





Disparagin (Code: BCS-EN88460) Regulation (EC) No L 197/2009 & Regulation (EU) No 283/2013 Discument WICA Section 8: Ectroxicological studies According to 198-Guidance Discument NACO/1018/1/2013 for applicants on green right downers for the applicant algebra applicants on green right downers for the applicant algebra applicants. 2018/04-19



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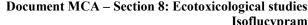
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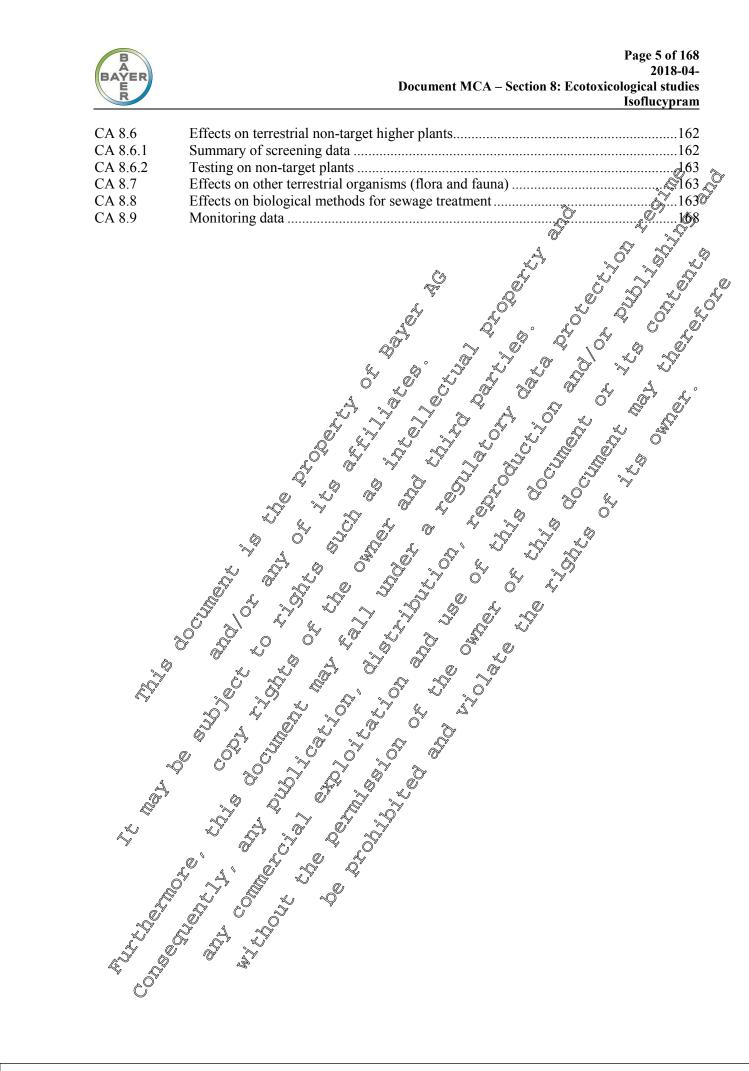


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CA8 ECOTOXICOLOGICAL STUDIES ON THE ACTIVE SUBSTANCE

INTRODUCTION

Isoflucypram (CAS-No. 1255734-28-1) is a new fungicidal active substance de cloped by Bayer. This document supports the application for regulatory approval of isofluo pram in Europe under Regulation (EC) No 1107/2009.

The document MCA Section 8 summarizes all ecotoxicological data and classification proposal, which are relevant for the approval of isoflucypram and the proposed intended uses, including the O representative uses, under Regulation (EC) No 1100/2009 in accordance with the requirements laid down in the Commission Regulation (EU) No 282/2013 and under Cassification regulation (EC) No 1272/2008.

Isoflucypram is a novel broad spectrum fungicide of the chemical class of N-cycloptopyl-Abenzylpyrazole-carboxamides with an outstanding efficacy against the major economically important fungal diseases of cereal crops (wheat, triticale, tye, basley and oats) and excerlent crop safety.

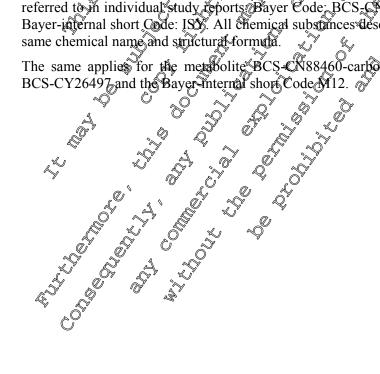
Since isoflucypram is an SDH inhibitor and thus assigned to the FRAC resistance Group 7 the application scope of isoflucypram-containing products on cereals with only one bliar spray at a maximum of 75 g a.s./ha supports in effective anti-resistance management strategy.

Tailor-made and broad spectrum isoffacypraph combinations show highly beneficial properties in terms of plant physiology beside the long-lasting and certain surative efficacy to control fungal diseases and to maximize the full yield potential of the cereal crops.

Details of the literature search undertaken are summarized in MCA Section 9. For isoflucypram and its metabolites, no publication and relevant scientifically peer-reviewed open literature reference has been identified which would indicate that a side-effect on human health, The environment and non-target species may exist which would then need to be considered in the risk assessment of this new active substance dossig

Throughout the development of isoflucypram the following synonyms may have been used and also referred to in individual study reports Bayer Code: BCS-CN88460-a.s., '460 and the Bayer-internal short Code: ISO. All chemical substances described by either of these codes refer to the

The same applies for the metabolite BCS-CN88460-carboxylic acid for which the Bayer Code is





CA 8.1 Effects on birds and other terrestrial vertebrates

CA 8.1.1 Effects on Birds

Studies on quail species, mallard ducks and wild canaries have been conducted with the active substance isoflucypram. Detailed information on acute, short-term and Jong-term effects isoflucypram on birds is presented in the following chapters.

Some studies were submitted which are not requested for European registration but mandato registration by US-EPA. The registration for a US registration includes an LD50 test, with passering (canary bird) according to OECD 223, two subacute studies (with quail and mallands) according to OECD 205 and two reproductions studies (quail and mallard) according to OECD 206. The quail reprotox study had to be repeated for registration purpose in the USA as the firstone did not fulfill all formal US standards (statistically significant difference at lowest test concentration at one test parameter). For further details, please also refer to Point CA 8 1.7.3.

Acute oral toxicity to birds CA 8.1.1.1

Acute oral toxicity to birds Table 8.1.1.1-1:

Test substance	Test design	Test (desint &		Reference
Isoflucypram	Acute toxicity	Bowhite L Quail L	7 (0	000 mg a.s./kg/	KCA 8	; M-635551-01-1 1 1 501 ; 2016; 57-01-1 1.1.1/02

Report: T.; 2015; M₂535551-01-1

Title: oxicity of BCS-CN88480 technical during areacute oral LD50 with the northern

bobwhite quail (Colimis virginianus).

Report No.: EBLNN006 M-53555 (201-1 Document No: Guideline(s). OCSPR \$50.210 Guideline deviation(s); Unot specified **GLP/GEP:**

Objective:
The aim of the study was to determine the acute oral LD₅₀ of BCS-CN88460 technical on Northern Bobwhite quail (Colinus virginianus).

Material and methods:

BCS-CN88460 tecknical, Origin batch No. 2013-006492, Purity 94.2 % w/w.

Northern Bobwhite quail 22-week-old adults were orally dosed with BCS-CN88460 technical based on body weight at a limit dose leve of 2000 mg a.s./kg body weight. A control group was run in parallel. Ten birds per treatment level (five males and five females) were randomized by body weight into the treatment level and control group on experimental Day -1. Birds were dosed with gelatin capsules of Day of following to hours of fasting and monitored for 14 days post-dosing. All feed and water was provided ad libitum.

The lards were individually housed in stainless steel cages which measured approximately 56 cm (length) × 28 cm (width) × 27 cm (height) and provided with an average temperature of 69.8°F (21.02° © at 53.1% relative humidity with a photoperiod of 8 hours light: 16 hours dark (116 lux).

Adult body weights were taken on experimental Day -1, Day 3, Day 7, and Day 14. Individual feed consumption was recorded for the first three days of the study and then for the Day 7 to 14 interval. Average feed consumption change (grams/bird/day) was calculated for Day 1 to 3, Day 4 to 7 and Day



8 to 14. Clinical observations occurred at least daily. Post-mortem examinations were conducted on all birds sacrificed at study termination.

The statistical analyses on body weight and feed consumption were conducted using TOXSTAT.

Findings:

Biological results

Test object	Northern Bobwhite quail
Test substance	BCS-CN88460 technical
	[mg a.s./kg bw]
LLD	> 2000
LD ₅₀	> 2000

Mortality & Clinical Observations

No mortalities and no clinical signs of toxicity were bserved in any bird All bard appearance and no effects of regurgitation were observed.

Body Weight & Feed Consumption

Body weight measurements (Day -1, Day 3, Day 7, and Day 14) and changes in Fody weight Day -1 to 3, Day 4 to 7, and Day 8 to 14) were statistically analyzed. There was a statistically significant decrease in body weight when expressed as body weight change at the 2000 mg as /kg body weight level for the Day -1 to 3 interval. However the birds in the 2000 mg a.s./kg body weight level recovered and regained bodyweight for the remainder of the study.

Individual food consumption measurements (Day 1 to 3, Day 5 to 7, Day 8 to 14) for the 2000 mg a.s./kg treatment group were not significantly different from the control group.

	(4)		y a' c	<u> </u>
	Body/weight desc	criptive statistics		&, ~
		d 3 ()		d 14
[mg a.s./kg bw]	Mean [g] n	Mean [g]	Mean [g] n ± SD.	Mean [g] ± S.D.
Control 👸	209.4 ± 9.4	204.4 ± 7.9 10	205.9 * 7.6 *	$\frac{208.0 \pm 8.0}{10}$
2000	2005 ± 8.4 10	200.3 7.2	205.7 ± 8.6 10	208.8 ± 10

 $\overline{SD} = standard deviation n = number of arviving birds$

~O	Body weight cha	nges O , O	V	<i>y</i>	
Treatment	Δd1-d3 3	Δ d3 d7		Δ d7-d14	
[mg a.s. fig bw]	Mean [g] n	Mean (g) ± S.O.	n	Mean [g] ± S.D.	n
Control	-4.9 ± 4.2 1.0 ×	1.4 41.2	10	2.1 ± 2.4	10
2000	^-9.2± 4.0* ↓0	5€5 ± 3,15√	10	3.1 ± 2.6	10

SD = standard deviation, a= number of surviving birds, *statistically significant decrease in body weight by a Kruskal-

	Food consul	Food consumption for intervals [g/bird/day]					
Treatment level	√4-3 €		d 4-7		d 8-14		
[mga.s./kg bw]	Mean [g] ± S.D.	n	Mean [g] ± S.D.	n	Mean [g] ± S.D.	n	
Contra	13.7 ± 1.8	10	14.8 ± 2.0	10	13.8 ± 1.8	10	
2000	12.6 ± 1.5	10	16.9 ± 1.7	10	16.7 ± 3.0	10	

SD = standard deviation, n = number of surviving birds



Validity criteria:

All validity criteria were met.

Validity criteria according to OCSPP 850.2100	Obtained in this study
Birds were randomly assigned to treatment groups	yes
Control mortality should be ≤ 10 %	0.0%
Minimum of 10 birds per treatment group	yes
Test substance was administered orally via capsule or gavage	yes
Definitive test was conducted with a minimum of five doses plus an appropriate control	no (limit test)

Conclusion:

The LD₅₀ of BCS-CN88460 technical for Northern Bobwhite quail was 2000 mg a Nkg body based on a limit-dose test. The Lowest Lethal Dose was ≥ 2000 mg a. s./kg body weight.

Report: KCA 8.1.1.1/02;

Title: Toxicity of BCS-CN88460 technical daring an acute

(Serinus canada)

Report No.: 044SRLS14©04 M-547051-01-1 @ Document No.: OCSPP \$50.2100 Guideline(s): Guideline deviation(s): not specified **GLP/GEP:**

Objective:

D₅₀ of BCSCN88460 technical on Canary The aim of the study was to determine the acuse (Serinus canaria)

Material and Method

BCS-CN88460 technical, Batch So. 2013-006492, Purply 94.2

Adult caparies (9 - 11 month and without body weight range of 196 - 26.0 g at study initiation) were orally with BCS CN8S 60 based on body weight at dos levels of 0 (control), 125, 250, 500, 1000, and 2000 mg/a.s./kg/Body weight Obw). Ten birds per dose level (five males and five females) were randomized by body weight into each treatment level on experimental Day -1. Birds were capsule dosed on Day of following sox hours of fasting and subsequently monitored for 14 days. All feed and water were provided ad libitum following dosing. Birds were individually housed in cages which provided an average temperature of 22.1°F (22.3°C) at 50 % relative humidity with a photoperiod of 8 hours light: 16 hours dark (227 wx). The birds were housed in commercial metal cages which measured approximately 27 cm (length) x 33 cm (width) x 31 cm (height). Birds were examined for mortality, clithical signs, body weight gain and food consumption. Adult body weights were measured on experimental Day -17, Day 7, and Day 14. Feed consumption measurements and clinical observations occurred daily

The statistical analyses on body weight were conducted using TOXSTAT.

Findings

Test object	Serinus canaria
Test substance	BCS-CN88460 technical
	[mg a.s./kg bw]
LD ₅₀	> 2000



Mortality and clinical observation

No mortalities were observed in any bird in any of the treatments. Lethargy (diminished hyporeactivity to stimuli) was observed in the 125, 500, 1000, and 2000 mg a.s./kg bw treatment groups in one, eight, ten, and ten birds respectively. All birds recovered from the observed symptoms by 1. No birds in the 250 mg a.s./kg bw treatment group were observed with any behavioral symptoms. No regurgitation was observed for any bird in the control or treatment levels. To mortality occurred during the course of the study therefore no birds were subjected to gross necrossy.

Body weight and feed consumption

yand there Body weight changes and food consumption were similar in all groups throughout the was no significant difference to the control

			4-	- //	1 1	
D.		Body weight Tescriptive statistics &				
Dose level	d -1		₽ d 7		d,14	. .
[mg a.s./kg bw]	Mean [g] ± S.D.	n	Mean [g] & S.D.	n	Meanag] ± S.D.	n
Control	22.2 ± 1.7	10 %	22.4°± 1.4°	10\$	23.5 ±Д.5	9_{10}
125	21.5 ± 1.3	10	21/.7 ± 1	90	22.4 1.4	1Q(
250	21.7 ± 1.2	D 0	<u>2</u> 2.2 ±1.6 ≥	10 ⋈	23°,3′ ± 1.7°°	JOV.
500	21.6 ± 1.1	\mathbb{P}_{10}	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	100	22.8 ± 52	NO.
1000	$21.8 \pm 1.2 \mathbb{Q}^{"}$	10	21.7 ± 029	Đố	22.8 1.1	10%
2000	22.1 ± 1.6	¥0 [*]	22.3 ± 3.3	10	23.5± 1.6.	10

SD = standard deviation; n = number of surviving birds

Dose level [mg a.s./kg bw]	A d-1-d7	Body weight changes A d7-644 Mean [g] S.D. n	Δ d-1-d14 Mean [g] ±	y S Yn
Control	S.D. 10 0.2 ± 0.7 10 0.2 ± 0.4 16	$1.1 \neq 0.5$	S.D.	10
250	\$\int_{0.5} \pm 0.8 10	1.1.±0.7 10	0 1.6, 20.9	10
1000	$0.1 \neq 0.9 < 10$ $0.0 \pm 0.5 < 10$	1.0 ± 0.0	10 ± 1.0 1.0 ± 0.6	10
2000	0.2 ± 0.9 10	1.2 ±0.5	→ 1.4 ± 1.1	10

SD = standard deviation = number of sprviving pirds.

		<u> </u>		<u>′</u>	
Q _n	FoodConsum	ption Descriptive	Statis	stics [g/bird/day]	
Dose leve	©	d 8-14	~ ~	d 1-14	
[mg a.s./kg bw]	Mean (g) ± n	Mean (g) ± 0 S.D.	n	Mean (g) ± S.D.	n
Control	A.1 ± 0.7	5.0 ± 0.9	10	4.6 ± 0.7	10
125	4.7 4.7.2 10	Q 4.8×0.9	10	4.7 ± 1.0	10
250	4.9 ± 0.8 10_{\odot}	5,2± 0.8	10	5.0 ± 0.7	10
500	4\3 ± 00°	28 ± 0.6	10	4.6 ± 0.5	10
1000	5.0 ± 3.3 10	\emptyset 5.6 ± 1.3	10	5.3 ± 1.3	10
2000	4.790.7510	5.2 ± 0.5	10	5.0 ± 0.5	10

rd deviation; n = numbe Oof surviving birds.



Validity criteria:

Validity criteria according to OCSPP 850.2100	Obtained in this study		
Birds were randomly assigned to treatment groups	yes	ð	
Control mortality should be ≤ 10 %	0.0%		
Minimum of 10 birds per treatment group	yes	21	
Test substance was administered orally via capsule or gavage	yes	\$\tag{\psi}	
Definitive test was conducted with a minimum of five doses plus an appropriate control	yes	Q Q	

Conclusion:

CA 8.1.1.2 Short-term dietary toxicits

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

Validity criteri	i <u>a:</u>						
All validity cri	iteria were met.						
Validity crite	ria according to OCSPP 850.2100	Obtained in this study					
Birds were ran	domly assigned to treatment groups	yes 💸					
Control mortal	lity should be $\leq 10 \%$	0.0%					
Minimum of 1	0 birds per treatment group	yes A S S					
Test substance	was administered orally via capsule or gavage	yes yes					
	was conducted with a minimum of five doses priate control	yes of the second secon					
CA 8.1.1.2	The LD ₅₀ of BCS-CN88460 technical for canary \$\infty \ 2000 mg as ./kg body weight						
Test substance	Test design Gest Figure En	dpoint					
Isoflucypram	Dietary toxicity (short-term)	360 mg/4s. //kg by 2014; M-507176-01-1					

Report: 2014@M-507176-01-1

oxicity of BCS (N8846) technical during a dietary LC50 with the mallard duck Title:

(Anas Atatyrhyn hos)

©044\$PØ\$14€06 Report M-507176-01-1 Document No.:

EU Dire ve 91/4/4/EEO Guideline(s):

Regulation (E@ No. 1107/2009 OCSPP 850,2200

OF D Guideline 20

Guideline deviation(s): not specified

GLP/GEP:

Objective:

The aim of the study was to determine the short-term dietary LC₅₀ of BCS-CN88460 technical on Mallard duck (Anas platyrhy@chos)

Material and methods;

BCS-CN\$8460 Cchnical, Batch code BCS-CN88460-01-06, Batch no.: 2013-006492, Purity 94.2 %. Six day old not lard total lings (Anas platyrhynchos) were fed for five days nominal dietary levels of 0 (control), 3,63, 625,1250,2500, and 5000 mg a.s./kg feed. The treatment levels, homogeneity, and stability comples were confirmed through the analysis of BCS-CN88460 in the feed. The mallard hatchlings were acclimated in brooder pens for five days and ten hatchlings per test level were randomized by body weight (initial 66 g - 88g into each treatment level on Day 0. The five day exposure period was followed by a 3-day subsequent recovery phase on the basal diet. All feed and water was provided ad libitum. Hatchlings were housed in brooder compartments which provided a



temperature range of 95° to 99 °F (35 to 37°C) at 51 % relative humidity with a photoperiod of 14 hours light: 10 hours dark (227 lux). The hatchlings were housed in galvanized steel brooders which measured approximately 91 cm (length) \times 76 cm (width) \times 25 cm (height).

Feed consumption, mortality and clinical observations for the hatchlings occurred daily. Hatching body weights were taken on Day 0, Day 5, and Day 8. Post-mortem examinations were conducted on the conducted of the conducted all control and high dose birds and on 40 percent of the other birds sacrificed at Study termination. The statistical analyses on body weight were conducted using TOXSTAT.

Findings:

Analytical results

The statistical allary	ses on body weight were conducted using TOASTAT.
The average concer	ntration of BCS-CN88460 was measured in feed on study initiation Day 🗗 and
Day 5.	unts of BCS-CN88460 were determined as control (0), 326, 643, 1323, 2529 and d representing a recovery range of 301 to 311 % of nominal. The mixing procedure was confirmed to be homogenous and stable under test conditions.
Findings:	
S	
Analytical results	
The measured amou	unts of BCS-CN88460 were determined as control (0), 326, 643, 1323, 2529, and
5554 mg a.s./kg fee	d representing a recovery range of 301 to 311 % of nominal. The mixing procedure
for BCS-CN88460	was confirmed to be homogenous and stable under text conditions.
Nominal dietary	Measured dietary Overall study O
concentrations	Measured dietary Overall study concentrations
[mg a.s./kg feed)]	[mg a.s./kg feed]
	Mean (SD) Mean % recovery
Control	ND ^a ND ^b
313	326 (10) \$ \$ 104% \$ \$
625	Measured dietary Overall study concentrations mg a.s./kg feed
1250	1329 (30) (3106)
2500	2529 (42)
5000	\$554 (286) \$\times 111\times \times 1
a ND = Not Detected (life	ant of governitation = 50 in a.s./kg feed)
Ö	ant of goantitation = 50 in a.s./kg feed)
Biological results	
	thered duck
Test substance BC	S-CINS 8460 technicative Or a Company of the contractive Or and the
	g a.s. (kg feet) (measured) [mg a.s./kg by) (measured)
LC_{50} > 5	555
LOEC > 5	\$54 \$\frac{1}{2} \frac{1}{2} \f

a ND = Not Detected (limit of grantitation)=

Biological results

Test object	Madaird duck
Test substance	BCS-CN88460 technicat
	[mg a.s./kg feed] (measured) [mg a.s./kg bw/ (measured)
LC ₅₀	> 5555
LOEC	> \$954 % \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$
NOEC	1 3 3 3 3 3 3 3 3 3 3

Mortality & Clinical Observations

One accidental mortality occurred in the 326 org a.s. Reg feed treatment level on Day 7. No birds died in the control group. No other clinical signs of toxicity or treatment-related mortalities were noted at any treatment level. Post-mortem examinations revealed no treatment related gross lesions or unusual observations.

Body Weight & Feed Consumption

Body weight data for the time points of Day 0, Day 5, Day 8, Day's 0 to 5, Day's 5 to 8, and Day's 0 to 8 were subjected to hootheses testing. No statistically significant effects for body weight or body weight change were observed for any treatment level as compared to the control. In addition, feed consumption was not reduced in any treatment level during the exposure and recovery periods.



Body weight descriptive statistics							
Measured dietary	Initiation (Day 0)		Day 5		Termination Day 8		
concentration [mg a.s./kg feed]	Mean [g] ± SD	n	Mean [g] ± SD	n	Mean [g] ± SD	n	
Control	72.9 ± 4.0	10	165.1 ± 13.7	10	238.0 ± 18.0	100	
326	72.9 ± 4.2	10	170.1 ± 5.5	10	245.5 ± 11.1		
643	72.1 ± 4.8	10	168.7 ± 12.6	10	238.0 ± 21.9	10	
1323	72.5 ± 4.7	10	168.7 ± 11.3	10	247.1 ± 14.8	10	
2529	72.5 ± 4.7	10	164.9 ± 12.4	_ (O	241.2 ± 17	10	
5554	72.6 ± 4.3	10	163.2 ± 9.3	10	235.5 ± 5.7	10	

R						Isoflucypram
Body weight descrip	ative statistics					
Dody weight descrip	Initiation Section				Termination	
Measured dietary	(Day 0)		Day 5		Day 8	
concentration	Mean [g] ±		Mean [g] ±		Mean [g] ±	
[mg a.s./kg feed]	SD	n	SD	n	SD	n 10 10 10 10 10 10 10 10 10 10 10 10 10
Control	72.9 ± 4.0	10	165.1 ± 13.7	10	238.0 ± 18.0	
326	72.9 ± 4.2	10	170.1 ± 5.5	10	245.5 ± 11.1	
643	72.1 ± 4.8	10	168.7 ± 12.6	10	238.0 ± 21.9 «	10
1323	72.5 ± 4.7	10	168.7 ± 11.3	10	247.1 ± 14.8	10
2529	72.5 ± 4.7	10	164.9 ± 12.4	_ (O)	241.2 ± 17.	
5554	72.6 ± 4.3	10	163.2 ± 9.3	\mathfrak{T}_0	235.5 ± 5.7	
= number of surviving	birds; SD = standa	rd dev	viation	1	"QA	
					Ŏ.	
Body weight change	S					
Measured dietary	Exposure period Recovery period Study per		Study period			
concentration	$(Day 0 - Day 5) \qquad (Day 5 - Day 8)$		(Day 0 - Day	8) 🔎 🤝 🐃		
[mg a.s./kg feed]	Mean [g] ±	n	Mean [g∤¥		Mean [g]	
[mg a.s./kg reeu]	SD	11	SD O	_N	S	
Control	92.1 ± 11.6	10		y 10	<u>)</u> 165. <u>1</u> €14.9 ©	
326	97.3 ± 7.1	10	v 75,9± 7.1,©	9,	7 1710°± 1126°	
643	96.6 ± 10.6	ÁM.		~Qr(165.9 ± 20.6	
1323	96.2 ± 10.2	\$ 10	Ø8.4±7∕1	¥ 10) \$74.6 ₹3.8 @	10 \$ \$
2529	92.4 ± 11.6	10		» 10	168,7 ^{9±} 17,90	100
5554	90.6 ± 6.7	, 16		N.	1630 ± 14.9	
=number of surviving b	irds; SD = standar	d devi	ation>	- N	9 1000 ± 14.4	<u> </u>
	~	¥		O		
Feed consumption s	ummary [g/biro	l/day		6		
			Regovery Pariod		/ . &	8
Measured dietary	Exposure peri		period &	\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\	uay perioa	
concentration	Day 0 Day 5		period & Day 5 Day &	M D	ay 0 - Day 8	*
[mg a.s./kg feed]	(Mean ± SAD)	*	/(Mean ± SD)		Dean ±SD) 📗	
Control	28.7 ± 8.2	& _y	46046.74	35	5.2 ± 1.4 «	
326		0	48.7 ± 5.9	© 36	5.34 12.0 _@	

		, W	· ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~
Feed consumption s	ummary [g/bird/day		
Measured dietary concentration	Exposure period Day 0 Day 5	Recovery period period Say 5 Day 8	Study period Day 0 - Day
[mg a.s./kg feed]	(Mean ± SA)	(Mean ± SD)	(Mean ± SD)
Control	28.7 ± 8.2 %	46,0 ± 6.7 %	35.2 ± 1√1.4 ≪
326	28.8 ± 0.1	48.7 ± 5.9	36.3 12.0 ₀
643	28.2 ± 6.9 ₺	45.4±5.6	34.6 ± 10.%
1323	30.44 ± 8.4	³ 50.7 ¥5.1 ≥	38.0 ± 12.6
2529	28.5 ± 867	44.9 ± 3.70	¥J34.7¸±Q0.9
5554	28.9 ± 70.8 «	A7.1 ± 4.1/	35.7€ 12.7

All validity criteria:

Validity criteria according to QCD 205	Obtained in this study
(adopted 04 April 1984) Q	
Mortality in the controls $\leq 10\%$	0.0 %
Conventration of the substance being tested should be	101 % - 111 %
at least 80 % of the nominal concentration	
Lowest treatment level should not result in compound-	yes
related mortality or other observable toxic effects	

The dietary Loso of SCS-CN88460 technical fed to the mallard duck was > 5554 mg a.s./kg feed or > 1360 mg/s.s./kg/body weight. Based on all parameters measured, the NOEC was 5554 mg a.s./kg feed (1360 mg a.s./kg body weight) and the LOEC was > 5554 mg a.s./kg feed (> 1360 mg a.s./kg body weight).



Report: M.; 2015; M-516743-01-1 KCA 8.1.1.2/02; Т · Title:

Toxicity of BCS-CN88460 technical during a dietary LC50 with the northern

bobwhite quail (Colinus virginianus)

Report No.: EBLNN008 Document No.: M-516743-01-1

Guideline(s): EU Directive 91/414/EEC

Regulation (EC) No. 1107/2009

OCSPP 850.2200 OECD Guideline 205

Guideline deviation(s): not specified

GLP/GEP: yes

Objective:

The aim of the study was to determine the short-term dietar Northern Bobwhite quail (Colinus virginianus)

Material and Methods:

BCS-CN88460 technical, Origin Batch No. 2013-006492, Purov 94.25

Ten day-old Northern Bobwhite quail hatchings were feat for five days nominal dietary levels of 0 (control), 313, 625, 1250, 2500, and 3000 ing a so (active ingredient) by feed. The treatment levels, homogeneity, and stability samples were confirmed for the analysis of BCS CN88460 in the feed. Ten hatchlings per test level were randomized by body weight in the each treatment level on Day 0. The five day exposure period was followed by a 3-day subsequent recovery phase on the basal diet. All feed and water was provided ad libitum. Hatchings were housed in brooder comportments which provided a temperature range of 90% to 99 % (32% 37%) with a photoperion of 14 hours light: 10 hours dark. The hatchlings were housed in galvanized steel brooders which measured approximately 91 cm (length) \times 76 cm (width) \times 25 cm (height).

Feed consumption and clinical observations for the haterlings occurred daily. Hatchling body weights were taken on Day 5, and Day 8 Post-morten examination were conducted on all control and high dose level birds and on 40 percent of the other birds sacrificed at study termination.

The statistical malyses on body weight were conducted using FOXSTAT.

The average concentration of BCS-CN88460 technical was measured in feed on study initiation (Day 0) and Day 5.

Findings:

Analytical results

The measured amounts of BC\$-CN88460 rechnical were determined as Control (0), 312, 618, 1235, 2546, and 508 mg a.S./kg feed representing a recovery range of 98 to 102 % of nominal. The mixing procedure for BCS-CN8\$460 was confirmed to be promogenous and stable under test conditions. Designations of treatment levels were based on the rocan measured concentrations.

concentrations	Measured dietwry & School Concentrations of BCS-CN88460	
[mg a.s./kg feed]	[mg a.s./kg/feed]	Percent of nominal
Control &	MD &	ND ^a
313	312(8)	100%
625	618 (22)	99%
1250	<u></u> 1 2 2 2 3 2 1 2 1 2 1 2 1 1 2 1	98%
2500	2546 (24)	102%
5000	5087 (306)	102%

^a ND = Not Detected (<LOQ = 50ppm).



Biological results

Test object	Northern Bobwhite quail	
Test substance	BCS-CN88460 technical	
	[mg a.s./kg feed] (measured)	[mg a.s./kg bw] (measured)
LC ₅₀	> 5087	> 487
LOEC	> 5087	> 937
NOEC	2546	487

Mortality & Clinical Observations

No mortalities and no clinical signs of toxicity were beserved in any revealed no treatment related gross lesions or unusual observations

Body Weight & Feed Consumption

a.s./kg feed level for any time-point.

Body weight was decreased at Day 5 and Dao 8 for the 5087 mg as /kg feed level as compared to the control group. In addition, body weight change was reduced in the exposure period (Day (100" Day 5)) and the study period (Day 0 to Day 8) for the 5087 mg a.s./kg feed to vels of compared to the control group. Feed consumption was also reduced in the 5080 mg at key feed during the study periods Body weight change was significantly reduced from the controls for the 618 and a.s. deg feed level at the Day 5 to Day 8 time-point. This appears to be a transient effect as there were no abnormal clinical observations or necropsy findings noted for the 618 mg a.s. of feed treatment level. Furthermore, no statistically significant effects for body weight or body weight change were observed for the 618 mg

a.s./kg feed level for any time-point.

There was an apparent reduction in feed consumption in the 5087 mg/a.s./kg feed treatment level during the exposure, recovery, and study periods based on empirical analysis.

		8	~	<u> </u>	/	
Body weight descrip	ptive statistics			0		
Measured dietary	Initiation	~ Q			Fermination	
concentration A	111/10/01/01/	<i>*</i>	7 3 3 A			
[mg a.s./kg feed]	Day 0				Day 8	
O'	Mean g ± SD	n 🦠	Mea@ [g] ±&D	10	Mean [g] ± SD	n
Control 🛴 👰	25.J ± 1.4	10,	42 1 ± 2.9°°	_{7,1} 10	4.7 ± 4.0	10
312	25(1) ± 1.4(2)	P 0	42.1 ± 201	10 ^	₹54.9 ± 4.7	10
618	29.0 ± 1.30	10 🔏	41.0±2.0	10	51.9 ± 3.0	10
1235	Q25.1 ±\1.3 €	100	40. 2 ± 3.1	40	52.1 ± 3.0	10
2546	25, <u>0</u> ± 1.4 ©	40	$40^{\circ} 2 \pm 3.5^{\circ}$	≽10	51.7 ± 3.8	10
5087	25Q0"± 1.45"	10 0	₹8.3 ±\$6*	10	48.0 ± 3.8*	10

n=number of surving birds; SD_standard deviation

^{*}Statistically significant difference as compared to the control group by Dunnett's Test.

	<u> </u>		°~' «					
Body weight descriptive statistics								
Measured dietary consentration [mg a.s./kg feed]	Exposure period (Day 0 – Day 5)	~	Recovery period (Day 5 – Day 8)		Study period (Day 0 - Day 8)			
Img assung reco	Mean 💋 ± SD	n	Mean [g] ± SD	n	Mean [g] ± SD	n		
Control	7.1 2.1	100	12.6 ± 1.7	10	29.7 ± 3.3	10		
312	17.10 2.2 🗶	70	12.7 ± 2.3	10	29.8 ± 4.2	10		
618	16.0 ± 1.7	10	$10.9 \pm 1.4*$	10	26.9 ± 2.8	10		
1235	45.1 ± 19	10	11.9 ± 1.3	10	27.0 ± 2.0	10		
2546	715.2°±,2.7	10	11.5 ± 1.9	10	26.7 ± 2.8	10		
5687	13.5 ± 3.2*	10	9.6 ± 1.0 *	10	$22.9 \pm 3.2*$	10		

n=number@f surviving birds; SD = standard deviation

^{*}Statistically significant difference as compared to the control group by Dunnett's Test.



Feed consumption summary					
Measured dietary	Exposure	Recovery	Study period		
concentration	[g/bird/d]	[g/bird/d]	[g/bird/d]		
[mg a.s./kg feed]	$(Mean \pm SD)$	$(Mean \pm SD)$	(Mean ± SD)		
	Day 0 - Day 5	Day 5 – Day 8	Day 0 - Day 8		
Control	6.3 ± 0.5	7.7 ± 0.5	68 ± 0.9		
312	6.3 ± 0.6	7.6 ± 0.6	8 ± 0 9		
618	6.2 ± 0.6	7.2 ± 0.6	6.6 ± 0.7		
1235	5.9 ± 0.6	7.3 ± 0.7	6.4 ± 1.0		
2546	6.2 ± 0.6	7.3 ± Q A			
5087	5.8 ± 0.5 *	6.2 ± 0.9*	6.0 ± 9 7*		
*Significantly different from the control.					
Validity criteria: All validity criteria were met.					
Validity criteria according to OECD 205 (adopted Obtained in this study					
04 April 1764)					
Mortality in the controls $\leq 10\%$ 0.0%					
Concentration of the substance being tested should be at least 80 % of the nominal concentration Lowest treatment level should not result in compound year related mortality or other observable oxic effects					
at least 80 % of the nominal concentration () A A A A A A A A A A A A A A A A A A					
Lowest treatment level should not result in compound yes? yes yes yes					
related mortality or oth	er observable oxic effects	ng-y yest, yest,			
	or observation governments				

^{*}Significantly different from the control.

Validity criteria:

Validity criteria according to OECD 205 04 April 1984)	(adopted Obtained in this study
Mortality in the controls $\leq 10\%$	\$\frac{1}{2} \frac{1}{2} \frac{1}{2}
Concentration of the substance being tested	Ishould be 98 % - 102 % 7 1 1
at least 80 % of the nominal concentration	
Lowest treatment level should not result in	compound yes? S
related mortality or other observable oxic	effects / S S S

Conclusion:

The dietary LC₅₀ of BCS-CN88460 technical for Northern Bobwhite quail is \$5087 mg a.s./kg feed or > 487 mg a.s./kg body weight. Based on all parameters measured, the NOEC was 2546 mg a.s./kg feed (487 mg a.s./kg body weight) and the LOEC was 5007 mg a.s./kg feed (937 mg a.s./kg body weight).

chronic and reproductive toxicity to b

Endpoint derivation for the chronic risk assessment in blods

Avian reproduction studies with isoflucypram were conducted with the Mallard duck (one study) and with the Bobwhite quail (two studies). All studies were conducted according to OECD Guideline 206 in the same laboratory. In order to clarify an unclear result the first reproduction study on Bobwhite quail was repeated. The unclear result was a statistically regular reduction of the 14-d chick body weight at 137, 333 and 1000 ppm (but not at 114 ppm). The magnitude of differences was small (5.83 -8.79 %), and without dose response.

In order to comply with requirements of non-European authorities and to verify this spurious pattern a new study was initiated which tested levels of 25, 250 and 2500 ppm, i.e. including not only a concentration (25 ppm) below the lowes Vievel of the initial study (37 ppm) but also a notably higher level of 2500 ppm. It was expected that such high concentration would result in a higher magnitude of 14-d chick bodyweight reduction in case of a true effect from isoflucypram.

But in the confrary; there were no differences in 14-d chick bodyweight in the second study at any test level, not wen in the top concentration of 2500 ppm. Thus, the finding in the initial study can be considered as false positive.

below body weight results for 14 days old quail chicks are depicted for both studies.



Study M-611590-01-1 (referred as to 'initial study')						
Concentration	Mean Body-	95% LCL	95% UCL	% effect to	P-value*	
[ppm]	weight [g]			control	<i>@</i> ,	
0	35.87	34.44	37.3	0.0	-	
37	33.19	31.97	34.41	7.84	0.010	
111	33.67	32.12	35.21	6.14	n.s.	
333	33.78	32.7	34.86	5.83	0.038	
1000	32.72	31.71	33.73	8.7,9	QQ9 01 X	
Study M-611653-	01-1 (referred as to	'second study')		\$\frac{1}{2} \gamma		
0	32.77	31.64	339	9 .0	- 3	
25	32.63	31.63	33.62	0.43	n SO V	
250	31.58	30.27	₹32.9 °	3.62	10 .s. \$	
2500	32.86	31.68	34.04	-0.28	n.s. O	

LCL = lower confidence level; UCL = upper confidence level? according to Dunnett 7 Multiple Comparison Test; n.s., not significant

Conclusion:

Body-weight values for 14 days old chicks clearly show no dose-response up to a concentration of 2500 ppm and this parameter can therefore be considered as not relevant for an endpoint derivation.

However, standard statistical analysis of the second study indicated very slight but statistically significant differences in the survival of 14 days old chicks at all treatment levels. However the maximum deviation of 2.06 % to the control can be rated as non-relevant,

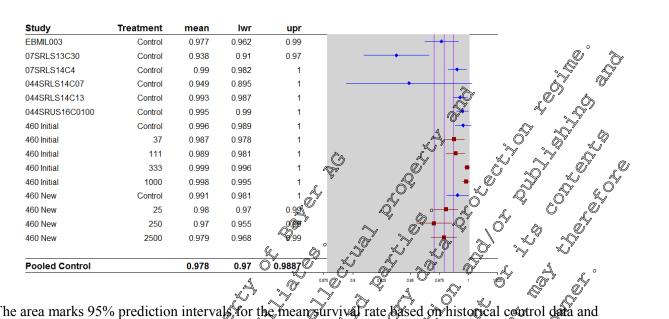
Study M-611590-01-1 (referred as to 'initial study')	, Ø ,			
Concentration [ppm]	P-value*			
survivors [%]				
	-5~			
37 98.8	% 9191			
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	0.9191			
	0.9191			
1000	0.9191			
Study M-611653-01% (referred as to 'second study')				
0 99.46 \$\times 00.0 \tilde{\ti	-			
25 0 98,04 0 1.03	0.0323			
250	0.0052			
2500 97.88 119	0.0271			

^{*} bold: values statistically significant conckheere-Terpstra Step-Down Test p-value)

According to DECD Guideline 206, Table 3, 'Normal values for percent of hatchlings that survive to 14 days' are given as between 35 and 20%. Mean survivor values in both studies (lowest value 97%), show values well above this 'normal range clearly indicating that the substance does not impact the survival of chicks.

Furthermore, survival values were compared to historical control data from the same laboratory (collected from several recent studies performed for the applicant):





The area marks 95% prediction intervals for the mean survival rate based on historical control data and the purple lines mark the estimated mean control survival rate and the 3% confidence interval. Blue color indicates point and confidence interval estimates from control and dark and color indicates those from treatment groups. '460 New' (i.e. the second study indicate the study with the statistical significance in the hatchling survivor rate. Note that I values of the relevant study are well within the confidence interval of the historical control

Overall assessment of NOEC OF 2500 ppm is proposed as endpoint for the exposure to quails. 1000 ppm@was defined representing the highest For the mallard dock an overall endpoint of concentration tested.

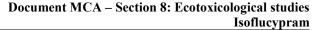
Table 8.1.1.3 1: Reproductive foxicity to biral

Test substance		Species S	Endpoint	Reference
Q.	23 weeks Reeding thronic reproduction	Maklard NOEC	1000 ppm 60 mg a.s./kg bw/d	T.; ; 2017; M-597500-01-
Isoflucypram	23 weeks feeding chronic reproduction	Bobymite NOEC	1000 ppm 64 mg a.s./kg bw/d	, C.; , J.; ; ; ; ; ; ; ; ; ; ; ; ; ; ; ; ; ; ;
	23 weeks reeding chronic, reproduction	~ Q \$7	2500 ppm 172 mg a.s./kg bw/d	, J.; , C.; , B.; 2018; M-611653-01-1 KCA 8.1.1.3/03

Therefore an overall endpoint of 1000 ppm (60 mg a.i/kg bw/d), derived from the Mallard duck reproduction study is appropriate for the reproductive risk assessment for birds.

