger range of  $\pm 25\%$  set by OECD guideline 216 at the 14 - 28 day determination. Differences to the control were 5.16% and 5.26% in the 1.4 mg and 6.9 mg test item/kg soil dry weight treatment, respectively, for the 14 – 28 day determination. The differences were not statistically significant for the 14 – 28 day determination for both test rates (Student t-test,  $\alpha = 0.05$ ).

The incremental soil nitrate formation rates did exceed the trigger range of  $\pm 25\%$  set by OECD guideline 216 at the 28 - 42 day determination for the low test rate. Therefore, the study was prolonged for 14 days. Differences to the control were 40.48% and -0.54% in the 1.4 mg and 6.9 mg test item/kg soil day weight treatment, respectively, for the 28 - 42 day determination. The difference was statistically significant for the 28 - 42 day determination for the low test rate (Student t-test,  $\alpha = 0.05$ ).

The incremental soil nitrate formation rates did not exceed the trigger range  $65 \pm 25\%$  set by OECD guideline 216 at the 42 - 56 day determination. Differences to the control were 7.95% and 47.38% in the 1.4 mg and 6.9 mg test item/kg soil dry weight treatment, respectively, for the 42 - 56 day determination. The differences were not statistically significant for the 42 - 56 day determination for both test rates (Student t-test,  $\alpha = 0.05$ ).

Table CA 8.5/03-5	Nitrogen transfor	mation test:	effects of the	testitem	onnitrate	formation rates
	(mean values)			× (	s o	Q

G 4168 kg soil dw
kg soil dw
×
$\bigcup \text{Dev. }\%^2$
-35.38*
215.52
16.61*
12.14
13.60
G 4168-
kg soil dw
Dev. % ²
-35.38*
-7.92
5.26
-0.54
17.38

1: CV, coefficient of variation

²:Dev., Deviation from control: dw dry weight;

³:Calculated from the mean values of NO3N content between the sampling date and day 0;

4: Calculated from the mean values of NO3-N content forween each sampling date;

*: statistically significant (according to Student t-test, two-sided,  $\alpha$ =0.05)

#### III. Conclusion

After 56 days, the test item had no hapact of nitrogen transformation (nitrate content, mineral nitrogen content and outrate formation rates) of coil microorganisms when applied at 1.4 mg and 6.9 mg test item/kg soft dry weight.

#### Assessment and conclusion by applicant:

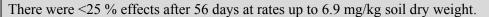
This is a new study that has not been previously evaluated.

Validitocriteria according to the current OECD 216 guideline (2000) were met:

• Variation between replicate control samples to be less than  $\pm 15\%$  (actual: max. 4.90%)

The study is therefore considered acceptable.





Data Point:	bid (M06) KCA 8.5/04
Report Author:	
Report Year:	
Report Title:	KWG 4168-carboxylic acid: Effects on the activity of the soil metroflora in the solution laboratory (nitrogen transformation)
Report No:	
Document No:	<u>M-688317-01-1</u>
Guideline(s) followed in study:	Regulation (EC) No 1107 2009 (2009) OECD-Guideline for the Testing of Chemical's Soil Microorganisms Nitrogen Transformation Test, Guideline 216, January 21, 2009
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted of the second s
GLP/Officially	Yes, conducted under GLR Officially recognised esting facilities
recognised testing facilities:	Yes, conducted under GLR Officially recognised testing facilitie
Acceptability/Reliability:	Yes Q , L' Q' , Y Q' , Q' , Q' , Q' , Q' , Q' , Q

#### **Executive Summary**

The purpose of this study was to assess the effects of the test item on the activity (nitrogen transformation) of the seil microflora in the laboratory.

KWG 4168-carbox of a circle was tosted a concentrations of 0 and 5.0 mg test item/kg soil dry weight.

After 28 days, the test item KWG 4168-varboxylic acid had no long-term impact on nitrogen transformation (nitrate content, noneral nitrogen content and nitrate formation rates) of soil microorganisms when applied at 0.5 mg and 5 mg test tem/kg soil day weight treatment.