Additional evaluation on avian reproduction studies regarding EC10 or EC20

According to "Outcome of the pesticide peer review meeting on general recurring issues in ecotoxicology" (EFSA 2015) it was agreed that for lower tier laboratory studies, until the typical





values for the standard guidelines are available, a statistical power analysis is not necessary. The following standard laboratory tests fell under this consideration:

Three avian reproduction studies were performed. The NOEC of the reproduction study with Bobwhite Quails (et al. 2018, M-611590-01-1) was determined to be at the highest test concentration (1000 mg a.s./kg food, corresponding to 65 mg a.s./kg b.w./day). At the highest test concentration the difference to control was always below 10%. With regards to the body weight of 14-day old survivors a significant effect was determined for all concentrations. At the highest test evel the effect amounted to 8.79 %. This did not follow a dose response and was therefore not considered substance related. The finding was verified by the second reprotoxicity study where this effect could not be detected at any test concentration up to 2500 mg a.s./kg food.

In a second reproduction study with Bobwhite Quails (et &d. 2018, M-6 (1653-01-1) the NOEC was determined to be at the highest test concentration of 2500 mg a.s./kg food corresponding to 174 mg a.s./kg b.w./day. At the highest test concentration the difference to control was always below 10%. For many parameters a dose response was not visible or the test groups behaved significantly better than the control. With regards to the hatchlong sur wal rate a significant effect was determined for all concentrations. At the highest test level the effect amounted to 1.19 9. It did not follow dose response which was therefore not considered substance related.

A reproduction study with mallards (2017, M-597500-01-1) revealed a NOEC at the highest test concentration of 1000 mg a.s./kg/food corresponding to 60 mg a.s./kg b.w./day. At the highest test concentration the difference to control was always below 10%. For many parameters a dose response was not visible or the test groups behaved significantly better than the control. For all three reproduction studies the low magnitude of the differences to the courtful and the missing of a dose-response relationship give reason not to perform an EC10 or an EC20 calculation.

2617; M-\$97500-01-1 Report:

Toxicity of BCS-CN88460 of reproduction in the manard duck (Anas platyrhynchos) 0448RLS14002 Title:

To evaluate to evaluate to evaluate to evaluate to the new the new to evaluate the new to evaluate to Report No.: Document No. Guideline(s)

Guideline deviation (s GLP/GEP:

Objective:

Objective The aim of the study was to evaluate the effects of dietary exposure to BCS-CN88460 technical on the healt and reproductive capacity of Mall and ducks (Anas platyrhynchos).

Material and Methods:

BCS-CN88460 technical, Quigin Batch No. 2013-006492, Purity 94.2 %.

The mallard reproduction study exposed adult Mallard ducks (Anas platyrhynchos) to BCS-CN88460 technical for approximately 22 weeks to nominal dietary concentrations of control, 111, 333, 1000 mg a.s./kg_leed. Mallard ducks were 21 weeks old at experimental start with 16 pairs of birds at each treatment level. Birds were observed for mortality, abnormal behavior and signs of toxicity; adult body weight and feed consumption were measured; gross pathology was conducted; reproductive parameters, as well as hatchling health, growth and survival, were examined. The biological portion of the study was conducted from 25 August 2015 to 1 March 2016.



Findings:

Analytical results

The measured amounts of BCS-CN88460 technical were determined as Control (0), 111, 333 and 2 1000 mg a.s./kg feed representing a recovery range of 92 to 96 % of nominal. The mixing procedure for BCS-CN88460 was confirmed to be homogenous and stable under test conditions. Designations of treatment levels were based on the mean measured concentrations.

	was confirmed to be homogenous and stable under test conditions. Designations of the based on the mean measured concentrations. Measured dietary concentrations of BCS-CN88460 mg a.s./kg feed Mean measured values (± SD) Percent of nominal NDa NDa
[mg a.s./kg feed]	concentrations of BCS-CN88460 [mg a.s./kg feed] Mean measured values (± SD) Percent of nominal NDa 106.7 (13.6) 96 % 313.0 (16.9) 94 %
(9	Mean measured values (± SD) Percent of nominal
Control	ND^a
111	106.7 (13.6)
333	106.7 (13.6) 96 % V V V V V V V V V V V V V V V V V V
1000	313.0 (16.9) 923.1 (46.8) 92 % 5 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7
a ND = Not Detected (<	
Biological results	ation Introduction Introduct
Dietary concentra	tion South A D D D D D D
The nominal amou	ints of BCS-CNX8460 in the dietary feed for the mallar preproduction study were
administered at le	vels of 0 (control) 111, 363, and 1000 mg 25/kg feed. The average measured
amounts of BCS-C	N88460 for Week 7 5 10 15 and 20 were described as 0° 0.7 δ 3 and 923 mg

administered at levels of 0 (control), 11, 363, and 1000 mg 35/kg feed. The average measured amounts of BCS-CN88460 for Week Y, 5, 10, 15, and 20 were determined as 0, 107, 513, and 923 mg a.s./kg feed representing percent nominal values of \$\infty\$6 %, 94 \infty\$ and \$\infty\$2 % mg a.s./kg feed, respectively. These values correspond to daily chetary dose levels of 8, 21 and 60 mg a.s./kg bw/day, respectively. A summary of the dietary concentrations is included in the following table.

Feed analysis summary for BCS (N88460				
Nominal dietary level ppm [mg a.s. @ feed	Mean measured dictary lovel Percent of ppm mg a Skg feed paminal (%)	Measured daily dietary dose [mg a.s./kg bw/day]		
0 (control)		-		
ŽÝ 11	2 107	8		
333	318, 0 94%	21		
1000	\$\frac{1}{2} \frac{1}{2} \frac	60		

Adult Bird Mortality & Clinical Observations

No mortality or significant linical symptoms were observed during the study in any treatment level. Feather loss for several female todds in the commol (30 inds), 333 ppm (5 birds), and 1000 ppm (1 bird) treatment revels were noted doe to normal eage wear for laboratory reared mallards. One female bird was observed with a kin abrasion on the head in the 1000 ppm treatment level. These findings were considered incidental and not treatment related

Adult Bird Bodyweight & Fred Consumption

The adult body weights in the malfard coproduction study were measured prior to dosing and every other week up to the egg production phase (i.e. Week 3, 5, 7, 9) and prior to adult sacrifice. Adult bird food consumpton was measured weekly during study. No statistical significance or biologically relevant effects occurred at any treatment level for adult bird body weight gain or food consumption.



Adult Bird Necropsy

Necropsy of the adult birds at study termination showed no apparent treatment related effects. In the control level, one female bird was observed with one follicle found to be solid and black in color. Additionally in the control level, one female bird was found with an enlarged spleen, fluid filed cavity, and right lobe of the liver discolored. In the 111 ppm level, one female bird had an enlarged spleen and one male had a fluid-filled sac in the abdomen, hard liver, and enlarged spleen. Feather loss for several female birds in the control (3 birds), 333 ppm (5 birds), and 1000 ppm (1 bird) treatment levels were noted due to normal cage wear for laboratory reared mallards. One female big was observed with a skin abrasion on the head in the 1000 ppm treatment level. These findings considered incidental and not treatment related.

Egg Reproductive Effects

There were no statistically significant adverse effects for the following egg perroductive endpoints number of eggs laid, percent eggs set of eggs land, number of eggs cracked percent eggs not cracked of laid, number of eggs set, and eggshell thickness.

Embryo Reproductive Effects

There were no statistically significant effects from the control for the number of viable embry of and the number of live embryos. No significant difference occurred for the percent wable embryos of eggs set and the percent live embryos of varieties embryos

Hatchling Effects

There were no statistically significant effects from the control for the initial number batched, percent number hatched of eggs laid, percent number hatched of eggs set, and 14-day hatchling survival.

Hatchling Body Weight

There were no statistically significant differences abany treatment level as compared to the control for initial hatchling weights and 14-day survivor body weights. No hatchlings produced from the study were observed to have any abnormal symptoms.

Validity criteria.

All validity criteria were met.

Validity criteria according to ECD 206 (adopted O Obtained in this study
04 April 1984)
Mortality in the controls $\leq 10\%$ \sim \sim \sim \sim \sim \sim \sim \sim \sim
The average number of 14 day our survivors per tren in 35
the controls should be a Yeast of for mallard cocks. O
The average egg shell thickness for the control group \$34 mm
should be at least 0.34 mm for manard duck.
Concentration of the substance being tested should be 92 % - 96 %
at least 80 % of the normal concentration &

Conclusion:

The No Observed Effect Level (NOEL) for parental toxicity and reproduction endpoints of Mallard duck exposed to BCS-CNS8460 technical was 1000 ppm (nominal test level) with a measured concentration of 223 mg@s./kg feed on the mean achieved dose of 60 mg a.s./kg bw/day. The Lowest Observed Effect Level (LOFF) was > 1000 ppm (nominal test level) equivalent to the measured concertration of 923 ppm of the achieved dose of 60 mg a.s./kg bw/day.



Report: KCA 8.1.1.3/02; C.; J.; J.; J.; 2018; M-611590-01-1 Title: Toxicity of BCS-CN88460 technical in the reproduction of the northern bobwhite

quail (Colinus virginianus)

Report No.: 044SRLS14C01 Document No.: M-611590-01-1

Guideline(s): EU Directive 91/414/EEC

Regulation (EC) No. 1107/2009

OCSPP 850.2300 OECD Guideline 206

Guideline deviation(s): not specified

GLP/GEP: ves

Objective:

The aim of the study was to evaluate the effects of dietary exposure to BCS-CN88460 technical on the health and reproductive capacity of the Northern Bobyshite Quail (Colinus virginiarus).

Material and Methods:

BCS-CN88460 technical, Origin Batch No. 2013-006492, Puroy 94 2%.

Adult bobwhite quail (Colinus virginianus) were exposed to BCS-CN 460 technical for approximately 23 weeks to nominal frietary levels of 0 control, 37, 411, 353, and 1000 ppm (mg a.s./kg feed). Bobwhite quail were approximately 32 weeks-old at experimental start with 8 pairs of birds at each treatment level. Birds were observed for mortality, abnormal behavior and signs of toxicity; adult body weight and feed consumption were measured; gross pathology was conducted; reproductive parameters, as well as hatelying health, growth and survival, were examined. The biological portion of the study was conducted from 21 April 2016 to November 2016.

Results:

Dietary Concentration

The nominal amounts of BCS-CN88460 technical in the dietary feed were administered at levels of 0 (control), 37, 110, 333, and 1000 ppm. The average measured amounts of BCS-CN88460 technical for Week 1, 5, 10, 15, and 20 were determined as 9, 37, 006, 327, and 974 ppm representing percent nominal values of 99%, 96%, 98%, and 97% respectively. These values correspond to daily dietary dose levels of 2, 7, 22, and 64 mg a 30 kg body weight/day, respectively. A summary of the dietary concentrations is included in the following table.

Feed analysis summary of BCS-CN88460				
Nominal dietary level ppm [mg a kg feed]	Measured Pescent of Cominal Apple [mg.a.s./kg/feed]	Measured daily dietary dose [mg a.s./kg bw/day]		
0 (control)		-		
37	Q 37	2		
111	10% 96	7		
333	\$327 Q \$\frac{1}{2} 98	22		
1000	974 97	64		

Adult Bird Oservations

Clinical observations of adult birds exhibited no treatment related signs of toxicity. Minor occurrences of feather loss on head/back and skin/head abrasions were observed on birds in the control and all treatment levels as associated with normal laboratory cage wear. One male bird (band no. 154 in the 111 ppm treatment levels was unable to maintain normal body position with its head; no observations indicated it was injury related. Adult observation started from 8 June 2016 to 28 September 2016, last day of adult in-life phase. However, bird no. 154 produced fertile eggs throughout the egg collection phase of the study. There were no significant clinical symptoms or compound related effects observed during the study.



Adult Bird Mortality

Adult mortality occurred during the test with one male bird (band no.148) in the control group and lesions (white spots) on the heart and liver. One female bird (band no. 259) in the 37 ppm treatment level was found to be emaciated with regressed ovaries. One male bird (band no.147) in the ppm level and one female bird (band no.261) in the 333 ppm level had mortality with no abnormal findings during necropsy. The deaths of the four birds in the study appeared to be the result of aggressive behavior from the corresponding pen mates.

Adult Bird Necropsy

Adult birds that died or were euthanized during the course of the study were subjected to gross necropsy. In the control group one female bird (hand no. 232) had observations resulting from pen mate aggression that resulted in euthanizing both pemale and mate pen mates. At the conclusion of the exposure period, all surviving birds were necropsied. Necropsy of the adult birds howed no apparent treatment-related effects.

Adult Bird Body Weight

The adult body weights in the quail reproduction study were measured prior to desing and biweekly up to the long photoperiod phase (i.e. Week 3.5, 7, and 9) and again prior to adult sacrifice. No effects were observed for adult male or female body weight gain in any test levels. The NOEC for the adult weight gain endpoint was determined to be 1000 ppm.

Adult Bird Feed Consumption

Adult bird food consumption was measured weekly during the 23-week adult phase of the study. There were no statistically significant differences for any treatment level as compared to the control for adult bird feed consumption. The NOEC for adult bird feed consumption was determined to be 1000 ppm.

Reproductive Pfects

Data for the egg production endpoints; eggs faid, percent eggs not cracked of laid, and eggshell thickness were evaluated. The embryo endpoints included percent viable embryos of eggs set, percent live embryos of viable embryos. There were no statistically significant differences for any egg production of embryo endpoint at any treatment level as compared to the control. The NOEC for these endpoints was determined to be 1000 ppm for this study.

Hatchling Effects

Data for percent number hatched of eggs set percent number hatched of live embryos, percent 14-day survivors of eggs set percent number 14-day survivors of total hatched, and hatchling body weight were evaluated. No statistically significant effects relative to the control group were observed except for the endpoint 14-day survivor weight. There was a statistically significant reduction for 14-day survivor weight in the 37, 333, and 1000 ppm treatment levels. The historical control range for the number of 16-day survivor hatching body weight endpoint is 29.7 grams to 38.5 grams, based on studies conducted from 2011 to 2015 (n=6). These statistical findings are not considered to be treatment related, as the treatment hatchling survivor body weight range of 32.7 grams to 33.8 grams falls within the historical control range 35.2 grams (\$\frac{1}{2}\$.1 g). Therefore the NOEC for hatchling effects was determined to be 1000 ppm.



14-Day survivor hatchling body weight			
Nominal treatment		Standard	
(ppm)	Mean [g]	Deviation [g]	
Control	35.9 ^b	2.7	
37	33.2ª	2.4	
111	33.7	3.0	
333	33.8ª	2.1	
1000	32.7ª	2.0	

^a Statistically significant difference as compared to control (P < 0.05)

Conclusion:

There were no treatment related effects on parental or offspring parameters in the study. The No Observed Effect Concentration (NOEC) for both parental toxicity and reproduction endpoints of northern bobwhite quail exposed to BCS-CN88460 technical was 1000 ppm (nominal test level) with a measured concentration of 974 ppm or the mean achieved dose of 64 mg as

BCS-CN88466 Technical: A reproduction study with the northern betwhite quail (Colines virginianus) Report: Title:

Report No.: M≈611653-01-1 Document No.:

LEU Dicective 🕬/414/BEC Guideline(s):

Regulation (EC) No. 1107/2009 OCSPP 8865200

OCSPP 850 2300 S ŒCD Ğündeline 206

Guideline deviation(s): not specified 🥾

GLP/GEP:

Objective:

The aim of the study was to evaluate the offects of dietary exposure to BCS-CN88460 technical on the health and reproductive capacit of the Northern Bob white Quail (Colinus virginianus).

Material and Methods:

Adult bobwhite quail colings virginianus were exposed to BCS-CN88460 technical for approximately 23 weeks to nominal obetary levels of 0 (control), 25, 250, and 2500 ppm (mg a.s./kg feed). Bobwhite quait were approximately 24 weeks-old at experimental start with 18 pairs of birds at each treatment level. Birds were observed for mortality, abnormal behavior and signs of toxicity; adult body weight and feed consumption were measured; gross pathology was conducted; reproductive parameters, as well as hatchling health, growth and survival, were examined. The biological portion weted from of the study was conducted from 23 March 2017 to 9 October 2017.

^b The historical control range for the number of 14-day survivor hatchling body weight endpoint is 290 grams to 38.5 prams based on studies conducted from 2011 to 2015 (n=6). Therefore, all levels fail within the historical control range and statistical findings are not considered treatment related.



Document MCA – Section 8: Ecotoxicological studies Isoflucypram

Results:

Dietary Concentration

The nominal amounts of BCS-CN88460 technical in the dietary feed were administered at levels of 0 of toxicity bserv. (control), 25, 250, and 2500 ppm. The average measured amounts of BCS-N88460 technical for Week 1, 5, 10, 15, and 20 were determined as 0, 24, 238, and 2377 ppm representing percent nominal values of 97%, 95%, and 95% respectively. These values correspond to daily dietary dose levels of 2, 18, and 172 mg a.s./kg body weight/day, respectively. A summary of the dietary concentrations is included in the following table.

Feed Analysis Summary of BCS-C\\$88460				
Nominal Dietary	Measured	Percent of	Measured Daily	
Level	Dietary Level	Nominal	Dietary Dose Q Amg a.ṣ̞̞/kg bw/day)	
ppm (mg a.s./kg feed)	ppm (mg a.s./kg feed)	(%)	(pang a.s./kg bw/day) ∌	
0 (control)	0 %			
25	24	₩97 (Ĉ		
250	238	95	<u>~</u>	
2500	2377	y 95 ^y .	₹ 6 ³ 174, ₹	

Adult Bird Observations

Clinical observations of adult birds exhibited no treatment related signs of toxicity. occurrences of feather loss on head/back as well as skin and head abraspons were observed on birds in the control and all treatment levels as associated with formal laboratory cage wear. One male bird (band no. 726) in the 25 ppm treatment level was unable to maintain nothinal body position with its head; no observations indicated it was injured related. Adult observation started from 29 June 2017 to 3 July 2017. Abrasions were observed on both feet during darry observation check. However, there were no significant sinical symptoms of compound related effects observed during the study.

Adult Bird Morbality 2

Adult mortality occurred during the test with one female bird (band to . 969) in the control group, two birds (female band no. 991 and male band no. 926) in the 25 ppm Level, and one female bird (band no. 938) in the 250 ppm devel. The deaths of the four birds in the study appeared to be the result of aggressive behavior from the corresponding pen mates.

Adult Bird Necropsy

Adult birds that died or were sufficient during the course of the study were subjected to gross necropsy. At the conclusion of the exposure period, all surviving birds were necropsied. Necropsy of the adult Dirds showed no apparent treatment-related effects. There was one pair (band no. 706 and 906) in the 25 ppm treatment level that mistakent contained two female birds; one had the phenotype of a male (band no. 706). The bird with band number 706 was a female with regressed ovaries.

The adult body weights in the quail reproduction study were measured prior to dosing and biweekly up to the long photoperiod phase one. Week 3, 5, 7, and 9) and again prior to adult sacrifice. No effects were observed for adult male or female body weight gain in any test levels. The NOEC for the adult weight gain spropoint was determined to be 2500 ppm.

Adult Bird Feed Consumption

Adult bird food consumption was measured weekly during the 23-week adult phase of the study. There were no statistically significant differences for any treatment level as compared to the control



for adult bird feed consumption. The NOEC for adult bird feed consumption was determined to be 2500 ppm.

Reproductive Effects

Data for the egg production endpoints are; eggs laid, percent eggs not cracked of laid, and eggshell thickness were evaluated. The embryo endpoints include; percent viable embryos of eggs set, percent live embryos of eggs set, and percent live embryos of viable embryos. There were no statistically significant differences for any egg production or embryo endpoints at any treatment leve as compared to the control. The NOEC for these endpoints was determined to be 2500 ppm for this study

Hatchling Effects

Data for percent number hatched of eggs set, percent number hatched of live embryos, percent 14 day survivors of eggs set, percent number 14-day survivors of total hatched, and hatchling body weight were evaluated. Statistically significant differences felative to the control were not observed for any treatment level or endpoint except percent number 14-day survivors of total hatche Dendpont. There was a statistically significant difference at all treatment levels for the percent number of 14 day survivors of total hatched. The historical control range for the number of 14-day survivors of eggs hatched endpoint is 93.8% to 99.5% based on studies conducted from 2014 through 2016 (n=7). An effect for this endpoint was not observed in the initial quail reproduction study, Study ID 044SRLS14C01), which was conducted on 21 April 2016 to November 2016. This statistical finding is not considered to be treatment related, as the preatment hat Alling survival range of 98.07% to 99.02% falls within the historical control range, the inhibition observed in this study did not follow a dose-response trend, and the associated inhibition compared to the control group is 2.0%. Therefore,

	Percent 14-days	urvivors of tot			
	Nominal treatment		Standard &		
	(ppm) 🏂 ,	Mean 1%]	Deviation [g]		
	Control 💆 为	99.1 🐇	, 0.019 &		
	25 👸	Ø8.0a ○	0.020		
ſ	250	**97.1%	<u> 1</u> 0.029° 7		
ſ	2 \$00	97.99	0.023		

Standard

Deviation [g]

99.1

0.029

250

97.1

0.029

250

250

97.1

0.029

250

Conclusion:

There were no treatment related effects on parer

Observed Effect Concentration (NOFC)

Forthern bobwhite quail exposed

neasured concentration

Owest Observed

Owest Observ There were no treatment related effects on parental or offspring parameters in the study. The No Observed Effect Concentration (NOEC) For both parental toxicity and reproductive endpoints of northern bobwhite quail exposed to BCSoCN88460 teconical was 2500 ppm (nominal test level) with a measured concentration of 23% ppm or the mean achieved dose of 174 mg a.s./kg bw/day. The measured concentration of 23 ppm for the mean achieved dose of 174 mg a.s./kg bw/day. The Lowest Observed Effect Concentration (LOEC) was >2500 mg a.s./kg food or >174 mg a.s./kg bw/day.



CA 8.1.2 Effects on terrestrial vertebrates other than birds

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

CA 8.1.2.1 Acute oral toxicity to mammals

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

Test species	Test design	Ecotoxicological	Endpoint	Reference	. W	2 2
Rat	Acute, oral	LD ₅₀ , male, female	> 2000 ms a.s./kg bw	KCA 3.2.1/01	4	\$\$872 <u>-</u> 07-

Long-term and reproduction toxicity to mammals **CA 8.1.2.2**

Endpoint derivation for the chronic visk assessment in mammals

This evaluation analyses the toxicity data available for isofluctoram (PCS-CN88460) under the aspect of relevance of findings for wild manufals long-term risk assessment. Is pecially findings related to the actual survival and the approductive performance are important for an ecotoxicological risk assessment. The 2-generation reproduction rat study is considered most relevant for the purpose, but other studies (the 90-day subchromic rat study and the developmental toxicity studies) were evaluated

The toxicological effects seep with BCS-CN88469 in a 90-day at study, in a rat reproduction study

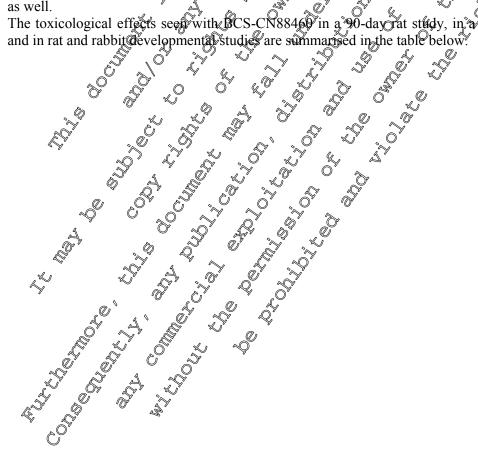




Table 8.1.2.2-1: Summary of subchronic, reproduction and developmental toxicity studies with Isoflucypram

130nacyprami Q					
Study type species dose levels tested	Overall NOAEL	Findings at Lowest Effect Level	Ecotox NOAEL	Ecotox relexant	Reference
90 day	300 ppm	1000 ppm: bw in both	1000 ppm	none	; 2017;
Wistar rat		sexes √, bilirubin	(63.5	<u>.</u>	M-487478-02-1
		concentration in both sexes	mg/kg	y	KCA 5.3.2/01
0 – 100 – 300 - 1000		 ↓ ,	bw/day		
ррт		liver: organ weight in	bw/day	4	
		females 1		Z ^O	
		kidney: hyaling droplets in	v . W	Q" (
		males		Ø 3	KCA 5:3 2/01
		thyroid stand: missimal follicular cell typertrophy			A. A
		10 noticular censoperitorny	Q , O	₽	
2-generation	1200 ppm	1200 ppin	12 0 0 ppm	none	, R
reproduction	(Wwer: Organ weight in Doth	92.9		20 18;
Wistar rat	Į Ž	SCACSO I	mg/kg bw/day)		M-612950-02-1 KČX 5.6.1/01
0 - 150 - 450 - 1200	w v		Dwygray)		KCA 5.6.1/01
ppm	Ţ,			O *	O
	6 - 0		128		
developmental rat	Maternal and	625 mg/kg mean food	· ~ //	delayed	<u>, P.;</u>
Sprague Dawley rat	fetal toxicity:	consumption between OD	ong/kg	ossoncat	2017; M-602126-01-1
0-25-125-625	325 m@kg 4 bw/day	6-8 , bilirubin 5 concentration and spean 0	©w/day√	ion	KCA 5.6.2/01
mg/kg bw/day	bw/may 5	alkaline phospharase	4 .	<i>y</i>	110110.0.2/01
mg/kg bw/day		activity Ψ ,			
		liver organ weight			
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~		delayed ossification			
developmental	Naternal '	500 mg/kg; one abortion,	©0 mg/kg	abortion	, I.;
rabbit	toxigity: 70 💝	me@y bw ₩, food 📞 🗳	bw/day		<u>2017;</u>
New Zealand White	mg/kg bw@ay	consumption √, ° >			M-588469-01-1
@,		<u>Tiver:</u> organ werght			KCA 5.6.2/02
0 - 10 - 70 $00$	development?				
mg/kg bw/day	500mg/kg				
O v	by/day				

V: decrease; ↑: increase:

bw = body weight

Bold: Endpoint used in risk assessment

In the 90 d subchronic study in rate some findings were reported at the 1000 ppm dose level, as a decrease in body weight a lower biliration concentration or findings of hyaline droplets in males. Bilirubin concentration and byaline droplets are not considered relevant parameters in an ecotoxicological context. Body weight differences between treatment and control animals were not statistically significant at the end of the recovery phase. Also, treatment-related changes noted at the clinical chemistry determinations and urinalysis were reversible and the differences from controls were not seen at the end of the recovery phase. The ecotoxicologically relevant endpoint in this study is taked as 1000 ppm (63.5 mg/kg bw/day). This value represents the highest dose tested.

The developmental rat study revealed some findings at 625 mg/kg/day, representing the highest dose tested. These were lower food consumption between GD 6-8 (GD = gestation day), lower mean



bilirubin concentration and phosphatase activity as well as a higher liver weight. Furthermore, fetal evaluation revealed a delayed ossification. As such, the ecotoxicologically relevant endpoint (NOAEL) is considered as 125 mg/kg bw/day.

In the developmental rabbit study maternal effects could be observed at the highest dose rate of 500 mg/kg/day (decrease in body-weight, mean food consumption and an increase liver weight. Due to the fact that at least one abortion was observed (indirectly related to treatment due to the poor health status of the animal) the relevant endpoint in this study might be considered as the next lower dose, 70 mg/kg bw/day. Note that the spacing between the two highest doses is quite high (next higher dose 500 mg/kg bw/day) and the magnitude of the effects at the highest dose is put to the higher dose.

The 2-generation rat study comprises the more comprehensive battery of test parameters as in the other study types described above and it involves both sexes. Thus, the wild mammal reproduction endpoint for isoflucypram should be based on this study. In this study the relevant NOAEL was established at a dose of 92.9 mg/kg bw/day, representing the highest dose tested i.e. no significant effects were observed). This NOAEL is well within the range of the other endpoints. Concluding, the endpoint of 92.9 mg/kg bw/day derived from the 2-generation study will be used for the risk assessment. This endpoint supersedes the 70 mg/kg bw/day of the developmental rabbit study as it follows a more appropriate study design (again, not that only low effects were found at the bighest dose rate of 500 mg/kg bw/day).

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

Substances with a high bioaccumulation potential could theoretically bear a risk of secondary poisoning for birds and mammals if feeding or contaminated prey like fish or earthworms. For organic chemicals, a log  $P_{OW}$  is used to trigger an in-depth evaluation of the potential for bioaccumulation. As the log  $P_{OW}$  of the active substance is flucy pram is above the trigger, an evaluation of secondary poisoning is conducted for the evaluation please refer to the Summary MCP Section 10, Point 10.1.1.2 of the product dossier.

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# CA 8.1.4 Effects of terrestrial vertebrate wildlife birds, mammals, reptiles and amphibians).

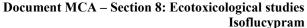
Risk to birds and mannals is a sessed in Document MCP, Section 10.1.

### Amphibians and Reptiles

Currently no test methods are established to assess the population relevant effects of chemicals to amphibians or reptiles. Since isoflucyprant is of the toward to be expected.

# CA 8.1.5 Fredoctine disrupting properties

There are no indications of endocrine-disrupting effects from the existing database for isoflucypram. All of the reports discussed in the following review are fully summarized under the appropriate data point within this dossier.





**Report:** KCA 8.1.5/01; L.; 2018; M-613376-01-1

Title: Evaluation of isoflucypram with regard to endocrine disrupting properties in non-

target vertebrates

Report No.: M-613376-01-1 Document No.: M-613376-01-1

Guideline(s): none
Guideline deviation(s): -GLP/GEP: no

# Summary of the assessment of endocrine-disrupting properties of isoflucypram in non-target vertebrates

From comprehensive toxicological investigations in mammals, isoflucypram does not raise concerns with regard to endocrine-related effects related to the EATS modalities, there is no exidence for direct effects in mammals, and indirect endocrine (thyroid)-related effects are not of concern for wild mammals. Because it has been demonstrated that the EATS pathways are highly conserved across vertebrates, it can be reasonably considered that in a first approach a simple testing strategy to confirm the absence of ED-related concern in non-target vertebrates other tran mammals would be sufficient, as long as an assessment of potential thyroid-mediated effects is included. This was addressed by including the results of a FELS study in the assessment strategy of potential endocrine disrupting properties of isoflucyprated. The PELS test with isoflucyprate was negative with regard to potential thyroid-mediated effects giving a first insight to the absence of ED-related concern in non-mammalian vertebrates.

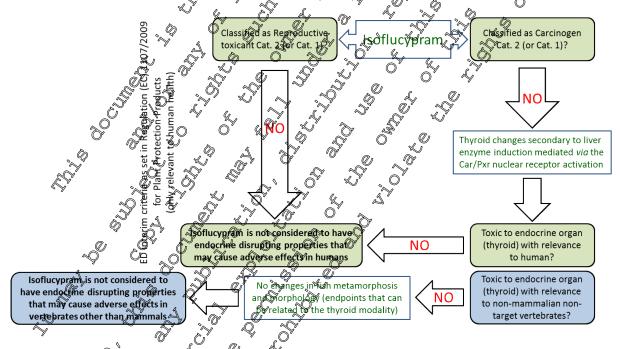


Figure 1: Decision the applied to the assessment of endocrine-disrupting properties of isoflucypram. The upper part (green cells) is based on the interim critical as decumentary by toxicological investigation in mammals; it therefore applies to wild mammals. The lower part (blue cells) concerns non-mammal vertebrates (i.e. wh) where the thyroid modality was investigated. Adapted from the document "Screening of dividiable endence of chemical substances for the identification of endocrine disruptors according to different options in the context of administration of endocrine disruptors according to different options in the context of administration of endocrine disruptors according to different options in the

Considering all available information from a comprehensive toxicological database, we do not find indications for endocrine disrupting properties through oestrogen, androgen, thyroid or steroidogenesis mode of action for isoflucypram. Also the available ecotoxicological studies did not contradict this conclusion and isoflucypram can be regarded as having no endocrine disrupting properties.



### **CA 8.2** Effects on aquatic organisms

Γable 8.2- 1:	Endpoints used in ris	k assessment and studies for isoflucy	pram
Test substance	Test species	Endpoint	Reference
	Fish, acute Pimephales promelas	96 h LC ₅₀ 0.081 mg a.s./L (nom) ^A	; 2018; 542897-02-1 KCA 8.2.1/0
	Fish, acute Oncorhynchus mykiss	96 h LC ₅₀ 0.098 mg a.s./L (pcm) ^A	0015; S M-54343 201-1 Q KCA & 2.1/02
	Fish, acute Cyprinodon variegatus	96 h LC ₅ (mfa)	2095; M-Q7137-Q1-1 QCA 821/03
	Fish, acute Pimephales promelas, Oncorhynchus mykiss, Cyprinodon variegatus	96 r LC ₅₀ 0.1628 mg(a.s./L ^B	Geometric mean acc to new aquatic guidance document (EFSA Journal 2013; E(7):3290)
	Fish, chronic (ELS)  Pimephales promelas	332 NOF (mom) 335./L (mom)	; 2017; MS80245-01-1 KCA 80:2.1/01/
	Fish, chronic (ELS)  Cyprinidon variegatus	35 NOE (0.0250ng a.s./L) (mm)	; ; ; ; ; ; ; ; ; ; ; ; ; ; ; ; ; ; ;
	Fish, chronic (EPS)  Pimerhales promelas  Cypinodor ariegans	350 NOEC 0.0197 mg &s./L ^B	Geometric mean acc. to new aquatic guidance document (ZFSA Journal 2013;11(7):3290)
Isoflu- cypram	Fish, BCF flow through Lepomis macrochirus	370 (kinetic BEF BCF lipic normalized and growth corrected)	; R.; 2017; M-610008-01-1 KCA 8.2.2.3/01
	Invertebrate, acute Daphaga magna	48 b C ₅₀ (gmto)	; 2016; M-574184-01-1 KCA 8.2.4.1/01
4	Invertebote, acute Americamysis Gahia	96 h EC ₅₀ (mm)	; ; 2016; M-547041-01-1 KCA 8.2.4.2/01
	Invertebrate, acute Daphaid magath, O Americamy & bahia	EC 0.233 mg a.s./L ^B	Geometric mean acc. to new aquatic guidance document (EFSA Journal 2013;11(7):3290)
Ğ	onvertebrate, chronic Daphnia magna	21 & EC ₁₀ 0.0661 mg a.s./L (mm)	; 2017; M-593961-01-1 KCA 8.2.5.1/01
	Thverte Frate, chronic Americamy & bahia	28 d NOEC 0.079 mg a.s./L (mm)	; ; ; ; ; ; ; ; ; ; ; ; ; ; ; ; ; ; ;
Ü	Invertebrate chronic  Leptocheirus plumulosus	28 d NOEC 11 mg a.s./kg (mm)	; 2017; M-601773-01-1 KCA 8.2.5.2/02



Test substance	Test species	]	Endpoint	Reference
	Invertebrate, chronic Crassostrea virginica	96 h EC ₅₀	0.170 mg a.s./L (mm)	; ; ; 2016 M-547035-01-1 KCA \$2.5.2/03
	Invertebrate, chronic Hyalella azteca	35 d NOEC	95 mg a.s./kg (mm) 44 mg a.s./kg (mm) 95 mg a.s./kg (mm)	; 2017; M <u>*</u> 585874-02-1 MCA 8.2.5.2/04
	Sediment dweller Chironomus dilutus		85 mg a.s./kg (mm) > 84 mg a.s./kg (mm)	M-596883 971-1 KCA 8, 25.4/01
	Green algae, Pseudokirchneriella subcapitata	<b>2</b> 0	2.02 mg a.s./L (gmm) > 2.02 mg a.s./L (gmm) > 2.02 mg a.s./L (gmm) > 2.02 mg a.s./L	; 2067; M-586715-01-1 KOA 8.20.1/01
	Blue green algae, Anabaena flos-aquae		A.8 mga s./L (gmm) (gmm) 3.4ang a s.l. (genm)	J. R.; J. R.; 2017; M&05074401-1 KCA & 6.2/0
	Marine diatom, Skeletonema costatom	7211-EbC	3.2 mg a.s./t./ (grim) 2.2 mg a.g./t./ (gmm)	J. R.; J. &; , K. H.; 2017; 14:604841-01-1 KCA 8.2.6.2/02
	Freshwater tijatom, Navicula pelliculosa	72 h-E _y 😘	> 2.0 mg a.s./L (grown) • 2.0 mg a.s./L (grown)	, J. R., J. W.; K. H.; 2017; 69-604809-01-1 KCA 8.2.6.2/03
	Aquate macrophyte	7,4-F. _r C ₅₀	> 3.02 mg f/s./L (grinn)	; 2017; 34-593965-01-1 KCA 8.2.7/01
BCS-	Fish, a stee		> 33\\$ mg psn./L \( (gmm) \)	; 2017; M-587655-01-1 KCA 8.2.1/04
CN88460- carboxylic acid (M12)	Invertebrate, acute Daphija magna	48 h & C 50	> 24 mg p.m. (nom)	; 2016; M-573296-01-1 KCA 8.2.4.1/02
. , ,	Green algás, S Pseudolóchneriália Subcapitata O	92h-E36%0	35.1 mg p.m./L (gmm)	; 2017; M-587659-01-1 KCA 8.2.6.1/02

Bold: endpoints used in risk assessment

Nom = nonwral concentrations, mm mean recasured concentration, gmm = geometric mean measured concentration ^A Endpoint corrected for purity. In study report uncorrected values are cited.

# Selection of endpoints for Fier 2 risk assessments with the active substance

The tier 2 decording to the Guidance of tiered risk assessment for edge of field surface waters (EFSA, 2013, is divided into tier 2A, tier 2B and tier 2C.

In case of isologypram the data requirements for Europe were exceeded. More species than required by underlying legislations were tested, to satisfy requirements outside the EU. If more species are investigated the data might allow the use of tier 2A, the geomean approach or the use of tier 2B, the Species Sénsitivity Distribution (SSD).

B Endpoint based on geometric mean of the given of evant endpoints of acute or chronic studies with the active substance. Detailed information given below under 'Selection of Edpoints for Tier 2 risk assessments with the active substance'



For the tier 2B the amount of data is not sufficient. The minimum number of species for fish (5) or for invertebrates (8) is not reached. For a fungicide as well an overall SSD might be considered. But here the data are not optimal as some of the existing values are unbound values. Therefore the tier 2B was not used. As the data were not sufficient for a robust tier 2B approach but exceeding the data requirements the tier 2A approach was chosen for the higher tier risk assessment.

### Fish acute

If more species are tested as required according to the data requirements land down in "COMMISSION REGULATION (EU) No 283/2013 of 1 March 2013" than it might be appropriate to use tier 2A. In case of isoflucypram the data requirements were exceeded for acute tish testing. Data for the different fish species: the rainbow trout (Oncorhynchus mykiss), the fathead minnow (Pimephales promelas) and the sheepshead minnow (Oppring acute tish testing).

The available data are not sufficient to use tier 21 the Species Sensitivity Distribution but allow the use of tier 2A, the geomean assessment factor approach.

If the 96h LC₅₀ values for the three species, based on the scal content of the scrive ingredient are chosen, the following is observed:

Table 8.2-2: Geometric mean for fish acute

Species	Species (Scientific name) 96 h LC 95 Confidence limits
Rainbow trout	Oncorhynchus mytgiss & 0 0074 - 0.131
Fathead minnow	Pimepholes promelas © \$\times \tag{Q081} \times \tag{V} \tag{V} \tag{068 \tag{0.093}}
Sheepshead minnow	Cypringdon variegans . 0.544° & 0.472°0.626
Geometric mean:	- 0 0.163

For the taxonomic group of fish the data requirements (the species) were exceeded. Data for three species are available. Therefore the geomean  $96 \, \text{kg} \cdot \text{C}_{50}$  for these three species was calculated.

Before this value can be used it has to be checked whether the geometric mean approach has been biased by introducing insensitive species. According to the Guidance on tiered risk assessment for edge of field surface voters (EFSA 2013) on assessment of this has to be made when the difference in sensitivity exceeds for 2 orders of magnitude. In case of fish chactor of 100 should not be exceeded.

The highest and the lowest 96 of LC derived for fish and isoflucypram differ by a factor of 6.7. Therefore the use of the geometrian approach for the tish across risk assessment is appropriate.

In addition the chronic fish data can be used to check the appropriateness of the tier 2A related Regulatory Acceptable Concentration (RAC) for acute fish of 0.001628 mg a.s./L. This RAC is a factor of 9.6 below the lowest observed chronic NOEC for ash, resulting from a Fish Early Life Stage (FELS) test with fathers minnow. The acute RAC based on tier 2A for fish is even a factor of 12.1 below the tied 2a chronic fish value based on the two existing FELS studies for fathead minnow and sheepshead minnow.

This demonstrates that the ties 2A fish acute 96 h LC₅₀ of 0.1628 mg a.s./L and the resulting tier 2A RAC of 0.00163 mg a.s./L is protective and can therefore be used within the aquatic risk assessment of isoflucypram.



### Crustacean acute

If more species are tested as required according to the data requirements laid down in "COMMISSION REGULATION (EU) No 283/2013 of 1 March 2013" it might be appropriate toguse tier 2A. In case of isoflucypram the data requirements were exceeded for acute testing of invertebrates, especially crustaceans. Data for two different crustacean species, the waterflea (Daphnia magna) and the mysid shrimp (Americamysis bahia) are provided. The available data are not sufficient to use the tier 2B, the Species Sensitivity Distribution but allow the use of the tier 2A, the geomean assessment factor approach.

The following EC₅₀ values (based on the real content of the active ingredient) for the available:

Table 8.2-3: Geometric mean for crustacean acute

Species	Species (Scientific name)	。 EC560°	95% confidence limits ing a.s./L]
Water flea	Daphnia magna 🙎		
Mysid shrimp	Americamysis bahia 🥍 🛚 🐾	0.270 (26h)	3 6230 - 0.420
Geometric mean:	- 4	0.239	

For the taxonomic group of crustageans the data requirements (one species) were exceeded Data for two species are available. Therefore the geomean EC5 for these two species was calculated.

Before this value can be used it has to be checked whether the grometor mean approach has been biased by introducing insensitive species. According to the Guadance on tiered risk assessment for edge of field surface waters (EFSA, 2013) this has to be assessed when the difference in sensitivity exceeds 2 orders of magnitude. In case of crustoceans a factor of 100 should not be exceeded.

The highest and the lowest EC derived for crusta cans in case of isoflucyprand differ by a factor of 1.34. Therefore the use of the geomean approach for the invertebrate (here crustacea) acute risk assessment, is appropriate.

In addition the chonic costacean data can be used to check the appropriateness of the tier 2A related regulatory acceptable concentration (RAC) for active crustaceaus of 6.00233 mg/L. This RAC is a factor of 33.9 below the lowest chronic NOEC for crustaceans which was observed for Americanysis bahia.

This demonstrates that the tier A for Frustaceans acute based on FC50 values for Daphnia magna and Americanysis bahia of 0.23 and the resulting the 2A RAC of 0.00233 mg/L is protective and can therefore be used within the aquatic risk assessment of soflucy pram.

### Fish chronic

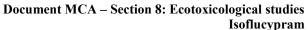
Fish chronic

If more species are tested as required according to the data requirements laid down in "COMMISSION REGULATION (EU) No 28\$\frac{1}{2}\text{013}\text{of 1}\text{ March 2013" than it might be appropriate to use tier 2. In case of isoflucy from the data requirements were exceeded for chronic fish testing.

Two Fish Early Life Stage (FELS) Tests are available. Studies for the two species fathead minnow (Pimerhales prometas) and sheep nead Minnow (Cyprinodon variegatus) are available.

As two and not only one FELS Test are available the data requirements are exceeded and the use of the tier 2A, the geomean assessment factor approach, might be appropriate.

Species Species	Species (Scientific name)	NOEC [mg a.s./L]
Fathead Winnow	Pimephales promelas	0.0156 (33d)
Sheepshead minnow	, Cypp Godon variegtaus	0.025 (35d)
Geometric mean:	- 🎾	0.0197





For the taxonomic group of fish the data requirements (one species) were exceeded. Data for two species are available. Therefore the NOEC for these two species was calculated.

Before the tier 2A approach can be used it has to be checked whether the geometric mean approach has been biased by introducing insensitive species. According to the Guidance on tiered risk assessment for edge of field surface waters (EFSA, 2013) this has to be assessed when the difference in sensitivity exceeds 1 order of magnitude. In case of chronic fish a factor of 10 should not be exceeded. The NOEC values of the two available FELS studies differ to a factor of 1.6 only. Therefore the use of the geomean approach for the chronic risk assessment for fish, is appropriate.

An additional possibility to check the protectiveness of the tier 2A is the comparison with the existing NOEC and LOEC values derived from the two chronic fish studies available. The observed tier 2A value of 0.0197 mg a.s./L is clearly below the LOEC of 0.050 mg a.s./L observed in the FELS test with the fathead minnow and is even below the NOEC of 0.025 mg a.s./L observed in the FELS test with the sheepshead minnow.

Not only the numerical numbers (NOEC and LOEC) need to be checked, it is important for the acceptability check of the geomean approach to prove it similar type of effects were observed in the studies. In case of isoflucypram the most sensitive endpoint in both FELS tests was larval survival.

Summarizing it can be stated that the Oronic tier 2A for fish and isoflucionam is reasonable as

- The difference between the NOEC values observed in the two studies is very low. The NOEC values differ by a factor of 1.6 only.
- The observed geomean NOEC is below any adverse effect observed in both studies.
- The two FELS tests do not differ with respect to the most sensitive endpoint

In the case of isoflucypram a chronic geomean approach for fish therefore seems to be reasonable.

# 

For the reason of planned global registration more than the European data requirements have to be fulfilled. Especially for the United States additional studies are needed for a submission. These studies need to cover (among others) as well additional studies with fish. As these data exist they have to be part of the submitted dossier and therefore have been submitted by the notifier for regulatory review.

**Report:** 268; M-52897-02-1

Title: Amendment no. 1. BCS-CN 88460 (tech.) - Acute toxicity to fish (Pimephales

promelan under Static canditions

Report No.: EBLN 356 0 Document No.: EBLN 356 0 M-542897.02-1

Guideline(s): EPA-FIFRA § 720/SEP-EPA-540/9-85-006 (1982/1985); OCSPP 850.1075 (Public

Draft, 1996); Council Regulation (EC) No 440/2008, C.1 (2008); OECD No. 203

(rev. 1992); JMAFF, 12 Nousan No. 8147 (2000); US EPA OCSPP 850.1075

Guideline deviation(s) Yes But acceptable

### Material and methods

GLP/GEP:

Test material	BCS-CN88460 techn. Batch BCS-CN88460-01-06; Origin batch 2013-006492; Specification number: 10200028196 purity: 94.2% w/w
Guideline(s) adaptation	None specified



Test species	Fathead minnow (Pimephales promelas)
Acclimation	More than 14 days Less than 5 % mortality was noted during the acclimatization period prior to the test initiation
Organism age/size at study initiation	Mean length: $2.5 \text{ cm} \pm 0.2 \text{ cm}$ (Mean $\pm \text{SD}$ ) Mean body weight: $0.2 \text{ g} \pm 0.1 \text{ g}$ (Mean $\pm \text{SD}$ )
Test solutions	Nominal concentrations: 0.0251, 0.002, 0.101, 0.201 and 0.401 mg a.s.1.  Nominal concentrations were corrected for purity Nominal values, which were not corrected for purity are stated in the study report.  Arithmetic mean measured concentrations: 0.0249, 0.0494, 0.0924, 0.215 and 0.4055 mg a.s./L  Samples were taken from all test chamber on day 0, day 2 and day 4.  Controls: reconstituted water  Solvent control: 0.1 ml/L dimethylformamide  Evidence of undissolved material: No precipitates during exposure were observed.
Replication	No. of vessels per control (replicates): 1  No. of vessels per solvent control (replicates): 1  No. of vessels per solvent control (replicates): 1  No. of organisms per vessel: 10
Organisms per replicate	No. of organisms per vessel: 100 100 100 100 100 100 100 100 100 10
Exposure	Static  Total exposure deration. 96 hours
Test Vessel Loading	G.050g fish/E fest modium
Feeding during test	None O O 4 40 47 5 5 5
Test conditions	Temperature: 20 2 – 22.0°C  Photoperiod: 16 hours light & hours dark  OH: 7.0 – 7.2  Dissolved oxygen saturation: 94 – 1126  Handness 40 – 60 mg CaCO 4
Parameters Measured Observations	Observations of mortality and signs of poisoning were made at 4, 24, 48, 72, 96-hours.  Discrete measurements of temperature, dissolved oxygen and pH were obtained at test initiation, 24, 48, 72, and 96 hours.
Chemical analysis	Analytical determination of test substance concentration (active ingredient) was performed with samples collected from each replicate test vessel after 0 hours, and after day 2 and day 4. They were analysed using HPLC-MS/MS
Data analysis	Depending on the suitability of the data set, LC ₅₀ values and the 95%-confidence intervals were calculated for each 24 hour interval using computer software FoxRat, which estimated the LC ₅₀ using one of three statistical techniques: moving average, logit analysis or Weibull analysis. The LC ₅₀ was determined by Weibull analysis.



Validity criteria	Required	Obtained
Mortality in control during test	<u>≤</u> 10%	0 %
Dissolved oxygen saturation	≥ 60%	94 - 112 %

Analytical results Measured conce	s: ntrations were 81 to	109% of nominal values and were stable throughout the test. based on nominal concentrations  **Day0** Day2** Day 4**  109** 97** 93** 103** 98** 99**
Therefore, the re-	sults of this study are	based on nominal concentrations
Nominal	Arithmetic mean	% of arominal concentrations Q O O
Concentration	measured concentration	Dawn Bary Day
(mg a.s./L)*	(mg a.s./L)	
0.0251	0.0249	109 6 97 93 93 95 103 7 98 1 99 7 6 97 6 97 6 97 6 97 6 97 6 97 6
0.0502	0.0494	103 98 99
0.101	0.0924	
0.201	0.2155** ©	¥ \$\\\ \delta \text{9}  \text{\$\sqrt{106} \text{\$\sqrt{0}}  \text{\$\sqrt{2}  \text{\$\sqrt{2} \q
0.401	0.4055** Q	
* Values corrected fo **Mean measured co	or purity (94.2%). Nomina oncentration (Dage, 2)	
Biological results	<u>s:</u>	
Observations		

Observations
At 4-hours, several sub-lethal effects were observed at 10 of 10 fish; in the two highest treatments with 0.201 and 0.401 mg a.s./L. After 24 hours several sublethal effects were observed in the next lower concentration with 0.101 mg a.s./L.

# Mortality 4

1.101 000110	A . A	~ _4(())·	A		
Exposure time (hours)	<b>4</b>	24 5	48	72	96
Nominal conc.	No of dead	No of dead	No of dead	No of dead	No of dead
(mg a.s./L)*		(%) (%)	<b>%</b> )	(%)	(%)
Control	\$\text{0}(0)\text{0}	~~0 (0)~°		0 (0)	0 (0)
Solvent Control	0 ( )		\$ 9 <b>@</b> )	0 (0)	0 (0)
0.0291	<b>(</b> 0)		(0)	0 (0)	0 (0)
₄ 0,0502	<b>₹</b> 0 (0) <del>\$</del>	0 (00° ×	0 (0)	0 (0)	0 (0)
0.101	000	2 (20)	6 (60)	9 (90)	9 (90)
0.201	10(0) Ø	(100)Q"	10 (100)	10 (100)	10 (100)
0.401	J 0 (QQ)	10 (160)	10 (100)	10 (100)	10 (100)

purity 34.2% Nominal values, which were not corrected for purity are stated in study report.



# Conclusion

	Isoflucypram
Conclusion The study meets the variable.	alidity criteria and the endpoints based nominal are:
LC ₅₀ 96 hours (95% C	0.081 mg a.s. / L* (0.068 – 0.093 mg a.s./L)
LOEC: lowest concentration with compared to the control	
NOEC: highest concentration weeffect compared to the o	rithout an significant 0.0502 mg a.s./L*
Values corrected for purit	y (94.2%). Values not stated in study Coport.
Report:	KCA 8.2.1/02; 2015; M-54344201-1, 7
Γitle:	BCS-CN88460 (tech.) - Acute to ncity to fish (Oncorhypchus mykiss) under static
Report No.:	EBLNN024
Document No.:	M-543443-01-1
Guideline(s):	EU Directive 9 14/14/Ed C S S S S S S S S S S S S S S S S S S
	US EPA OCSPP 850/1075
	EPA-FIFR \$ 726)/SEP-6PA-540/9-85-006 (1982/19850)
	OCSPP \$50.1075 (Public Draft 1996) Council Regulation (ES) No 440/2008, C.1
	(2008) OECD No. 200 (rev. 1992) JMAFF, 12 Nous to No. 8147 (2000)1
Guideline deviation(s):	none of the contract of the co
GLP/GEP:	
5	

^{*} Values corrected for purity (94.2%). Values not stated in study port.

# Material and methods

Test material	BCS-QN88460 techn.
8	Specification number: 10200028196 Specification number: 10200028196 Specification number: 2013-000492;
~ \$	purity 94.2% w/w
Guide (s) adaptation	None specified State of State
Test species	Rainbow trout (Oncornynchus mykiss)
Acclimation	More than 4 days
	More than 4 days Compared than 5 % mortality was poted during the acclimatization period prior to the test initiation Compared
Organism	Mean length: 4.2 * 0.6 cm (Mean ± SD)
age size at	Mean body weight: 0.0 ± 0.4% (Mean ± SD)
study initiation	
Test solutions	Nominal Concentration 9. 0.0400, 0.0801, 0.160, 0.320 and 0.641 mg a.s./L
Test solutions	Nominal concentrations were corrected for purity. Nominal values, which were not corrected for purity are stated in the study report.
	Corresponding arithmetic mean measured concentrations: 0.0397, 0.0760, 0.190,
	0.359 and 0.726 mg a.s./L.
	Controls: reconstituted water
V	Solvent control. 0.1 mi/L dimethyl formamide
	Evidence of undissolved material: No precipitates during exposure were observed.
Replication	No. of vessels per concentration (replicates): 1



	No. of vessels per control (replicates): 1 No. of vessels per solvent control (replicates): 1
Organisms per replicate	No. of organisms per vessel: 10
Exposure	Static Total exposure duration: 96 hours  0.20 g fish/L test medium
Test Vessel Loading	0.20 g fish/L test medium
Feeding during test	None V O O O
Test conditions	Temperature: 13.0 – 13.5°C Photoperiod: 16 hours light /8 hours dark Light intensity: 751 - 822 lux pH: 6.9 – 7.2. Dissolved oxygen: 91 – 402% Hardness: 40 – 60 mg/caCO/L Observations of mortality and signs of porsoning were shade at 4, 24248, 72 and 96 hours.  Discourage of the control of
Parameters Measured / Observations	Observations of mortality and signs of porsoning were made at 4, 24048, 72 and 96 hours.  Discrete measurements of temperature, dissolved oxygen and pH-value were obtained at test initiation and at 24048, 72 and 96 hours.
Chemical analysis	Analytical determination of test substance concentration (active ingredient) was performed with samples colleged from each replicate test vessel after 0 hours, and after day 2 and day 4. They were analysed using HPLC MS/MS.
Data analysis	Depending on the suitability of the dataset, LC, valves and the 95 % confidence intervals were calculated for each 24-hour interval using computer software ToxRat Pro Version 2.10, which estimated the LC4 using Weibull analysis.

Validity criteria			Required		Obtained
Mortality in control	during tes	¥ _%	[∞] ≤10%	& i	0 %
Dissolved oxygen	aturaţion		£60%	0.	91 - 102 %

Recoveries were between 81 and 124% (see table below). The analytical results confirm a correct dosing and the stability of the lest item within the nominal range of 80 to 120% (with the exception of one value slightly above 120%). Therefore all results of this study are based on nominal concentrations.

Nominal Concentration	Atithmette mean measured concentrations*	% (	of nominal	concentra	tions
(mg a.s./L	(mg/a.s./L)	Day 0	Day 1	Day 2	Day 4
0.0400	<u>4</u> • 0.0397	115	-	98	84
0.0861	0.0760	112	-	92	81
0.460	0.190	113	124	-	-
0.320	0.359	112	-	-	-
0.641	0.726	113	-	-	-

^{*} Values corrected for purity (94.2%). Nominal values, which were not corrected for purity are stated in study report.

^{**} Mean values given under Day 0, Day 1, Day 2 and Day 4



# **Biological results:**

## **Observations**

In the controls (negative and solvent) no mortalities or sub-lethal effects were observed within the whole test period. At 0.160 mg a.s./L severe sub-lethal effects were observed in all fish after 4 hours of exposure. At test termination (96 hours) five of the remaining eight fish at the 0.0801 mg a.s. Detest level showed sub-lethal effects in terms of labored respiration and four of them were additionally dark in coloration and remained at the bottom of the aquarium for unusually long periods of time

### **Mortality**

level showed sub-						tionally dark
in coloration and	remained at the	bottom of the	e aquarium 🕼	unusually long	g periods of tin	•
Mortality			4			
Exposure time (hours)	4	24	48	72 ©	2, 96	
Nominal conc.*	No of dead	No of dead	No of dead	No of dead	ONo of dead	
(mg a.s./L)	(%)	(%)	Y (%) X		<b>(%)</b>	¥ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~
Control	0 (0)	0 (0)		Q0 (0)	0(0)0	
Solvent Control	0 (0)	0 (00)	0 (0)	, O O	0 (0)	
0.0400	0 (0)	90(b) /k				Õ
0.0801	0 (0)		× (20)	×2 (20)	2 (20°)	Z.
0.160	0 (0)	10 (1900)	(\$\frac{10}{2}(10))	) 10 <b>(10</b> 0) »	10(\$00)	<b>Y</b>
0.320	10 (100)	10 (100)	109(100)	<b>100</b> (100)	10 (100)	
0.641	10 (100)	×10 (100)	70 (10 <b>0</b> )	10 (100)	10 (1090)	

^{*} Values corrected for purity (94,2%)

### Conclusion

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

LC50 96 hours (95% C.I.): 0.098 mg @ (0.074 0.131 m	s./L* @
	g a _s ,/L)"
lowest concentration with an significant effect 0.0801 mg as	©″ .ε./I *
compared to the control	%>./ L
NOEC:	
highest concentration without an significant 0 0.0400 mg a.	.s./L*
NOEC: highest concentration of hout of significant of 0.0400 mg a effect compared to the control	

^{*} Values corrected for purity (94.2%). Values not stated to study, report.

Report: ; 2015; M-537137-01-1

Acuto oxicito of BCOCN88460 technical to the sheepshead minnow (Cyprinodon Title:

variegatus) under static conditions

EBLNN923 Report No ∰-537**‡**97-01-1 Document

EU Directive 91/414/EEC Guideline Regulation (EC) No. 1107/2009 LES EPA OCSPP 850.1075

Guideline deviation(s): none GLP/GEP: yes



# Material and methods

Material and m	,
Test material	BCS-CN88460 techn.
	Origin batch ID: 2013-006492;
	Specification number: 10200028196;
	purity: 94.2% w/w
Guideline(s)	None specified
adaptation	
Test species	Sheepshead Minnow (Cyprinodon variegatus)
Acclimation	More than 14 days No mortalities during 48 hours prior to testing, no deatments for disease
Organism	
age/size at	Mean length: 29.6 mm $\pm$ 2.1 mm Mean body weight: 0.579 g $\pm$ 0.563g
study	
initiation	
Test solutions	Nominal concentrations; \$0.0625, \$0.125, \$0.250, \$0.500, and 1.00 mg a.Q./L
	Nominal concentrations: 0.0625, 0.125, 0.250, 0.500 and 1.00 mg a.s./L  Corresponding mean neasured concentrations: 0.0462, 0.0886, 0.496, 0.395 and 0.869 mg a.s./L.  Samples were taken from all test chambers on day 0 and day.  Controls: water  Solvent control: 0.1 ml/L dipactly formans de
	0.869 mg a.s./L.  Samples were taken from all test chambers on day 0 and day 4.  Controls: water  Solvent control: 0.1 ml/L dimethyl formamide  Evidence of andissolved material: No precipitate during exposure were observed.
	Samples were taken from all test chambers on day 0 and day 4.
	Controls: water
	Solvent control: 0.1 m/L dimethyl formamide
	Evidence or undissolved material. To precipitate southing exposure were observed.
Replication	1 to: of vegsels per contextuation, (reprisates).
	INO. OF ACSSETS DEL COMMON (TERMICALES). I
	No. of vessels per solvent control Geplicates): k
Organisms	Nogor organisms per vessel: 195
per replicate	
Exposure	Station Statio
Test Vessel	0.19 g fish/L fest medium
Loading	
× 7	NO 17 2 O XY & A
Feeding during test	Nighe 4 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5
Test	Tennerature: 21.6 22.10 0
conditions	Photoperod: 16 hours bight / Chours dark
Q ^y	Light intensity 751 822 have pH: 79 – 8.9
	Dissolved oxygen. 6.2 mg/L ~ 8.8 mg/L (81-93% oxygen saturation)
4	Gentle Fration was added to each test chamber to achieve > 60% saturation
6	throughout the test @
S.	Salinity: 22 % Z
Parameters	Observations of mortality, sublethal symptoms and behavioral effects, such as
	Wertical orientation, on bottom, erratic behavior, labored respiration and dark
Observations	coloration, were made at 4, 24, 48, 72, 96-hours
	Discrete measurements of temperature, dissolved oxygen and pH were obtained at
K Z	test initiation, 24, 48, 72, and 96 hours.
Chensieal	Samples of each test concentration were taken at day 0 and day 4 of the testing
analysis	period. They were analysed using Liquid Chromatography-Mass
-	Spectrometry/Mass Spectrometry (LC-MS/MS).



Data analysis

The LC₅₀ values were calculated using CETIS statistical software and were determined by the characteristics of the data, i.e. the number of concentrations in based on an 600 abov which survival was between 0 and 100 percent and the 95% confidence intervals of the NOEC and LOEC were empirically determined based upon observation data in a late of the local state including lethal and sublethal effects.

### Results

Validity criteria	Required	<b>Obtained</b>
Mortality in control during test	≤ 10%	0 %
Dissolved oxygen saturation	≥ 60%	81 93%

# Analytical results:

Recoveries were between 61 and 90% (see Table Below). Therefore results are based on anithmetic mean measured concentrations of BCS-CN88460. No residues of BCS-CN88460 above the LOQ (0.005 mg a.s./L) were found on day 0 and day 4 in the control samples.

Nominal concentration (mg a.s./L)	Day 0 Measured concentration (mg a.s./k)	Day®% Nordinal	Day 4  Measured Concentration (mg a.s./L)	Day 4 %	Arithmetics meath measured concentration (mg a.s./L)	measured
Control	< 0.005	NAS NAS	Ø 0.00 <i>5</i> Ø	NA «C	×0.005,©	NA
Solvent Control	< 07.905	N 🖗	\$ < 0. <b>6</b> 05 \$	NA NA	0.005	NA
0.0625	<b>Q</b> .0513 <b>G</b>	<b>\$2</b> %	00411	66%	0.0462	74%
0.125	0.101	© 81%©	50.0764	61%	D″ 07. <b>9</b> 7886	71%
0.250	\$ 0. <b>2</b> 97	83%	√ 0° <b>18</b> 9 √	74%	<b>©</b> 0.196	79%
0.500	<b>20.434</b>	<b>%</b> 87%	y 0.255 S	70%	€ 0.395	79%
1.000	\$0.896 ⁰	090%	©0.841©	\$4% @	0.869	87%

Observations Common carp in the Control solver control, 0 962 ng a.s./L and 0.0886 mg/L groups appeared healthy and normal throughout the test. After hours of exposure, two fish in the 0.196 mg a.s./L treatment group showed tark colorations. In the 0.395 mg a.s./L treatment group one fish was dead and eight fish showed dark cotoration. After 66 hours. In the 0.869 mg a.s./L treatment group all fish and eight his showed dark coloration. After 96 how were dead at test termination (see table below).



### **Mortality**

Exposure time (hours)	4	24	48	72	96
Arithmetic mean measured conc. (mg a.s./L)	No of dead (%)	No of dead (%)	No of dead (%)	No of dead	No of dead
Control	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Solvent Control	0 (0)	0 (0)	0 (0)	0.40)	0
0.0462	0 (0)	0 (0)	0 (0)	<b>(0)</b>	,0°(0) ~~
0.0886	0 (0)	0 (0)	<b>V</b> 0 (0)	<b>(0)</b>	
0.196	0 (0)	0 (0)	0 (0)	0 (0)	( 0 (B)
0.395	0 (0)	0 (0)	0 (0)	0(0)	1(10)
0.869	0 (0)	0 (0)	4 (40)/	Ø10 (100)/	\Q^{(1000)}

# Conclusion

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

LC50 96 hours (95% C.I.):	Ž, v	0.544 mg f.s. / L 6 6(0.4726 0.626 mg a.s. L)
LOEC: lowest concentration with an compared to the control	significant effect	0.196 mg a s. L. &
NOEC: highest concentration without effect compared to the control	at an significant	0.0886 mg a 5 / L

Title: BCS-CN88460 arboxy@c-acid (BCS-@Y2649@) - Acute toxicity to rainbow trout

Oncorpynchus mykiss) under static conditions - Final report

Report No.: EBLAN193.

Document No.: M-\$8765501

Guideline(s): BPA-FIRRA § 72-1/SEP-EPA-540/9-85006 (1982/1985)

OCSP5 850. 1975 (Public Draw, 1995) OECD No. 203 (rev.1992) JMAFF, 12 Nousan

No \$447 (2000)

Guideline deviation(s): note GLP/GEP; yes

# Material and methods

Test material	BCS-CN 88460 carboxylic-30rd (BCS-CY26497)
· (C)	√lot/batch SE √1263 √219-9 √
6	Tox-No. 20054-00
	Purity 98.8% w/w
Guideline(s)	None specificat
adaptation S	
Test species	Rainbow trout (Oncorhynchus mykiss)
Acclimation	At least 14 days, fed daily with commercial trout food
110110	Health during acclimation: less than 5% mortality

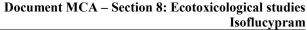


Organism	Mean length: $3.9 \pm 0.4$ cm
Organism age/size at	Mean body weight: $0.5 \pm 0.2$ g
study	
initiation	
	Nominal concentrations: 6.18, 12.4, 24.7, 49.4 and 98.8 mg p. (b)/L
Test solutions	Geometric mean measured concentrations: 6.69, 12.9, 25.0, 26.3 and 33.5 for
	p.m./L
	Controls: water and solvent control
	Evidence of undissolved material. At testistart undissolved test material was " " "
	observed at the nominal test concentration of 24.7 mg p.m./L. In the two highest
	test concentrations with nominal 49 4 and 98.8 mg p.m./L undissolved tost material &
	was observed over the whole exposure period.
Replication	No. of vessels per concentration (replicates):
	No. of vessels per control (replicates): 1
	was observed over the whole exposure period.  No. of vessels per concentration (replicates): 1  No. of vessels per solvent control (coplicates): 1  No. of organisms per vessel: 10  No. of organisms per vessel: 10
Organisms	No. of organisms per vessel: 10
per replicate	No. of organisms per vessel: 10  Static conditions  Total exposure duration: 6 hours  0.13 g fish/L test medium
Exposure	Static conditions Total exposure duration: 6 hours  0.13 g fish/L test medium
	Total exposure duration: 6 hours 7
Test Vessel	0.13 g fish/L testomedium
Loading	
Feeding	No food 48 before and during study
during test	Temperature: 43.1 - 13.9°C
Test	Temperature: 43.1 - 13.9°C  Photoperiod: 16 hours light / 8 hours dark  Light intensity: that specified
conditions	Photoperiod 16 hours light / 8 hours dark
Conditions	Light intensity: not specified & &
	pH: 6,607.3 7 4 2 2 2
, C	Water hardness: 40%-60 mg/CaCO3/L
8	Dissolved Oxygen 90 - 96% saturation
	Conductivity: ②10 μS/cm Fish were observed for mortalities and signs of intoxication for the first four hours
Parameters	
Measured /	after start of exposure and then daily thereafter Dissolved oxygen, wated temperature and phovalues were determined daily.
Observations	Daysolved oxygen, water temperature and prevalues were determined daily.
Chemical	The chemical analysis of BCS-CN88460 carboxylic-acid (BCS-CY26497) (in
analysis $\mathbb{Q}$	wator by HPLC (UV) was performed in all test levels after 0 hours, on day 2 and on
· · · · · · · · · · · · · · · · · · ·	day 4 of the exposure period.
Data analysis	Calculation of the geometric mean freasured concentrations was performed according to DECD 23.
p.m. = pure metabol	
p.m. pure metabol	
Results	
77 1: 1: 4 : 2° :	

Validity criteria	Required	Obtained
Mortality in confrol during test	<u>≤</u> 10%	0%
Dissolved oxygen saturation	≥ 60%	≥ 90%

# Analytica results:

The chemical analysis of BCS-CN88460-carboxylic-acid (BCS-CY26497) resulted in recoveries between 96 and 109 % of nominal for the three lower test concentrations of 6.18, 12.4, 24.7 mg p.m./L over the whole study duration although slight amounts of undissolved test material was observed at





nominally 24.7 mg p.m./L at test start. In the two higher treatment groups of nominally 49.4 and 98.8 mg p.m./L, the solubility of the test item in the medium was exceeded.

At the nominal concentration of 49.4 mg p.m./L the analytically measured recoveries ranged between 32 % at test start and 90 % of nominal at day 4. Over the whole exposure period undissolved test material was observed in the aquarium. However, the analytical measurements on day 2 and 4 showed that parts of the undissolved substance were dissolved within the exposure period. At day 2 and 4 nominal concentrations were detected in the water samples. At the nominal concentration of 98.8 mg p.m./L undissolved test material was observed over the whole testing period. Additionally the analytical measurements proved the exceedance of the test substance solvibility in the medium. The analytical results ranged between 10 % of nominal at test start and 43% of nominal at day. Also in the highest concentration some of the undissolved substance, observed at test start was dissolved over the exposure period.

Considering the low recoveries in the two highest test item concentrations the results were based on geometric mean measured concentrations of BOS-CN88460 carbox fic-acid (BCS-CY26497). The calculation of the geometric mean measured concentration resulted in a lower exposure concentration in the highest concentration than in the concentration below (nominally 49.4 org p.m./L). Therefore the concentrations were re-ordered according their actual exposure concentrations for the effect evaluation.

Nominal Concentration (mg p.m./L)	Geometric mean & measured concentration (mg p.m./lx)	Day (b)	Day		Day 4
6.18	6.69	1007	108	1978	108
12.4	12.9 🐇 💍	\$103 g	\$104 <b>\</b>	\$\$\) 104	104
24.7	25.0	96,	√ 104€)*	104	101
49.4		5° 3\$' 6°	<b>48</b> 6		69
98.8	\$\ \tag{\sqrt{08.3}} \ \tag{\sqrt{0}}	<b>N</b> 0 <b>N</b>	039 😽	£ 39	31

Average of two detections (presented or rounded values, all calculation were done with Microsoft® Excel)

# Biological results

### Observations

In the controls no mortalities of sub-left al effects were observed within the whole test period. Lethal effect were observed in the geometric mean measured concentration of 33.5 mg p.m./L. One fish was dead after 48 hours of exposure. No further mortalities were observed during the test.

No sub-lethal effects were observed in all test concentrations over the test duration of 96 hours.

### Mortality

Exposure time (hours)	4 3	24	48	72	96
geometric mean [mg/pl.m./L]	No. of dead	Nø of dead	No. of dead (%)	No. of dead (%)	No. of dead (%)
Control	0 °C	7, 0	0	0	0
Solvent control		<b>Q</b> 0	0	0	0
6.69		<b>@</b> 0	0	0	0
1129	0 0	0	0	0	0
25.0	1 20 D	0	0	0	0
28.3	* , <u>~</u> 0	0	0	0	0
335	<b>3</b> 0	0	1 (10)	1 (10)	01 (10)

# Conclusion

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



LC ₅₀ 96 hours (95% C.I.):	> 33.5 mg p.m./L (not determined)
LOEC: lowest concentration with an significant effect compared to the control	33.5 mg p.m./L
NOEC: highest concentration without an significant effect compared to the control	25.0 mg p.m./L

# Long-term and chronic toxicity to fish **CA 8.2.2**

### CA 8.2.2.1 Fish early life stage toxicity

I.C. 06 h	0/ (CL):	> 33.5 mg p.m./L	1
LC ₅₀ 96 hours (95	% C.I.):	(not determined)	
LOEC: lowest concentration compared to the co	on with an significant effect	33.5 mg p.m./L	80247 OF-1 fish (Emephales projectas)
NOEC:			
	on without an significant the control	25.0 mg p.m./L	
		, T	
CA 8.2.2	Long-term and chroni	c toxicity to fish	
CA 8.2.2.1	Fish early life stage to	cicity test	
	Long-term and chroni Fish early life stage to	A & A	
Report:	KCA 8.2.2.1/0;	; 200°; M-5	<b>8</b> 0247- <b>6</b> 5-1 & 6
itle:	Early-life stage toxicity	of BCS-CN88460 stech.)	fish (Emephales promelas)
eport No.:	EBLNN029 JQ		
ocument No.:	M-580247-01-4		<u> </u>
buideline(s):	EU Dwective 91/414	EC L L L L	
	Regulation 107/2009 (	Eur <b>©</b> pe) "O" ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~	
	US EPA OCSPP \$50.14		
	s): yes, see report (		
GLP/GEP:	Syes O S		
Taterial and met	Rods O S	67428-2-5 460-01-05	
Test material:	BOS-CN88460-Vech ) 0	674-28-2-5 460-01-05 27	
1 est materia	Frigit Ratch No. NI I 8	67\$P8-2 & O . W	
, Ø	Bateh Code: BCS-CN88	460-01-05 a, m	
	BCS-CN88460 (rech.) O Origin Batch No: NLL 8 Batch Code: BCS CN88 purity: 980% www	\$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$	
	Language Contraction of the Cont		
Guideline(s)	None specified .		
adaptation 🏻 🌋			<u> </u>
· <i>U</i> ,		, .	
· · · · · · · · · · · · · · · · · · ·	Fathead Tinnow (Pimen	, .	
Test species:	Fathead on innow (Pime)	hale Grometas)	
Test species:  Organism Age	Embroos less than 24 h c	hale Grometas)	
Test species: Organism Age at Experimental	Fathead innow (Pime) Embroos less than 24 h c	hale Grometas)	
Test species: Organism Age at Experimental	Embroos less than 24 h c	hale Grometas)	
Test species:  Organism Age at Experimental Start:	Embigos less than 24 h c	hale Sprometas)	0 μg a.s./L
Test species: Organism Age at Experimental Start:	Embigos less than 24 h c	hale Sprometus)  (A8, 1.53, 4.88, 15.6, 50.	
Test species: Organism Age at Experimental Start:	Embigos less than 24 h c	hale Sprometus)  (A8, 1.53, 4.88, 15.6, 50.	0 μg a.s./L 51 – 1.36 – 4.35 – 14.9 – 48.8
Test species:  Organism Age at Experimental Start:	Embigos less than 24 h c	hale Sprometas)  048, 1.53, 4.88, 15.6, 50. asured concentrations: 0.4	51 - 1.36 - 4.35 - 14.9 - 48.8
Test species:   Organism Age at Experimental  Start:  Test solutions	Embigos less than 24 h c	hale Sprometas)  148, 1.53, 4.88, 15.6, 50.  Sured concentrations: 0.4  and solvent control (dimeth	51 - 1.36 - 4.35 - 14.9 - 48.8
Test species: Organism Age at Experimental Start:  Test solutions	Nominal concentrations: Corresponding mean menting a.s./If Controls: water confol a Evidence of undissolved	hale Sprometas)  148, 1.53, 4.88, 15.6, 50.  Sured concentrations: 0.4  and solvent control (dimethematerial: Not reported	51 - 1.36 - 4.35 - 14.9 - 48.8
Test species: Organism Age at Experimental Start:  Test solutions	Nominal concentrations: Corresponding mean ments a.s./l Controls: water control at Evidence of undissolved	hale prometas)  1348, 1.53, 4.88, 15.6, 50.  25 a sured concentrations: 0.4  26 and solvent control (dimethematerial: Not reported entration (replicates): 4	51 - 1.36 - 4.35 - 14.9 - 48.8
Test species: Organism Age at Experimental Start:  Test solutions	Nominal concentrations: Corresponding mean menting a.s./If Controls: water confol a Evidence of undissolved	hale prometas)  1348, 1.53, 4.88, 15.6, 50.  25 a sured concentrations: 0.4  26 and solvent control (dimethematerial: Not reported entration (replicates): 4	51 - 1.36 - 4.35 - 14.9 - 48.8
Test species: Organism Age at Experimental Start:  Test solutions	Nominal concentrations: Corresponding mean ments a.s./l Controls: water control at Evidence of undissolved	hale prometas)  0.48, 1.53, 4.88, 15.6, 50. asured concentrations: 0.4  nd solvent control (dimethematerial: Not reported intration (replicates): 4  1 (replicates): 4	51 - 1.36 - 4.35 - 14.9 - 48.8
Test species: Organism Age at Experimental Start: Test solutions Replication:	Nominal concentrations: Corresponding mean ments a.s.// Controls: water control a Evidence of undissolved No. of vessels per control No. of vessels per solven	hale prometas)  (A8, 1.53, 4.88, 15.6, 50. asured concentrations: 0.4 and solvent control (dimethematerial: Not reported intration (replicates): 4 (replicates): 4 t control (replicates): 4	51 - 1.36 - 4.35 - 14.9 - 48.8
Test species: Organism Age at Experimental Start: Test solutions Replication: Organism per	Nominal concentrations: Corresponding mean ments a.s.// Controls: water confeol a Evidence of undissolved No. of vessels per concentrol of vessels per controls.	hale prometas)  (A8, 1.53, 4.88, 15.6, 50. asured concentrations: 0.4 and solvent control (dimethematerial: Not reported intration (replicates): 4 (replicates): 4 t control (replicates): 4	51 - 1.36 - 4.35 - 14.9 - 48.8
Test species: Organism Age at Experimental Start: Test solutions Replication:	Nominal concentrations: Corresponding mean ments a.s.// Controls: water control a Evidence of undissolved No. of vessels per control No. of vessels per solven	hale prometas)  (A8, 1.53, 4.88, 15.6, 50. asured concentrations: 0.4 and solvent control (dimethematerial: Not reported intration (replicates): 4 (replicates): 4 t control (replicates): 4	51 - 1.36 - 4.35 - 14.9 - 48.8



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	Total exposure duration: 33 days (5-day-hatch and 28 d post-hatch)
Test Vessel Loading:	At the end of the test: 0.0054 – 0.0065 g fish/L/day
Feeding during test	Newly hatched larvae were fed live brine shrimp nauplii ( <i>Artemia sp.</i> ) three times per day, except on weekends when food was added 2 times per day. Feeding was stopped one day prior study termination.
Test conditions:	times per day, except on weekends when food was added 2 times per day. Feeding was stopped one day prior study termination.  Temperature: 23.8 to 25.3°C Photoperiod: 16:8 light:dark Light intensity: 400-774 lux pH: 6.8 to 7.4 Water hardness: 36.7 to 53.0 mg CaCO ₃ /L Dissolved oxygen (% saturation): 84 to 106% Conductivity: 96.9 - 116 µS/cm  Water temperature was measured and recorded hourly by a data logger in two
Parameters Measured / Observations	replicates of the control and in two replicates of the solvent control during the whole test.  Dissolved oxygen (to percent saturation), pH and the water temperature were measured in one afternating replicate of all test levels on days 0, 7, 95, 21, 28 and 33.  Total hardness was measured in one alternating replicate of four test levels (control, solvent control, towest and highest test levels on study days 0, 7, 15, 21, 28 and 33 Conductivity (in µS/cm²) of the used test water was measured and documented hourly by a data logger.  Every day all incubation cups were observed for embryo mortality until all embryos were katched or dead. Hatched large were also recorded.  During the largel phase observations on mortality were done daily and abnormal
	of the mouth to the tip of the candal perfuncte. Wet weight of control and solvent softrol fish was recorded for evaluation of test system biomass loading. The dry weights of individual rish were measured two days later.
Sampling for chemical analysis	The actual concentrations of BCS-CN88460 were analytically determined in samples of all dose levels taken on study day -1/-2, 0, 7, 15, 21, 28 and 33. BCS-CN88460 was measured by HPLC-MS/MS.
Data analysis:	Biological data (e.g. hatching success, time to hatch, larval survival and larval growth) were statistically analysed. Replicate means were used for statistical analysis based on the design of the test system each test chamber (aquarium) is the experimental unit. For each parameter analysed the following statistical tests
	were conducted:  Student t-test to determine if replicates A-D of the control and the solvent control could be peoled.  Shapiro Wilk's test to check the normality of the data set and - Levenes or Cochran's test for homogenecity of variances  For the evaluation of the NOEC and the LOEC the William's Multiple Sequential t-test was used. All statistical analyses were conducted using a computer program (TOXRAT® Professional) developed by



Validity criteria	Required by OECD 210, 1992	Required by OECD 210, 2013	Obtained °
Dissolved oxygen concentration throughout the test (% saturation)	60% - 100%	≥ 60%	©≥ 84 -106%*©
Water temperature difference between test chambers or between successive days at any time during the test	± 1.5°C max	₹1.5°C max	The water temperature ranged  between 23.8° and 26,3° C  and did not differ by more than ± 1 5° C between test  chambers or between test  successive days at any time  during the test.
Analytical measure of the test concentrations	Compulsory	© Compulsory	Done, Done
Hatching success of controls (Control/solvent control)	360% 37	> 70%	83%/91%
Post-hatch survival of controls	Q > 70% ×	75%	₹°00% ©

^{*}The measured oxygen saturations above 100% are not in line with the given range for this validity criterion, but do not influence the outcome of the study. Therefore, the outcome of the study is still robust

# Analytical results:

The recoveries are between 82 and 107%. Therefore results of the study are based on nominal test concentrations. No residues of BCS-CN88460 were measured in the controls above 0.0674 µg a.s./L which was used as the lowest standard concentration during this study.

Nominal conc (μg a.s./L)	Arithmetic mean measure Oconcentration (µg a.s.#L)	Range mean measured concentration [age a.s./L]	Range % of nominal
0.487		@417 <del>*</del> @504 *>	87 - 105
4.53	\$\tag{9\?36}\tag{\tag{8}}	1.28 - 1.56	84 - 102
4.88	\$\frac{1}{2}\tau^2 \frac{1}{4.35}\tau^2 \tau^2	309 – 4.53	82 - 93
15.6		13.5 - 6.4	87 - 105
50.0		\$\infty \infty 44.0\tilde{\$^53.7}	88 - 107

Time to hatch and hatching success
The hatching of larvae started on day a success (based on the hund) The hatching of larvae started on day 4 and tasted until day 5. On post hatch day 0 the mean hatching success (based on the number of inserted legs) ranged between 77 and 91% in all dose levels. Post hatch day 0 was reached of day 5, when 99% of all fertilised and living embryos in the control and 98% in the solvent control had latched. The endpoint hatching success on day 5 (post hatch day 0) resulted in a NQEC  $\geq$  50 µg aQ./L and a LOEC > 50 µg a.s./L.



Nominal concentrations (µg a.s./L)	Mean cumulative % hatching success (day 4)	Mean cumulative % hatching success (day 5)
Water control	32	91
Solvent control	17	83
0.48	13	83
1.53	11	84
4.88	22	91
15.6	30	84
50.0	29	77 🛴

L <b>arval survival</b> Mean larval survi arval survival res	val at test termination	on ranged from 15 to 100% in all treatment groups. The endpoint 15.6 µg a.s./L.
Nominal concentrations (µg a.s./L)	Larval survival	
Water control	97	
Solvent control	100 Q	
0.48		
1.53	92	
4.88	<b>25</b> 0	
15.6	95	
50.0		
Growth  n the highest test	concentration (50)	pig a soll) only a law number of fish was available at the end of lity. The growth of fish os density dependent. Therefore it was

Nominal ×	Mean total length	Mean wet weight
concentrations "	(mm) ± SD	$\bigcirc$ (mg) $\pm$ SD
(μg a.s./L)		
Water control	\$\tilde{\pi}\) 17.5 \tilde{\pi}\] 1.26 \tilde{\pi}	$4.3 \pm 3.31$
Solvent control C	17.9/± 1.22/	$15.2 \pm 3.18$
0.48	0 9.5 ± 178 0	$15.4 \pm 4.69$
1.53	Q17.6 ±1.64	$14.9 \pm 4.52$
4.88	180°± 1.00°	$15.6 \pm 2.64$
15.6%	17.9 ± 1.44	$15.8 \pm 3.73$
50.0	\$16.2 <b>2</b> .17	$9.4 \pm 4.10$

SD = Standard de Mation &

# Morphological and behavioral effects

Between study day and lest termination on study day 33 (post hatch day 28) the following morphological and behavioral symptoms were observed:

Up to test concentration 15.6 µg a.s./L and including the control and solvent control only a few fish fish showed symptoms like "fish lying on side or back on the bottom", "loss of equilibrium, tumbling during swimming", "undernourished, too small for their age", "brighter coloration". In addition at test



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concentration 15.6  $\mu$ g a.s./L one fish was observed with a haematoma on the head on study day 32 and 33.