0

I. Materials and Methods A. Materials

Test Material 🔊	KWG 4168-carboxycyl@ acid
Lot/Batch #:	AE 1344313-01-03 5 5
Content:	2-(2-{[ethy](propy])amino]methyl}-1,4-dioxaspiro[4.5]dec-8-yl)-2-
	methylpropionic acid: 20.6% w/w
Description:	Purbid brown liquid
Stability of test	Sufficient@ased Sn expiration date
compound:	
Reanalysis/Expiry	13 march 2021
date:	
Density:	Not reported
Treatments	© Not reported 0.5 and 5.0 mg test item/kg soil dry weight
Test Pates	
Test Tates:	0.5 and 5.0 mg test item/kg soil dry weight
Solvent/vehicle:	Acetone
Solvent/venicie.	Actione



Analysis of test concentrations:	No
Test design	
Test vessel:	Disposable plastic boxes, approximately 0.5 L incolume
Test substrate:	According to the guideline, taken from fallow grassland (location; finder Speyerer Hohl ", No. 977). No pesticides or organic or mineral fertiliser had been used on the soil for at least four yets prior to test initiation. Three per treatment group and control 28 days $20 \pm 2^{\circ}$ C 7.3 - 7.4 Darkness 49 to 50%/WHC was to assess the effects of the test item on the activity (nitrogen increfiora in the laboratory).
<b>Replication:</b>	Three per treatment group and control
Duration of test:	28 days $\sqrt{2}^{4}$ $\sqrt{2}^{4}$ $\sqrt{2}^{4}$ $\sqrt{2}^{4}$
Environmental test conditions	
Temperature:	$20 \pm 2^{\circ} C$ $\downarrow$ $\uparrow$ $\uparrow$ $\uparrow$ $\uparrow$ $\uparrow$ $\uparrow$ $\downarrow$
pH:	$7.3 - 7.4 \overset{\text{O}}{=} \overset{\text{O}}{} \overset{\text{O}}{=} \overset{\text{O}}{} $
Photoperiod:	Darkness & A L A A A A A
Water content:	49 to 50% @WHC 2 & 6 & 6 & 6
B. Study Design	
The purpose of this study transformation) of the soft m	was to assess the effects of the test item on the activity (nitrogen nicroflora in the laboratory
Test rates were 0.5 and 5.0	ng test item/kg soil de weight. There were three replicates per treatment
Test units were disposable pl 0.5 L and the donensions we	lastic boxes with 300 g soil (dry veight) the box volume was approximately ere 0.10 × 0.10 × 0.065 m.
deviations (<2 hours) from disturbances of the test perfo	C in the dark. Test conditions (temperature) were recorded continuously doctrimented in the raw data and reported in the final report. Short-term the recommended temperature range do normally not result in major primance and were not reported.
KWG 4168-carboxylic acid by dissolving 50.0 mg KW applied onto Quartz Sand.	was soluble in acctone: therefore, a stock solution in acctone was prepared 4168 carboxyfic acrif in 20 mL acctone and appropriate amounts were filer evaporation of the acctone overnight, the quartz sand and additionally a solution weight) was mixed into the soil by means of a laboratory mixer.
WHCL 25	the soil was ventilated and the soil water content was adjusted to 49% of
To the control, acetone treat soil dry weight, was mixed i	ed quartz sand (coaporated) and additionally 0.5% lucerne meal (based on into the spil.
	adjusted to \$9% of WHC. The soil water content was determined in one
	g date (7, 14 and 28 days). The nitrogen content was determined in each l soils.

# sample of reated and control soils.

Validity criteria according to the OECD 216 guideline were met:

• Variation between replicate control samples to be less than  $\pm 15\%$  (actual: max. 1.90%)



The study is therefore considered acceptable.

No adverse effects of the test item on nitrate content in soil were observed at day 28. At day 28, 3 differences to the control were 0.75% and -4.12% in the 0.5 mg and 5 mg test item/kg soil dry weight treatment, respectively.

At sampling point day 28, the difference was statistically significantly difference compared to the control for the high test rate (Student t-test,  $\alpha = 0.05$ ).