At the test concentration of 50.0 µg a.s./L in total fifty-one from primary 60 fish died during arval exposure between study day 6 and 25. In most cases observations of symptoms as "undernoorished," too small for their age", "fish lying on side or back on the bottom" and/or loss of equilibrium, tumbling during swimming" were previously made. It was remarkable that the majority of hatched larvae were extremely small in size, which resulted in association with other symptoms or most cases in death or in an obviously retarded development.

The behavioral and morphological observations resulted in a NOEC of 5.6 µg a.s./L and a LOEC of 50.0 µg a.s./L.

### Conclusion

The test fulfilled the validity criteria of the underlying guideline and even for the actual valid version of the OECD 210 from 2013, with the exception of one minor case. A diort-term incident (a breakdown of stock solution delivery in test level 15.0 µg a s./L) was observed during the study without resulting in any influence on the results and/or on the biological outcome of the study. Based on morphological and behavioral observations and the statistical analysis of hatching success, larval survival and larval growth (correspond as dry weight and total lengths the lest revealed the following NOEC, LOEC, MATC and EC₁₀ (based on nominal concentrations of BCS CN88460):

Hatching Larval Growth Success (day 5) (day 23) Length)	Growth Dry Q Weight	Morphological & Behavioral effects
LOEC [µg a.s./L]:   lowest concentration with an significant effect compared to the control   50   50   515.8	\$\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2}\frac{1}{2	50
NOEC [µg a.s. 6: highest concentration without an significant effect compared to the control $2.50\%$	≥ 15.6	15.6
EC ₁₀ (95% CL) [µg a.s. L] п.d. п.d. п.d.	n.d.	n.a.

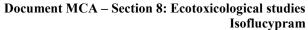
n.d. = not determined n.a. = not applicable

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For this study  $CC_{10}$  and  $EC_{20}$  calculations were not possible or applicable due to the following reasons: For the endpoint "Time to hatch" the observed NOEC was the highest concentration of 50  $\mu$ g a.s./L. An  $EC_{10}$  of  $EC_{20}$  calculation therefore is not possible.

For the endpoint "lawal subvival" only one test item concentration (50  $\mu$ g a.s./L) resulted in effects. With only one effect concentration no reasonable EC₁₀ or EC₂₀ calculation could be performed. At the NOEC the larval survival reached 95%. The observed survival thus differed to the control by 2% and to the solvent control by 5% only. It can therefore be stated that an EC₁₀ would have been a higher concentration as the presented NOEC.

For the endpoints "length" and "weight" only one effect concentration was observed (50  $\mu$ g/L): At the NOEC there was no effect detected compared to the controls. It can therefore be stated that an EC₁₀ would have been a higher concentration as the presented NOEC. For these reasons no EC₁₀ or EC₂₀ values were reported.





Report: KCA 8.2.2.1/02; ; 2016; M-575119-01-1

# Material and methods

Title:	Early life stage toxicity of BCS-CN88460 technical to the sheepshead minnow		
11110.	Early life stage toxicity of BCS-CN88460 technical to the sheepshead minnow (Cyprinodon variegatus) under flow-through conditions 044SRLS15C20 M-575119-01-1 EU Directive 91/414/EEC Regulation (EC) No. 1107/2009 US EPA OCSPP 850.1400 s): None Yes  BCS-CN88460 (tech.) Origin Batch No: NLL 8674-28-2 Batch Code: BCS-CN88460-01-5 Specification No.: 10200028196 purity 94.2% w/w None specified  Sheepshead minnow (Cyprinodon varjegatus)		
Report No.:	044SRLS15C20		
Document No.:	M-575119-01-1		
Guideline(s):	EU Directive 91/414/EEC		
C:11: 1::::	Regulation (EC) No. 1107/2009 US EPA OCSPP 850.1400		
Guideline deviation(	s): None		
GLP/GEP:	Yes		
Material and met	hods		
Test material:	BCS-CN88460 (tech.) Origin Batch No: NLL 8674-28-2 Batch Code: BCS-CN88460-01-5 Specification No.: 10200028196 purity 94.2% w/w None specified		
	Origin Batch No: NLL 8674-28-2		
	Batch Code: BCS-CN88460-01-5		
	Specification No.: 10200028196 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2		
	purity 94.2% w/w		
Guideline(s)	Origin Batch No: NLL 8674-28-2 Batch Code: BCS-CN88460-01-5 Specification No.: 10200028196 purity 94.2% w/w None specified		
adaptation	Trone speciment		
Test species:	Purity 94.2% w/w None specified  Sheepshead minnow (Cyprinodon varjegatus)  24-48 hour of leggs in the merula stage  Nominal concentrations: 3 23, 6.25, 12.5, 25.0 20.0 μg a.s./Ly		
Organism Age	24-48 hour of Reggs in the merula stage 2		
at Experimental			
Start:			
Took as lestions	Namifal an Outration 2 th 6 25 12 5 25 0 20 0 12 5		
Test solutions	1 101 mga Consent apons. 3/3/5, 0.25, 12.5, 25.0./5/0.0 pc/sa.5./1x/		
	Arithmetic mean measured concentrations: 2,92, 6.13 11.3, 25.0, 45.8 μg a.s./L Controls water control and solvent control drieth dene glycol 0.1 mL/L)		
	Evidence of indissolved material: No precipitations observed		
Replication:	No of vessels per concentration (replicates):		
	No of vessels per control (replicates): 4		
	No. of vessels per solvent control (peplicaes): 4. V		
Organisms per	No of fertifized eggs/embryos per vessel: 20		
replicate.			
Exposure:			
LAposure.	Flow-through Total exposure diffation 55 days (6-day hatch and 29 d post-hatch)		
. 0.			
Test Vessel	of the end of the test: 0.026 g fish/L day		
Loading: "			
Feeding during	Ecoding with brine shripp (Arigmia salina) starting on Day 5		
test			
	Temperature 24.0 to 24.6 C		
Test conditions:	Light intensity: @5 to 778 lux		
	pH. 8.1 8.2 8.2		
	Saling range: 20 % to 21%		
	Dissolved oxygen (% saturation) range: 73% to 91%		
Parameters >	Temperature was measured continuously throughout the exposure in a centrally		
Measured /	Tocated test vessel. Dissolved oxygen, pH and salinity were measured at		
Observațions	experimental start and at least weekly thereafter.		
. O			
Č	Biological parameters measured were fish hatchability, sublethal effects,		
	survival and growth (length, wet weight and dry weight for all surviving fish on		
	day 35). Visual observations made were total lengths, wet weights and dry		



	weights. Hatching observations made daily during hatching phase, observations for sublethal effects and survival made daily, growth determinations made at the end of the exposure.
Sampling for chemical analysis	end of the exposure.  Two alternating replicates for each level were taken on a weekly basis.  BCS-CN88460 was measured by using Liquid Chromatography-Mass Spectrometry/Mass Spectrometry (LC-MS/MS).
Data analysis:	The replicate test vessels were considered to be the smallest experimental unit based on the design of the test system, and hence replicate means were used for statistical analysis of each endpoint. Raw data from the control and solvent control groups were compared for equal variance using the Equal Variance Two-Sample t test to determine if the data sets were poolable. Appropriate tests were used to determine if the data had equal variances and normal distribution (i.e. Bartlett's Test, and Shapiro-Wilk's test). If normality and homogeneity of variance were demonstrated for the raw of transformed values, then parametric analyses were conducted using analysis of variance (ANOV) followed by Dunnett's test (p=0.05). If normality and/or lomogeneity of variance were not demonstrated on raw or transformed values, nonparametric procedures were used. ECx values were calculated where applicable using linear interpolation.

	uscu. ECA values y	merc caregulated writing	Schhudshic rights u	mega micropolatic
Results	\$\frac{\partial \text{q}}{\partial \text{q}}.\text{\text{\text{q}}}		sppintage using in	
Validity criteria		Required by OPPTS 850.1400, 1996		
Dissolved oxygen co	oncentration	60% - 100%		
Water temperature of test chambers or bed days at any time of the state of the stat	ween socessive	± 1.5 °C max	U ± 1,0°C max,	
Concentrations of te solution throughout		#20 % of mean measured values	Yœs	
Hatching success of		75%	<b>9</b> 75%	
Post-hatch survival	of controls,	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	> 80%	

Analytical results:
Recoveries were between 74 and 113% so the results are based on arithmetic mean measured concentrations.

Nominal conc.  Arithmetic mean  measured concentration		% of nominal concentrations*					
(μg a.s./L)	s measured coacentration s	[⊮] Day 0	Day 7	Day 14	Day 21	Day 28	Day 35
3.13	2.92	81	105	94	98	84	97
6.25	$\mathcal{C}$ 6.13	95	110	104	86	94	99
13/5	¥1.3	87	94	109	74	92	87
25.0	25.0	89	113	109	85	99	103
500	45.8	76	103	106	88	85	92

^{*} Mean two replicates used for calculations



# Biological results:

### **Observations**

Observations of fish were recorded daily throughout the study. All fish including the controls appeared normal during the course of the study.

# Time to hatch and hatching success

The percent of the embryos that hatched by day 6 was analysed statistically to determine therewere any related adverse effects as compared to the pooled controls. Day 6 represented the day in which > 90% of the viable eggs completed hatching and was the most representative day for the time to hatch data analysis and EC_x calculations, respectively. The Day 6 mean percent hatch ranged from 829 to 6 Terent from Poole, 92.9%. Statistical analysis indicated that percent hatch was not significantly different from Poole controls in any test level.

Arithmetic mean measured concentration (μg a.s./L)	Mean % hatching success on day 6
Water control	87.9
Solvent control	87,9
2.92	<b>Q</b> 2.1 <b>(Y</b>
6.13	£ 92.1 € *>
11.3	8963
25.0	\$2.9
45.8	( 90.0° )

Alevin survival on day 6 American from 87.9 92.9%. Statistical analysis indicated that alevin

Mean percent a	levin survivat i	ranged fron	n 87.9	92,9%.	Statistical	analysi
survival was not	significantly di	ifferent from	h pooled c	omtrols i	any test	level. [™]
Mean percent a survival was not  Arithmetic mea concentration		Mean % sur	xival			
Water control	<i>**</i>	© 8749				
Solvent control		\$ 807.9			~	
2.92		§92.1 ₂	*/ * O*			
6.13	\$ 4	92,0		<b>&amp;</b> '	3"	
11.3	S A &	<b>89</b> .3				
25.0 _{@/}		\$ \$\disp\ 92.9 \( \)		T'		
45.8	U Q	<b>№</b> 90,0	Ď	ð		
- 4				U)		

# Fry survival on day 38

Mean percent fry sarvival anged from 90 to 98 8%. Statistical analysis indicated that fry survival was significantly different from pooted controls in the highest level.

Arithmetic mean measured concentration (µg.a.s./L)	♥ ♥ ♥ Mean % sarvival
Water commol O	98.8
Solvent control ~	98.8
<b>2</b> .92	97.6
6.13	96.3
11.3	98.8
25.0	95.0
45.8	90.0



### Growth

At test termination (study day 35), the fish were sacrificed and measured for total length, wet weight, and dry weight. The mean lengths ranged from 19.1 to 19.9 mm. Mean dry weights for fish reged from 24.4 to 28.1 mg. Mean wet weights for fish ranged from 101.0 to 115.9 mg. Statistical analysis indicated that length and weight were not statistically different from pooled controls in any test levels

Arithmetic mean measured concentration (µg a.s./L)	Mean total length (mm)	Mean wet weight  107.5  105.7  101.0
Water control	19.6	107.5
Solvent control	19.2	© 105.7 € © Q © Q
2.92	19.1	101.0
6.13	19.4	107.40
11.3	19.4	
25.0	19.5	2 14.7 0° 5° 6° 5° 5° 5°
45.8	19.9	N15.9 A S
Conclusion		19.7
Conclusion The study is considered to concentrations are:	to be valid and the	e endpoints based on anothmetic mean measured

# Conclusion

	% Time	Alevin	y Fry©	Total	Wet
	to Hatch	survival S	survival	length	weight
LOEC [µg a.s./L]:	% ,			,	,
lowest concentration with an significant $\sqrt{2} > 45.8$	<b>≱</b> 45.8_ °	> 45.8	<b>45.8</b>	45.8	45.8
effect compared to the control		L C	,	43.6	45.6
NOEC [µg a.s./IC):	1				
highest concentration without an \( \infty \) 45.8\( \infty \)		45.8	25.0	45.8	45.8
significant effect compared to the control		2			

For the endpoints "time to hatch" Chatching success" "mean survival" and "growth" (total length and wet weight) no effects up to and including the highest concentration (45.8 µg a.s./L) were observed. An EC₁₀ or EC₂₀ calculation therefore is not possible.

For the endpoint "fro survival on day 35" less than 10 % difference to the control at the highest test item concentration of 45. Pug a L were observed. Thus the number of effect concentrations and the observed offect size were not sufficient for a reasonable EC10 and EC20 calculation.

# Fish Full life cycle test CA 8.2.2.2

Please refer to Section CA 2.2. Based on the triggers state in the EU-directive and the Aquatic Guidance Document, a fish full life cycle (FFLC) study is not required.



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

Report: KCA 6.2.5/01; , R.; 2017; M-610008-01-1

[pyrazole-4-14C] BCS-CN88460 - Aqueous exposure bioconcentration fish tes Title:

biotransformation in fish (Lepomis macrochirus)

EBLNN359 Report No.: Document No.: M-610008-01-1

EU Directive 91/414/EEC; Regulation1107/2009 (Europe); OECD Test Guideline(s):

305; US EPA OCSPP 850.1730

Guideline deviation(s):

According to the guideline and the study protocol, the measurement of the total organic carbon (TOC) will be performed 48 and 24 nours prior to the study protocol, the measurement of the total organic carbon (TOC) will be performed 48 and 24 nours prior to the total organic carbon (TOC) will be performed 48 and 24 nours prior to the total organic carbon (TOC) will be performed 48 and 24 nours prior to the total organic carbon (TOC) will be performed 48 and 24 nours prior to the total organic carbon (TOC) will be performed 48 and 24 nours prior to the total organic carbon (TOC) will be performed 48 and 24 nours prior to the total organic carbon (TOC) will be performed 48 and 24 nours prior to the total organic carbon (TOC) will be performed 48 and 24 nours prior to the total organic carbon (TOC) will be performed 48 and 24 nours prior to the total organic carbon (TOC) will be performed 48 and 24 nours prior to the total organic carbon (TOC) will be performed 48 and 24 nours prior to the total organic carbon (TOC) will be performed 48 and 24 nours prior to the total organic carbon (TOC) will be performed 48 and 24 nours prior to the total organic carbon (TOC) will be performed 48 and 24 nours prior to the total organic carbon (TOC) will be performed 48 and 24 nours prior to the total organic carbon (TOC) will be performed 48 and 24 nours prior to the total organic carbon (TOC) will be performed 48 and 24 nours prior to the total organic carbon (TOC) will be performed 48 and 24 nours prior to the total organic carbon (TOC) will be performed 48 and 24 nours prior to the total organic carbon (TOC) will be performed 48 and 24 nours prior to the total organic carbon (TOC) will be performed 48 and 24 nours prior to the total organic carbon (TOC) will be performed 48 and 24 nours prior to the total organic carbon (TOC) will be performed 48 and 24 nours prior to the total organic carbon (TOC) will be performed 48 and 48 nours prior to the total organic carbon (TOC) will be performed 48 and 48 nours prior to the total org hours prior to test initiation. This deviation has no negative impact on the outcome of the study, since there results of both measurements were reasonable < 2 mg/L)

GLP/GEP:

### Material and methods:

Material and me	
Test material	Non-radiolabelled test item:  Radiolabelled test item:  Radiolabelled test item:  BCS-CN88460  BCS-CN88460  Ratch code: BCS-CN88460
	BCS-CN88460  BCS-CN88460
	BCS-CN88460
	Specification Nov. 10200028196
	Purity: 94.2% w/w @ F F O CHO CONTROL OF F
	Purity: 94.2% w/w Radiochemical purity: >99%
	Non-radiolabelled test item:  BCS-CN88460  Batch code: BCS-N88460-01-06  Specification Nov. 10200028196  Purity: 94.2% w/w  Radiochemical purity: > 99%
	* * * * * * * * * * * * * * * * * * *
Guideline(s)	Norther specificed State
adaptation	Tropie specifical
Test species	Bluegiff sunfish (Lepomis macrochirus) & & Q
<u> </u>	Fish were acclimated to the test dilution water for $\geq 10$ days prior to initiation of
Acclimation	Fish were accumated to the test didding water for \( \) A decrease with a test initiation by the fight for
, O	teeting. No mortality was noted 14 days prior to the test initiation by the fish for
, Q	the bioconcentration part. The fish for the biotransformation part had a mortality
D-4-:18 - 44	of 0.7% noted in the range of 14 days prior to the test initiation.
Details on test	Bioconcentration part:
organisms	Wean body weight a Study initiation: 2.8 \$
(	Length at study initiation: 9.7 cm
Q _J	- Lipper content at test initiation: 4:86 % (w/w) of whole fish
~~~~	
4	Biotraits formation part: - Mean body weight at study initiation: 12.9 g
Q"	- Mean body weight at study in tration: 12.9 g
T 48 1 4	- Length at study initiation: 9.10m
Test solutions	Rour agraria (A) B, CD) were used in the test:
,	
	Bioconcentration part:
Q'	Solvent control (Aquarium A): Active substance dissolved in acetonitrile in 0.1
	onL/L Simethylformamide
	Nothing Last concentrations (A quarium D and C): 0.5 and 5 us DCS CN99460/I
	Nominal lest concentrations (Aquarium B and C): 0.5 and 5 µg BCS-CN88460/L
	Mean measured test concentration 0.478 and 5.05 μg BCS-CN88460/L
	Piotronaformation part:
C C	Biotransformation part: Naminal concentration: 5 ug BCS CN88460/I
	, •
	Mean measured concentration: 4.88 μg BCS-CN88460/L
	Evidence of undissolved material: not reported



D on 1: +:	No of coasele non contention (continue to the content of the conte
Replication	No. of vessels per concentration (replicates): 1
0	No. of vessels per solvent control (replicates): 1
Organisms per replicate	No. of organisms per vessel: 70 Test type: Flow through Route of exposure: aqueous Total exposure duration: 28 days
Exposure	Test type: Flow through
	Route of exposure: aqueous
	Total exposure duration: 28 days
	Total depuration duration: 14 days
Test Vessel	Biomass loading rate: 0.133 – 0.645 g fish (wet weight) per litre of test medium
Loading	per day
Test	Temperature: 21.8 – 22.8°C (continuous measurements), 22.3 – 22.9°C (discrete
conditions	Temperature: 21.8 – 22.8°C (continuous measurements), 22.3 – 22.9°C (discrete measurements)
	Photoperiod: 16.8 nours (30 migrate transfert period) \sim
	Light temperature: warm-white fluorescent jumps
	Light temperature: warm-white fluorescent tamps pH: 7.1-7.8 Water hardness: 2.7 ± 0.3 dH (German hardness)
	Water hardness: 2.7 ± 0.3 ° dH (German hardness)
	Oxygen saturation: 73,79%%. The test water was agrated to reach oxygen
	TOC: < 2.0 mg/L in Gilution violet
	Conductivity: < 100 S/cm
Feeding	Oxygen saturation: 73, 97%. The test water was agrated to reach oxygen saturation. TOC: < 2.0 mg/L in dilution water Conductivity: < 10 µS/cm Fish were fed daily with a commercial fish diet at grate of 1 to 2% of body weight. Based on the mean body weight of sampled fish the amount of food was
during test	weight. Based on the mean body weight of sampled fish the amount of food was
during test	recalculated at regular intervals.
Parameters	The temperatures in all treatment groups were measured at test start (day 0) and
Measured /	then once a week. Additionally, the temperature was measured continuously in the
Observations	control aquatium. The pH values on all treatment groups were measured at test
	start then once a week. The concentration of dissolved oxygen in all treatment
	groups were measured at test start and then once a week. The total organic carbon
ي	Sonten was ineasured prior to test start (test water without test item), at test start
, O	(day 3, -1 and 0 before addition of the fish), day 7, 14, 21, 28, 35 and 42.
Ö	Observations of fish for any signs of conormalities or mortalities were made 2-4
, Q	hours after addition to test vessels, and then daily. On day 0, 28 and 42 four fish
	were sampled out Caquarium A-C in order to determine the lipid content of the
C1: f	whole fish The length and weight were also measured from each individual fish.
Sampling for	Stock solution analysis of the radioaction measurements were taken at day – 4
chemical	(aquaria B) and (aquaria B), on Day 14 (aquaria D) and day 28 (aquaria B and C).
analysis	Stock solution symples for the stability measurements of the test item in the stock
2	Stock solution samples for the stability measurements of the test item in the stock solutions were taken at day 4 (aquaria B, C), on day 14 (aquarium D) and 28
	(aquaria B.). Out of each stock solution (without solvent control) 500 μ L were
E	taken.
, ~	
~	Wateranalysis
0	Water samples for the radioactivity measurements were taken at day -1, 0, 1, 3, 7,
, O	10, 14, 26, 28, 29, 31, 35, 38 and 42 (aquaria A-C) and on day -1, 0, 1, 3, 7, 10, 14
L. C	From againium D.
, Ž	Water samples for the characterisation of residues in water (biotransformation
	part) were taken at day 0, 1, 3, 7, 10, 14, 21, 28, 29, 31, 35, 38 and 42 from
	aguarium C and on day 7 and 14 from aquarium D. Only the samples of day 0, 1,
The Salar	28 arr 29 (aquarium C) and the samples of day 7 and 14 (aquarium D) were
	analysed.
_	
	Fish analysis For the bioconcentration part on day 1, 3, 7, 10, 14, 21, 28, 29, 31, 35, 38, and 42
	For the bioconcentration part on day 1, 3, 7, 10, 14, 21, 28, 29, 31, 35, 38 and 42



four fish per aquarium (A-C) were blotted dry and killed by a neck cut. The length and weight of each individual fish was measured and documented to link the analysed chemical concentration to the individual fish. The sampled fish were dissected into edible tissues (fillet = body muscle, skin and skeleton) and visceta / non-edible parts (viscera = head, fins and internal organs) and transferred into preweighed vials. The radioactivities (expressed as disintegrations per minute dpm) were measured in order to determine the TRR (total radioactivity residues) in fish. For the biotransformation part 15 fish were sampled (aquarium D) after 7 days and 14 days, respectively. The length and weight were measured and the fish were dissected into edible tissues (fillet = body muscle, skin and skeleton) and viscera / non-edible parts (viscera = head, fins and internal organs). The coarse pieces of the edibles or viscera of each day were combined and homogenized. From these samples, a sub-sample was taken for extraction and analysis.

Parent compound and metabolites in the extracts of water and fish samples were analysed by HPLC with radiodetection. They were identified in isolated fractions from representative extracts by HPLC and TLC conchromatography with radiolabeled reference compounds.

Results:

Validity criteria Required (OE© 3054912)	Obtained
Water temperature variation over the whole test period $\pm 2^{\circ}C$	22.3 – 22.9°C
Dissolved oxygen % saturation in all test vessels	> 73%
Concentration of test substance in test chambers maintained within required range of the mean of the measured $\pm 20\%$	82.2 - 118% (Aquarium B: 0.5 μg a.s./L)
Concentration of test substance in test chambers maintained within required	② 90.6 − 119%
Concentration of test substance in test chambers maintained within required range of the mean of the measured values during the uptake phase	(Aquarium C: 5 µg a.s./L)
values during the uprake phase	83.8 – 139%*
	(Aquarium D: 5 μg a.s./L)
The concentration of the test substance is Test concentration water	Yes**
below its limit of sombility in test water solubility of test item in test water	_
Mortality or other adverse effects disease in control and preated fight	< 10%

^{*} In test aquasium D We concentration of the test substance was temporary (day 14) above 20% of the mean of the measured values. The excellence was not induced the outgoing of the BCF calculations because aquarium D was used for the determination of the metabolism of the test substance in fish.

** Water Solubility of BCS CN88460 at pH 5 = 1.8 mg/L

Anatytical results:

Mean measured water concentrations during the uptake period was $0.478 \pm 0.06 \,\mu g/L$ [\$^4C]-BCS-CN88460 equivalents at the low freatment (0.5 \mu g/L) and $5.05 \pm 0.442 \,\mu g/L$ [\$^4C]-BCS-CN88460 equivalents at the light treatment (5.0 \mu g/L). This represented 96% of the low nominal concentrations and 101% of the high nominal concentration. Water concentrations ranged from 0.393\mu g/L to 0.568 \mu g/L in the low treatment and 4.58 \mu g/L to 6.0 \mu g/L in the high treatment through the uptake phase. No radioactivity was detected in the tank of the solvent control. Mean measured water concentration from day at to day 2 of depuration showed a clear decrease in [\$^4C]-BCS-CN88460 equivalents in both treated tanks. On day 3 of depuration, no radioactivity was detected in the low and high treatment.

Average daily concentrations of total radioactivity in water (mg/L, expressed as [14C]-BCS-CN88460 equivalents) during the uptake phase in the aquaria B, C and D are given below.



Study phase	Study day	Nom. concentration: 0.5 μg/L (Aquarium B)	Nom. concentration: 5.0 μg/L (Aquarium C)	Nom. concentration: 5.0 μg/L (Aquarium D)
	0	0.457	4.75	5.0
	1	0.442	4.58	\$\tag{4.42} \tag{\varphi} \tag{\varphi}
	3	0.393	4.79	4.34
e	7	0.565	4.82	4.6
Uptake	10	0.457	<i>5</i> 45	4,09
U	14	0.464	3 .17	\$\tilde{\
	21	0.568	6.00	
	28	0.481	5.11 Q	
	Mean	0.478	5.05	Q4.88, Q Q

Nom. = Nominal, - = No measurement

Mean total residues expressed as mg/kg of [14C/BCS CN88460 equivalents in edible tissue, whole fish or viscera parts in the low and high treatment in are given in the following table.

Study	Nom. o	concentration: 0.	.5 μg/L 🦮	Nom	oncentration S.	0 μg/🗜
day		(Aquarium B)	8 8 2		(AgnariunCC)	
			kg of [[©] C]-B€S-	CN88460 equiva	lenes S	<u>'</u>
1	$0.0758 \pm$	041800 ±	2850 _s ±	0.3580 ± 📞	2 1.4900 ±	2.9500 ±
1	0.0408	0.007	S 0.0690	© 0.0398 🔊	%2170 ©	0.1890
3	$0.0492 \pm$	°>√0.1590 ±	0 3880 + 6	0,5480 ±√	₹.5000	3.0900 ±
3	0.0187	0.0282	00450	° 00.07 6 3,	0.3590	1.0000
7	0.0740 ± 2	0@560 ±€°	0.4920	√ 0.53®± ~	1.5400 ±	2.9300 ±
/	0.0149	0.584	k©> 0.1180 ⊗) 0 ₀ 159	0.5610	1.1500
10	0.0493	0.2269 ±	0.53/70 ±	1 20100 4 5√	2.8800 ±	1.6900 ±
10	0.00\$35 %	0.1750 🌭	0.5440	0.3970	∜ 0.8980	0.5420
14	0.0008 ± 20	Ø1480 ±	\$ 0.289 6 ¥	© 0.7890± 0	1.7200 ±	$3.0400 \pm$
14	0.0114	0.0387	≾ 0. 12 000 ∂	0.2040 🗸 🗸	0.4250	1.1100
21 .	°>0.0526 ±	0.1360 ± 0	0.2580 ±	€0300±0°	$3.8800 \pm$	$2.2000 \pm$
21	0.0057	0000352	0.0180	<>> 0.51 20 °	1.5800	0.8800
28	0.0707 ±	0.21650.€	0.3240×± «	0.6 03 0 ±	$1.9500 \pm$	3.9400 ±
26	0.0215	⁷ 0.07 §	√ 0. k9 90 ⊘	⁰ 0.0859	0.7390	1.9100
29	0.023©±	0.0 5 76 ±	0.9909 ±	9.3700 ±	$0.7480 \pm$	$1.2900 \pm$
23	0.0033 💍	0.0876 ± 0.08759 0.00873	0.0802	® 0.1520	0.4150	0.8050
31	0:90572 ±	©00843¥		$0.0683 \pm$	$0.0927 \pm$	$0.1300 \pm$
31	△ 0.000421	0.000	\$ 0. 00	0.0204	0.0320	0.0499
35	@0.00548 ±	0.0 67 74 ± ©	QQ/108±	$0.04400 \pm$	$0.0585 \pm$	$0.0816 \pm$
	0.00123	0.00172	(0.002 \$Q *	0.00772	0.0118	0.0167
38	0.00443€	Ø.0063 ر	0.008 3 7 ±	$0.0430 \pm$	$0.0600 \pm$	$0.0876 \pm$
30%	0.000486	© 0.000 882	[™] 0 ,00 0118	0.0289	0.0231	0.0146
42	0.0 333 °±	0.0\\37 ±	Ø.0586 ±	$0.00335 \pm$	$0.00428 \pm$	$0.00596 \pm$
72	030104	3 0.011 2 0	0.0154	0.00128	0.00167	0.00224

Bioconcentration factors (BCF) were determined during the uptake and depuration period by dividing the [14C]-tissus radioactivity by the mean [14C]-water radioactivity up to and including that day for each fish. Results based on four sampled fish for the high and low treatments are given in the following table.



Study day	Nom. concentration: 0.5 μg/L (Aquarium B)			Nom. o	Nom. concentration: 5.0 μg/L (Aquarium C)		
	BCF edible	BCF viscera	BCF whole	BCF edible	BCF viscera	BCF whole°	
	part	part	fish	part	part	fish	
	(Mean ± SD)	(Mean ± SD)	(Mean ± SD)	(Mean ± SD)	(Mean ± SD)	(Mean ♣SD)	
1	169 ± 90.8	634 ± 154	356 ± 16.9	76.8 ± 8.54	634 ± 2 0.5	319@46.6	
3	114 ± 43.4	785 ± 105	368 ± 65.4	117 ± 16.2	657£ 213	319 ± 75.2	
7	159 ± 32.2	1060 ± 255	551 ± 126	112 ± 33.5	<u>6</u> 19 ± 244	325 ± 19	
10	107 ± 20.6	1161 ± 1175	489 ± 379	209 ± 82.4	597 ± 186	¥ 350 ¥ 112 €	
14	131 ± 24.6	623 ± 258	319 ± 83.7	$\sqrt[8]{162 \pm 41.8}$	U 624 ± 228 C	3.54 ± 87.2°	
21	110 ± 11.9	541 ± 37.6	284 ± 7.37	204 ± 102 0	770 ± 3 13	438 ± 175 Q	
28	148 ± 44.9	678 ± 417	346 ± 157	120 ± 170	~781 ±379 √	ຼັ387⊕ັ147 ູ©	
29	48.3 ± 6.91	190 ± 168	108 ± 75 0°	73.4 🛳 30.1 . (ຶ້ 256 ≨160 ູ 🌣	148 ± 82.00	
31	12.0 ± 0.88	27.2 ± 9.56	17.6¢± 2.96	° 13,5 4.0 5	25,8 ± 9,89°	\$18.4 ± 6.34	
35	11.4 ± 0.257	22.7 ± 5.27	16.©± 3.61°	8.71 ± 1,53	26.2 ±2€31	11.6 # 2.33 。	
38	9.27 ± 1.02	17.9 ± 2.47	13.2 ± 1.03	@8.51 ± \$,73	17.4 ≠ 2.90 [©]	119 ± 4.57	
42	7.00 ± 2.68	12.5 ± 4.69	£ 8.95 € 3 .49 ^	√ 7.00 ± 2.07€	118 ± 3.64	8.66 ± 2 22	

To calculate the steady-state biocopeentration factors based on parent substance, the measured TRR concentration was corrected for the amount of BCS-CN88460 found at day 44 in the measurement of the metabolism part of the study. In the edible part 12% % and in viscera 5.70 % of TRR, respectively, could be identified as BCS-CN88460 was only slightly metabolized in water. According to the measurements, the amount of BCS-CN88460 in the TRR in water was > 90 % in each sample. Therefore the concentrations in water used for the calculation of the parent based steady-state BCF values were not corrected to the amount of BCS-CN88460. The BCF s.p for the whole fish was calculated using the adapted values for edible part and viscora under consideration of the different weight portions and the whole fish weight. Results for the steady-state bioconcentration factors based on parent substance are given in the following two tables.

	0.500 μg/L (aqua Gum B) Q						
sampling	BCF edib	le par 🍳 🙏	BCF Wisc	era part 🍣	BCF wh	nole fish	
day	mean* 💍	SD S	mean	SP SP	mean*	SD	
1	33.0 » e	× 18 ₂ 2,	¥ 62√6 (₹1 \$7.2	45.2	5.1	
3	249 25.8	/ 9,36 °	₹3.4 O	11.11	47.0	9.42	
7	25 .8	\$5.21	84.5	20.34	51.3	10.89	
10	21.3	© 4.12 [©] √) 1149ž (115.57	54.6	38.75	
14	25.8) 484 Q"	6 0.4 D	25.01	39.0	7.53	
21	18.3	₹.98 Ø	× 44.2×	3.07	28.7	1.28	
28	29.0	√8.8 Q	65Q ¹	40.19	42.2	17.87	

	5.00 pc/L (aquarium C)								
Sampling	Sampling BCF editor par		BCF viscera part		BCF whole fish				
day &	mean	SD	mean*	SD	mean*	SD			
1	22.5	1.7	62.6	4.0	35.9	4.6			
3,\$		3.14	62.6	20.30	37.5	7.26			
	21.7	6.47	59.0	23.18	37.3	13.25			
\$\forall 10 \text{\$\text{\$\gamma\$}}	38:0	15.17	54.1	16.90	43.9	14.23			
14.0	30.0	7.77	57.1	20.82	41.2	7.97			
21	33.7	16.81	62.7	25.49	45.6	17.42			
28	23.3	3.31	74.9	36.31	44.1	14.74			



Biological results (Bioconcentration part):

The lipid content was not conducted on all sampled fish, therefore, a mean lipid value was used to normalise the BCF. The used mean lipid value is 5.13% which is the mean value from day 0 to 28 considering all treatment groups. This value was used to calculate the lipid normalization factor and the respective corrected bioconcentration factors. Substance uptake, depuration constants and bioconcentration factors are given in the table below.

	0.500 µg [pyrazole-4- ¹⁴ C] BCS-CN88460			\$.00 μg [pyrazote-4-140] & BCS-CN88460/L			
Cw		e	A,	Q.	, Ø _ ^	Š. Ž	
Chemical concentration in	0	0.478 ± 0.060	00	O *	505 ± 0.442		
water considering whole			. 4	Q° Z			
exposure period [μg L ⁻¹]							
$C_{\rm w}$					582 + 0.407	∜ Ů*	
Chemical concentration in water at steady state [µg L ⁻¹]	. 4	904±09055		8	63 ± 0.497		
water at steady state [µg L]	edible	viscera	whole	edible	viscera	whole	
	tissue (tissue	rish S	tissue	tissuc	Tish	
Cf	Ø:0614 Ø	0.290 ±	0.150/±	\$ \$\$.807 ≠ \$	3 4 5 + V	Ø 1.96 ±	
Chemical concentration in fish	0.0014	Ø.0329	0.13.50	0.213	9.502 ×	0.240	
at steady-state [mg kg ⁻¹]	~~``	~ ~~		r e	S &		
BCFss	130 ±	614 ±	316♣	\$62 ± 65	725 ±	393 ±	
Steady-state BCF [L kg ⁻¹] 🗞	18.90	% 8.9 "	316 31.2	42.2	\$ 7.8	42.3	
BCFssl	<i>b</i>			. *	8		
Lipid normalized steady-state BCF IL kg-1 *	\$127	£9 9	3080	M58	707	383	
BCF [L kg-1]*	5 . T						
BCF _K	(127 ± ^	75) (¥	₹71 ±	137 ±	674 ±	361 ±	
Kinetic BCF & kg-1	(127 ± ^ (13.4)	7 5.6	33.2	14.6	39.2	20.0	
k ₁	b 4	7 0 0 0 1 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0		ý .			
	148 4	861 ±	25.50 ±	118 ± 11.5	700 ± 40.8	350 ± 19.4	
Overall optake rate constant (C) kg day []	412.3		25,30 27	11.3	40.6	19.4	
l ka		1.15 ±	205	0.505	1.04	0.050	
Overall depuration rate	0.932 ± \$	1.15 ± 0.0522	0.05 ± 0.0885	0.785 ± 0.0715	1.04 ± 0.0445	0.970 ± 0.0523	
constant [day 4]	Sy.1300'	0.9022	© 10.0883	0.0713	0.0443	0.0323	
BCFKL &	Y B						
Lipid-normalized kinetic BCI	2 24	732	362	147	657	352	
		, Q"					
BCF _{Kg}		\$					
Growth-corrected kinetic BCK	Ø29	762	379	155	686	370	
[L kg ⁻¹]	\$\frac{1}{2}\frac{1}{2						
Growth Fate constant [day-12.*	n.d.	n.d.	0.018	n.d.	n.d.	0.023	
luay 30" S							
k _{2g} y y y							
Growth-corrected depuration	0.914	1.13	1.03	0.762	1.02	0.947	
rate constant [day-1]							
t _{1/2g}	0.758	0.613	0.673	0.910	0.680	0.732	



	0.500 μg [pyrazole-4- ¹⁴ C] BCS-CN88460/L			5.00 μg [pyrazole-4- ¹⁴ C] BCS-CN88460/L		
Growth-corrected half-life [day]						w°
BCF _{KLG}						
Lipid-normalized growth-cor- rected kinetic BCF [L kg ⁻¹]*	126	743	370	151	669	361

n.d. = not determined

Conclusion

For the whole fish, the lipid normalized steady-state bioconcentration factor (BCF_{SST}) was calculated to be 308 L/kg and 383 L/kg for the treatment level of 0.5 and 5.0 ug/L, respectively. For the whole fish, the lipid normalized and growth corrected kinetic Diocorrectnation factor (BCF_{KL}) was calculated to be 370 L/kg and 361 L/kg for the treatment level of 0,5 and 500 μg/δ, respectively.

CA 8.2.3 Endocrine disrupting propertie

There are no indications of endocine-disrupting effects from the existing catabase for isoflucypram. All of the reports discussed in the following review are fully suppriarized under the appropriate data point within this dossier.

; 20**0**8; M-653376-01-1 Report: KCA 8,≇3/01;

Evaluation of soflucypram with regard to emocrine disrupting properties in non-target vertendates. Title:

V4-813376-01-1√ Report No .: Document No .: Guideline(s): Guideline deviation(s)

GLP/GEP; 👰

of endocone-disrupting properties of isoflucypram in non-target Summary of the assessment vertebrates

From comprehensive exicological divestigations in manimals, isoflucypram does not raise concerns with regard to endowine-retaited effects plated to the EATS modalities; there is no evidence for direct effects in mammals, and indirect endocrine (thyrodd)-related effects are not of concern for wild mammals Because it has been demonstrated that the EATS pathways are highly conserved across vertebrates, it can be reasonably considered that in a first approach, a simple testing strategy to confirm the absence of ED-related concern in mon-target vertebrates other than mammals would be sufficient, as long as all assessment of potential thyroid-mediated effects is included. This was addressed by including the results of a FEQS study in the assessment strategy of potential endocrine disrupting properties of isoflucypram. The FELS test with isoflucypram was negative with regard to potential thyroid-mediated effects, giving a first insight to the absence of ED-related concern in nonmammalish vertebrates.

^{*}Lipid normalization factor = 0.975 (based on mean lipid fraction (wet weight) of 5.13 %

^{**}growth rate constants were only calculated for the whole fish but also used for the calculations of edible and vi



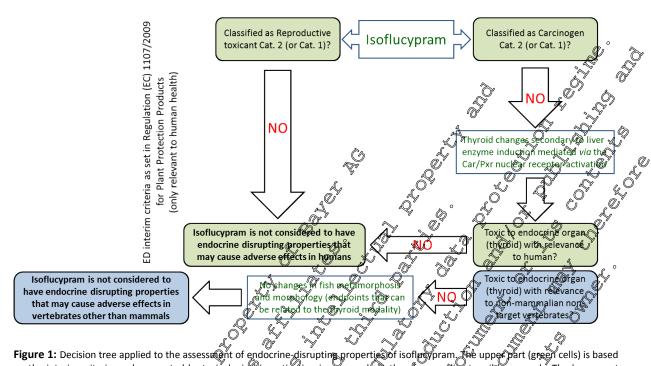


Figure 1: Decision tree applied to the assessment of endocrine-disrupting properties of isoflery pram. We upper part (green cells) is based on the interim criteria as documented by toxicological investigation in mammals. It therefore applies to with mammals. The lower part (blue cells) concerns non-mammal vertebrates (i.e., fish) where the thyroid modality was investigated. Adapted from the document "Screening of available evidence on chamical substances to the identification of endocrine disruptors according to different options in the context of an Impact Assessment - Specific Contract SANIF 2015/53/S12.706218" prepared for the European Compassion in 2016.

Considering all available information from a confirencisive toxicological database, we do not find indications for endocane disrupting properties through oestrogen, androgen, thyroid or steroidogenesis mode of action for isothicypram. Also the available ecotoxicological studies did not contradict this conclusion and isoflucypram can be regarded as having no endocrine disrupting properties.



CA 8.2.4 Acute toxicity to aquatic invertebrates

CA 8.2.4.1 Acute toxicity to Daphnia magna

Material and methods

CA 8.2.4	Acute toxicity to aquatic invertebrates
CA 8.2.4.1	Acute toxicity to Daphnia magna
Report: Title:	KCA 8.2.4.1/01; 2016; M-574184-01-1 Acute toxicity of BCS-CN88460 (tech.) to the waterflead Daphnia magna in a static laboratory test system - Final Report
Report No.: Document No.: Guideline(s):	KCA 8.2.4.1/01; 2016; M-574184-01-1 Acute toxicity of BCS-CN88460 (tech.) to the waterflew Daphnia magna in a static laboratory test system - Final Report EBLNN033 M-574184-01-1 OECD Guideline No. 235 (Guideline for Testing of Chemicals, Chironomus sp., Acute Immobilisation Test., Dopted July 28, 2011) US EPA OCSPP 850.SUPP on(s):
Guideline deviation GLP/GEP:	yes A A A A A A A
Material and m	nethods & & Z Z Z Z Z Z Z Z Z Z Z Z Z Z Z Z Z
Test material	on(s): none yes BCS-CN88460 tech., Origin batch ID: 2013-006492 Batch BCS-C888466-01-06 Specification No. 102000028196 Purity 94.2% w/w None specified Water flea (Daphnia mogna)
Guideline(s) adaptation	None specified
Test species	Water flea (Daphria magna)
Organism age/size at study initiation	First instar neonates, less than 24 hours old
Test solutions	Nonanal concentrations: 50.0, 100, 200, 400 and 800 µg a.s./L Corresponding geometric mean concentrations: 59.9, 116, 226, 433 and 853 µg a.s./L Controls: Elendt M7 medium Solvent control: 0 in ml/L Dimethylformamide Evidence of undissolved material: No remarkable observations, clear media.
Replication	No. of vessels per control (replicates): 6 No. of vessels per control (replicates): 6 No. of vessels per control (replicates): 6
Organisms per replicate	No. of organisms per vessels 5
Exposure	Static S S S S S S S S S S S S S S S S S S S
Feeding during test	None



Test	Temperature: 19.4 – 23.2°C
conditions	Photoperiod: 16 hours light / 8 hours dark at max. 1200 lux
	pH: 7.9 – 8.0. Water hardness: 214 mg CaCO ₃ /L Dissolved oxygen: 8.6 - 8.9 mg/L Conductivity: 555 uS/cm
	Water hardness: 214 mg CaCO ₃ /L
	Dissolved oxygen: 8.6 - 8.9 mg/L
	Conductivity: 555 µS/cm
	Alkalinity: 53 mg CaCO ₃ /L
Parameters Measured / Observations	Visual comparison of untreated control animals and treated animals, performed after 24 and 48 hours of exposure Temperature in test solutions was measured at the start of exposure 4.5 boost start
	and at the end of the test. Oxygen saturation and put-values were determined of the start and the end of the test.
Chemical	The content of BCS-CN88460 in exposure modia was measured for verification of
analysis	the test item concentrations and HPLC-MS/MS (LOQ = 625 µg/L).) at test
•	initiation and termination.
Data analysis	The EC ₅₀ value was calculated by probit analysis, fitted by an iterative weighed
	linear regression according for the maximum likelihood principle with the software
	ToxRat-Professional Version 2.10)

Validity criteria	J,) L	Required	Qb@ined_
Mortality in control during	Çest C			≤10%	\$0.0 X
Dissolved oxygen concentra	ition (al , th	ne end of th	réfiest 🧳	$\emptyset \ge 3$ $\log L$	$\langle \rangle \geq 8.6 \text{ mg/L}$

Analytical results:
The accompanying chemical analysis of BCS-CN 8460 in the reshly prepared test solutions at test initiation ranged between 105% and 114% (mean: 110%) of the aspired nominal concentrations. The corresponding concentrations of the aged test solutions at the end of the 48 hours exposure period ranged between 107% and 122% (mean: 115%) of nominal demonstrating stability in the test system. Since some analytical results exceeded the defined limits of 80-20% for nominal range, all results are based on geometro mean-measured concentrations. No contaminations of BCS-CN88460 were detected in samples from untreated water control.

Nominal Concentration (µg as./L)	Day & Measured Concentration Jug a.QL)		Concentration (μg a.s./L)	Day 2 % Nominal	Geometric mean measured concentration (µg a.s./L)
Control	2 625 °		< 0.625	-	-
Solvent control	@ 0.62\$\dots	, * , 0'	< 0.625	-	-
50.0	A 500 J	₽14	61.1	122	59.0
100	1 3 🛴 .	J 113	120	120	116
200	221	111	231	116	226
400	\$ 48	105	448	112	433
8000	© \$\frac{2}{849}	106	856	107	853



Biological results:

Immobility

R		Document West	Isoflucypram
Biological results	<u>:</u>		
Immobility			
Exposure time (hours)	24	48	
Nominal conc. (μg a.s./L)	No of immobilised daphnids (%)	No of immobilised daphnids (%)	
control	0 (0)	0 (0	
solvent control	0 (0)	0 (8)	
50.0	0 (0)	(0)	
100	1 (3.3)	2 (6.7)	
200	4 (13.3)	18 (60.0)	
400	24 (80.0)	39 (10 9)	
800	30 (100)	30 (1 6 0) Q	
Conclusion The study meets concentrations are	24 (80.0) 30 (100) s the validity enteria a e: % C.I.): 316 ag a.s. / (5% C.I.): 201 ag a.s. / (1	and the endpoints base	don geometric mean-measured
EC ₅₀ 24 hours (95	% C.I.): 316 ag a.s. /d	(273 4,365 μ _β ,a.s. /L)	
EC ₅₀ 48 hours (95	5% C.I.): 201 µg a.s. I	L (176 – 229 μg a.s./L)	
Report: Fitle:	KGA 8.2.4 02; Agute to selty of BC Daphnia magea in a selection of BC EBLON198 © M-\$7329601-1	2016: M-5733 S-6N88460 carbocolic-acid	296-01-1 (BCS-Y26497) to the waterflea
Guideline(s);	EBLON198 O M-5/73296-01-1 EU Directive 91/114 Regulation110 2009 US EVA OCSPP 856	S-Cys8460-carboxync-acresiant labeliatory test system (EFC) (Europe) (1010)	
Guideline deviation	none s		

Conclusion

EC ₅₀ 24 hours (95% C.I.):	316 μg a.s. / (273 4365 μg a.s. /L)
EC50 48 hours (95% C.I.);	201 μg a.s. L (176 – 229 μg a.s. /L)

Material and methods

EC50 48 nours (95% C.14; 201 µg a.\$ L (176 – 229 µg a.\$ /L)
	KGA 8.2. 6702; 2016; M-573296-01-1 Aguite to serity of BCS-CN88460 carboxolic-activ (BCS-CY26497) to the waterflea Daphnia magga in a staric laboratory test system EBLON198
Report:	KGA 8.2.45/02; 2016: M-573296-01-1
Title:	Aguite toxicity of BCS-CN88460 carboxolic-acid (BCS-CY26497) to the waterflea
	Daphnia magga, in a staric laboratory test system
Report No.:	Daphnia magna in a stanc laboratory test system EBLON198 M-5-73296-01-1
Document No.:	© M-5₹3296-01-1
Guideline(s)	EU Directive 91/4/EBC
	Regulation 110 2009 (Europe)
∜ >>	US DA OCSPP 850 1010 V
Guideline deviation	on (Spinofite Spinofite)
GLP/GEP:	Aes & S
Q ₁	
Material and m	nethods & S & & &
	EBLON198 O M-5-73296-01-1 EU Directive 9 1714/EEC Regulation110 2009 (Europe) US EVA OCSPP 850 1010 on one methods BCS-CN88460-carboxylic-acid (BCS-CY26497) Origin barch: SUS 12631-19-9
Test material	BCS CN88460 Arthorythe acid BCS CV26407)
Test material	Origin barch: SES 12601-19-9
~	Batch code: BCS-CX26497-01-02
A S	TOX 2005&00 Q
	Parity: 988%
Guideling (s)	None specified
odonto (Xn	Whole specified
adaptation	
Test species	Water (Lea (Daphnia magna)
Organion	First instar neonates, less than 24 hours old
age/size at	, 1000 01011 - 110011 0 010
study	
initiation	



Test solutions	Nominal concentrations: 1.5, 3, 6, 12 and 24 mg p.m./L Corresponding arithmetic mean measured concentrations: 1.77, 3.45, 6.87, 13.3 and 26.6 mg p.m./L Control: water and solvent control (100 µL dimethylformamide/L)
Replication	No. of vessels per concentration (replicates): 6 No. of vessels per control (replicates): 6
Organisms per replicate	No. of organisms per vessel: 5
Exposure	Static Total exposure duration: 48 hours
Feeding during test	None Q Q Q Q Q Q
Test conditions	26.6 mg p.m./L Control: water and solvent control (100 μL dimethylformamide/L) No. of vessels per concentration (replicates): 6 No. of vessels per control (replicates): 6 No. of organisms per vessel: 5 Static Total exposure duration: 48 hours None Temperature: 20.3 - 22.4°C Photoperiod: 16 hours light / 8 hours dark Light intensity: max. 1200 lux pH: 7.6 - 7.8 Water hardness: 213.6 mg €aCO ₃ L Dissolved oxygen 8.6 - 8.9 mg/L (> 95.9 saturation) Conductivity: 535 μS/cm Alkalinity: 533 ng CaCO ₃ /L Counting of mobile daphrids. Visual comparison of untreated control animals and treated animals, performed after 24 and 48 hours of exposure. Measurement of pH-value and measurement of dissolved oxygen, both determined for all freshly prepared solutions (batch, sample) and again in the aged solutions (composite eplicates) at the end of exposure. Water temperatures within the test system were recorded at start and end of exposure from one vessel of the untreated control group and of the highest treatment group.
Parameters Measured / Observations	Counting of mobile daphneds. Visual comparison of untreated control animals and treated animals, performed after 24 and 48 hours of exposure. Measurement of pH-value and measurement of dissolved oxygen, both determined for all freshly prepared solutions (batch samples and again in the aged solutions (composite replicates) at the end of exposure. Water temperatures within the test system were recorded at start and end of exposure from one vessel of the untreated control group and of the highest treatment group.
Chemical analysis	Froshly propared test media: Sampling immediately before distribution to the test vessels, from batch preparation for each treatment and control group. Aged test media: Sampling immediately after termination of exposure as composite from all replicates of a treatment group and control group. All samples were measured by HPL -UV.
Data analysis	Probit analysis, fitted by an iterative weighted linear regression according to the Machium kelihood principle. For calculations Tox-Rat-Professional (Version 3.2.1) and Excel 2010 were used.

p.m. = pure potabolite

Results

Validity criteria	Required	Obtained
Mortality in control during tes	<u>≤</u> 10%	0%
Dissolved exgen concentration at the end of the test	≥ 3 mg/L	\geq 8.6 mg/L

Analytical results:
The recoveries were between 108 and 118%. Therefore results of the study are based on nominal test concentrations. No contaminations of BCS-CN88460-carboxylic-acid (BCS-CY26497) were detected in samples from untreated water control.



Nominal test concentration (mg p.m./L)	Day 0 Measured Concentration (mg p.m./L)	Day 0 % Nominal	Day 2 Measured Concentration (mg p.m./L)	Day 2 % Nominal	Arithmetic mean measured concentration* (mg p.m./L)	% Mean measured concentration
1.5	1.77	118	1.76	117	1.70	10/8
3.0	3.42	114	3.47	116	36A5	¥.15, \$
6.0	6.82	114	6.92	115	₫ 6.87	\$ 1.150
12.0	13.2	110	13.4	112	13.3	
24.0	25.9	108	27.2	113	ළු 26.6 වි	Y.11 , Q ,
concentration (mg p.m./L) 1.5 3.0 6.0 12.0 24.0 * Mean measured fi Biological result Observations No immobilities exposure. Immobility Exposure time (hours) Nominal test concentration (mg p.m./L) Control	s or other effe	cts on beh	avor occurred	ir untreat	ed control with	hours, an EC ₅₀
Exposure time (hours) Nominal test concentration (mg p.m./L)	No of immobility	d Jimn	A8 S No of the control of the contro			
Control	Q ₀ (0)		0 (0)	,		
Solvent control	(0)		040)			
1.5	0 (0)		0 (0)		\$ \$ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \	
3.0	((0) .	8 3	0(0)	()	
6.0			(3.3)		Z	
12.0		O W	(6.7)		~~ ?n	
24.00	40(0)	, Q A	0 (0)		<i>U</i>	
Conclusion The study meets As the highest	the validity crit concentration of d not be perform	Fra. The price of	m./L caused not EC ₅₀ Q4 and 48	hours) for	BCS-CN88460	hours, an EC ₅₀ -carboxylic-acid

^{*} Mean measured figure not given in report; calculated on the basis of concentrations on day 0 and 2χ

Biological results:

Observations

Immobility

Exposure time (hours)	24	48
Nominal test	No of	Q No of
concentration (mg p.m./L)	immobilized	mmobilized 7
Control	% (0)	\$\text{0}(0)\text{\$\tilde{\text{0}}\$}
Solvent control	0 (0)	
1.5	0 (0)	
3.0	6 (0) 5	\$\int 0 (0) \tag{\tag{\tag{0.00}}
6.0		1(3/3)
12.0		(6.7)
24.00	(0) (0)	

Conclusion

The study meets the validity criteria.

As the highest concentration of 24 mg p.m./L caused no immobilization after 48 hours, an EC₅₀ As the highest concentration of 24 mg p.m/L caused no immobilization after 48 hours, an EC₅₀ calculation could not be performed and the EC₅₀ (24 and 48 hours) for BCS-CN88460-carboxylic-acid (BCS-CY26497) was stated to be higher than 24 mg p.m./L nominally



CA 8.2.4.2 Acute toxicity to an additional aquatic invertebrate species

A study with an additional aquatic invertebrate species (mysid shrimp) was conducted for registrations outside the EU. This study was previously evaluated in the EU. A summary of the study is provided below.

teport: KCA 8.2.4.2/01; 547041-01-1 BCS-CN88460: A 96-hour static-renewal acute to scity test with the saltoater mixed (Americamysis bahia) eport No.: 149A-257B M-547041-01-1 US EPA OCSPP 850.1035 mone gLP/GEP: yes Material and methods Test material BCS-CN88460 Batch Code: 203-006492. Purity 94.29 w/w. Guideline(s) adaptation Test species Saltwater mixed (Americaniysis bahia) Organism age/size at study initiation
Test material BCS-CN88460 Batch Code: 2693-006492. Purity 94.2% w/w Guideline(s) adaptation Test species Saltwater mysid (**prericonysis bahia*) Organism age/size at Javenile mysids less than 24 hours old
Test material Guideline(s) adaptation Test species Saltwater nevid (**americanysis gahia**) Organism age/size at Javenile mysids less than 24 hours old
Test material Guideline(s) adaptation Test species Saltwater nevid (**americanysis gahia**) Organism age/size at Javenile mysids less than 24 hours old
Test material Guideline(s) adaptation Test species Saltwater nevid (**americanysis gahia**) Organism age/size at Javenile mysids less than 24 hours old
Test material Guideline(s) adaptation Test species Saltwater nevid (**americanysis gahia**) Organism age/size at Javenile mysids less than 24 hours old
Test material Guideline(s) adaptation Test species Saltwater nevid (**americanysis gahia**) Organism age/size at Javenile mysids less than 24 hours old
age/size at
study initiation 🗘 🦠 📡 🌣 🎺 🎺 🎺
Test solutions Nominal concentrations: 0.05%, 0.11, 0.23, 0.45 and 0.90 mg a.s./L
Mean measured concentrations: 0.037, 0.11, 0.23, 0.42 and 0.82 mg a.s./L
Controls, institutat intered and aerated segment obtained at indian kivel inter,
Delaware Delaware Company of the Com
Solvent control 0.1 m/L dimethylformamide
Evidence of indissolved material: No precipitates observed.
Replication No of vessels per concentration (replicates): 2
No. of ressels per control (replicates): 2
No. of vessels per solvent control (replicates): 2
Organisms per No of organisms per beaker: 19
replicate 5 5 5
Exposure Semi-static renewal after 48 hours Total exposure duration: 96 hours
Feeding during None O S
test of the second of the seco
Test Temperature: $25 \pm 2^{\circ}$ C Photogeriod: 16 hours light / 8 hours dark at 681 lux pH: $8.0 - 8.2$ Dissolved oxygen: $\ge 6.7 \text{ mg/L}$ ($\ge 91\%$ of the saturation value)
Photogeriod: 16 hours light / 8 hours dark at 681 lux
pH: 8.0 – 8.2
, , , , , , , , , , , , , , , , , , ,
Salinity: 20 ‰



Parameters Measured / Observations	Observations of mortality and treatment related effects were made at 3.5, 24, 48, 72 and 96 hours. Measurements of temperature, pH, salinity and dissolved oxygen of the water in the test chambers were performed daily. At time point of renewal, measurements were conducted prior and after renewal of the test media
Chemical analysis	Test concentrations were determined from samples of test water collected from each treatment and control group at the beginning of the test, before and after renewal at the approximate mid-point of the test (approximately 48 hours) and at the end of the test by high performance liquid promatography (HPVC) with ultraviolet absorbance detection at a wavelength (220 nm. LOO was 0.0313 mg a.s./L.
Data analysis	The mortality data were analyzed using the computer program of C. E. Stephan. The program was designed to calculate the LCs value and the 95% confidence interval by probit analysis the moving average method, and finomial probability with nonlinear interpolation. Based on the mortality pattern in this study, probit analysis was used to calculate the 48 and 72-hour ΦC_{50} values. Nonlinear interpolation was used to calculate the 96-hour LC ₅₀ value and binominal probability was used to calculate the 95% confidence interval. Due to the method used to calculate the 96-hour LC ₅₀ value, the slope of the concentration response curve could not be calculated.

	·	≥ .v		
Validity criteria according	gto OPPTS 85	500 1 035	Required	Op tained *
Mortality of mysids in contr	ols at rest end		J 10%, O	Negative control: 5% Solvent control: 0%
Dissolved oxygen of ar-satu	ration 🦃 į	Ş, Ş	[*] ≥ 60%	<u></u> 291% √

Analytical resorts:

Recoveries were between 86.9 and 107% (see table below). Nevertheless biological results are based on arithmetic mean measured concentrations. No residues of BCS-CN88460 above the LOQ were measured in the controls.

Nominal Concentration (mg a.s./L)	Arithmetic mean % of nominal measured concentrations concentrations (mg a.s./L)	Range of individual measurements (% of nominal)
0.056	0.050	97.6 - 107
0.11	Q1 Q1 > 000	96.2 - 105
0.23	0.23	94.2 - 107
0.45	0.42 93.3	87.9 – 99.3
0.90	<u>√</u> 0.82 √ Q 91.1	86.9 – 95.2

One dead mysod in the negative control group at test termination was recorded. All other mysids in the negative and solvent control groups appeared normal throughout the test. All mysids in the 0.057 and 0.17 mg & ./L treatment groups also appeared normal throughout the test, with no mortalities or overt signs of joxicity observed.



Mortality

Exposure time (hours)	3.5	24	48	72	96	<u> </u>
Nominal conc. (mg a.s./L)	No of dead	No of dead	No of dead	No of dead	No of dead	
Control	0	0	0	0	\$ 0	
Solvent control	0	0	0	0	0 6	F S
0.057	0	0	0 💍	0	0 💸	
0.11	0	0	0, 8	0,Q,	00	\$ 2
0.23	0	2	4 Q Y	, S	S C	
0.42	0	0	1 5	17	Q 20 O	
0.82	0	8	20 °	\$\tag{\text{7}} 20\text{7}	© 200 ·	

Conclusion

The study meets the validity criteria and concentrations are:

LC ₅₀ 96 hours (95% C.I.):	0.27 mg a.s. L (0.23 mg a.s./L - 0.42 mg	».s./L
NOEC:	© 11 mg a.s./L 0	

to aquatic invertebrates **CA 8.2.5**

Long-term and chronic toxicity rouse toxicity states for aquatic inv ertebrates are provided under CA 8.2.5.1 and Long-term and chrome toxicity CA 8.2.5.2.

Reproductive and development toxicity to Daphnia magna CA 8.2.5,12

,2017<u>(</u>M-593**2**61-01-1 Report:

Effects of BCS-CN8846@(tech.) On development and reproductive output of the Title:

waterfle Daplana magna in acstatic renewal laboratory test system

Report No.: M₂Ø3961√01-1 Document No.

Guideline(s) EU Directive 9144/EB

Guideline deviation(s)

GLP/GEP:

Material gasti ille	anous. Or work and a second se
Test malerial:	BCSCN88460 (tech.)
	BQS bate@code: BCS-CN88460-01-06
	Specification No: 102000028196
	Purity 94.2% w/w
Guideline(s) adaptation	None specified
Test species:	Water flea (Daphnia magna)





Organism Age at Experimental Start:	1 st instar neonates less than 24 h old
Test solutions	Nominal concentrations: 0, 4.5, 9.0, 18, 36, 72 and 144 µg a. L Corresponding time weighted average concentrations: 5.33 ¥0.7, 20.3, 40, 4, 80, and 162 µg a.s./L Controls: water control and solvent control (0.1 mL dimethylformamide/L test solution) Evidence of undissolved material: No andissolved matter visible
Replication:	No. of vessels per concentration (replicates): 10 replicates No. of vessels per control (replicates): 10 replicates
Organisms per replicate:	No. of organisms per vessel: 160°
Exposure:	Static-renewal conditions (approx. 3 water renewals for week) Total exposure duration, 21 days
Test Vessel Loading:	100 mL of test solution in 250 mL@lass beakers
Feeding during test	Three times perweek with living cells of the green algae Desmodesmus, subspicatus
Test conditions:	Temperature: 19.7 – 21 C Photoperiod: 16.8 hours light dark Light intensity: 1000 21200 dax pH: 7.4 – 7.9 Water hardness: 13 to 14 (°dH) German degrees) Dissolved oxygen: 8,2 – 9.2 mg/L Alkalmity: 2, – 4 (°dH, German degrees, as carbonate hardness)
Parameters Measured Deservations	Measurement of water temperature, total hardness, alkalinity, pH and dissolved on vere conducted on day 0, 2, 5, 9, 9, 2, 14, 96 and 19. As endpoints, the total number of living offspring per parental animal, the parental age by first offspring emergence as well as the rate of parental survivors and their body-length and dry body mass at the end of the study was recorded.
analysis 🐠	For verification of the actual rest item concentrations during exposure, water- samples from start and end of 3 representative exposure-intervals were analysed. BCS-CN8\$460 was measured by HPLO-MS/MS
Data analysis:	For consideration whether or not recorded mortality of parent animals follows a concentration response pattern, the Cochran-Armitage trend test was used to detect if there is a significant repression of the response versus test concentration
	with a positive slope (non-GLP). If applicable, at least the EC ₁₀ including the associated 95 percent confidence timits for parental immobilisation and total living offspring was calculated by Probit analysis.



Validity criteria according to OECD 202	Required	Obtained
Mortality of the parent animals in control at the end of the test	≤ 20%	0%
Mean number of living offspring produced per parent animal surviving in control at the end of the test	> 60	> 80

Analytical results:

Validity criteria according to OECD 202				Required	Obtain	ed		<i>@1</i>	*
Mortality of the parent animals in control at the end of the test			nd of	≤ 20%	0%	~	,		
Mean number of living offspring produced per parent animal surviving in control at the end of the test			rent	> 60	> 80	Ő	Z,		ත
Validity criteria according to OECD 202 Required Obtained Mortality of the parent animals in control at the end of the test ≤ 20% 0% Mean number of living offspring produced per parent animal surviving in control at the end of the test > 60 > 80 Analytical results: No residues of BCS-CN88460 above the LOQ were parasured in the controls. No minal Time weighted mean Time weighted Time weight									
Nominal concen- trations (μg a.s./L)	Time weighted mean measured concentrations (µg a.s./L)	% of nominal concentrations	Day 0	Day 2 Aged	Day 9	<u> </u>	tions 19 (1	
4.5	5.33	118	126	U 124	,918 ×	113	11.7	ÕÎ16	
9.0	10.7	€ وال	-126	£ 24 €	7 1175	1\$2	J18 &	119	
18.0	20.3	Q 113	119	1200	64	Q 05 E	113	112	
36.0	40.4	© 112 4	120\\$		1 12 C	1040	Q12	110	
72.0	80.1	ill sy	120	¥19 (1110	$10\tilde{2}$	0112	108	
144	162 👸	1125	A 19	0 118	403	302 3106	111	111	

Biological results:

Observations

No dose related behavioral effects were noted for any test level, including the control group.

Length and body weight at test termination

Time weighted mean	Les	igth J	Dry bod	y weight
measured concentrations (µg a.s./②)	Mean ± Sp (mm)	% Deviation from solvent control	Mean ± SD (g)	% Deviation from pooled controls
Control	© 3.89 € 0.2 €		0.852 ±	-
Solvent control	$4\cancel{6}$ $\pm 0.\cancel{2}$	22, 2, E	0.803 ±	-
Pooled control	3.98 £ 9.2	~ , Q · -	0.828 ±	-
5.33	\$\frac{4.06}{2} \neq 0.1 \qua	-0.1	0.874 ±	+ 5.6
10.7 ^	3.0A ± 0.2	-3.1	0.935 ±	+ 13.0
20.3	.91 ±0.2	-3.9	0.817 ±	- 1.3
400 4	3.95₹ ≠ 0.1 √	-3.0	0.844 ±	+ 2.0
8 0.1 0	$0 + 4.01 \pm 0.1$	-1.4	0.709 ±	- 14.3
1625	7.72 ± 0.1	-8.6*	0.693 ±	- 16.3

^{*} Statistical significant infference from solvent control (verified by Williams Multiple Sequential t-test Procedure on a 5% level of significance at one-sided smaller).