Very low nitrite and ammonium contents below 0.6 mg/kg dry weight were measured at day 28 ir control and the test item treatments.

The mineral nitrogen contents in soil were within the trigger range of  $\pm 25\%$  set by EPPQ and SETAC guidelines at day 28. At day 28, differences to the control were 0.75% and - 4.05% in the 0.5 mg and 5 mg test item/kg soil dry weight treatment, respectively.

At sampling point day 28, the difference was statistically significantly different compared to the control for the high test rate (Student t-test,  $\alpha = 0.05$ ).

The variation between replicate control samples was below the validity criterion of 15% of the OFCD test guideline 216.

Table CA 8.5/04-1 Nitrogen transformation test? effects of the sest item on anymonium- (mean values)

		0		<u>s s</u> õ		
	Control	*	, 625 mg KWG A	168- 🔊 K	5.0 mg KWG 4	168- [%]
			0.5 mg KWG A carboxycylicae	id/kg soil d	5.0 mg KWG 4 carboxycylic ac	id/kg soil dw
Day	NH4-N mg/kg	CV ¹ %	NHA-N mg/kg	Dev. % ²		<b>Dev. %</b> ²
_	dry weight	ô ô	doy weight	10°	dry weight	
0	5.532	4.95	9.281	r -4.54	5,223 ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	3.45
7	0.548	Q.00 💭 . Öğ	0.548	0.00 5	0.548 S	0.00
14	0.548	0.00	0.548	\$000 U	0.548	0.00
28	0.548	0.00	\$\$48	0.00	0.548	0.00
1 01	CC	· (.) · · · · · · · · · · · · · · · · · · ·				

1: CV, coefficient of variation; "

2:Dev., Deviation from control: dw: dry weight

*: statistically ignificant (according to Student t-testotwo-sided, α=0.05) .

Table CA \$5/04-2 Nitrogen transformation test: effects of the test item on nitrite- (mean values)

	Control 7 7 0,5mg KWG 4168- xarboxxcylic acid/kg sól	5.0 mg KWG 4168- if dw carboxycylic acid/kg soil dw
Day	NO ₂ -N mg/kg QV ¹ % NO ₂ -N mg/kg Dev ² %	² NO ₂ -N mg/kg Dev. % ² dry weight
0	0.4040 0 12.13 ~ 0.485 ~ 20.05*	0.476 17.82*
7	0.347 0.00 0 40 347 0 0.00	0.347 0.00
14	0,047 0.00 0.00 0.00	0.347 0.00
28	6347 0.00 0.347 0.00 0.00	0.347 0.00

1: CV coefficient of variation

²:Dev., Deviation from coord ol: dw dry weight;

*: statistically significant (according to Student Fiest, two-sided,  $\alpha$ =0.05)

#### Table CA 8.5.04-3 Nitrogen transformation test: effects of the test item on nitrate- (mean values)

Control Control		0.5 mg KWG 4168- carboxycylic acid/kg soil dw		5.0 mg KWG 4168- carboxycylic acid/kg soil dw		
Day A	NO3 mg/kg dry weight	CY ¹⁶ %	NO3-N mg/kg dry weight	Dev. % ²	NO3-N mg/kg dry weight	Dev. % ²
0 🗞	18:065	0.19	17.909	-0.86	17.960	-0.58
7	Q <b>1</b> 8.626	0.86	19.432	4.33	18.697	0.38
14	27.227	1.77	28.580	4.97*	27.647	1.54
28	40.779	1.90	41.084	0.75	39.098	-4.12*



	Control	0.5 mg KWG 4168- carboxycylic acid/kg soil dw	5.0 mg KWG 4168- carboxycylic acid/kg soil dw _@ •	
¹ : CV	, coefficient of variation;			` 🏷

²:Dev., Deviation from control: dw: dry weight;

*: statistically significant (according to Student t-test, two-sided,  $\alpha$ =0.05)

Table CA 8.5/04-4 Nitrogen transformation test: effects of the test item on N_{min}-content (mean values)