Immobility

Time weighted mean measured concentrations (μg a.s./L)	No of immobilised daphnids (%)	
Control	0 (0)	
Solvent control	0 (0)	\$\tag{\psi}\$
Pooled control	0 (0)	
5.33	0 (0)	
10.7	0 (0)	
20.3	0 (0)	
40.4	0 (0)	
80.1	0 (0)	
162	0 (0)	
Effects on reproduction		
Time weighted mean	Total offspring per	Average daily offspring Rge at First offspring per surviving pagental comergence
measured	surviving parental	per surviving parental S emergence

Effects on reproduction

Time weighted mean measured concentrations (µg a.s./L)	Total offspring per surviving parental \$\sqrt{\text{Temale}}\$ (Mean(\frac{1}{2}SD) \$\sqrt{\text{SD}}\$	Average dails offspring per surviving parental female (Mean ± SD)	Age at Arst offspring Emergence Mean = SD)
Control	85.5¥ 17.3	6.8 ±0.2 6	1022 ± 0.97
Solvent control	96.5 ± 1992	© 7.8 ± 1.6 €	Ø10.32 ± 1.08
Pooled control	91.0±98.7	7.3 ± 1.5	10.27 ± 1.00
5.33	95.9± 7.4 °	7.3 ± 9.5 %	9.72 ± 0.32
10.7	\$\tag{9.6 \pm 1.05}	7.1 ± 0.9 °	9.92 ± 0.32
20.3	78.3 ±27.0 ~	(5).4 ± 2.5	10.62 ± 1.03
40.40	92.2± 13.4	7.3 10.0	10.22 ± 0.70
80.0	92.2 ± 13.8	8.D± 1.2, V	9.92 ± 0.74
√ 62 ×	35.4 + 20.9	5.4 ± 2.0°	12.52 ± 1.49

Conclusion:

For this study the EC₁₅ values were reported in statistically applicable cases. For the endpoints "dry weight" and "total number of offspring per surviving female" this was the case.

For the endpoint "length" less than 10 % infference to the control was observed at the highest test item concentration of 162 µg a.s./L. The number of effect concentrations and the observed effect size is not sufficient for a reasonable ECQ and EC₂₀ calculation.

For the endpoint "weight" only the two highest concentrations resulted in a reduced body weight (14.3 and 16.3 % reduce@compared to the controls, respectively). The number of effect concentrations and the observed effect size were low. An EC₁₀ was determined to be 66.1 µg a.s./L. At the NOEC no weight reduction compared to the controls was observed.

In the study no immobilities were observed. Therefore no ECx calculations can be performed.

Reproduction: For the endpont "total number of offspring per surviving parental female" an EC₁₀ of 97.1 de/L was calculated.

At the NOTEC of 80.1 µg/k no reduction of reproduction compared to the control was observed.



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

	Length	Dry body weight	Immobili- sation	Age at first offspring emergenees	Total offspring per surviving parental comale
LOEC [µg a.s./L]: lowest concentration with an significant effect compared to the control	162	162	> 162	162	\$ 162, \(\frac{1}{2}\)
NOEC [µg a.s./L]: highest concentration without an significant effect compared to the control	80.1	80.1	∑ ≥ 162	80.1	80.15
EC ₁₀ [μg a.s./L]	n.a.*	66 01	n.a.*	n.a.*	97.1 °

^{*}Not applicable due to absence of effects

CA 8.2.5.2 Reproductive and development toxicity invertebrate species

Americams sis babia, Leptocheirus Chronic studies with additional aquatic invertebrate species plumulosus, Crossostrea virginica and Hyaello aztear) webs confected for registration outside the EU. These studies are summarised below.

Title:

BCS-(N88460): A flow-through life cycle texticity test with the saltwater mysid

(American (Sis bahla))

Report No.:

Document No.:

Guideline(s):

Guideline deviation(s):

GLP/GEP:

Wes

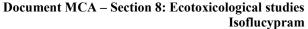
Material and methods:

Test material Report: K. CA 8.2.5 9/01;

matchiai and in	
Test material	
~~	BCS-CN88460 Batch 2013-006492. Purity 942% www. None specificat
Guideling(s)	None specified & San
adaptation	
Test species	Sattwater mysic Americamysis bahia)
Organism	Juvenile mysids, less than 24 hours old
age/size at	
study initiation	
^~	Nonlinal concentrations: 22, 44, 88, 175 and 350 μg a.s./L
Test solutions	Another the mean measured concentrations: 20, 37, 79, 146 and 299 µg a.s./L
	Controls: natural ozonated and filtered seawater
, O	Controls, natural ozonated and intered seawater
	Solvent control: 0.1 mi/L triethylene glycol
	Evidence of undissolved material: No precipitations observed.
Replication	No. of compartments per concentration (replicates): 4



	No. of compartments per control (replicates): 4 No. of compartments per solvent control (replicates): 4
	Toot 1: No. of organisms per compartment: 15
Organisms per replicate	Test 1: No. of organisms per compartment: 13 Test 2: No. of organisms per compartment: 10
Exposure	Flow-through Total exposure duration: 28 days
Feeding during test	Live brine shrimp nauplii (Artemia sp.) daily
Test conditions	Temperature: $24.8-26.8^{\circ}\text{C}$ Photoperiod: 16 hours light / 8 hours dark at 115 fex pH: $7.8-8.0$ Dissolved oxygen: ≥ 5.2 mg/L ($\geq 62\%$ of the saturation value) Salinity: 20%
Parameters Measured/ Observations	No. of compartments per solvent control (replicates): 4 Test 1: No. of organisms per compartment: 15 Test 2: No. of organisms per compartment: 10 Flow-through Total exposure duration: 28 days Live brine shrimp nauplii (<i>Artemia</i> sp.) daily Temperature: 24.8 − 26.8°C Photoperiod: 16 hours light / 8 hours dark at 115 dax pH: 7.8 − 8.0 Dissolved oxygen: ≥ 5.2 mg/L (≥ 62% of the saturation value) Salinity: 20 ‰ Observations of mortality and climical signs of toxicity in G1 mysids were made daily throughout the exposure period. Following pairing, all second-generation (G2) mysids produced in each reproductive compartment were counted and removed daily. Collected G2 offspiring were observed for approximately 96 hours post-release. During this period observations of mortality were made daily. At the conclusion of the 96-hour observation period, the surviving G2 mysids were discarded. Test water samples were collected from one test chamber of each treatment and control group two and four day prior to the start of exposite to confirm
anarysis	Test water samples were collected from one test chamber of each treatment and control group two and four days prior to the start of exposure to confirm concentrations after conditioning the diluter system for six and four days, respectively. Test water samples also were collected from one replicate test chamber in each treatment and control group at the loginning of the test, approximately weekly during the test and of the end of the test to measure concentrations of the test substance. An additional sample was collected on Day 2 due to a small leak discovered in the toxicant delivery to the 44 μg a.s./L treatment group and again at the end of the day to confirm concentrations.
EG .	Concentrations of BCS-CN88466 in the samples were determined using an Agilent Model 1100 or 1200 high performance liquid thromatograph equipped with an Agilent Series 1700 or 1200 pariable wavelength detector.
Data analysis	Data for survival and the percent of surviving females producing young are considered to be discrete ariable data. Data for the number of young per reproductive day, number of young moduced per surviving female and growth are considered to be confinuous. Variable data.
	Discrete-variable data were analysed using Chi-square and Fisher's Exact tests to identify treatment groups that showed a statistically significant difference from the pooled control. Continuous-variable data were examined to determine whether the concentration-
	response was fundamentally monotonic (trending in one direction, e.g. response not trending op) and then down as concentration increase) or non-monotonic. All continuous-variable data consistent with a monotonic concentration response were analysed using the Jonckheere-Terpstra trend test applied in a step-down procedure. The data were also evaluated for normality using the Shapiro-Wilk's test and for homogeneity of variance using Levene's test. All of the data passed the assumptions of normality and homogeneity of variance. Therefore, those treatment means that were significantly different from pooled control means were identified using Dunnett's test.
	using Dunnett's test. All statistical tests were performed using a personal computer with SAS software.





Results

Validity criteria according to OPPTS 850.1035	Required	Obtained
Mysids of the first-generation in the controls died between the start and end of the test	≤ 30%	< 30%
Females of the first-generation in the controls failed to produce young	<u>≤</u> 25%	≤ 2.7
The average number of young produced by first-generation in the controls	≥ 3	≥ 8.8 ± 3.98

Analytical results:

Recoveries were between 71 and 98% (see table below). Therefore results were based on another mean measured concentrations. No residues of BCS-CN88460 were found in the control and solvent control samples above the LOQ (limit of quantification = 0.0125 mg/a/s./L).

Nominal Concentration (µg a.s./L)	Arithmetic mean measured concentration (µg a.s./L)	% of nominal concentrations	Range of individual of the control o
22	20 ± 1.3	√ _~ \$91 \$\$	80 :3 – 9 3
44	37 ± 3.€ (*)	820 E	71.1 98.4
88	79 # 3.2		82.6-97.2
175	140 ± 7.0	§ 83 _©	\$\frac{1}{2}\text{\$\times 3.3 - 8\times 2}\$
350	299 ± P	\$ \$ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \	77.3€90.5€

Biological results:

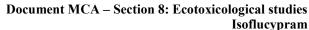
After 13 days of exposure, survival in the pooled control group and in the 20, 37, 79, 146 and 299 µg a.s./L treatment groups was 92.5, 95.0, 91.7, 96.7, 95.0 and 98.7%, respectively. Fisher's Exact test indicated there were no statistically significant decreases in survival in any treatment group when compared to the pooled controls (p > 0.05).

Survival from pairing on Day 14 to G1 most determination on Day 28 in the pooled control group and in the 20, 37, 79, 146 and 299 μg a.s./L. Freatment groups was 76.8, 70.8, 65.9, 54.3, 66.7 and 18.0%, respectively. Fisher's Exact test indicated there were statistically significant decreases in survival in the 79 and 299 μg a.s./L treatment groups in comparison to the pooled controls ($p \le 0.05$). While the decrease in survival was statistically significant in the 79 μg a.s./L treatment group in comparison to the pooled control, it was not considered to be treatment-related since it was not dose-responsive.

The mean number of young produced perceproductive day in the pooled control group and in the 20, 37, 79,146 and 299 µg a.s. It treatment groups was 0.699, 0.991, 0.659, 0.555, 0.378 and 0.000 young per day, respectively. Dunnet is test indicated there were statistically significant decreases in reproduction in the 146 and 299 µg a.s./L treatment groups in comparison to the pooled control (p \leq 0.05). According to the one-sided Jonckheere-Terpstra step-down trend test, a statistically significant, concentration-related trend was no longer evident when the data from the 146 and 299 µg a.s./L treatment groups were excluded from the calculations (p \leq 0.05).

The recan total length of female mysids in the pooled control and the 20, 37, 79, 146 and 299 µg a.s./L treatment groups was 7.95, 7.96, 8.04, 8.16, 7.72 and 7.51 mm, respectively.

The mean dry weight of females in the pooled control and the 20, 37, 79, 146 and 299 µg a.s./L treatment groups was 1.25, 1.30, 1.17, 1.18, 1.12 and 1.09 mg, respectively.





Mortality

Exposure time	Juvenile Initiation			Survival g on Day 13	Adult St Day 14 to		to Test To	vival Day 14 ermination ° Day 28
Mean measured conc. (μg a.s./L)	No of exposed	Percent survival	No of exposed	Percent survival	No alive at pairing ¹	Percent survival	No alive at pairing ¹	Percent Surviva
Control	60	98.3	60	90.0	49	85.T	49 🔎	, T Ø.6 «
Solvent	60	98.3	60	95.0	<u>څ</u> 50	# 8.0	50¢	76.0
Pooled	120	98.3	120	92.5	99	Q 81.8	. 99	76.8
20	60	96.7	60	95.00	48 🔏	81.3	48 ~Q	70.8
37	60	98.3	60	91	44	79 .7	5 44 S	65.9
79	60	98.3	60	9 6.7	46	%71.7 _∞	46	54.30
146	60	98.3	60	S 95.0	¥2 X	⁷ 78 %	42	66.7
299	60	100	60 ,	98.3	© 50 °	400*	50	\$8.0*\(\circ\)

* Statistically significant decrease in survival in comparison to the project control using Fisher Exact test $(p \le 0.05)$.

1 The number alive at pairing may be less than the number surviving to Day 13 due to the social control of the surviving to Day 13.

2 While the decrease in survival was statistically significant in comparison to the pooled control of was not dose-responsive.

Penroduction The number alive at pairing may be less than the number surviving to Da 13 due to the fact that extra females that wannot

Keproduction		<u> </u>
Arithmetic mean measured conc. (µg a.s./L)	Females Producing Young Po	Number of er Surviving le ± SD ¹
Control	9582 ± 6270	± 3.98
Solvent control Pooled control	0.816 0.132 94 94 12.2	2 ± 2.04
	0.699 ± 0.233 99.3	5 ± 3.46
20		5 ± 3.88
37	0.659 = 0.106 929 0 10.8	3 ± 1.19
79	$0.535 \pm 0.366 $ 9.6	± 2.98
146	0.378 ± 0.084*0	± 1.64* ²
299	△ 0.00€ 0.00€ 0.00€ 0.00€ 0.00€ 0.00€	± 0.00* ²

Statistically significant secrease in mean number of young produced per reproductive day and mean number of young per surviving from in comparison to the pooled control using Dunnett's test ($p \le 0.05$).

Statistically significant decrease in percent of urviving femals, producing young in comparison to the pooled control using Figure's Exact test $(p \le 0.05)$.

Calculated based on the total number of surviving females present at test termination. Females that died prior to test

termination and the soring that they produced year excluded from the calculation of the mean percent of females producing young and the mean number of young per ternale.

proflucing young and the man number of young per ternale.

According to the one-side Conckhere-Terpstra step down trend test, a statistically significant concentration related trend was no longer evident when the date from the 146 and 299 μg a.i./L treatment groups were excluded from the calculations (Δ) = 0.051



Growth parameters after 28 days

Arithmetic mean	Mean total length ± SD (mm)		Mean dry weig	ght ± SD (mg)
measured conc. (μg a.s./L)	Males	Females	Males	Females &
Control	7.62 ± 0.155	7.83 ± 0.167	0.97 ± 0.067	1.22 ± 0.098
Solvent control	7.79 ± 0.153	8.06 ± 0.074	0.93 ± 0.021	1.27 ± 0.143
Pooled control	7.70 ± 0.169	7.95 ± 0.172^{1}	0.95 ± 0.049	1.25 + 0.116
20	7.77 ± 0.147	7.96 ± 0.091	0.87 ± 0.050	1.30 ± 0.175
37	7.85 ± 0.185	8.04 ± 0.185	0.99 ± 0.991	©17±0753 ©
79	7.95 ± 0.442	8.16 ± 0.151	1.03 0.109	
146	7.80 ± 0.141	7.72 ± 0.240	0.98/± 0.039	1.12 ± 0.144
299	7.47 ± 0.225	7.51 ± 6.365*	0.90 ± 0.173	1.09 ± 0.212^{2}

While there was a statistically significant difference found between the pregative and solvent control groups in the female total length data (p = 0.0442) the difference between the groups was slight (2.9%) and likery due to the natrow standard deviation within each group of data. Therefore, the control cata were pooled for comparison among the

Conclusion

reproduction. The The study meets the validity criteria. The most sensitive endpoint wa results, based on arithmetic mean measured concentrations, are:

NOEC: SO
highest concentration without an significant officet \$\times 79 \mu g a.s./\tilde{\text{D}}
compared to the control & S
LOEC:
lowest concentration with an significant effect compared 146 fig a.s./L
to the control of the
MATC: Q Q Q Q Q Q Q Q Q Q Q Q Q Q Q Q Q Q Q
maximum Acceptable Toxicant Concentration
7 - day C ₅₀ Survival 2 299 ng a.s./L
13 - day LC ₅₀ Surviv@l
21 - day LC ₅₀ Surxival Δ
28 - day LC ₅₀ Survival Q [*] S [*] S [*] S [*] S [*] 247 μg a.s./L

The mysid chronic study was performed according to the US EPA OCSPP 850.1350.

The variabilities in chronic mysid studies are high in general the control variability for reproduction is around 30%. For this study type therefore an EO₁₀ or EC₂₀ calculation is not biologically meaningful as both values lay within the control variability. Nevertheless the different endpoints will be shortly addressed in the following:

For the endpoint "prenile survival, day an ECx calculation was not applicable as no effects were observed. The NOTC was the highest test item concentration at which 100% survival was observed on

For the endpoint "in venile survival, day 13" an ECx calculation was not applicable as no effects were observed. The NOEC was the highest test item concentration at which 98% survival were observed on day 13 (wintrol= 90%, solvent control = 95%, poled controls = 92.5%).

For the endpoint "adult survival, day 14 – day 21" an ECx calculation was not applicable as only the highest test item concentration resulted in a reduced survival of 44%. At the NOEC of 146 µg a.s./L no effect was observed.

ureaument groups.

² According to the one-sided Jonckheere-Terpstra step-down trend jest, a statistically significant consentration related brend was no longer evident when the data from the 999 µs. 1./L treatment group was excluded from the calculations (p 0.05).

* Statistically significant decrease in comparison to the pooled control using the Dunnett, test (\$\subsetext{L} \cdot 0.05).



For the endpoint "adult survival, day 14 – day 28" an ECx calculation was not applicable due to a lacking clear dose response. At the highest test item concentration of 299 µg a.s./L a clear effect (18%) survival) was observed.

For the growth related endpoints the highest difference compared to the controls was observed to the endpoint mean total length of females. At the highest test item concentration of 299 µg as L the observed percentage difference in length compared to the control was 5.5% only. At the NOE of 146 μg a.s./L only 2.9% difference to the controls was observed. A potential EC₁₀ would numerically exceed therefore a NOEC. The reported NOEC therefore represents a worst case endpoint?

Material and methods

	KCA 8.2.5.2/02: 2017: M-6017/3-0161
Report:	KCA 8.2.5.2/02; BCS-CN88460 - 28-day to writy test exposing estuarine amphipods (Leptocheirus plumulosus) to a test substance applied to sediment under static-tonewal conditions following EPA test methods M-601773-01-1 M-601773-01-1 EPA Test Method EPA/600/R-01/020 (2001) n(s): none yes Name of substance: BES-CN88460 Batch No: 2013-006492
Title:	BCS-CN88460 - 28-day to bity test exposing estuarine amplipods (Leptocheirus)
	plumulosus) to a test substance applied to sediment under static-onewal conditions
	following EPA test methods & South Andrews S
Report No.:	M-601773-01-1
Document No.:	M-601773-01-1
Guideline(s):	EPA Test Methods/EPA/600/R-01/020 (2001)
Guideline deviation	n(s): none
GLP/GEP:	yes of the state o
	M-601773-01-1 M-601773-01-1 EPA Test Methods EPA 600/R-01/020 (2001) n(s): none yes
Material and me	ethods of the control
Test material	Name of substance: B&S-CN88460
	Batch No: 2013-006492 CAS No: 125573428-1
	CAS No: 125573428-1 0
	CAS No: 125573428-1
Guideline(s)	plumulosus) to a test substance applied to sediment under static-onewal conditions following EPA test methods M-601773-01-1 M-601773-01-1 EPA Test Methods EPA 600/R-01/020 (2001) n(s): none yes Name of substance: B&S-CN88460 Batch No: 2013-006492 CAS No: 1255734-28-1 Purity: 942% None specified Leggocheirus plumulosus
adaptation	5 Or Specifical Control of the Contr
Test species ©	Louth chairman plumid a cultivation of the chair
Acclimation	Leptocheirus plumulosus Tost species were acclimated to test conditions
Organism	- Age 7 to 14 days and at exposure initiation
age/size at	
study initiation	
Test solutions	Nominal consentrations: 3.8, 7.5, 15, 30 and 60 mg a.s./kg sediment dry weight
<u> </u>	Arithmetic mean measured concentrations
	- iposediment: 3,15.4, 10, 22, and 43 mg a.s./kg sediment dry weight
, 7	- in sediment pore water: 0.022, 0.036, 0.086, 0.16 and 0.27 mg a.s./L
	Controls: Water control
	Evidence Rundissolved material. All stock solutions had no visible undissolved
& 1	test substance following preparation.
Replication	No. of ressels per concentration (replicates): 6
Q ₁	No. of vessels per control (replicates): 6
4	No. of vessels per solven control (replicates): 6
Organisms	No. of organisms per Vessel: 20
replicate	
Exposure S	Static-renewal:
	Total exposure duration: 28 days
Feeding doring	The amphipods were fed a diet consisting of a flaked fish food suspension
test S	prepared in natural, filtered sea water.
Test O	Temperature: 23-25°C
conditions	Photoperiod: 16 h light: 8 h darkness
	Light intensity: 510 – 1000 lux
L	



	pH: 7.6 – 9.3
	Dissolved oxygen: 5.7 – 7.1 mg/L (control), 4.7 – 7.0 mg/L (solvent control), 4.2
	-7.2 mg/L (test concentrations)
	Salinity: 20-22‰
	Ammonia as Nitrogen: 0.10 – 0.59 mg/L
Parameters	At exposure initiation and termination, dissolved oxygen concentration, satisfy,
Measured /	temperature and pH were measured in the overlying water of each remaining
Observations	replicate vessel of each treatment level and control used for biological nonitoring
	during the 28-day exposure. On test days 1 through 27 dissolved oxygen, salinity
	pH and temperature were measured in one alternating replicate of each treatment
	level and control. In addition, temperature was continuously monitored in an
	auxiliary vessel in the temperature controlled water bath used @ house the test
	vessels throughout the study. Aminonia concentration of the overlying water was
	monitored at exposure initiation and termination in each treatment level and
	control.
	Observation of organism mortality and annormal behavior were made at exposure
	initiation and at daily intervals Wereafter, until exposure termination (day 28). At
	exposure termination (the total number of surviving adult and young amphipods was determined. At exposure termination also the gender and growth of the
	surviving adults was determined.
Sampling for	Dosed sediments were sampled during the mixing equilibration period, prior to
chemical	the allocation of the codiments in the conficute exposure vessels. In addition
analysis	the allocation of the sediments into the policate exposure vessels. In addition, subsamples of the dosing stock colutions used to dose the sediments were also
unurysis	analyzed for test substance concentration.
	During the in-life phase of the definitive study, sediment pore water, and
	overlying water samples were removed and analyzed for BCS CN88460
	concentration on test days 0, 14 and 28. On days 0%14, and 28, samples were
	removed and analyzed from replicate vessels G, I, and J, respectively, for each
	Freatment level and the controls. 9 6 4
	The sediment and aqueous samples were analyzed for BCS-CN88460 using liquid
8	chromatography with tandem mass spectrometry detection (LC/MS/MS).
Data analysis	An Equal Variance Two-Sample Tost was used to compare the performance of the
	negative control organisms with that of the solvent control organisms in order to
Ky .	determine there were any statistically significant positive or negative effects.
	Shapiro Wilks Pest for normality (U.S. EPA, 2002) was conducted to compare the
(bserved sample distribution with a normal distribution. As a check on the assumption of homogeneity of variance implicit in parametric statistics, data were
_@	assumptions of the manufacture of the qualifying tests
~Q~	analyzed using Bartlett's Test Based on the results of the qualifying tests described above, data for all endpoints met the assumptions of normality and
4	homogeneita Consequently, Duranett's Multiple Comparison Test, a parametric
	statistical procedure, was used to assess treatment-related effects for all endpoints.
<u> </u>	Statistical procedure, was used assess treatment related effects for an enapoints.

Results:

Validity criteria according to EPA/600/R-01/020	Required	Obtained
Average survival Camphinods in controlo	≥ 80%	88%
Survival in single replicates	> 60%	≥ 70%
Temperature A	± 3°C	23-25°C
Salighty O 6	20‰ ± 3‰	20-22‰

Analytical results:

No BCS-CN88460 residues were measured in the sediment, pore water or overlaying water controls above the limit of quantification (LOQ).



Nominal sediment concentration	Arithmetic mean measured sediment concentrations		%	of no	minal conce	ntration	
(mg a.s./kg)	(mg a.s./kg)		Day	y 0	Day 14	Day 28	
3.8	3.1		10)5	81.6	60.5	
7.5		5.4		10)5	73.3	≥38.7
15		11		10	00	73.3	\$50.0
30		22		10	00	70.0	50.0
60		43		93	.3	78.3	41.7
					•		
Nominal sediment concentration	Measured (concentration	n (mg a.s	e	A	Arithmetic m	ntration
(mg a.s./kg)	Day 0	Day 14	Day	28		mg as./L	
			© Pore V	Vater	Ŋ,		D D
3.8	0.032	0.021	0.00	Ă j	Ž,	0.02 0 0.036	
7.5	0.064	0.030	200 01	4 0	4	Q, <u>0</u> ,036	
15	0.12	0.080	0.05 0.40	i3\y	J.	0.086	
30	0.23	,845 <u>(</u>	0.409	2		0.166	Ũ
60	0.33	00.29	20 719	00	*	Q-270	
		Q'	Ogerlyin	wate			
3.8	0.0025	0.0013	0.00	88	.W"	0.00156	,
7.5	0.0086	0.0044		17	4	0.06490	Ö
15	0.00000	0 0.0120	0.00	50		200900 ×	y _{?

Nominal sediment concentration	Measured o	concentration	(mg a.s.L)	Arithmetic mean with measured concentration
(mg a.s./kg)	Day 0	Day 14	Day 28	(mg as s./L)
			Pore Water	
3.8	0.032	0.021 (0.00/1	\$\tag{9.020}
7.5	0.064	0.030	10 014 ©	Q 0.036
15	0.12	0.080	0.053	\$\int_{\infty} \frac{\sqrt{0}}{2}.086 \sqrt{0}' \times
30	0.23	Ø 15 &	0.4092	0.160
60	0.33	O _{0.29}	×0.190 🕡	(
		Q' ,	Oyerlying wat	er 🍣 🎺 💍 💍
3.8	0.0025	Q.0013	0.00088	♥ \$\tilde{\pi}0.0015\text{\$\text{\$\text{\$\pi}\$}\$}
7.5	0.0086	0.0044	0.0017	0.06490
15	Õ. ® 00	0.012	0.0050	\$\tag{\partial}{\partial}\tag{\partial}{\partial}\tag{\partial}\par
30	0.0160 🚄	0.0180	0,0074	0.01380
60	0.023	3 0.0490	0.0290	O 0.08366

Biological results

growth rate and number of offspring per surviving female amphipod at the 28 day exposure with BCS CN88460 and amphipods (Leptocheirus Mean percen survival, grow exposure termination of the plumulosus).

(mg a.s./kg)	Mean Pêrcent Survival (SD)	Mean Male Growth Rate in my day (SD)	Mean Female Growth Rate in mg/day (SD)	Mean number of offspring per surviving female amphipod (SD)
Control	Q 88 (12)	© 0.092×(0.0051)	0.051 (0.0057)	20 (5.2)
Solvent control	\$ 88 (10) D	0.0068)	0.056 (0.0057)	17 (5.9)
3.1	≥94 (5) °Q	0.088 (0.0069)	0.053 (0.0059)	15 (2.8)
5.4	\$ 83 QQ	0.094 (0.0130)	0.056 (0.0036)	16 (1.6)
11	94(6)	0.087 (0.0097)	0.053 (0.0086)	20 (7.4)
22	№ (6) №	0.087 (0.0110)	0.053 (0.0360)	12 (3.6)*
43	92 (7)	0.089 (0.0071)	0.052 (0.0037)	10 (2.6)*

^{*} Significantly reduced compared to the negative control, based on Dunnett's Multiple Comparison Test SD = Standard Deviation

Conclusion

For the endpoint "survival" no effect was observed. The survival rate at the highest test item concentration slightly exceeded the controls. Therefore no ECx calculation is possible.



For the endpoints "mean male and mean female growth rate" no adverse effect were observed. Only minor differences compared to the controls were observed.

Males: At the highest concentration of 43 mg a.s./kg a difference of 7.1% was observed compared to the solvent control and 3.3% difference compared to the control. These differences were not statistically significant.

Females: At the highest concentration of 43 mg a.s./kg a difference of 9.2% was observed compared to the solvent control and a by 1.9% better growth compared to the control. These differences were statistically significant.

Therefore neither for males or females a biologically meaningful EC₁₀ could be calculated

For the endpoint "mean number of offspring per surviving femals amphipod," no EC10 or EC calculation was performed. Only the two highest testatem concentrations resulted in a reduced pumb of offspring (12 respectively 10). The observed data do not allow a meaning of EC calculation. At the reported NOEC of 11 mg a.s./kg for this endpoint no reduction at all was observed.

the reported NOEC of 11 mg a.s./kg for this endpoint no reduction at all was observed.
Endpoints based on arithmetic mean measure Concentrations are
Amphipod percent survival (day 28 endpoint)
Amphipod percent survival (day 28 endpoint) Arithmetic mean Arithmetic mean measured Endpoint measured sediment pore water (ing a.s./kg) (ing a.s./kg)
LC ₅₀ (95% C.I.):
LOEC: lowest concentration with an significant effect compared to the control.
NOEC: highest concentration without an significant effect compared to the control 0.27

NA = Not applicable. LCs Calue was empirically estimated. Therefore, corresponding 95% confidence intervals could not be determined.

Amphipod male growth

	· /	
Endpoint 3 A S A S A S A S A S A S A S A S A S A	Akithmetic mean	Arithmetic mean measured
Endpoint S A S S	measured sediment	pore water
	(mg@r.s./kg)	(mg a.s./L)
Endpoint	♥	> 0.27 (NA)
LOEC:	> 43	
lowest concentration with an significant effect	> 43	> 0.27
compared to the confrol	y [*]	
LOEC: lowest concentration with an significant effect compared to the control	,	
I highest concentration without afteriorities and etter I	43	0.27
compared to the control & &		
NA = Not applicable. C value was empirically estimated;	therefore, corresponding 95%	confidence intervals could not be
determined of S		
compared to the control NA = Not applicable. FC value was empirically stimated; determined		
$oldsymbol{\mathbb{C}}$		



Amphipod female growth rate (day 28 endpoint)

Endpoint	Arithmetic mean measured sediment (mg a.s./kg)	Arithmetic mean measured pore water (mg a.s./L)
EC ₅₀ (95% C.I.):	> 43 (NA)	> 0.27 (NA)
LOEC: lowest concentration with an significant effect compared to the control	> 43	
NOEC: highest concentration without an significant effect compared to the control	43	0.27

NA = Not applicable; EC value was empirically estimated. Therefore, corresponding 95% confidence intervals could not be calculated.

Amphipod reproduction (day 28 endpoint)

Endpoint	Arithmetic mean Anthmetic mean measured measured sedimont pore viater (mgass./kg) (mgass./L)
EC ₅₀ (95% C.I.):	3 (NA) 5 20.27 (NA)
LOEC: lowest concentration with an significant effect compared to the control	22 \$ \$\tilde{\text{\$\tilde{\text{\$\tilde{\text{\$\tilde{\text{\$\tilde{\text{\$\tilde{\text{\$\tilde{\text{\$\tilde{\text{\$\tilde{\text{\$\tilde{\text{\$\tilde{\text{\$\tilde{\text{\$\tilde{\tilde{\text{\$\tilde{\tilde{\text{\$\tilde{\text{\$\tilde{\text{\$\tilde{\text{\$\tilde{\tilde{\text{\$\tilde{\text{\$\tilde{\text{\$\tilde{\text{\$\tilde{\text{\$\tilde{\text{\$\tilde{\text{\$\tilde{\text{\$\tilde{\text{\$\tilde{\text{\$\tilde{\text{\$\tilde{\text{\$\tilde{\text{\$\tilde{\text{\$\tilde{\text{\$\tilde{\text{\$\tilde{\text{\$\tilde{\tilde{\text{\$\tilde{\text{\$\tilde{\text{\$\tilde{\text{\$\tilde{\tilde{\text{\$\tilde{\tilde{\text{\$\tilde{\tii
NOEC: highest concentration without an significant effect compared to the control	0.086

was emphically estimated. Therefore, corresponding 95% confidence intervals could not be NA = Not applicable; determined.

; 2016; M-547035-

Title:

-how shell deposition test with the eastern oyster (Crassostrea

Report No .: Document No.

Guideline(s): Guideline deviation(s)

GLP/GKP:

Material and methods

THE COLUMN THE STATE OF THE STA	remous A, V) 14
Test material	BGS-CNS9460 Technical
	Batch code BCS-CN88460-01-06
\$	Origin batch No.: 2013-006492
	Purity 94,20%
Guideline(s)	None specified
adaptation	Specifica Specifica
Test species	Eastern oyster (Crassostrea virginica)



Culturing	The oysters were held in filtered saltwater from the same source and at
conditions/	approximately the same temperature as used during the test. During the 12-day
Acclimation	holding period immediately preceding the test, water temperatures in the culture
110011111111111111111111111111111111111	ranged from 20.5 to 21.7°C, the pH of the water ranged from 7.9 to 8.2 and the
	dissolved oxygen concentrations were ≥ 7.3 mg/L ($\geq 90\%$ of saturation). The
	salinity of the water on the day of organism receipt was 12 (%), was raise (%)
	16‰ approximately 24 hours after receipt, and ranged from 20 to 21 (‰) for the
	remaining 10 days of holding. During the 7-day period immediately preceding the
	test, the oysters in the lot used for the test showed no signs of disease or stress and
	there was <2% mortality
Organism	Initial valve height: $35.8 \pm 2.4 \text{ mm}$
age/size at	
study initiation	
Test solutions	Nominal concentrations: 0.056 0.11, 0.23, 0.45 and 0.90 mg a.s. N.
	Corresponding arithmetic mean measured concentrations 0.049, 0.091, 0.22, 0.37
	and 0.88 mg a.s./L Controls: dilution water (natural seawater)
	Solvent control: 0.1 nd/L dimethyfformanide
Replication	Controls: dilution water (natural seawater) Solvent control: 0.1 ntl./L dimethylformanide No. of vessels per concentration (replicates): 1 No. of vessels per control (replicates): 1 No. of vessels per solvent control (replicates): 1 No. of organisms per vessel? 20
Replication	No. of vessels per control (replicates):
	No. of vessels per solvent control (replicates). 1
0	No of oresplans whitesoff 20 & S
Organisms per replicate	No. of organisms per vessel? 20 5 4 5 5 5 5
Терпсас	
Exposure	Flow through O
	Total exposure duration: 98th & S
Test Vessel	Test chambers were 54-1 glass aquarta filled with approximately 27 L of test water. The deput of the test water in a representative chamber was 14.7 cm.
Loading	water. The depth of the test water in a representative chamber was 14.7 cm.
Feeding during	Marine microalgae at a target rate of 2.9 × 10 cells/overter/day
test &	o o was a second to the constant of the consta
	Temperature: 48.8 – 19.1°C
Test	Photoperiod 16.8 Mours light: darkness
conditions	$1 \cdot 1000$ interpolity $301 \cdot 100 \times 100 \times 100$
	pp. 7.646 8.0 5 0 4 4 4
	Dissolved oxegen: 70 - 8.1 mg/L \(\begin{array}{c} 85\% \text{ of saturation} \\ \text{Scatter} \\ \text{200} \\ \
07	Salphy, 2000 29 29 39
Parameters	Observations of mortality and other signs of toxicity were made approximately
Measured♣, /	3.5, 24, 48, 72 and 90 nours after test initiation. Measurements of shell deposition
Observations	for the oyster's were made at 96 hours.
₩	Temperature was measured in each test chamber at the beginning and end of the test. Measurements of the water temperature were done continuously in one
¥	negative control vessel. Dissolved oxygen was measured in each test chamber at
(P)	the beginning of the test approximately 24-hour intervals during the test, and at
S. S.	the end of the test. Measurements of pH were made in each test chamber at the
	Deginging of the test at the approximate mid-point of the test (~ 48 hours), and at
Ž Ő	the end of the test.
Sampling for	Test concentrations were measured in samples of test water collected from each
Sampling for chemical	Treatment and control group at the beginning, the approximate mid-point and the
analysis	end of the test for verification of the aspired exposure concentrations. The
, S. C.	analytical method consisted of high performance liquid chromatography (HPLC).
Doto 2001	
Data analysis	The shell deposition data from the negative control and solvent control groups
	were compared using a t-test. Since no significant differences were detected



between the two control groups (p > 0.05), the control data were pooled for comparison of growth inhibition in the treatment groups. The EC₅₀ value was calculated using linear interpolation.

The shell deposition data were evaluated for normality and homogeneity of variance (p = 0.01) using the Chi-Square and Levene's tests, respectively. Since the assumptions of normality and homogeneity of variance were not met, all attempt was made to correct the condition by square root transformation of the data. Data transformation did not correct the problem for homogeneity of variance, so the data in the treatment groups were compared to the pooled control data using the Wilcoxon's rank sum test with a Bonterroni adjustment to dentife any significant differences (p = 0.05). The no-observed-effect concentration (NOEC) was determined from the attistical analyses of the data and are assessment of the concentration response pattern. Statistical analyses were conducted using a personal computer with TOXSTAT software

Results:

Validity criteria		Required	Q brained
Mortality of the oysters in the control(s		< 10%	
The dissolved oxygen concentration		≥60 %	
Evidence of spawning	i de la	D No	No D Y
The mean shell growth observed in the	control group(s)	≥ 25mm (Control: 5 mm Solvent control: 2.8 mm

Analytical results:

Measured concentrations of the samples ranged from approximately 76 to 127% of nominal. Therefore results of the study were based on mean measured concentrations. No residues of BCS-CN88460 were found in the control and solvent control samples above the limit of quantification (LOQ = 0.0313 mg a.s./L).

Nominal /	Arithmetic mean			
Concentration	measured concentration	% %of no	minal concen	trations
(mg a.s./L)	(mg a.s./Ly			
0.056	© 30.049	×89.4	\$ 9.5	85.2
0.11		0 76.40°	®87.2	84.1
0.23	9.22	95,6	7 101	87.7
0.45	0.375	×79.2 ×	84.4	81.8
0.90	0.88	(F) 127(5) T	81.7	84.1

Observations of the control of treatment group during the test, and all oysters, appeared normal throughout the test. An absence of fecal matter in the 0.88 mg a.s./L test chamber during the lest suggested that the oysters in this treatment group were not actively feeding. When the shell deposition data for the negative control was compared with the solvent control, no statistically significant differences were found at the 95% level of confidence. Therefore, the control data were pooled for comparison with the treatment groups.



Mortality

Arithmetic mean	Exposed			No. dead			Cumulative
measured concentration (mg a.s./ L)	specimen (=100%)	~ 3.5 h	24 h	48 h	72 h	96 h	percent of mortality
Negative control	20	0	0	0	0	0	0 7
Solvent control	20	0	0	0	0	00	0 0
0.049	20	0	0	0	0	\$\vec{y}{2}	0 😽 🛴
0.091	20	0	0	0	0	₄ 0	
0.22	20	0	0	0	0 &	7 0	
0.37	20	0	0	© 0	0	0	
0.88	20	0	0 ~	0	.0	0	
Arithmetic mean	Mean Sh	ell Deposi	ition	Shell/Gr	owth		
Shell Deposition and She		all Danasi	+:467	ShallaCra	owthat		
measured concentration	± Standa	ard Devia	tion	Inhibitio	on is		
(mg a.s./ L)		(mm) 🖔)
Negative control	2.5						
Solvent control	2.8						
Pooled control	2.7	2.7 \$1.15, \(\) \(
0.049	2,	25 1.49 × 8 × 0 × 0					
0.091	200	± 0.97*	- Q	1, v ~~~~			
0.22	€ 0.8	$\pm 0.82*$,	69		Ö	
0.37	0.2 ± 0.08 ± 0.08 ± 0.00 ± 0.0						
0.88 0.00* 0 1000							
Shell growth inhibition was calculated relative to the pooled control.							
Shell growth inhibition was calculated relative to the pooled control. 2 96-hour EC ₅₀ (95% confidence interval) 0.17 ms a.s./L 60.13 − 0.21 mg a.s./L). * Statistically significant difference (p≤0.05) from the pooled control using the Wilcoxoft rank time test (with Bonferroni							
Statistically significant difference (p<0.05) from the profiled control using the wilcoxoff rank after test (with Bonferroni adjustment 1 tailed)							
Shell growth inhibition was calculated relative to the pooled control. 96-hour EC ₅₀ (95% confidence interval) 0.17 ms a.s./L (0.13 – 0.21 mg a.s./L). Statistically significant difference (p≤0.05) from the profiled control using the Wilcoxon rank from test (with Bonferroni adjustment, 1 tailed)							
		<i>\@</i>	,\$	<i>,</i> 0	O V	4,	
	Ç Ö				٦.	Ø n	

Shen Deposition and Sher	i Olowiii 🔟	
Arithmetic mean	Mean Shell Deposition	Shell Growth Inhibition
measured concentration	± Standard Deviation	. Inhibition far
(mg a.s./ L)	(mm) 👋 🧳	
Negative control	2.5 ± 0.80	
Solvent control	2.8 ± 1 = 3	~ ~ 4
Pooled control	2.7 4 1.15	N
0.049	2,5 ± 1.49.	
0.091	200°± 0.9°7*	240 25
0.22	Ø.8 ± 0.82*	
0.37	0.2_90.46*	P 0593 L
0.88	020 ± 0.00* 0	1000

Conclusion

All validity criteria were met. Endpoints of the study are based on nominal concentrations and are:

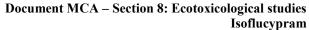
O O					
EC50 96 hours				0.170 ng	g a.s. /10
LOEC	, , , ,		,		~O`
lowest concentration wit	h an şignifics	unt effcot		0 €091 m	ga.s./L
compared to the control			.0	0′ 🦠	0
NOEC:		, ® %	<	J.	
highest concempation w	thout an sign	ificant eff	ect ॄ ○	0.049 m	g a.s. / L
compared to the control	<u>~</u> 0 ~			Õ	

This study type is wandled within the advantic book assessment as an acute invertebrate study. The observed EC₅₀ in combination with an assessment factor for acute studies is used to derive a RAC. Therefore it is questionable whether the calculation of an EC₁₀ is reasonable.

No mortalities were observed within the study period. Therefore no ECx calculation for this parameter is applicable

For the Hell growth a reasonable dose response relationship is existing. A calculation of an EC₁₀ should be possible, but was not performed yet.

At the reported NOEC the observed difference to the pooled controls was 8%. Nevertheless the reported standard deviations for this endpoint should be considered as well (>50% for the solvent control Compared to the negative control no difference was observed at the NOEC of 0.049 mg a.s./L.





Report: KCA 8.2.5.2/04; ; 2017; M-585874-02-1

42-day toxicity test exposing freshwater amphipods (Hyalella azteca) to BCS-Title:

42-day toxicity test exposing freshwater amphipods (Hyalella azteca) to BCS-CN88460 applied to sediment under static-renewal conditions following EPA test methods - Amended final report - 13798.6406
M-585874-02-1
US EPA Test Method 100.4
OCSPP 850.1770 (In Preparation) none
yes

Report No.: Document No.:

Guideline(s):

Guideline deviation(s): **GLP/GEP:**

Material and methods

Test material	Name of substance: BCS-CN8 960 \sim 0 0 0
	Batch No: 2013-006492
	CAS No: 1255734-28-1
	Purity: 94.2%
Guideline(s)	Name of substance: BCS-CN88460 Batch No: 2013-006492 CAS No: 1255734-28-1 Purity: 94.2% None specified Hyalella azteca - Age: 8 days old at exposure initiation Nominal concentrations 6.3, 12, 25, 50 and 100 mg a.s./kg sediment dry weight Arithmetic mean measured concentrations
adaptation	
Test species	Hyalella azteca 🖧 💸 👸 👸 👸
Organism	- Age: 8 days old at exposure initiation \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \
age/size at	
study	
initiation	
Test solutions	Nominal concentrations 6.3, 10, 25, 50 and 100 mg a.s./kg sediment dry weight
	- in sediment 5.8, 10, 22, 44 and 5 mg. a.s./kg sediment dry weight
	- in sediment pore water 0.15, 0.31, 0.72, 1.1 and 1.8 mg a.s./L
	Controls Water control, solvent control
	Evidence of undissolved material All stock solutions had no visible undissolved
- D 1:	test substance following preparation.
Replication	No of vessels per concentration (replicates): 92
	No. of vessels per control (replicates): 12 @ O No. of vessels per solvent control (replicates): 6
0	No. devesses per solvent control replicates). (2
Organišms per replicate	No of organisms per vessel: 107
Exposure	
Exposure	
Feeding	During testing 15 mL of flaked fish Good suspension (YCT) was added daily to
during test	each test vesser, as well as an additional 0.5 mg of ground flake fish food in an
	aqueous suspension.
Test∠』	Temperature: 22 23°C Daily measurements), 21-24°C (Definitive study)
conditions	Photoperiod: 10/8 hours, 686 940 lux
(ηβΗ: 6.2 – 7.2
A S	Dissolved xygen; 3.1 – 8.0 (control), 3.1 – 8.1 (solvent control), 2.7 – 8.4 (test
	concentrations),
	Xmm@na: ≤010 – 0.45 mg N/L
79 2	Hardness: 52-96 mg CaCO ₃ /L
	Alkalinity 20-44 mg CaCO ₃ /L
	Conductivity: 640 μS/cm
Parameters	Dissolved oxygen concentration; temperature, pH, total nardness, alkalinity and
Measured /	conductivity were measured in the overlying water of each replicate vessel of
Observations	each treatment level and control used for biological monitoring during the 42-day
	exposure. pH and ammonia (as nitrogen) concentration were measured in a pore





	water sample of the control and the highest treatment level (100 mg/kg). In addition the temperature was continuously monitored in an auxiliary vessel in the temperature controlled water bath used to house the test vessels throughout the
	study.
	Daily observations of organism behavior (e.g., adverse effects) were made and amphipod survival and growth (length) was determined. Reproduction of adult
	amphipods was measured until end of the study.
Sampling for	amphipods was measured until end of the study. Dosed sediments were sampled during the mixing/equilibration period prior the
chemical	allocation of the sediments into the represent test vesses. In addition, subsamples
analysis	of the dosing stock solutions used to dose the sediments were also analyzed for test substance concentration. Resulte of these pretest analyses were used to
	confirm that sufficient quantities BCS-CN88460 had been applied during the
	dosing process.
	During the in-life phase of the definitive study, overlying water, fore water, and sediment samples were removed and analyzed for BCS CN88460 concentration