	Control		0.5 mg KWG 41 carboxycylic aci		50 mg KWG 41 carboxycylic aci	68- d/kg soil dw
Day	N _{min} -N mg/kg dry weight	CV ¹ %	Nmin-N mg/kg dry weight	<b>Dev. %</b> ²	Nmin-N mg/bg dry weight	<b>Dev.</b> %2 4
0	24.000	1.01	23.674	-1.36 🥿	24.159°° 0	0.665
7	19.521	0.82	20.327	A.13 0	19.5992	0.36
14	28.122	1.71	29.475	4.8.6	28,542	1.49
28	41.674	1.86	41.979 📞	0,03	89.993∖	-4.03 °

¹: CV, coefficient of variation;

²:Dev., Deviation from control: dw: dry weight;

*: statistically significant (according to Surdent & test, two-sided, a

The cumulative soil nitrate formation rates did not exceed the trigger range  $3i \pm 25\%$  set by OECD guideline 216 at the 0 - 28 day determination for both test rates. Differences to the control were 2.10% and -6.91% in the 0.5 mg and 5 ong test item/kg soil dry weight treatment, respectively

The difference was statistically significantly different compared to the control for the high test rate (Student t-test,  $\alpha = 0.05$ ) for the cumulative nitrate rate at the 0 - 28 day determination.

The incremental soil nitrate formation rates and not exceed the trigger range of ± 25% set by OECD guideline 216 at the 10-28 they determination. Differences to the control were 7.75% and -15.50% in the 0.5 mg and 5 mg test item/kg soil dry weight treatment, respectively.

The difference was statistically significantly different compared to the Sontrol for the high test rate (Student t-test a = 0.05) for the incomental nitrate rate in the 14-28 day determination. S

× 1

Table CA <b>8.5</b> /04-5		
ES 1	(mean values) a b b b b b b b b b b b b b b b b b b	

	<u> </u>					
	Control		0.5 mg KWG4	168- 🔊	5.0 mg KWG 4	168-
	, N a	c Oĭ	f carboxycylie a	cid/kg soil dw	carboxycylic a	cid/kg soil dw
D	Mean mg NØ	N/kgSoil drow	eight per day ³	Q.		
Day	mg day	CW % ¹ %	mg/day O	Dev. % ²	mg/day	Dev. % ²
0 - 7	0.080	£.50 × "	0.2180	172.50*	0.105	31.25
0 - 14	<b>40,654</b>	5.35	0.762	16.51*	0.692	5.81
0 - 28	0.811	3.58	0.828	2.10	0.755	-6.91*
2.	Control	A	0.5 mg KWG 4	168-	5.0 mg KWG 4	168-
$\sim$		~~~~ (C	Carboxycvlic a	cid/kg soil dw	carboxycylic a	cid/kg soil dw
Davi	Mean mg NO ₃	N/kg soil dry w	eight per day⁴			
Day	mg/day 🔬 🔪	$CV/0^1 \ll$	mg/day	Dev. $\%^2$	mg/day	Dev. $\%^2$
0 - 7	0.030 ~~~	25.50	0.218	172.50*	0.105	31.25
7 - 14	229	03.91 🔬 🔍	1.307	6.35	1.279	4.07
14 - 28	Ø.968 C	7.95	0.893	-7.75	0.818	-15.50*

¹: CV coefficient of variation

²:Dev, Deviation from control: dw: dry weight;

³ Calculated from the mean values of NO3-N content between the sampling date and day 0;

⁴:Calculated from the mean values of NO3-N content between each sampling date;

*: statistically significant (according to Student t-test, two-sided,  $\alpha$ =0.05)



#### III. Conclusion

After 28 days, the test item KWG 4168-carboxylic acid had no long-term impact on nitrogen transformation (nitrate content, mineral nitrogen content and nitrate formation rates) is soil microorganisms when applied at 0.5 mg and 5 mg test item/kg soil dry weight.

#### Assessment and conclusion by applicant:

This is a new study that has not been previously evaluated.

Validity criteria according to the current OECD 216 guideline (2000) were met:

• Variation between replicate control samples to be less than  $\mathbb{P}15\%$  (actual max.  $\cancel{6}90\%$ 

The study is therefore considered acceptable.

There were <25 % effects after 28 days at rates up to 5.0 mg/tog soil dry weight.

#### Relevant literature on soil micro-organisms

No relevant scientifically peer-reviewed open-literature could be found on spiroxamine of its major metabolites, from an ecotoxicological perspective, of soil pricro-organisms. Details of the literature search undertaken can be found in the M-CA Section 9 of the current submission.

## CA 8.6 Effects on terrestrial non-target higher plants

### CA 8.6.1 Summary of screening data

Screening data are not available but GDP seeding emergence (QCD 208) and vegetative vigour (OECD 227) data are available using the representative formulations. Please refer to Document-M-CP Section 10 for the representative formulations.

#### CA 8.6.2 Festing on non-target plants

There are no data available with spiroxamine technical but seedling emergence (OECD 208) and vegetative vigour (OECD 220) data are available using the representative formulations which have been presented in Document M-CP Section 10.

#### Relevant literature on mon-target to restrial plants

No relevant scientifically peer-reviewed open literature could be found on spiroxamine or its major metabolites, from an ecotoxicological perspective, on non-target terrestrial plants. Details of the literature search undertaken can be found in the M-CA Section 9 of the current submission.

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### CA 8.7 Effects on other terrestrial organisms (flora and fauna)

No data are available on the effects of spiro amine on other terrestrial organisms. Additional data are not considered to be necessary.

# CA 8.8 Effects on biological methods for sewage treatment

An activated studge despiration inhibition test (ASRIT) study has been conducted using spiroxamine technical. The endpoint is summarised if the table below and full details of the study are provided in the summary.

Table CA 8.8-0	Summary of studies on biological methods for sewage treatment with spiroxamine
it O	

Test item	Test type	EU endpoint	Reference
Spiroxamine	Activated sludge, respiration inhibition test	EC ₅₀ 191.1 mg a.s./L EU	<u>M-298672-01-1</u>

EU: previously evaluated as part of the original EU review and listed in EFSA conclusion and DAR



Data Point:	KCA 8.8/01			
Report Author:				
Report Year:				
Report Title:	Activated sludge, respiration inhibition test with Spiroxaphine, tech. substance			
Report No:	2008/0013/01			
Document No:	<u>M-298672-01-1</u>			
Guideline(s) followed in	Council Directive 67/548/EEC; Annex V, Method C.11 Activated sludge			
study:	Council Directive 67/548/EEC; Amer V, Method C.11 Activated sludge respiration inhibition (1988). This test method is equal to OECD Guide The 209 (1984).			
	(1984). <u>A</u> O ^V <u>A</u> <u>A</u> O ^V			
Deviations from current	None A A A A A A A A A A A A A A A A A A A			
test guideline:				
Previous evaluation:	yes, evaluated and accepted			
	RAR (2010), RAR (2017)			
GLP/Officially	Yes, conducted under GLP Officially recognised resting facilities			
recognised testing				
facilities:				
Acceptability/Reliability:	$Yes \qquad \qquad$			
Executive Summary				
Activated sludge was exposed to spiros amine technical at afferent concentrations in a 3-hour test in				
order to assess effects on	respication rates and respiration ishibition.			
Exposure to spiroxamine shower 90.32% respiration inhibition of activated slugge at a test item				
concentration of 1000 mg/E.				
The EC ₅₀ and EC ₁₀ weite determined to be 191.1 and 53.9 mg a.s. L, respectively.				
I Matavials and Mathada				
I. Materials and Methods				
A. Materials				

Test Materia OSpiroQamine technical Lot/Batch #: H004650 Light brown Purrity: **Description:** Stability of test compound: **Reanalysis/Expiry** date **Density:** treported Ś Treatments 00, 180, 320, 560 and 1000 mg a.s./L Test rate ~~ Analysis of test None concentrations: Test design Test vessel: 300 mL glass Erlenmeyer flasks Test medium: Mixed population of aquatic microorganisms (activated sludge) from aeration tank of a domestic sewage treatment plant (Municipal STP Cologne-Stammheim)