	on test days 0 (exposure, initiation), day 44 and 28 (termination of sediment phase)
	of the exposure).
	All aqueous and sediment samples were analyzed for BCS-CN88460 using a liquid chromatographic system equipped with mass spectrometry detection.
	(LC/MS/MS) based on methodology validated at Smothers Viscient
Data analysis	Determination of adverse effects on Sercent survival was determined after
	transformation (e.g., arcsine square root percentage) of the data. An Equal Variance t Two-Sample Test or Wilcoxon's Rank Sum Two Sample Test was
	conducted on all surviyal, growth and reproduction rata to compare the
	performance of control organisms with that of which is the second of control organisms of the second or control or con
	solvent control organisms in order to determine if there were any statistically
	significant positive or negative iffects Shapiro Wilks Lest for normality was conducted to compare the observed sample
	Chistribution with a normal distribution for all enthoints
8	As a check on the assumption of homogeneity of variance implicit in parametric
	statistics, data were analyzed using Bartlett's Test Dercent survival data (day 28 and 42) and day 42 reproduction did not refet the assumption for normality,
	therefore, Social's Many-Que Rand Sum Vest, a Jon-parametric statistical
. /	procedure, was used to establish treatment
	effects for the endpoints. Dunnett Multiple Comparison Test, parametric statistical procedures, was used to establish treatment effects for all remaining
	endpoints CETISTA Version 1.27 was used to perform the computations.
·	

Validity criteria Required	Obtained
Recovery in sediment samples 70 - 120%	103 ± 3.92 %
Recovery in agreeous sample 80 220 %	$110 \pm 7.29 \%$

Analytical/results.

No BCS-CNS 460 residues were measured in the sediment, pore water or overlaying water controls above the manimum detectable limit.



Nominal sediment	Arithmetic mean	% of no	minal con	centration
concentration (mg a.s./kg)	measured sediment concentrations	Day 0	Day 14	Day 28
6.3	5.8	92	92	92
13	11	88	88	88 奏
25	22	104	76	88
50	44	84	90	292
100	95	97	95	₹ 95

Nominal sediment		hmetic mean	% of no	ominal con		
concentration (mg a.s./kg)		ured sediment ocentrations	Day 0	Day 14	Day 28	0
6.3		5.8		92	92	
13		11	88	88	88	
25		22	104	76	88	4 . 4
50		44	84	90	Q 2	
100		95	97	95	2 95	
			T.	<u> </u>	V 2,	
Nominal sediment concentration	Arithm conce	netic mean measu ntration (mg a.s./	red T	Arithme	tic Mean	
(mg a.s./kg)	Day 0	Day 14	9ay 28 ₀	<i>ି</i> (mg ବି	toation ♥ ks./L)@	
		Por	e Wayter 🐇			
6.3	0.18	0.13	∂ .14 €	Q 0.	? &LV	
13	0.33	0.30	0.27		371 ° 0'	
25	0.84		0:62	y & 0.	72 👸	
50	1.2	[] 1.2 % °	√1.1 ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~	⇒ r		
100	2.1	1.60	1.70		.8 0	
		verly Qverly	ing water		<u> </u>	
6.3	0.0130	0.004 V	¥.0037©	U.AS	9 70	Į Į
13	0.0360	0.0140	0.0958	©°° 0.0	1860	7
25	≈ 0.047 0	(0340)	Q9150 ×	0.0	3 20	
50	0.0750	0.0486	0.0350	0.0	527 " [*]	
100	V.900	0.0800	0.0430	F Ø.1	043\$	

Biological results:

Biological results:

Mean percent survival of adult amphipods and prean amphipod growth (length) during the chronic exposure of amphipods (Hyatella azteca) to BCS CN88460 on test day 28

Arithmetic mean measured sediment concentration (mg a.s./kg) Mean Percent Survival	Mean Length per Amphipod in mm (SD)
Control (3) (9) (9) (1)	5.55 (0.06)
Solvent control 99,69	5.71 (0.16)
5.8 4 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	5.59 (0.29)
11 9 96 (14)0	5.49 (0.17)
22 96 8	5.44 (0.33)
44 95 (7)	5.75 (0.50)
95 86 (14)	5.50 (0.17)



Mean percent survival of adult amphipods and mean number of offspring released per female amphipod during the chronic exposure of amphipods (*Hyalella azteca*) to BCS-CN88460 on test day 35 and 42.

Arithmetic mean measured sediment	Mean P Surviva		Mean Length per Amphipod in mm (SD)		Mean Number of Offspring Released per	
concentration (mg a.s./kg)	Day 35	Day 42	Day 35	Day 42	Day 35	Day 42
Control	93 (10)	91 (10)	n.a.	5.95 (0.35)	1.1 (3.2)	\$\ \ 9 .8 (7. 2)
Solvent control	98 (5)	96 (5)	n.a. 💍	5.94 (0.16)	2.2 (3.1)	4.3(7.3)
5.8	91 (8)	89 (8)	n.a.	5.88 (0.53)	5.3 (2.6)	1 🗣 (7.9)
11	99 (4)	95 (9)	n,	6.24 (0.20)	3.8 (3.8)	Q,8 (9.35)
22	95 (8)	90 (13)	na.	5.82 (0.44)	° 3.9 (2/.4)	§ 9.3 (5.4)
44	96 (7)	95 (8)	n.a.	6 19 (0.26)	2.8 (2.5)	9,6(4.9)
95	81 (14)*	81 (14) 🖇	n 🔊	3 .09 (3 .94)	0.74 (1.4)*	~3%7 (5.9°)

SD = Standard deviation

Conclusion

For the endpoint "mean survival" only at the highest concentration of 5 mg/3.s./kg/3 difference to the controls was observed. At the NOEC of 44 mg/a.s./kg/3 to difference to the controls was observed. The number of effect concentrations as not sufficient for Feasonable FO₁₀ and EC₂₀ calculation.

For the endpoint "length" no dose response was observed. The mean length at the highest test item concentration even exceed slightly those of controls and solvent controls bue to absence of effects a reasonable EC₁₀ and EC₂₀ calculation cannot be performed.

For the endpoints "pean number of offspring stay 35 and day 425 only at the highest test item concentration a decrease in number was observed. No adverse effects were observed at the NOEC of 44 mg a.s./kg. The number of effect concentrations is not sufficient for a reasonable EC₁₀ and EC₂₀ calculation.

Endpoints based on arithmetic rocan measured concentrations are:

Amphipod percent survival (day 28 endpoint)

Endpoint Control of the control of t	Arithmetic mean measured sediment (mg a.s./kg)	Arithmetic mean measured pore water (mg a.s./L)
LC ₅₀ (95% C.I.):	© > 95 (NA)	> 1.8 (NA)
LOEC: lowest concentration with an significant effect compared to the control	> 95	> 1.8
NOEC: highest concertration without in significant effect compared to the control	95	1.8

NA = Not applicable C₅₀ value was empirically estimated. Therefore, corresponding 95% confidence intervals could not be calculated.

^{*}Significantly reduced compared to the control, based on Dumett's Multiple Comparison Test



Amphipod percent survival (day 35 endpoint)

Endpoint	Arithmetic mean measured sediment (mg a.s./kg)	Arithmetic mean measured pore water (mg a.s./L)
LC ₅₀ (95% C.I.):	> 95 (NA)	> 1.8 (NA)
LOEC: lowest concentration with an significant effect compared to the control	95 (*)	
NOEC: highest concentration without an significant effect compared to the control	44	1.10

NA = Not applicable; LC50 value was empirically estimated. Therefore, corresponding 3% complence in ervals could not be calculated.

Amphipod percent survival (day 42 endpoint)

Endpoint	Acithmetic mean Apithmetic mean measured measured sediment pore water (mg/a.s./L)
LC ₅₀ (95% C.I.):	> 95 (NA)
LOEC: lowest concentration with an significant effects compared to the control	\$ 95 \(\text{\$\frac{1}{2} \text{\$\fin}} \text{\$\frac{1}{2} \text{\$\frac{1}{2} \text{\$\frac{1}{2} \text{\$\frac{1}{2} \text{\$\frac{1}{2} \text{\$\frac{1}{2} \$\fr
NOEC: highest concentration without an significant effect compared to the control.	1.8

NA = Not applicable; I value was empirically estimated. Therefore, corresponding 95% confidence intervals could not be calculated.

Amphipod growth as Tength (day 28 endpoint)

Endpoint EC ₅₀ (95% C.I.)	Arithmetic mean Omeasired sediment (mg a.s.kg)	Arithmetic mean measured pore water (mg a.s./L)
EC ₅₀ (95% C.I.)	> 9©(NA)	> 1.8 (NA)
LOEC: lowest concentration with an significant effect compared to the control	I (()): 4/0F	> 1.8
highest concentration without an significant offect	95	1.8
compared to the control of NA = Not applicable, EC ₅₀ value was empirically estimated calculated.	d. Therefore, corresponding 95%	6 confidence intervals could not be



Amphipod growth as length (day 42 endpoint)

Endpoint	Arithmetic mean measured sediment (mg a.s./kg)	Arithmetic mean measured pore water (mg a.s./L)
EC ₅₀ (95% C.I.):	> 95 (NA)	> 1.8 (NA)
LOEC: lowest concentration with an significant effect compared to the control	> 95	
NOEC: highest concentration without an significant effect compared to the control	95 0	

NA = Not applicable; LC₅₀ value was empirically estimated. Therefore, corresponding calculated.

Amphipod reproduction as offspring per female (day 5 endpoint)

Endpoint	Acithmetic mean Apithmetic mean measured measured sediment pore water (mg/a.s./L)
EC ₅₀ (95% C.I.):	52 (26 73) 1.2 (0.31 41.5)
LOEC: lowest concentration with an significant effects compared to the control	95 4 9 91.8
NOEC: highest concentration without an significant effect compared to the control	44 4 4 1.1

NA = Not applicable; 1650 value was empirically estimated. Therefore, corresponding 95% confidence intervals could not be calculated.

Amphipod reproduction as offspring per female (day

Endposint Om	Arithmetic mean easinged sediment (mg a.s.kg)	Arithmetic mean measured pore water (mg a.s./L)
EC ₅₀ (95% C.I.)	O NIO(ND)	ND (ND)
LOEC: lowest concentration with an significant effect compared to the control	> 95	> 1.8
NOEC: highest concentration without an significant effect compared to the control	95	1.8

ND = Not determined. A > 50% reduction was evident at 95 mg/kg: however, an upper confidence limit could not be calculated and therefore the results as considered unreliable. Consequently, the EC50 for 42-day reproduction is not determined.

uetermined.

NA = Not applicable A C₅₀ value was empirically estimated. Therefore, corresponding 95% confidence intervals could not be calculated.



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

Isoflucypram is an active substance with fungicidal activity. Specific effects on insect growth and development are not expected.

CA 8.2.5.4 Sediment dwelling organisms

A chronic study with an additional aquatic invertebrate species (Chiropomus dilutes) was for registration outside the EU. This study is summarised below.

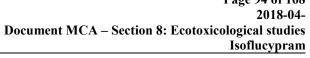
KCA 8.2.5.4/01; Report: ; 2017; M-59**©**\$83-01.-1

Life-cycle toxicity test exposing midges (Chironomas dilutus) to BOS-CN88460 Title:

technical applied to sediment under static onewal conditions following EPA tests methods
13798.6405

Material and methods

	technical applied to sediment under static enewal conditions following EPA test methods 13798.6405 M-596883-01-1 US EPA Test Method 100.5 OCSPP 850.1760 (In Brevaration) on(s): not specified yes nethods Name of substance: BCS-CN88460 Batch Nov: 2013-006492 Purity 94.29 w/w Nore specified Midge (Chironomus dilutus) First instal larvae, three days old at exposure initiation A 10 mL volume of each dosing stock solution was applied to 0.05 kg of fine silica and placed in glass Petr dishes and the solvent was allowed to evaporate for 90 minutes till draviess. The dry sand, containing the test substance, was added to the 3.0 kg of west sediment (2.112 kg fir) sediment) in individual glass jars. Test vessels contained 600 mL (approximately 4.0 cm layer) of spiked sediment (equivalent to 155 g wer weight per vessel of 107 g dry weight per vessel). Waterused during study was laboratory well water (final test water volume per vessel: 0.175 L) Nominal sediment concentrations: 6.3, 13, 25, 50 and 100 mg a.s./kg Arithmetic mean measured sediment concentrations		
	methods of the second s		
Report No.:	13798.6405		
Document No.:	M-596883-01-1		
Guideline(s):	US EPA Test Method 100.5"		
	OCSPP 850.1760 (In Preparation)		
Guideline deviation	on(s): not specified of the specified of		
GLP/GEP:	yes of the second secon		
M 4 1 1			
Material and n	1ethods V		
Test	Name of substance: BCS-CN88460		
material:	Batch Nov.: 2013-00649/2		
	Purity 94.2% w/w © O O O O O O O O O O O O O O O O O O		
Guideline(s)	None specified & & & & & O' &		
adaptation			
T			
Test species;	Midgo (Chironomy diluta)		
Organism	First instar larvae, three days old at exposure initiation		
age:			
age:	A 10% I - 00		
Preparation	A symbol vorume of each gosing stock solution was applied to 0.05 kg of fine silica		
of spiked	and placed in glass Perredishes and the solvent was allowed to evaporate for 90		
sediment	minutes till drydess. One dry sand, containing the test substance, was added to the		
0	3.0 kg of web sediment (2.182 kg bry sediment) in individual glass jars. Test vessels		
\ \\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\	contained 600 ml (approximately 4.0 cm layer) of spiked sediment (equivalent to		
4	155 g wed weight per vessel of 10% ary weight per vessel).		
Ø"	Water used doving study was laboratory well water (final test water volume per		
	vesself. 0.1.75 L) \(\sqrt{1} \qquad \qq		
Test	Nominal sediment consentrations: 6.3, 13, 25, 50 and 100 mg a.s./kg		
solutions	Arithmenic mean measured sediment concentrations		
(- in sectiment 5.5, D, 21, 42 and 85 mg a.s./kg		
	in sediment pore water: 0.12, 0.26, 0.47, 0.91 and 1.4 mg a.s./kg		
	Water control: 2.112 kg dry sediment + 0.05 kg fine silica		
Ü	Solvent control: 10 mL acetone per 2.162 kg wet sediment		
	Evidence Quindissolved material: Stock solutions were observed to be clear and		
	colorless		
Replication:	Vessels to measure biological response:		
Copincarion.	No. of vessels per concentration (replicates): 12 (A-L)		
<u> </u>	No. of vessels per control (replicates): 12 (A-L)		
	riot of vessels per conduct (represented). 12 (11 2)		
	Vessels designated for auxiliary male production:		
L	ressets designated for administy mate production.		





-	
	No. of vessels per concentration (replicates): 3 (M-P)
	No. of vessels per control (replicates): 3 (M-P) Vessels for chemical analysis: No. of vessels per concentration (replicates): 3 (Q-S) No. of vessels per control (replicates): 3 (Q-S) Vessels for measuring representative pore water characteristics: No. of vessels for 100 mg/kg treatment level (replicates): 3 (U-W) No. of vessels per control (replicates): 3 (U-W) No. of organisms per vessel: 12 (Replicates which were established for analytical and pore water quality measurements on test day, were not injusted with any larvae) Static-renewal conditions: Daily renewal of 300 mL water or each test vessel, i.e. seven cycles providing 50 mb of water per cycle Renewal of 700 mL at test day 9 due to relatively low dissolved on gen measurements. Total exposure duration of days Larvae were fed a dieconsisting of a finely ground flaked fish tood suspended in laboratory well water (4 mg/mL). During exposure the food was introduced at a
	v°
	Vessels for chemical analysis:
	No. of vessels per concentration (replicates): 3 (Q-S)
	No. of vessels per control (replicates): 3 (Q-S)
	Vessels for measuring representative pore water characteristics:
	No. of vessels for 100 mg/kg treatment level (replicates). 3 (U-W)
	No. of vessels per control (replicates): 2 U-W)
Organisms	No of organisms per vessel: 12 (Replicates which were established for abalytical
per replicate:	and nore water quality measurements on test day were not initiated with any
per reprieute.	larvae)
Exposure:	Static-renewal conditions: Daily renewal of 390 mi, water of each test vessel, i.e.
	seven cycles providing 50 mg of water per cycle, weneval of 700 mL at test day 9
	due to relatively low dissolved oxygen measurements.
ļ	Total exposure duration of days
Feeding	Larvae were fed a die consisting of finel ground flaked fish food suspended in
during test	laboratory well water (4 mg/mL). During exposure, the food was introduced at a
	rate of 1.5 ml of Alaked fish suspension per test vessel per day
T	Water temperature: 22 to 24% (daily measurements), 22 to 25% (continuous measurements) Photoperiod: 16:& light: dark Light intensity: 500 to 340 lux pH: 6.1 to 7.64 Water hardness: 72 to 92 mg/kg as CaCo Dissolved oxygen (mg/L): 2.5 - 8.8 Conductivity (as/cm): 490 - 620
Test	measurements
conditions:	Photoporio 16. 2 light day
	Light int facitive foo to a for love
	Light intensity. 900 to 940 tuy.
	DII. 0.1 to /.04
	water nardaess: /2 to 92 mg/kg as CaCo3
	Dissolved oxygen (mg/ls/. 2.5 ~8.8 ~
	Sonductivity (1887cm)*490 620 7
	Alkannty (CaCO3) (12 – 28)
Ö	Light intensity: 500 to 340 lux pH: 6.1 to 7.6 Water hardness: 72 to 92 mg/kg as CaCo ₃ Dissolved oxygen (mg/L): 2.5 \$8.8 Sinductivity (as cm): 490 620 Alkalipity (CaCO ₃): 12 - 28 Ammonia as N (mg/L): 0.37-0.50 (test day 0) \$\frac{1}{2}\$ 0.10 (test day 61) Artificial sediment: 3.0 kg sphagnum peac (5%) 12 kg kaolin clay (20%) 45 kg fine and (5%) 160 g powder CaCO ₃ (0.3%)
Sediment 🗞	Artificial sediment:
	3.0 kg/sphagnum peat (5%)
	12 kg/kaolin@lay (20%)
	45drg fine sand (75%), O' & & A'
Parameters	Dissored oxygen concentration, comperature, total hardness, alkalinity,
Measured 🔊	conductivity and pH were measured in overlying water of each replicate vessel of
Observations	each treament level and control used for biological monitoring. In addition, the
	temperature was continuously measured in an auxiliary vessel in the temperature
	controlled water bath used to home the test vessels throughout the study.
~~	Daily observations of mortality and abnormal behavior were made.
"Y	Four of the replicate test cases were randomly selected prior to day 16. Midge
e ⁽	Charval surviyar and Fowth measured as ash-free dry weight was assessed.
*	
	emerged from each replicate test vessel was observed and recorded.
W W	Number of eggs produced in each primary egg mass laid by female midges in each
	treatment level and control by replicate were counted the day the egg mass was laid.
	Harching success was determined by subtracting the number of unhatched eggs
&p. ~3	from the original estimate of egg numbers from that egg mass.



Sampling for chemical analysis	Dosed sediments were sampled during the mixing/equilibration period, prior to the allocation of the sediments into the replicate exposure vessels. In addition, subsamples of the dosing stock solutions used to dose the sediments were also analyzed for test substance concentration. During the in-life phase of the definitive study, sediment, pore water, and overlying water samples were removed and analysed for BCS-CN88460 concentration in test days 0, 16 and 61. On days 0, 16, and 61 samples were removed and analyzed from replicate vessels Q, R and S, respectively for all treatment levels and the controls.
Data analysis:	LOEC and NOEC values were determined. An Equal Variance Two-Sample to Test or Wilcoxon's Rank Sum Two-Sample Fest was conducted on all survival growth, emergence and reproduction data to compare the performance of regative control organisms with that of solvent control organisms. Shapiro-Wilks' Test for normality was conducted to compare the observed sample description with a normal distribution for all apdpoints. For check on the assumption of homogeneity of variance, data for each endpoint were analyzed using Bartlett's Test. Based on the results of the qualifying tests described above, the following analyses were used for the determination of treatment related effects. Wilcoxon's Test with Bonferroni's Adjustment was used to establish treatment effects for percent hatch and days to oviposition. Steel's Many-One Rank Sum Test was used to establish treatment effects for female time to death and eggs per egg mass. Bonferroni's Adjusted t-Test of Dumett's Multiple Comparison Test was used to establish treatment effects for all remaining endpoints. CETISTM was used to perform the computations.

Analytical results:
No BCS-CN88460 desidues were measured in sediment, the werlaying water and sediment pore water of the control above the limit of quantification. of the control above the limit of quantification

Nominal sediment Concentration (mg a.s./kg) Arithmetic mean measured sediment concentration (mg a.s./kg) 6.3 5.5 5.5	t	minal conce	ntration
concentration (mg a.s./kg) concentrations (mg/a.s./kg)	Day 0	Day 16	Day 61
6.3 5.5 F	94	84	83
13	100	92	72
25 27 27 27 27 27	92	84	76
13	86	88	80
400 0 1 2 2 85	93	81	80
13			



	Measured concentration (mg a.s./L)			
Nominal concentration (mg a.s./kg sediment dry weight)	Day 0	Day 16	Day 61	Arithmetic mean
	Over	rlying water		
6.3	0.018	0.0026	0.00081	0.007* 0.007*
13	0.072	0.0039	0.0012	0.026*
25	0.063	0.0077	0.0027	0.025*
50	1.3	0.016₺	0.0067	0,341* 7
100	0.24	0.029%	0.011	Ø.093*Q* V
	Sedimo	ent port/water	10 ×	
6.3	0.14	40 ,090	Q 3	° & 20,12 ° &
13	0.30	© 0.23	≫ 0.24 ©	0.26
25	0.51 🐇	0047	0. 4 4	\$\times 0.4\times \times \times \tag{7}
50	0.95 O*	√93 °C	6 85 %	Ø 1 0,91 🔬 .
100	1,6	0 1.2 V	\$1.3 a	Q 01.4 Q Q

^{*}Mean measured values not given in report; calculated on the basis of concentrations on day of day o

Biological results:

Midge larvae survival and growth (test day 16):

Midge larvae survival and growth (test day 16):

Midge survival and growth observed during the 16 days of exposure met the minimum standard criteria established by EPA Test Method 100.5 (e., 70% survival and 0.48 mg ash free dry weight per midge larvae). As demonstrated by the negative control and solvent control organism performance, the exposure system provided test conditions that were appropriate for promoting acceptable survival and growth of Chiropomus dilutus

On test day 16, survival observed among midge exposed to the \$5, 1,521, 42, and 85 mg/kg mean measured treatment evels overaged 96 92, 88, 98, and 96%, respectively. Statistical analysis (Dunnett's Multiple Comparison Test) demonstrated no significant reduction in survival among midges exposed to any of the treatment levels lested compared to the negative control (96%).

On test day 16, growth (ash free dry weight) animng the midge exposed to the 5.5, 11, 21, 42, and 85 mg/kg mean measured treatment levels averaged 1 \(\text{D2} \), 1.87, 2.09, 1.85, and 2.26 mg ash-free dry weight per midge larvae, respectively. Statistical analysis (Dunnett's Multiple Comparison Test) demonstrated to significant reduction in growth in any of the treatment levels tested compared to the negative control (2.27 mg/sh-free/dry worlght por midge larvae).

Arithmetic mean measured sediment concentration (mg/a.s./kg)	Mean percent Orvival (SD)	Mean Ash-Free Dry Weight Per Larvae in mg (SD)
Negative Control	3 ©(5)	2.27 (0.56)
Solvent Control	Q 4 (8)	2.68 (0.56)
Q 5.5 V	@ 96 (5)	1.72 (0.34)
	92 (12)	1.87 (0.15)
21	88 (14)	2.09 (0.40)
\$\tag{\text{O}} 42\text{\$\tilde{\text{V}}\$} \tag{\text{V}}	98 (4)	1.85 (0.08)
\$ 85 £	96 (5)	2.26 (0.47)

SD = Standard deviation



Mean percent emergence and mean emergence rate during the life-cycle exposure with BCS-CN88460 and midge (*Chironomus dilutus*).

The mean cumulative percent emergence on day 61 in the negative control and solvent control met the minimum standard criteria established by EPA Test Method 100.5 (i.e., ≥ 50% emergence), when percent emergence among midges exposed to the 5.5, 11, 21, 42, and 85 mg/kg mean measured treatment levels was 73, 82, 81, and 66%, respectively. Statistical analysis (Dunnett's Multiple Comparison Test) determined no significant reduction in percent emergence among midges exposed to any of the treatment levels tested compared to the negative control (71%). △

Mean emergence rate among male midges exposed to the 5.5, 11,21, 42, and 85 mg/kg mean measured treatment levels was 0.0544, 0.0519, 0.0542, 0.0538, and 0.0493, respectively. Statistical analysis (Bonferroni's Adjusted t-Test) determined to significant veduction in the measured treatment levels exposed to any of the treatment levels tested compared to the regative control (0.0496).

Mean emergence rate among female midges exposed to the 55, 11 21, 42 and 85 mg/kg mean measured treatment levels was 0.0473, 0.0493, 0.0454 0.0478, and 0.0459 respectively statistical analysis (Dunnett's Multiple Comparison Test) determined no significant difference in unean emergence rate among female midges exposed to any of the treatment levels tested compared to the negative control (0.0447).

Arithmetic mean measured sediment concentration	Mean Percent	Mean Male	Mean Female
sediment concentration (mg a.s./kg)	Emergence (SD)	Emergence Rate (SD)	Emergence Rate (SD)
Negative Control &	(14) V	0.0496 (0.06\$1)	9.6 447 (0.0051)
Solvent Control	86 (8)	0,6\$18 (0.0058)	©.0496 (0.0032)
5.5	√ 73 (12) ×	20.0544 (V.0054)	0.0473 (0.0037)
11 0	\$ (8) \$	\$ 0.0519 (0.0020)	0.0493 (0.0020)
215	×82 (117) ₂ ° ≤	0.0342 (0.0936)	0.0454 (0.0035)
	\$ 81 (b)	0.0538 0.0041)	0.0478 (0.0056)
85 8	66/(9)	\$0.049\$\(\text{(0.09\$)}\)	0.0459 (0.0049)

SD = Standar Queviation

Mean trays to death during the life-cycle exposure with BCS-CN88460 and midge (Chironomus dilutus).

The mean number of days to death among male midges exposed to the 5.5, 11, 21, 42, and 85 mg/kg mean measured treatment levels was 3.4, 3.4, 3.2, 3.4, and 3.3 days, respectively. Statistical analysis (Bonferroni's Adjusted t-Test) determined no significant difference in mean number of days to death for males in any of the treatment evels tested compared to the negative control (3.3 days).

The mean number of days to death among temale midges exposed to the 5.5, 11, 21, 42, and 85 mg/kg mean measured treatment devels was 3.3 3.2 3.1, 3.4, and 3.5 days, respectively. Statistical analysis (Steel's Many-One Rank Sum Dest) determined no significant difference in mean number of days to death for females exposed to any of treatment levels tested compared to the negative control (3.1 days).



7 (8 (° 20)
per of eggs per mated female,

percent hatch and days to oviposition during the life-cycle exposure with BCS CN88460 and midge (Chironomus dilutus).

The mean number of eggs per egg mass among midges in the negative control and solvent control was 1077 and 1107, respectively. The mean percent hatch among egg masses in the control and solvent control was 79 and 87%, respectively. These data met the minipuum standard criteria for reproductive endpoints established by EPA based on test method 100.5 and the latest revisions based on discussions with regulatory scientists (≥ 80 @eggs, per egg mass $\neq 80$ % hatch?

The mean number of eggs per egg mass smong midges exposed to the 5.5 \$1, 21642, and 85 mg/kg mean measured treatment levels was \$247,\$077,\$157,\$1204, and \$158, respectively. Statistical analysis (Bonferroni's Adjusted t-Test) determinent no significant difference in the mean number of eggs per egg mass in any of the treatment levels tested compared to the negative control (1077).

Mean percent hatch among midges exposed to the 5.5, 19, 21 242, and 85 mg/kg mean measured treatment level was 95, 95, 95, 98, and 93%, respectively. Statistical analysis (Wilcoxon's Test with Bonferroni-Holm's Adjustment) determined no significant difference in mean percent hatch in any of the treatment levels tested compared to the negative control (79%).

The mean number of egg masses per mated female among midges in the negative control and solvent control was 0.67 and 0.90, respectively. The plean number of egg masses per mated female among midges exposed to the 55, 11, 1, 42 and 85 mg/kg mean measured treatment levels was 0.74, 0.95, 0.83, 0.89, and 0.93, respectively. Statistical analysis (Stoel's Many-One Rank Sum Test) determined no significan difference in the mean number of egg masses per mated female in any of the treatment levels tested compared to the negative control (\$\vec{v}\$.67). \$\vec{v}\$

The mean number of eggs per mated female among midges exposed to the 5.5, 11, 21, 42, and 85 mg/kg mean measured treatment levels was 911, 1034, 961, 1077, and 1109, respectively. Statistical analysis (Bonferron Adjusted 1 Pest) determined no significant difference in the mean number of eggs per mated demake in an prof the treatment levels tested compared to the control (828 eggs pe Temate).

The mean number of days to viposition was 1.0 and 1.1 among midges in the negative control and solven control respectively. The mean number of days to oviposition among midges exposed to the 5.5, 1, 21, 42, and 85 mg/kg mean measured treatment levels was 1.0, 1.1, 1.0, 1.0, and 1.1, respectively. Statistical analysis (Wilcoxon's Test with Bonferroni-Holm's Adjustment) determined no significant difference in the mean number of days to oviposition in any of the treatment levels tested compared to the negative control (1.0).



Arithmetic mean measured sediment concentration (mg a.s./kg)	Mean Eggs per Egg Mass (SD)	Mean Percent Hatch (SD)	Mean Egg Masses per Mated Female (SD)	Mean Number of Eggs per Mated Female (SD)	Mean Days to Oviposition (SD)
Negative Control	1077 (120)	79 (35.2)	0.67 (0.36)	828 (3.5)	1.0 (@080)
Solvent Control	1107 (117)	87 (14.1)	0.90 (0.14)	1002 (210)	1,1 (0.14)
5.5	1247 (264)	91 (3.4)	0.74 (0.060)	(155)	, (Q.
11	1077 (131)	95 (4.1)	0.090)	Ø1034 (191)	1.5(0.16)
21	1157 (130)	95 (3.7)	0.83 (0.19)	961 (271)	Q.0 (0.00) \$
42	1204 (187)	98 (1.2)	0.89 (0.10)	Ø077 (Z53)	\$\frac{1.0}{0.060}\$
85	1198 (116)	93 (8.3)	0.93 (0010)	7 110%(127)	, 1A (0.11)

SD = Standard deviation

Conclusion

No adverse effects up to and including the highest concentration (100 mg α /kg) were observed therefore the calculation of EC₁₀ and EC₂₀ values is not possible.

Endpoints based on arithmetic mean measured sediment concentrations are

	Arithm	etic mean r	neasured s	diment		tic mean m		
Endpoint	~ C	oncentratio	nýmg a S/k	kg) 🛴 🛴	, Liè	oncentratio	m∕mg a.s./I	٦)
	NOEC	EOEC	L©	©EC50	NOEC	LOEC	LC50	EC50
Midge Larval Survival	\$ 85.54	\$35 ·	\$\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\		0 1.4 0	Ø1.4	> 1.4 (NA) ^A	-
Midge Larval Growth		> 850	4	(NA)	2 .4	> 1.4	-	> 1.4 (NA) ^A
Percento emergence	85%	\$\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\	- 5 ⁷	5€5 €NA)^\$		> 1.4	-	> 1.4 (NA) ^A
Male Emergence Rate	85	Y > 85	, 5 ⁹ -` ;;	> 85 (NAV) ^A	₹1.4	> 1.4	-	> 1.4 (NA) ^A
Female Emergence Rate	86	85 0		> 85 (NA)	1.4	> 1.4	-	> 1.4 (NA) ^A
Male Days of Death	85 %	285 - 285	Q - Q	>85 (X A) ^A	1.4	> 1.4	-	> 1.4 (NA) ^A
Female Days to Death	85	Q> 85		> 85 (NA) ^A	1.4	> 1.4	-	> 1.4 (NA) ^A
Number of Eggs per Egg Mass	85 8	\$\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\	4 - 5	> 85 (NA) ^A	1.4	> 1.4	-	> 1.4 (NA) ^A
Percent hatch	\$\hat{8}^{\hat{5}}	© > 85°	Q'	> 85 (NA) ^A	1.4	> 1.4	-	> 1.4 (NA) ^A
Egg masses per mated emale	850	\$\times 85	-	> 85 ^B (NA) ^A	1.4	> 1.4	-	> 1.4 (NA) ^A
Eggs per Matos Vemale	\$85 \\	> 85	-	> 85 ^B (NA) ^A	1.4	> 1.4	-	> 1.4 (NA) ^A
Daysto Ovigosition	85	> 85	-	ND ^B (NA) ^A	1.4	> 1.4	-	ND ^B (NA) ^A

ANA = Not applicable. LC/EC₅₀ values were empirically estimated. Therefore corresponding 95% confidence intervals could not be determined.

 $^{^{}B}$ ND = Not determined. Given the nature of this endpoint, an EC $_{50}$ estimate is not appropriate.



CA 8.2.6 Effects on algal growth

CA 8.2.6.1 Effects on growth of green algae

Report: KCA 8.2.6.1/01; ; 2017; M-586715-01-1

Title: Pseudokirchneriella subcapitata growth inhibition test with BCS-CN \$8460 (tech.)

Report No.: EBLNN050
Document No.: M-586715-01-1

Guideline(s): EU Directive 91/414/EEC; Regulation (EC) No. 1107/2009; U.S. EPA Pesticide

Assessment Guidelines, Subdivision J, §122-Q, 123-2; OCSPQ Guideline 8504500

(January 2012)

Guideline deviation(s): According to OCSPP 850.4500 the measured test substance concentration at tests

initiation is considered appropriate to use for unstable test items. However, in this study the ECx calculations after 96 hours were performed using the mean measured.

values to follow the recommendations from OPPTS 850.100

GLP/GEP: yes

Material and methods

Test material	BCS-CN8846@(tech.) O S S S
T est material	Batch code: BCS-CN88460-01-06
	Specification: 102000028196 Or "O" Specification:
	Purity: 94.2% w/w & & & & & & & & & & & & & & & & & &
C: 1-1:(-)	According to OCSPB 850.4500 the measured test substance concentration at test
Guideline(s)	initiation is considered appropriate to use for unstable test items. However, in this
adaptation	stady the EC _x calculations after 96 hours were performed using the mean measured
	walues to follow the recommendations from OPP \$850,000.
- · »(Freshwater green alvae (Posydokiv hnavjella suscanitata)
Test species	Strain SAG 61.81
, Q	400 μL of a 7-10 day 31d stock culture was transforred into a 300 mL cotton
Culturing	plugged Erlenmeve Clask containing about 100 mL of nutrient medium every 7-10
conditions	plugged Erlenmeyer flask containing about 100 mL of nutrient medium every 7-10 days. To ensure that the argae used as inoculum were exponentially growing, a pre-
	culture was prepared 3 days before the start of the test and cultivated under the same
	Conditions as on the main test.
Organism «	Precoultures were prepared from stock cultures 3 days before the start of the test
age/size at	Precultures were prepared from stock cultures 3 days before the start of the test using OECD medium.
study 💇	
initiation	
, &	
Test	Nominal concentrations: 0.0238, 0.0763, 0.244, 0.781, 2.50 and 8.00 mg a.s./L
solutions	Corresponding geometric mean measured concentrations (0 – 72 h): 0.020, 0.062,
- X	0.126, 0.528, 1.82 and 2.02 mg a.s./L
	Corresponding arithmetic mean measured concentrations (0 – 96 h): 0.0201,
	©:0633 0.198 0.608, 1.61 and 1.82 mg a.s./L
.79 .	Controls: water and solvent controls (dimethylformamide at 0.1 µL/mL)
	Evidence of undissolved material: In the nominal test concentrations of 2.50 mg
	a.S./L undissolved test item was observed at the water surface from day 2 onwards.
	At the highest concentration of nominally 8.0 mg a.s./L undissolved test item was
C	found on the surface of the test media over the whole test period.
i	



Replication	No. of vessels per concentration (replicates): 4
	No. of vessels per control (replicates): 4
	No. of vessels per solvent control (replicates): 4
Evnogura	Static
Exposure	Total exposure duration: 96 hours
Initial cell	Static Total exposure duration: 96 hours 10 ⁴ cells/mL at test initiation Temperature: 22.2 – 23.4°C Photoperiod: continuous light Light intensity at surface of test vessels: 4520 to 4950 lux pH of controls (0 – 72 h): 7.9 – 84 Growth medium same as culture medium: Yes Type of light: artificial (Cool white duorescent lamps)
density	
T. 4	Temperature: 22.2 – 23.4°C
Test conditions	Photoperiod: continuous light
Conditions	Light intensity at surface of test vessels: 4520 to \$\tilde{\phi}\$50 \text{lux}
	pH of controls (0 – 72 h): 7.9 – 84
	Growth medium same as culture medium: Yes (7) (7) (7)
	Type of light: artificial (Cook white Tuorescent larsps)
Parameters	Temperature was determined by a continuous measurement in one additional
Measured /	incubated glass vessel filled with the same amount of deignised water as in the test
Observations	vessels. The pH was reasured at the start of the study and additionally after 20 and
	after 96 hours in all Dest levels and the control. The light was deasured once during
	the test. Cell numbers per volume (as a surrogate for Fiomass per volume) were
	estimated photometrically.
~ ·· ^	
Sampling for	Samples were analysed for the sexual concentration of the B&S-CN88460 present in
chemical	the test medium of all meatment levels and the controls after 0, 72 and 96 hours.
analysis	
Data analysis	Exvalues (e.g. = 50) and confidence intervals were calculated for the standard
Data anarysis	exposure period, using a commercial program (ToxRatPro 3.2.1).
	0.122, 27, 27, 27, 27, 27, 27, 27, 27, 27,

p.m. = pure metabolite

Results

		ALV %		
Validity criteria aco			Required	Obtained
The biomass in the co	ntrol cultures shou	May have		
increased exponential	ly By a factor of at	least to within	3 16	77
the 72-hour test period				
The mean coefficient	of variation for se	ection-by-section	P	
specific wwwwwwwwwwwwwwwwwwwwww	(days 0-0, 1-2	and 2-3) in the	< 35%	14.8%
control cultures must i				
The coefficient of ya	riation of average	specific growth		
rates during the 72-ho	our test period in	replicate control	< 7%	1.3%
cultures must not exce	ed 7%. 🎺 🏽 🧷			

Analytical results:

Some recoveries were not within the range of 80 – 120% of nominal (see table below). Thus biological results, after 2 hours and 96 hours are based on geometric and arithmetic mean measured conceptrations of BCS-CN88460, respectively. No residues of BCS-CN88460 were found in the control and solvent control samples above 0.000626 mg/L, which was used as the lowest standard concentration during this study.



Nominal Concentration	Geometric mean measured concentrations after 72 hours	Arithmetic mean measured concentrations after 96 hours	% of nomi	nal concer	ntrations*
(mg a.s./L)	(mg a.s./L)	(mg a.s./L)	0-hour	72-hour	96- C our
0.0238	0.0200	0.0201	79.8	88.2	8 4.9
0.0763	0.0620	0.0633	76 T	86.6	86:07
0.244	0.196	0.198	<u>_</u>	83	\$2 .4
0.781	0.598	0608	72.6	80.8 ×	₹80.3€
2.50	1.82	¥.61	102	©52.00	393
8.00	2.02	1.82	26.8	23.9	Q7.8
* Values not given in study report; calculated on the basis of mominal concentrations and concentrations measured after the report of the property of the prop					
Biological results No morphologica	s: al change in algae was obser	ved in any test concentration	n. 5		
72 hours					
Geometric mean	measured Mean	n cell number Inhib	ition of ave	rage speel	fic

Geometric mean measured concentrations (mg a.s./L)	Mean cell number Insibition of average specific growth rate (%)*
Water Control	4. 77.0 . 4 . 6
Solvent Control	0 2 70.4
0.0200	\$74.9 \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$
0.0620	76.5° ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~
0.196	√
0.598	67.2 0 3.2#
1.82	52.2 7 9.0#
2.02	5.7#

[#] Significantly (α 0.05 one-sided smaller) reduced, based on Williams multiple sequential t-test procedure

	"()"
Geometric mean measured Vield Concentrations (cells/mL 104)	Inhibition of yield (%)
concentrations (colls/mL 104) (colls/mL 104)	1
Geometric mean measured concentrations (cells/mL 10 ⁴) Pooled control 0.0200	
Pooled control 76.2	0
	3.0
0.0020	0.9
0.196	2.8
0.598	13.2#
1.82	32.8#
2.02	21.3#
Significantly ($\alpha = 0.05$, one-sided smaller) reduced, based on Williams multiple	le sequential t-test procedure
C T	
0.196 0.598 1.82 2.02 Significantly (α = 0.05, one-sided smaller) reduced, based on Williams multiple	32.8# 21.3# le sequential t-test procedure



Geometric mean measured concentrations (mg a.s./L)	Area under the growth curve (biomass integral)	Inhibition of Biomass integral (%)
Pooled control	1438.1	0
0.0200	1394.8	3.0
0.0620	1445.4	3.8 3.8
).196	1383.6	© 3.8
0.598	1272.1	11.5# 0
1.82	998.5	30.6# 🗸 🧳
2.02	1138.3	20.8#%
Significantly ($\alpha = 0.05$, one-sided sn $\frac{6}{100}$ hours	naller) reduced, based on Williams multiple	
6 hours Arithmetic mean measured	Mean cell members	
5 hours Arithmetic mean measured concentrations		Inhibition of average specific growth rate (%)*
6 hours Arithmetic mean measured	Mean cell members	Inhibition of average specific growth rate (%)*
6 hours Arithmetic mean measured concentrations (mg a.s./L)	Mean cell in manber (cells/mi/×104)	Inhibition of average specific growth rate (%)*
Arithmetic mean measured concentrations (mg a.s./L) Water Control Colvent Control 0.0201	Mean cell member (cells/ml × 10°) 220.0 219.3 209.5	Inhibition of average specific growth rate (%)*
Arithmetic mean measured concentrations (mg a.s./L) Water Control Solvent Control 0.0201	Mean cell mimber (cells/mt, × 10*) 220.0 219.3 209.5	Inhibition of average specific growth rate (%)*
Arithmetic mean measured concentrations (mg a.s./L) Water Control Solvent Control 0.0201 0.0633 0.198	Mean cell member (cells/ml, × 10°) 220.0 219.4 209.5 31.8	Inhibition of average specific growth rate (%)*
Arithmetic mean measured concentrations (mg a.s./L) Water Control Solvent Control 0.0201	Mean cell mimber (cells/mt, × 10*) 220.0 219.3 209.5	Inhibition of average specific growth rate (%)*

Arithmetic mean measured concentrations (mg a.s./L)	Mean cell member Inhibition of average specific growth rate (%)*
Water Control	
Solvent Control	219.4 5 5 5 6
0.0201	209.5
0.0633	
0.198	210.60° & 0.8
0.608	\$\times 19\k4 \times \frac{1}{2} \times 2.6\frac{\pi}{2}
1.61	0 7 7 150.8
1.82	\$\frac{40.1}{2} \times \frac{1}{2} \times \frac{1}{

[#] Significantly ($\alpha = 0.05$, one sided smaller) reduced, based on Williams multiple sequential t-test procedure

Arithmetic mean measured	Yield Y S (cells/mL Y 104)	Infibition of yield (%)
(mg a.s./L)	cels/mL 104	
Pooled control	(3 A) (3/8.7 (7)	0
0.0201	208.50	4.7
0.0633	2108	3.6
0.198	200.6	4.1
0.608	\$\tag{90.4}\$	12.9#
1 1 61	149	31.5#
1.82	139/1	36.4#

[#] Significantly (\alpha = 0.05, one-sided smaller) reduced, base on Williams multiple sequential t-test procedure

Arithmetic mean measured concentrations	Area Wider the growth curve biomass integral)	Inhibition of Biomass integral (%)
concentrations (mg a.s./L)	© &	integral (70)
Pooled control A	4976.5	0
0.0201	4783.6	3.9
0.0633	4881.1	1.9
0.198	4787.9	3.8
0.60\$	4350.2	12.6#
1.61	3411.0	31.5#
1.82	3526.8	29.1#

[#] Significantly ($\alpha = 0.05$, one-sided smaller) reduced, based on Williams multiple sequential t-test procedure



Conclusion

The study meets the validity criteria and the 72 hours endpoints based on geometric mean and the 96 hours endpoints based on arithmetic mean concentrations are:

•	
E _r C ₅₀ 72 hours (95% C.I.):	> 2.02 mg a.s./L (n.d.)
E _r C ₂₀ 72 hours (95% C.I.)	> 2.02 mg a.s./L (n.d.)
E _r C ₁₀ 72 hours (95% C.I.)	> 2.02 mg a L (n.d.)
LOE _r C 72 hours: lowest concentration with an significant effect compared to the control	0.508 mg a.s./L
NOE _r C 72 hours: highest concentration without an significant effect compared to the control	0.190 mg a S/L
E _y C ₅₀ 72 hours (95% C.I.):	2.02 mg a.s./L\(\frac{1}{2}\)n.d.)
E _y C ₂₀ 72 hours (95% C.I.):	1.15 mg a.s./1 (0.65 to
E _y C ₁₀ 72 hours (95% C.I.):	0.42 mga.s./L (0.08 to 0.70 mg a.52)
LOE _y C 72 hours: lowest concentration with an significant effect compared to the control	0.598 60g a.s./L
NOE _v C 72 hours: highest concentration without an significant effect compared to the control	9.196 ang a.s./L
ErC50 96 hours 05% (2).):	> 1.82 mg a.s./L (n.d.)
LOE _r C 96 hours: lowest concentration with an significant effect compared to the control	0.608 mg ag./L
NOE _r C 96 hours: highest concentration without an significant effect compared to the control	0.198 mg a.s./L
E _y C ₅₀ 96 hours (95%C.I.)	>1.82 mg a.s./L (n.d.)
LOE _y C 96 hours: lowest concentration with an significant effect compared to the control	000 mg a.s./L
NOTE C 96 hours: highest concentration without an significant effect compared to the control	0.198 mg a.s./L
E _b C ₅₀ 96 hours (95% C.I.)	> 1.82 mg a.s./L (n.d.)
LOEbco hours lowest concentration with an agnificant effect contrared to the control	0.608 mg a.s./L
NOE _b Coo hours: highest concentration without an significant effect compared to the control	0.198 mg a.s./L
	. 1 .

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



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; 2017; M-587659-01-1 KCA 8.2.6.1/02; Report:

Title: Pseudokirchneriella subcapitata growth inhibition test with BCS-CN88460-

carboxylic-acid (BCS-CY26497)

EBLNN290 Report No.: Document No.: M-587659-01-1

OECD Guideline 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Guideline(s):

Material and methods

Guidenne(s).	(July 28, 2011) OCSPP Guideline 850.4500: Algal Toxicity (January 2012)
Guideline deviat	ion(s). A goording to OCSDD 850 4500 the maggired test substance concentration at test
Guidennie de viai	
	study the ECx calculations after 96 hours were performed using the mean measure of the study of of the s
	values to follow the recommendations from OPPT 850,1000.
GLP/GEP:	yes a variable of the contraction of the contractio
	initiation is considered appropriate to use for unstable test items. However, it his study the ECx calculations after 96 hours were performed using the mean measured values to follow the recommendations from OPPT 850.1000. yes nethods
Material and n	
Test material	BCS-CN88460-carboxylic-acid (BC\$-CY26497)
1 ost material	Batch code: SES 12631-19-9 & C & C & C & C & C & C & C & C & C &
	Sample description: TO \$20054.01
	Purity: 98.8% w/w
Guideline(s)	According to OCSPP 850.4500 the measured test substance concentration at lest
` '	
adaptation	study the EC _x calculations after 96 hours were performed using the mean measured
	values to follow, the recommendations from OPPTS 850 \$600.
т .	Freshwater green algae (<i>Pseudokirchneriella subcapitata</i>)
Test species	Strain SAG 61.8 () S
	1000 με of a 7-10 day cold stock culture was transferred for a 300 mL cotton