<b>Replication:</b>	None	0
<b>Duration of test:</b>	3 hours	
Environmental test conditions		
Temperature:	19.1 – 19.5°C	
рН	7.6 - 7.8	
B. Study Design	ч. Ф	
This study was conducted in activated sludge in a 3-hour trange-finding test.	n order to assess the effects on resp est. Test concentrations were selected	iration of spinoxamine technical on
compound) was incubated for aerated through a glass tube a	nsumption, 250 mL of sludge with 3 hours in 300 mL closed Etlenmeyer at 50 to 1004/h with clean oil-free air	r flasks (with air inlet and outlet) and
compound used was 3.5-Did mg/L. The test item concentra	concentrations of 100, 180, 520, 560 hlorophenol and was applied at cond ation in physico-chemical oxygen cons m were used, one at the start and the o	centrations of 2.553, 10, 20 and 40 supption Control was 1000 mg a.s./L.
A synthetic wastewater feed water: 16.0 g peptone, 11, 69 7H ₂ O, and 2.8 g K ₂ HPO ₄ .	was made by dissolving the followin mean extract, 3.0 g urea, 0.7 g NaCl,	g amounts of substance in 1 litre of $g$
bottles and O ₂ -content was a measured before the use of th II. Results and D	It of the Erlenmeyer flasks was com neasured with an O ₂ -meter (redex el ne activated sludge as well as at the en scussion	ectrode) Temperature and pH were
Validity criteria according to	the study report wore met	vy W V starsla 2,50()
• $EC_{50}$ 3.5-LOP show	be between S mg/L to 40 mg/L fact	ual: 5 to 30 mg/L)
highest concentration of 100		
Respiration inhibition wasoo	osecced at a concentrations of test ite	m when compared to the control.
The respiratory rates of the tw	Q controls differ less than 15%.	
Table CA 8.8/01-1 Respir substa	atory rate and Inhurition on activated s	sludge exposed to spiroxamine tech.
Nominal concentration	Respiratory rate test item	Inhibition (%)
(mg a.s./L)	(mg/l/h)	<u> </u>
Control (mean)	38.8	-
100	0 30.0	22.58
	* 19.7	49.12
3200 0 0	10.3	73.46
560 2	6.0	84.52
1000 💍	3.8	90.32
<u> </u>		



#### III. Conclusion

Activated sludge was exposed to spiroxamine technical at different concentrations in a 3-hour test in order to assess effects on respiration rates and respiration inhibition.

The EC₅₀ was determined to be 191.1 mg a.s./L of spiroxamine technical, with 95% confidence limits between 151.5 and 233.4 mg a.s./L.

The EC₁₀ was determined to be 53.9 mg/L of spiroxamine technical, with 95% confidence the between 25.5 and 79.8 mg a.s./L.

#### Assessment and conclusion by applicant:

This study was previously evaluated and accepted in the RAR (2000), RAR (20

Validity criteria according to the current OECD 209 guideline 2010 were considered to be met bu one of the criteria could not be verified from the data valiable in the report.

- Oxygen uptake rate in the blank control >20 mg of gen/gactivated sludge in an hour factual; cannot verify from data in study report.
- Coefficient of variation of oxygen uptake rate in control replicates 230% at the end of the test (actual: 4.6%)
- EC50 3.5-DCP should be between mg/b to 40 mg/L: (actual 3 to 30 mg/L)

The validity criteria used in the study report vere achieved Although it is not possible to verify the first criterion above, taken from the current OECD209 test guideline, it is believed that the study is still valid as sufficient sensitivity was seen in the reference treatment and the control variation was within acceptable limits.

The study is therefore considered acceptable

The EC50 was determined to be 197.1 mga.s./L

Data Point: KCA'8.8/02 A KCA'8.8/02
Data Point: KCA'8.8/02 A A A A A A A A A A A A A A A A A A A
Report Year $1989 \sqrt{3}$ $\sqrt{3}$ $\sqrt{3}$
Report The: Oxygen consumption test with activated shidge KWG 4168
Report No: 5 BA-898270 5 5 6
Document No: $\sqrt{1-009945-01}$
Guideline(s) followed in A. ISO 192-1986 IB (ETAD 103/OE D 209)
Study. $a_{\mu} \cap a_{\mu} \otimes \cdots \otimes \cap a_{\mu} \otimes \cdots \otimes a_{\mu}$
Deviations from current bes 2 2 2
test guideline. Detailed methods unavailable to assess deviation.
Previous valuation: yes Valuated and Classified DAR (1997), RAR (2010) RAR (2017)
DAR (1997), RAC (2010) RAR (2017)
source and the source was not performed under OEF conditions, a new study was
performed.
GLP/Officially
recognised testing A
facilities:
Acceptability/Reliability: Supportive only

### Executive Summary

Activated studge was treated with KWG 4168 in a 3-hour test to assess effects on oxygen inhibition and respirated rate.

A control, a reference item and five concentrations of the test item were used in this study.



At the highest test concentration, 1000 mg/L, oxygen consumption was inhibited by 87% when compared to the control.

The  $EC_{50}$  was determined to be 159 mg a.s./L with confidence intervals between 90 and 222

#### **Materials and Methods** I. out of the contract of the con A. Materials **Test Material** KWG 4168 Lot/Batch #: 17001/89 **Purity:** 94.2% **Description:** Not reported Stability of test Not reported compound: **Reanalysis/Expiry** Not reported date: **Density:** Not reported Treatments 60 and 1000 **Test rates:** Analysis of test concentrations: **Test design** reported **Test vessel:** 3 hours Duration of test Environmental tes conditions Temperature: B. Study Design K) Activated sludge (6.0 g total solution/L) was treated with KW(\$4168 in a 3-hour study to assess the effects on respiratory rate and oxygen consumption

The test item was added ( 8.0 m) of purient medium, 12.5 mL of activated sludge and 229.5 mL of water.

The test substance was applied in concentrations of 100, 180, 320, 560 and 1000 mg/L. A control was used and a the reference substance was 55-dictorophenol applied at 1 mg/L.

No replication was documented in the study report.

Initial oxygen values, were recorded, in mg, and after 3 hours were recorded again.

#### Results an Discussion II.

Validity Eriteric according to the OECD 209 guideline (2010) could not be assessed.

The table shows repriration rates decreasing from 20 mg/L h in the lowest test item sludge to 4 mg/L h at the highest concentration of the test item.

At the Highest test concentration, 1000 mg a.s./L, oxygen consumption was inhibited by 87% when compared to the control.



Nominal concentration of test substance (mg/L)	Respiratory Rate (mg/L h)	Inhibition (%)	
Control	27	-	<u>6</u> 0
100	20	24	
180	10	63 🐨	
320	6	78 1	à à
560	5	82 5	
1000	4	8.5	Í ÖÍ "O
1 (Reference)	26	Q Q Z	
20 (Reference)	6	A 78 6 Q	

Oxygen inhibition and respiration rate in activated sludge treated with KWG 4168 Table CA 8.8/02-1

#### III. Conclusion

At the lowest test concentration, 100 mg a.s./L, oxygen consumption was inhibited by 24% when compared to the control. This rose to 87% at the highest test conceptration 1000 mg a.s./L.

The EC₅₀ was determined to be 159 mg a st with confidence intervals between 90 and 222 mg a st

#### Assessment and conclusion by applicant

The study was not performed to GLP or to a recognised test guideline atthough the test methodology used would appear to be consistent with current methods. The validity criteria according to the OECD 209 Guideline (2010) could not be assessed one to insufficient details in the study report.

considered to be consistent The study is therefore considered as supporting information only but is with the other available GLP data

C

The EC₅₀ was determined to be 159 mg a.s.

## Relevant literature on biological methods for sewage treatment

No relevant scientifically peer reviewed open literature could be found on spiroxamine or its major metabolites, from an ecotoxicological perspectives on biological methods for sewage treatment. Details of the literature search undertaken can be found in the M-CA Section 9 of the current submission.

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CA 8.9 Monitoring data with effects of spicolamine in the EU are available or are considered to be required.