Culturing	plugged Erlenneyer flask containing about 100 mL of nutrient medium every 7-10
conditions	days To ensure that the algae used as inoculum were exponentially growing, a pre-
	culture was prepared 3 days before the start of the test and cultivated under the same
	conditions as in the main test.
Organism 💍	Preculture overe prepared from stock cultures days before the start of the test
age/size at	using OECD medium.
study 🤝	Pre-Culture Owere prepared from Mock Culture of days before the start of the test using OECD medium.
initiation	
,	
Test	Nominal concernations: 3.13, 6.25, 62.5, 25.0 and 50.0 mg p.m./L
solutions	©orresponding geometric mean measured concentrations (0 – 72 h): 3.46, 6.65,
	13.4026.3 and 35. Umg p.m./L 0
, ~	13.426.3 and 35.12mg p.m./L O Corresponding anithmetic mean measured concentrations (0 – 96 h): 3.43, 6.63,
	13.4, 26.2 and 5.3 mg/p.m./Q
	Controls: water and solvent controls (dimethylformamide at 0.1 µL/mL)
Evidence of undissolved material not mentioned	
Replication No. of vessels per concentration (replicates): 4 No. of vessels per control (replicates): 4	
F	Sanc & Q
Exposure	Antal exposure duration: 72 hours with a prolongation to 96 hours
	10,000 cells/mL at test initiation
Initiakæell	10,000 cells/mL at test initiation
density 0	
	45"



Test conditions	Temperature: 21.8 – 22.7°C Photoperiod: continuous light Light intensity at surface of test vessels: 4.620 to 4940 lux pH of controls (0 – 72 h): 7.5 – 8.1 Growth medium same as culture medium: Yes Type of light: artificial (Cool white fluorescent lamps)
Parameters Measured / Observations	Temperature was determined by a continuous measurement of one additional incubated glass vessel filled with the same amount of deionised water as in the test vessels. The pH was measured at the start of the study and additionally after 2 and after 96 hours in all test levels and the control. The light was measured once during the test. Cell numbers per volume (as a surrogate for biomass per volume) were estimated photometrically.
Sampling for chemical analysis	Samples were analysed for the actual concentration of the BCS-CY26497 present in the test medium of all treatment levels and the controls after 0, 72 and 96 hours.
Data analysis	EC _x values (e.g. x = 50) and confidence intervals were calculated for the standard exposure period, using a confinercial program (Too Rat Pro 3.2.1)

p.m. = pure metabolite

Results:

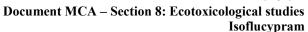
Validity criteria acc. to OECD TG 201	Required	Obtained
The biomass in the control coltures Chould have	\ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \	
increased exponentially by a factor of at least 16 within	(\$76 , °	₹ 72.3 ₹ \$
the 72-hour test period 🔊 🔊 💆 🔘		&. ~
The mean coefficient of variation for section by-section	2	0, 4,
specific growth rates (days 0-1, 92 and 2-3) in the	~ %	1,0%
control cultures must not exceed 3.5%.	S O	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
The coefficient of variation of average specific growth rates during the whole test period in replicate control	\$\int\{\partial\}\\ \int\{\partial\}\\ \tag{\partial\}\\ \par	
rates during We whole test period in replicate control	~ < 7%	2 1.3%
cultures must not exceed 7%.	r a, &	Į

Analytical results:

Some recoveries were not within the range of 80–120% of nominal (see table below). Thus biological results after 72 hours and 96 hours are based of geometric and arithmetic mean measured concentrations of BCS-088460-carboxylic acid (BCS-026497), respectively. No residues of BCS-088460-carboxylic acid (BCS-026497) were found in the control and solvent control samples above 0.000626 mg/L, which was used as the lowest gandard concentration during this study.

Nominal Concentration (mg p m /I)		concentrations weasured concentrations		% of nominal concentrations*		
(mg p.m./L)	(mg/j.m./L)	(mg p.m./L)	0-hour	72-hour	96-hour	
3.13	3.46	3.43	1.11	0.99	0.98	
6.2 5	\$ 0 6505 °	6.63	1.07	0.99	0.99	
22.5	3 .4	13.4	1.08	0.99	0.99	
\$\frac{1}{25.0}\$	26.3	26.2	1.06	1.00	0.99	
500	35.1	35.3	0.70	1.01	1.01	

Valued not given in study report; calculated on the basis of nominal concentrations and concentrations measured after 0, 72 and 96 hours.





Biological results:

72 hours		
Geometric mean measured concentrations	Mean cell number	Inhibition of average specific growth rate
(mg p.m./L)	after 72 h per mL	
Water Control	723000	
Solvent Control	708000	
3.46	719000	-0.1 0
6.65	676000	1.3
13.4	722000	-0.2Q*
26.3	773000	
35.1	662000 ,	
(mg p.m./L)		
Daalad aantual	70.46	
Pooled control	7 7000)	
3.46	Q ,76.9 0	0.50
3.46 6.65	© 70.9 ° 66.6 ° 71.3 °	5 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
3.46 6.65 13.4	76.6 70.9 70.9 71.2 71.2	5.8° 5.8° 5.8° 5.8° 5.8° 5.8° 5.8° 5.8°
3.46 6.65 13.4 26.3	66.6 70.2 70.2 70.2	5.6 4 -0.50 4 -0.9 4 -0.9 4 -0.9 7.6#
3.46 6.65 13.4 26.3 35.1 # Significantly (a = 0.05 are-sid	76.9 76.6 71.2 76.3 65.2 65.2	-0.50 -0.9
3.46 6.65 13.4 26.3 35.1 # Significantly (α = 0.05, me-sid Holm	76.9 66.6 71.2 76.2	-0.50 5.8 -0.9 -0.9 -8.1 -7.6# Multiple sequedially-rejective Welsh-t test after Bonferroni
3.46 6.65 13.4 26.3 35.1 Esignificantly (α = 0.05, pre-sid Holm		Inhibition of average specific growth rate % -0.1 -0.1 -1.8 # ols hhibition of weld (%)
Geometric mean	Area under the	Inhibition of Biomass
Geometric mean measured concentrations	Area under ine	Inhibition of Biomass
Geometric mean measured concentrations (mg p.m./L)	Area under the growth carve (biomass integral)	Inhibition of Biomass integral (%)
Geometric mean measured concentrations (mg p.m./L) Pooled control	Area under the growth curve (biomass integral)	Inhibition of Biomass integral (%)
Geometric mean measured concentrations (mg p.m./L)	Area under the growth curve (biomass integral)	Inhibition of Biomass ointegral (%)
Geometric mean measured concentrations (mg p.m./L) Pooled control	Area under the growth carve (biomass integral)	Inhibition of Biomass integral (%)

Geometric mean measured concentrations (mg p.m./L)	Yield Anhibition of yeld (%) (cells and x 40°)
Pooled control	706 Q Q S S O S
3.46	0 76.9 0 5 0 -0.50 0 0
6.65	66.6
13.4	71.2
26.3	760 4 4 8.1 4
35.1	65.2 ° 7.6¢

Geometric mean 🔊	Area under the Inhibition of Biomass
measured concentrations	
(mg p.m./L)	(biomass integral)
Pooled control	1296.6
3.46	(a) 1267.3 (b) W 0.0°
6.65	1215 D & A.3
13.4	© 1295.5 © -2.1
26.3	\$56.1 \$\times 6.8
35.1	11898 6.3#



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96 hours

Arithmetic mean measured concentrations (mg p.m./L)	Mean cell number after 96 h per mL	0-96 h average specific growth rates [days	Inhibition of average specific growth rate [%]
Water Control	2015000	1.326	- 0 . 1
Solvent Control	1997000	1.324	- 2 2
3.43	2031000	1.328	- 0.2
6.63	1931000	1.316	6 7 2 7
13.4	2010000	1.325	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
26.2	2014000	1.326	\$ 0.0° \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \
35.3	1845000	1.304	136#

[.] after Bonferron. - % inhibition = increase in growth relative to the pooled control α = Significantly (α = 0.05, one-sided smaller) reduced, based on Multiple sequentially-rejective Welsh-t test after Bonferrom

Arithmetic mean	Yield Y Inhibition of vield (%)
measured concentrations	(cells/mL × 10 ³)
(mg p.m./L)	
Pooled control	1996
3.43	202.1 4 7 -1.3
6.63	(D)2.1 × 3.80 ×
13.4	200.0°
26.2	2004 9 00.4 9
35.3	183.5 0° 8.1° 8.1° 1

[#] Significantly ($\alpha = 0.05$, one-sided smaller) reduced based on Multiple sequentially-rejective Welsh-t test after Bonferroni Holm Holm

Arithmetic mean 🎣	Area ander the Inhibition of Biomass growth curve Integral (%)
	growth curve S integral (%)
(mg p.m./L)	(biomass integral)
Pooled control \O	4511.6
3.430	\$43.5,0° \$\tag{9}\$ -0\$\tag{9}\$
6.63	4319.47 Q Q.3
×3.4 ×	45,50.5 0 0 -0.90
26.2	\$\\ \delta \\ \d
35.3	√ ~41740° √

^{-%} inhibition: Increase in growth relation to the pooled control

Conclusion

The stude meets the validity oriteria and the 72 hours endpoints based on geometric mean and the 96 hours endpoints based on mean measured concentrations are:

[#] Significantly (\alpha = 0.05, one sided smaller) reduced based on Multiple sequentially-rejective Welsh-t test after Bonferroni Holm



E _r C ₅₀ 72 hours (95% C.I.):	> 35.1 mg p.m./L (n.d.)
E _r C ₂₀ 72 hours (95% C.I.)	> 35.1 mg p.m./L (n.d.)
E _r C ₁₀ 72 hours (95% C.I.)	> 35.1 mg p.m./L (n.d.)
LOE _r C 72 hours: lowest concentration with an significant effect compared to the control	35.1 mcp.m./L
NOE _r C 72 hours: highest concentration without an significant effect compared to the control	26.3 mg p.m./L
E _y C ₅₀ 72 hours (95% C.I.):	n.dx
E _y C ₂₀ 72 hours (95% C.I.)	in and a second
E _y C ₁₀ 72 hours (95% C.I.)	
LOE _y C 72 hours: lowest concentration with an significant effect compared to the control	35 mg p. m/L
NOE _y C 72 hours: highest concentration without an significant effect compared to the control	26,3 mg p.m./L
E _b C ₅₀ 72 hours (95% C.I.):	S Md. O'
E _b C ₂₀ 72 hours (95% C.1.)	n.d.
E _b C ₁₀ 72 hours (95% E.I.)	Sh.d.
LOE _b C 22 hours: lowest concentration with an stemificant effect compared to the control	35.1 mg p.m.H.
NOE _b C 72 hours highest concentration without an significant effect compared to the control	6.3 mg p.m./L
ErC50 96 pours (95% C.J.):	> 35.3 mg p.m./L (n.d.)
E _r C ₂ C ₂ 96 hours (95% C.I.)	n.d.
E_rC_{10} 96 hours $(25\% C.I.)$	n.d.
LOE _r C 96 hours: lowest concentration with an significant effect compared to the control	35.3 mg p.m./L
NOE 96 hours: Whighest consentration without an significant effect compared to the control	26.2 mg p.m./L
E _y C ₅₀ 96 hours (95% C.I.):	> 35.3 mg p.m./L (n.d.)



		•
E _y C ₂₀ 96 hours (95% C.I.)	n.d.	
E _y C ₁₀ 96 hours (95% C.I.)	n.d.	
LOE _y C 96 hours: lowest concentration with an significant effect compared to the control	35.3 mg p.m./L	
NOE _y C 96 hours: highest concentration without an significant effect compared to the control	26.2 mg p.m./L	
E _b C ₅₀ 96 hours (95% C.I.):	> 35.3 mg p.m./L (n.d)	
E _b C ₂₀ 96 hours (95% C.I.)	n.d.	
E _b C ₁₀ 96 hours (95% C.I.)	o n.d.	
LOE _b C 96 hours: lowest concentration with an significant effect compared to the control	35 3 mg p.m./L	
NOE _b C 96 hours: highest concentration without an significant effect compared to the control n.d.: not determined due to mathematical reasons or in	26.2 mg p.m. L	
~	''' i	, s Co

Only at the highest test item concentration of 35.1 mg p.m./L avgrowth reduction was observed. The observed effects on growth inhibition were below the 10% leven No growth reduction occurred at the NOEC of 26.3 mg p.m./L.

The number of effect concentrations and the observed effect size were not sufficient for a reasonable EC₁₀ and EC₂₀ calculation.

CA 8.2.6.2 Effects on growth of an additional algal species

Report: KCA 8.26-2/01; R.; R.; J. R.; J. R.; J. R.; K. H.; 2017;

Title: BCSc N88460: A 96 hour toxicity (est with the cyanobacteria (Anabaena flos-aquae)

Report No.: 14.9-111 Document No.: M=60507-01-14

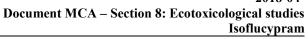
Guideline(V): QDECD 201

EU Directive 92/69ÆEC, Method C.3. U.S. EPA OCSPP Vumber 850.4550

Guideline deviation(s): none

Material and methods:

*Y	
Test material	PBCS-CN88400 (tech)
. Y . S	Batch number: 2013-006492
	CAS number: 1255734-28-1
	Purity: 94.2% w/w
Guideline(s)	None specified
adaptation	
Test species	Freshwater blue-green algae (Anabaena flos-aquae)





Culturing conditions	The algal cells were cultured and tested in freshwater AAP medium. Algal cells used in this test had been actively growing in culture medium under the same
	environmental conditions as used in this test for at least two weeks prior to test initiation.
Organism age/size at	Algal cells for this study were taken from a culture that had been transferred to fresh medium three days prior to test initiation. Nominal concentrations: 0.024_0.076_0.24_0.78_2.5 and 8.0 mg a s.//
study	The sin medium times days prior to test initiation.
initiation	
Test solutions	Nominal concentrations: 0.024, 0.076, 0.24, 0.78, 2.5 and 8.0 mg a.s./v.
	Corresponding geometric mean measured concentrations (0-72 h): 0021,0075,
	0.24, 0.76, 2.2 and 5.1 mg a.s./L.
	Nominal concentrations: 0.024, 0.076, 0.24, 0.78, 2.5 and 8.0 mg a.s. //. Corresponding geometric mean measured concentrations (0-72 h): 0.021, 0.075, 0.24, 0.76, 2.2 and 5.1 mg a.s./L. Corresponding time weighted mean measured concentrations (0.06 h): 0.020, 0.072, 0.23, 0.73, 2.1 and 4.8 mg a.s./L Controls: water and solvent control (dimethyltorman) de at 0.1 µL/mL) Evidence of undissolved material: Visible particulates in 8.0 mg a.s./L test solutions
	0.072, 0.23, 0.73, 2.1 and 4.8 mg a.s./L
	Controls: water and solvent control (dimethy) formand de at 0.1 µL/mL)
5 1: .:	at test initiation and in test chambers throughout the 96 your exposure period
Replication	No. of vessels per concentration (replicates): 4
	No. of vessels per control (replicates) 4 No. of vessels per solvent control (replicates): 4
Exposure	Static Static
Laposure	No. of vessels per concentration (replicates): 4 No. of vessels per solvent control (replicates): 4 No. of vessels per solvent control (replicates): 4 Static Total exposure doration: 96 hours 10 ⁴ cells/mL at test infration Temperature: 23.4—23.80C Photoperiod: continuous light Light intensity, 1940 to 23.80 lux pH of controls (0 96 hours): 73—9.5 Growth medium same as culture medium: Yes
Initial cell	10 ⁴ cells/mL at test initiation
density	
Test	Temperature: 23.44—23.80°C
conditions	Temperature: 23.4—23.8°C Photoperod: continuous light Light in the internal and a second se
	Light intensity, 1940 to 2350 lux V
	pH of controls (0 496 hours): 7,3 - 9.5, 7 0 4 37
-	Spe of ight; artificial (Cool white Puorescent light)
Parameters Measured /	Temperature was continuously monitored throughout the study. The pH of the
Measured / Observations	medium in each treatment and control group has measured at test initiation and at
Observations	exposure termination (96 hours). The light was measured once during the test. Cell densities were monitored at approximately 24-hour intervals during the test by
£9"	conducting call counts using a hemacytometer and a microscope. At the end of the
> %	exposure period algae were examined microseopically for atypical cell morphology
	Ge.g., changes in cell shape. See or color) Cells in the replicate test chambers also
	were assessed for aggregation or Docculation of cells, and adherence of the cells to
	the dest chamber. Y
Sampling for	Sample were of lected from the batches of test solution prepared for each
chemicato **	treatment and control group at the beginning of the test, from surrogate replicates
analysis	included for analytical sampling at 72 hours, and from test solution pooled from the
anarysis	remaining biotic replicates of each treatment and control group at the end of the lest
	to determine concentrations of the test substance.
Data analysis	The calculation of area under the growth curve, growth rates, yield and percent
0.	indivition values, as well as all statistical analyses, were conducted using 'The SAS
	System or Windows The results of the statistical analyses, as well as the Evaluation of the concentration-response pattern, were used to determine the NOEC
, Š	for each pagameter at 72 and 96 hours.
	of equal parameter at 72 and 90 nours.



Results:

Validity criteria acc. to OECD TG 201	Required	Obtained	_ 0
The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period.	16	87	
The mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3) in the control cultures must not exceed 35%.	< 35%	29.8%*	
The coefficient of variation of average specific growth rates during the 72-hour test period in replicate control cultures must not exceed 7%.	\$\frac{\infty}{\sqrt{2}} < 10\%	Ø6.17%*	dy reports of the
* Values not given in study report; calculated on the basis of cell Analytical results: Some recoveries were not within the range of 00 - 12) 🐃 W
results after 72 hours and 96 hours are based on geo concentrations of BCS-CN88460, respectively. No control and solvent control samples above the limit of was used as the lowest standard concentration during	metac mean a residuer of B quantification	nd time weight	teOmean@neasufed were found in the
Coometrie manife Times	cighted mean		

Nominal Concentration	Geometric mean measured concentrations after 72 hoos	Time weighted means measured concentrations after 96 hours	% of nor	ninal Conce	ntrations
(mg a.s./L)	after 72 hoors (mg a.s./L)	(mg a.s.A)	0€bour ⊲	72-hour	96-hour
0.024		0,02	\$\sqrt{90.5}\gamma'	82.6	69.9
0.076	Q.075 S	©.072 @ s.	106	90.5	81.3
0.24	0.24	0.23	T 06	91.0	79.1
0.78			99.7	94.6	73.3
2.5 📞	2.2	2.1 _Q	91.5	81.4	67.7
8,90	Ö 55 8	\$ 4.85	68.2	59.6	36.6

Biological results

No morphological change in algae was observed in any test concentration. No adherence of cells to the test chambers or flocculation or ascregation of cells was observed.

72 hours

Geometric mean measured concentrations (mg a.s./L)	Mean Cell number after 72 h (cells/mL × 10 ⁴)*	Inhibition of average specific growth rate (%)
Pooled control	√ 77.4	-
0.021	√ √ 72.7	1
0.075	69.9	4
0.24	76.2	2
0.767	61.0	5
2.2	71.3	1
5.1	2.85#	77

^{*} Values not given in study report; calculated on the basis of single replicate values given in study report.

[#] Treatment group was significantly reduced (Dunnett's Test, p < 0.05) when compared to the pooled control mean



Geometric mean measured concentrations (mg a.s./L)	Yield (cells/mL)	Inhibition of yield (%)
Pooled control	764000	0
0.021	717000	6
0.075	688500	100
0.24	752000	2 ⁰
0.76	599500	22
2.2	703000	8
5.1	18500#	98 0

Geometric mean measured	Yield (cells/mL)	Inhibition of yield (%)	
concentrations			
(mg a.s./L) Pooled control	764000	0	
		0	
0.021	717000	6	
0.075	688500	100	
0.24	752000	201	
0.76	599500	22	
2.2	752000 599500 703000 18500# antly reduced (Dunnett's Test p < 0.05 Area under the growth curve (biomass integral) 15726000 13228000	8 2	
5.1	18500#	Q 98 Q 3	
Treatment group mean was significa	antly reduced (Dunnett's Text) $p < 0.05$) where compared to the pooled conti	rol poean
Geometric mean measured	Area under the growth	Inhibition of Biomass	
concentrations	curve (biomass integral)	integfal (%) 🛴	
(mg a.s./L)			4
Pooled control	k5726000 @/		
0.021	2270000		
0.075	0 13248000	115	
0.24	(\$\sqrt{40}1600\sqrt{2}2	7 11 V	©
0.76	12222000		J
2.2	13728000		
5.1	\$\frac{1}{2}\$5000 [#]	\$ 97 O	
Treatment group mean was signification	antly Educed Dunnet Test, & 0.05) when compared to the pooled conti	rol mean
96 hours			
Time weighted mean			
maggired concentration office	· Maan Vall namban aften 06 6	Whibitian of average	

Time weighted mean measured concept ation after	Mean vell number after 96.6	Inhibition of average
96 hours (mg) a.s./L)	(cells mL × 19 ⁴)*	specific growth rate (%)
Pooled Control	Q <u>A</u> 257.9	-
0.02	246.5	2
0.072	318.20	-4
0.23	258.6	1
0.73	293.0	-2
2.1	255, 10	0
4.8	2105	89
Values not given in study report; cal	Cylated on the basis of sixele replicate	values given in study report.
% Inhibition: Increase in growth rela		
	educed (Dunnew's Test $p < 0.05$) when	n compared to the pooled control me
	8.	T 1.11.4. 6 . 11.40/)

Time weighted mean measured concentrations after	Yield (cells/mL)	Inhibition of yield (%)
96 Hours (Hig/a,sa/L)		
Pooled Control &		-
0.02	2455000	4
0.072	3178750	-24
0.23	2576250	0
0.23	2920000	-14
2.1	2541250	1
4.8	1.4750#	99

^{- %} Inhibition: Increase of yield relative to the pooled control

[#] Treatment group mean was significantly reduced (Dunnett's Test, p < 0.05) when compared to the pooled control mean



Time weighted mean measured concentrations after 96 hours	Area under the growth curve (biomass integral)	Inhibition of Biomass integral (%)	
(mg a.s./L)		I I	
Pooled Control	55726500	-	
0.02	50334000	10,0	,0' d
0.072	59655000	-10"	
0.23	53955000	<u></u>	
0.73	54456000	2 × 2	
2.1	52659000	Q 6 Q 3	
4.8	795000#	99 💆 🔾	
0.072 0.23 0.73 2.1 4.8 % Inhibition: Increase of biomass inte * Treatment group mean was significant *Conclusion The study meets the validity cr 96 hours endpoints based on tim	iteria and the 72 frours endpo	points based on seomethic me	and the
E _r C ₅₀ 72 hours (95% C.I.):	4.8 mg a s. /L (4	1.76 4.9 mg a.s.45)	S. S
E _r C ₂₀ 72 hours (95% C.I.):	\$\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\		
E _r C ₁₀ 72 hours (95% C.I.):		1.3 to 4 (mg a:s. L)	
LOE _v C 72 hours:			

					♠	7/
0.23	539	955000	, A	3		
0.73	544	156000		2 🐇		Ţ
2.1	526	559000	Q	6		
4.8		5000#		9 0	Q Ô	
- % Inhibition: Increase of biomass in	tegral relative to the	ne pooled control			y O	
*Treatment group mean was signification	ntly reduced (Dun	p < 0.05	when compared	to the poole	d control mea	ın⊘ʻ ⊘
		4 6° 5), Š.,	% 4	Ĭ
- % Inhibition: Increase of biomass in # Treatment group mean was significa Conclusion The study meets the validity 96 hours endpoints based on ti	(4 4	م الم
The study meets the validity	criteria and the	e 72 Prours Endpo	oints based or	geometr	ic mean an	dine
96 hours endpoints based on ti	me weighted m	ean concentration	os are:			A
E_rC_{50} 72 hours (95% C.I.):		4% mg a & L (4	7 to 4.9 mig a.s			
F.C. 701 (059/ CL)	- 4				***	
E _r C ₂₀ 72 hours (95% C.I.):		© 4.6 mg a.s./I@4	.6 to 4.7 mg@.s	./L		
E _r C ₁₀ 72 hours (95% C.I.):		∯3 mg aØs./L (4	.3 to 4 Amg a.s	(L)		
LOF _v C 72 hours:		A	· ************************************			
LOE _y C 72 hours: lowest concentration with an sign compared to the control	nifican@ffect \$		ng a.s./L &			
compared to the control		\$ 2		4		
NOE _r C 72 hours:						
highest concentration without an	Significant	ž 2.2 r	ngazs./L			
effect compared to the control	Significant &					
E _b C ₅₀ 72 hours (95% C.k.):		3.5 mg a.s./L Ø.	4 to \$5.1 mg a	.s./L)		
LOE _b hours:						
lowest concentration with an sig	nificant effor	5.1 f	ng a.s./L			
compared to the captrol	-0 4 "					
NOE _b C 72 hours: highest concentration without an	signature O		/*			
effect compared to the control	Signarcane	2.2 r	ng a.s./L			
E _y C ₅₀ 72 hours (95% C.I.):		3.4 mg a.s./L (1.:	5 to > 5.1 mg a	.s./L)		
			20.4 - 5.1	/T. \		
E _y C ₂ / ₃ 72 hours (95% C.I.)		2.9 mg a.s./L (0.8	39 to > 5.1 mg a	s./L)		
E _y C ₁₀ 72 hours (95% & I.):		2.6 mg a.s./L (0.6	58 to > 5.1 mg a	s./L)		
LOE _r C 96 hours:	2, an					
lowest cocentration with an sign	ificant effect	5.1 r	ng a.s./L			
compared to the control						
NOS C 96 bours:						
highest concentration without an effect compared to the control	significant	2.2 n	ng a.s./L			
checkeyinpared to the control						



E _r C ₅₀ 96 hours (95% C.I.):	3.7 mg a.s./L (1.8 to > 4.8 mg a.s./L)
E _r C ₂₀ 96 hours (95% C.I.):	3.0 mg a.s./L (0.89 to > 4.8 mg a.s./L)
E _r C ₁₀ 96 hours (95% C.I.):	2.8 mg a.s./L (0.62 to > 4.8 mg a.s./
LOE _r C 96 hours: lowest concentration with an significant effect compared to the control	4.8 mg a.s./L
NOE _r C 96 hours: highest concentration without an significant effect compared to the control	3.7 mg a.s./L (1.8 to > 4.8 mg a.s./L) 3.0 mg a.s./L (0.89 to > 4.8 mg a.s./L) 2.8 mg a.s./L (0.62 to > 4.8 mg a.s./L) 4.8 mg a.s./L 2.1 mg a.s./L 2.1 mg a.s./L 4.8 mg a.s./L
E _b C ₅₀ 96 hours (95% C.I.):	30 mg a.s./L (2,3/to 4.0 mg a.s./L)
LOE _b C 96 hours: lowest concentration with an significant effect compared to the control	il 8 mg (S./L)
NOE _b C 96 hours: highest concentration without an significant effect compared to the control	H mg &s./L
E _y C ₅₀ 96 hours (95% C.I.)	2.9 mg/a.s./L, 2.2 to \$.8 mg/a.s./L)
E _y C ₂₀ 96 hours (95% C.I.):	24 mg as./L (1.740 3.44mg a.s./D)
E _y C ₁₀ 96 hours (95% C.I.): LOE _r C 96 hours: lowest concentration with an significant effect compared to the control NOE _r C 96 hours: highest concentration without an significant effect compared to the control	2.2 mg a.s./L (1.5 to 3.2 mg d.s./L)
Growth inhibition was observed only at the	e bighes Otest item concentration. The
Growth inhibition was observed only at the oncentrations is not sufficient or a reasonal	



, J. R.; , J. R.; Report: KCA 8.2.6.2/02; , K. H.; 2017;

M-604811-01-1

marine diatom (Skeletonema Title: BCS-CN88460: A 96-hour toxicity test with the marine diatom (Skeletonema

costatum)

Report No.: 149P-113 Document No.: M-604811-01-1 Guideline(s): OECD 201

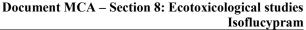
EU Directive 92/69/EEC, Method C.3.

U.S. EPA OCSPP Number 850.4500

Guideline deviation(s): none **GLP/GEP:** yes

Material and methods

T 4 1 1	BCS-CN88460 (tech.) Batch number: 2013-006492 CAS number: 1255734-28-1 Purity: 94.2% w/w None specified Marine diatom (Skatetonema costatum) The algal cells were cultured and tested in altiwater algal medium. Algal cells used in this test had been actively growing in culture and time under the same
Test material	Batch number: 2013-006492 \$\times 0 \times 0 \ti
	CAS number: 1255734-28-10
	Purity: 94.2% w/w
Guideline(s)	None specified & A A A A A A A A A A A A A A A A A A
adaptation	
Test species	Marine diatom (Skaletonema costatum) y y S S S
1 est species	
Culturing	The algal cells were cultured and tested in saltwater algab medium. Algal cells used
conditions	in this test is a feet were growing in culture allegated to saute
Conditions	environmental condition cas used in this test for at least two weeks prior to test
	initiation of the state of the
Organism	Algal cells for this study were taken from a culture that had been transferred to fresh
age/size at	medium three days prior to test initiation?
study	
initiation	medium three days prior to test initiation?
Test	Nominal concentrations: 0.024, 0.076, 0.24, 0.78, 2, and 8.0 mg a.s./L
solutions	Corresponding geometric mean measured concentrations (0 – 72 h): 0.017, 0.060,
	0.22, 668, 1. Sand 22 mg a.s./L Some concentrations (0 – 96 h): 0.016,
* *	Corresponding time-weighted ragan measured concentrations (0 – 96 h): 0.016,
	0.036, 0.20, 0.65, 1.7, 2.9 mg a.s./L
	© ontrols: water and solvent controls (dimethylformamide at 0.1 μL/mL) Evidence of indissolved material. All test solutions appeared clear and colorless.
	Small particulates were visible on the bottom of the flask in the 8.0 mg a.s./L
4	treatment group 4 0
Replication	No. of wessels per concentration (replicates): 4
repriessor	No of vessels per control (replicates): 4
2	No. of vessels per solvent control (replicates): 4
J	Static C. S.
Exposure	Total exposure duration: R hours
, , , , o	10 cells at test init ation
Initial cell	10 vells at test init ation
density	
i	





Test conditions	Temperature: 18.8 – 19.5°C Photoperiod: 14 hours light / 10 hours dark Light intensity at surface of test vessels: 3880 to 4730 lux Salinity: 32 – 34‰ pH of controls (0 - 72 hours): 8.1 – 8.9 Growth medium same as culture medium: Yes Type of light: artificial (Cool white fluorescent lamps)
Parameters Measured / Observations	Light intensity at surface of test vessels: 3880 to 4730 lux Salinity: 32 – 34‰ pH of controls (0 - 72 hours): 8.1 – 8.9 Growth medium same as culture medium: Yes Type of light: artificial (Cool white fluorescent lamps) Temperature of a container of water adjacent to the test chambers in the environmental chamber was measured continuously. Light intensity was measured at test initiation at test solution level at the locations corrounding the test plasks. The pH of the medium in each treatment and control group was measured at test initiation, at approximately 72 hour and at exposure termination (96 hours). Cell counts were performed at approximately 24-hour intervals using a hemacytometer and a microscope. At the end of the exposure period algae were examined microscopically for atypical cell morphology (e.g., changes in cell shape, size or color). Cells in the replicate test chambers also were assessed for aggregation or flocculation of cells, and otherwise of the cells to the test chambers.
Sampling for chemical analysis	Samples of the test solutions were collected at approximately 0, 72 and 96 hours to measure concentrations of the test substance. At test initiation samples were collected from each cest concentration and control solution prior to distribution into the test chambers. At 72 hours, samples were collected from the single sacrificial replicate for each test concentration and control groups. At test termination the biological replicates from each respective test concentration and control solutions were pooled and then sampled.
Data analysis	The calculation of area under the growth curve, growth rates, yield and percent inhibition values, as well as all statistical analyses, were conducted using 'The SAS System for Windows'. The results of the statistical analyses as well as the evaluation of the concentration-response pattern, were used to determine the NOEC for each parameter at 72 and 96 hours.

Validité criteria acc. to OECO TG 260 Required	Obtained
The biomass in the control cultures should have increased exponentially by a factor of at least 16 within 16	120
the 72-hour test period.	120
The mean coefficient of variation for Section by-section	22.60/
specific growth rates (day 0-1, 1/2 and 2-3) in the <35% control cultures must not exceed 3%.	23.6%
The coefficient of variation of average specific growth	
rates during the 72 h test period in replicate control < 7%	3.4%
cultures must not exceed 7% 0	

Analytical results.\(\)
Some recoveries were not within the range of 80 – 120% of nominal (see table below). Thus biological results after 72 hours and 96 hours are based on geometric and time weighted mean measured concentrations of BCS CN88460, respectively. No residues of BCS-CN88460 were found in the control and solvent control samples above 0.0024 mg a.s./L, which was used as the lowest standard concentration during this study.



Nominal Concentration Concentration Geometric mean measured concentrations after 72 hours		Time weighted mean measured concentrations after 96 hours	% of nom	inal conce	ntrations
(mg a.s./L)	(mg a.s./L)	(mg a.s./L)	0-hour	72-hour	96- hæ ær
0.024	0.017	0.016	91.4 %	55.0	<i>3</i> 90.4
0.076	0.06	0.056	96.1	65.7	41.2
0.24	0.22	0.20	1208	76.3	56.6
0.78	0.68	0.65	× 96.4	79.9	3 6.0
2.50	1.8	1.7	91.0	\$9.8	9 38.00
8.00	3.2	Ø/2.9	47.3 _(33.5 [©]	201
<u>2 hours</u>	flocculation or aggregation				
Geometric mean concentrations a (mg a.s./L) Pooled control 0.017 0.06	n measured Mainter 72 hours Company	n cell number Ind lls/mL × 100 * spectors 120.3 110.5 110.5 100.8	distion of a fire growth	verage raite (%)	
0.22 0.68 1.8		90.30	© 0 % © 5% D 18	* -	
3.2	\$\frac{\frac}{\frac{\frac{\frac{\frac{\frac{\frac{\frac{\frac{\frac{\frac{\fin}}}}}}{\fint}}}}}}}}}}}}}}}}}}}}}}}}}}}}}}}}}}}}	44.7 [#] Q & C	<u> </u>		

Geometric mean measured concentrations after 72 hours (mg a.s./L)	Mean cell y imber Inhibition of average (cells/mL × 100)* specific growth rate (%)
Pooled control	120.3 5 5 5
0.017	(4, D) 1165 0 4 27 , 20 0
0.06	
0.22	18.0 Ø 8 0 Ø
0.68	90,30 % 67
1.8	\$ \$\infty\$ 5.038 \$\times\$ \$\times
3.2	44.7# \$\infty \tag{\pi} \ta

^{*}Mean cell number after 72 Innot presented in study report but calculated on the casis of single replicate values.

[#] Treatment group was significantly reduced Dunner Test p < 0.05), when compared to the pooled control

Geometris mean measured Yield (cells/mL)	Inhibition of yield (%)
(mg a.s./L)	
Pooled control	-
1001/ ~ 0 ~ 109 5 000 ~ 1	8
0.06	9
0.22	2
0.68	25
1.8 4 498250#	58
3.2 7 737250#	63

[#] Treatment group mean was significantly reduced (Depenett's Test, p < 0.05) when compared to the pooled control mean



Geometric mean measured concentrations (mg a.s./L)	Area under the growth curve (biomass integral)	Inhibition of Biomass integral (%)
Pooled control	57519000	
0.017	53436000	7
0.06	55950000	3
0.22	56436000	2
0.68	50220000	13 ,0 0
1.8	32250000#	44 🗸
3.2	28920000#	\$ 500 \$

(mg a.s./L)	Area under the growth curve (biomass integral)	Inhibition of Biomass integral (%)
Pooled control	57519000	- 2 2
0.017	53436000	7 8
0.06	55950000	3 ()
0.22	56436000	
0.68	50220000	13 0 0 0
1.8	32250000#	44 2 7 69 01
3.2	$28920000^{\#}$ y reduced (Dunnett's Test, $p < 0.05$) when	2 500 2 4
6 hours		13 0 44 44 44 44 44 44 44 44 44 44 44 44 4
Time weighted mean measured concentrations after 96 hours	Mean cell number (cells/ml * 104)	Inhibition of average
Time weighted mean measured	(cells/mf × 10 ⁴)	Inhibition of average specific growth rate (%)
Time weighted mean measured concentrations after 96 hours (mg a.s./L)		Inhibition of average specific growth wite (%)
Time weighted mean measured concentrations after 96 hours (mg a.s./L) Pooled control	(cells/mL 104)	Inhibition of average specific growth wite (%)
Time weighted mean measured concentrations after 96 hours (mg a.s./L) Pooled control 0.016	(cells/mf × 10 ⁴); (cells/mf × 10 ⁴); (150.5) (159.5)	Inhibition of average specific growth wite (%)
Time weighted mean measured concentrations after 96 hours (mg a.s./L) Pooled control 0.016 0.056	(cells/mf × 10 ⁴); (cells/mf × 10 ⁴); (150.5) (159.5)	Inhibition of average specific growth wite (%)
Time weighted mean measured concentrations after 96 hours (mg a.s./L) Pooled control 0.016 0.056 0.20	(cells/mf. 10 ⁴); (cells/mf. 10 ⁴); (cells/mf. 10 ⁴); (cells/mf. 150.5); (cells/mf. 15	Inhibition of average specific growth wite (%)

^{- %} Inhibition: Increase in growth relative to the pooled control of the project control of

Time weighted mean measured Yield (cellson) Yield (cellson)	Inhibition of yield (%)
concentrations after 96 hours (mga.s./L)	
Pooled compol 4 1600000	<u>√</u> -
© 0.016 © © 0.495000 © 0	7
0.056 0 0 152000 0	5
0.20	2
0.65	-9
1.7 2 2 2 1230000 2	23
2.9 0 106000#	34

^{- %} Inhibition Increase of yield relative to the posted control p and p are treatment of the posted control p and p are treatment of the posted control p and p are treatment of the posted control p and p are treatment of the posted control p and p are treatment of the posted control p and p are treatment of the posted control p and p are treatment of the posted control p and p are treatment of the posted control p and p are treatment of the posted control p and p are treatment of the posted control p and p are treatment of p are treatment of p and p are treatment of p are treatment of p and p are treatment of p are treatment of p and p are treatment of p are treatment of p and p are treatment of p are treatment of p and p are treatment of p and p are treatment of p and p are treatment of p are treatment of p and p are treatment of p are treatme

Time weighted mean measured concentrations after 96 fours (mg a, s./L)	Area inder the growth curve (biomass integral)	Inhibition of Biomass integral (%)
Pooled control	57519000	-
0.016	53436000	7
0.056	55950000	3
0.20	56436000	2
0.65	50220000	13
0.65	32250000#	44
2.9	28920000#	50

^{- %} Inhibition: Increase of biomass integral relative to the pooled control

[#] Treatment group mean was significantly reduced (Dunnett's Test, p < 0.05) when compared to the pooled control mean



Conclusion

R	Document MCA – Section 8.	Isoflucypram
Conclusion The study meets the validity criteria and the hours endpoints based on time weighted recommendations.	ne 72 hours endpoints based on gemean concentrations are:	ometric mean and the
E _r C ₅₀ 72 hours (95% C.I.):	> 3.2 mg a.s./L (n.d.)	
E _r C ₂₀ 72 hours (95% C.I.)	2.8 mg a.s./L (2.3 to 3.5 mg a.s./L)	
E _r C ₁₀ 72 hours (95% C.I.)	1.1 mg a.s./IC00.73 to 1.6 mg/a.s./L)	
LOE _r C 72 hours: lowest concentration with an significant effect compared to the control	0.68 mg a.s.	
NOE _r C 72 hours: highest concentration without an significant effect compared to the control	0.22 prig a.s./L.\	cometric mean and the
E _b C ₅₀ 72 hours (95% C.I.):	2.2 mg a.s./L 3 (1/7) to 2/20 mg ass./L)	
LOE _b C 72 hours: lowest concentration with an significant effect compared to the control	\$ 0.68 mg.45./L	
NOE _b C 72 hours: highest concentration without an significant effect compared to the control		
E _y C ₅₀ 72 hours (95% C.I.):	1.8 mg a.s./L (1.3 to 2.4 mg a.s./L)	
E _y C ₅₀ /2 hours (95% C.1.):	055 mg a.s./L (029 to 10 mg a.s./L)	
E _y C ₁₀ 72 hours (\$3% C _y):	0.30 mg a. QL (0.12 to 0.72 mg a.s./L)	
LOE _y C 72 hours: Solution with an significant effect compared to the control of	0.682mg a, s.702	
NOE _y C 72 hours: highest concentration without an significant effect compared to the control	0.22 mg a.s./L	
ErCso 96 horos (95% C.I.):	>2.9 mg a.s./L (n.d.)	
E _r C ₂₀ 96 6 ours (95% C.12)	2.9 mg a.s./L (n.d.)	
E _r C ₁₀ 96 hours (95%C.I.)	> 2.9 mg a.s./L (n.d.)	
LOE _r C 96 hours lowest concentration with an enificant effect compared to the control	2.9 mg a.s./L	
NOE _r C % hours highest concentration without an significant effect compared to the control	1.7 mg a.s./L	
E _b C ₅₀ 96 Frours (95% C.I.):	> 2.9 mg a.s./L (n.d.)	
	•	4



LOE _b C 96 hours: lowest concentration with an significant effect compared to the control	1.7 mg a.s./L	n° &.
NOE _b C 96 hours: highest concentration without an significant effect compared to the control	0.65 mg a.s./L	
E _y C ₅₀ 96 hours (95% C.I.)	> 2.9 mg a.s./L (n.d.)	
E _y C ₂₀ 96 hours (95% C.I.):	(< 0.01% to > 2.0 mg a.S./L	
E _y C ₁₀ 96 hours (95% C.I.):	1.1 mg a.s./LO* (\$\infty\$0.016 to 2.0 mg/a.s./L),	
LOE _y C 96 hours: lowest concentration with an significant effect compared to the control	2.9 mg/m.s./L	
NOE _y C 96 hours: highest concentration without an significant effect compared to the control	2.9 mg a.s./L) 1.7 mag a.s./L) 7. R.; J.R.; J.R.; hour fexious feet with the freshwater dia	
	(Ø. R.;)	
Report: KCA 8.76.2/03*	;9. R.;; J.K.;	, K&H. 2017;
Title: BCS-CN88460: A 96-1	hour fexicity test with the freshwater dia	tom(Navicula
pelficulosa) 🗳		
Report No.: 149P-14A		
Document No.: M-604809-01-7		
Guideline(s): OECD 20 C ED Directive 92469/EE U.S. EPA OCSPP Num	E.C. Metho©C.3.	
Guideline deviation(s): none		
GLP/GEP: yes yes		
Materia and methods		
D C C C C C C C C C C C C C C C C C C C	~ %/ / ₂ & //	

Test material	BCS CN88460 (Ech.) O 4 4
T CSt IIIateriai	Batch number 2013-006492 0 0
e e	CAS number \$1255734-28-1
~0	Piii*KV: 99 19/0 w/kw & S & S
Guideline (s)	None specified
adaptation	
auaptarion	
T & .	Freshwater diatom (Nasicula pelliculosa)
Test/species	
	The algal colls were cultured and tested in freshwater AAP medium with silica
Culturing 5	constituents. Algal cell used in this test had been actively growing in culture
conditions	medium under the same environmental conditions as used in this test for at least two
	Weeks prior & test initiation.
	Algal cells for this study were taken from a culture that had been transferred to fresh
Organism 0	modium three days prior to test initiation.
age size a	inequality aree days prior to test initiation.
study O	
initiation	
111111111111111111111111111111111111111	





	Nominal concentrations: 0.024, 0.076, 0.24, 0.78, 2.5 and 8.0 mg a.s./L
Test	
solutions	Corresponding geometric mean measured concentrations (0 – 72 h): 0.014, 0.053,
	0.20, 0.67, 1.8 and 2.0 mg a.s./L
	Corresponding time-weighted mean measured concentrations (0 – 96 h): 0.014 0.050, 0.19, 0.63, 1.7, 2.0 mg a.s./L
	0.050, 0.19, 0.63, 1.7, 2.0 mg a.s./L
	Controls: water and solvent controls (dimethylformamide at 0 µL/mL)
	Evidence of undissolved material: All test solutions appeared clear and colorless
	Small particulates were visible on the bottom of the flask in the 8.0 mg & ./L
	Small particulates were visible on the bottom of the flask in the 8.0 mg s./L treatment group.
Replication	No. of vessels per concentration (replicates): 4
	No. of vessels per control (replicates): 4
	No. of vessels per solvent control (replicates): 4
Exposure	Static Static
Exposure	Total exposure duration: 96 hours
T:4:-111	Total exposure duration: 96 hours 10 ⁴ cells/mL at test initiation Townserture: 24.0 24.29 C
Initial cell	10 ⁴ cells/mL at test initiation
density	10 ⁴ cells/mL at test initiation
	Temperature: 24.0 – 24.3°C Photoperiod: Continuous light Light intensity at surface of test vessels 3870 to 4600 flux pH of controls (60/2 h): 7.5 – 9.9 Growth medium same as culture medium: wes
Test	Photoperiod: Contingous light & A & & & & & & & & & & & & & & & & &
conditions	Light intensity at surface of test wessels 3870 to 4600 flux
	pH of controls (@22 h); 7.5 – 9.9
	Growth medium same as culture medium; wes
	Type of light artificial (Cool white fluorescent Camps)
	Temperature of a container of water adjacent to the jest chambers in the
Parameters	environmental chamber was measured continuously. Light intensity was measured
Measured /	at test initiation at test solution level at nife locations surrounding the test flasks.
Observations	The OH of the medium in each treatment and control group was measured at test
	initiation at approximately 72 hours and at sposure termination (96 hours).
	Cell counts were performed at approximately 24 hour intervals using a
	hemacytometer and a microscope. At the end of the exposure period algae were
8	examined microscopically for any pical cell morphology (e.g., changes in cell shape,
Ò	size or color). Cells in the redicate test chambers also were assessed for
	aggregation of flocculation of cells, and wherence of the cells to the test chamber.
	Samples of the test solutions were collected at approximately 0, 72 and 96 hours to
Sampling for	measure concentrations of the test substance. At test initiation samples were
chemical	collected from each fest concentration and control solution prior to distribution into
analysis	the test chambers. Of 72 hours, somples were collected from the single sacrificial
	replicate for each test concentration and control groups. At test termination the
4	biological replicates from each respective test concentration and control solutions
	were pooled and the sampled.
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	
Data analysis	The calculation of area under the growth curve, growth rates, yield and percent
**	inhibition values, as well as all statistical analyses, were conducted using 'The SAS
e C	System for Window. The results of the statistical analyses, as well as the
Ó	evaluation of the concentration-response pattern, were used to determine the NOEC
	for each parameter at 3 and 96 hours.
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	The calculation of area under the growth curve, growth rates, yield and percent inhibition values, as well as all statistical analyses, were conducted using 'The SAS System for Window.' The results of the statistical analyses, as well as the evaluation of the concentration-response pattern, were used to determine the NOEC for each parameter at 74 and 96 hours.
. J. J	
Ď	
\bigcirc	



Results:

Validity criteria	acc. to OECD TG 201	Required	Obtained	a
	he control cultures should have entially by a factor of at least 16 within period.	16	359	
	cient of variation for section-by-section rates in the control cultures must not	< 35%	31.5%	
	of variation of average specific growth 72 h test period in replicate control texceed 10%.	© 10%	0.28%	
المصلحة المصادمة	~·	. *		
esults after 72 concentrations of	were not within the range of 80 – 120 hours and 96 hours are based on of BCS-CN88460, respectively. Hent control samples above 0.0024 mg ring this study.	geometric and residues of Bu a.s./L which	time weighted CS CN88460 v was used as th	d mean measu vere found in ne lowest stand
Some recoveries esults after 72 oncentrations control and solvoncentration du	hours and 96 hours are based on of BCS-CN88460, respectively. So the ent control samples above 0.0024 magning this study. Geometric mean measured concentrations after 72 hours (mg as L) (mg	geometric and residues of Bo a.s./L which ghted mean oncentrations 6 hours a.s./L)	time weighted CS CN88460 v was used as the	d mean measu vere found in ne lowest stand
Some recoveries esults after 72 concentrations control and solve concentration du	hours and 96 hours are based one of BCS-CN88460, respectively. So nent control samples above 0.0024 magning this study. Geometric mean measured concentrations after 72 hours (mg as L) (mg	geometric and residues of Bo rays./L which ghted mean oncentrations of hows	time weighted CS CN88460 v was used as the	oncentrations 96-hour

Nominal Concentration (mg a.s./L)	Geometric mean measured concentrations after 72 hours (mg as L)	i i i i i i i i i i i i i i i i i i i	% of Hom	inat conce	ntrations 96-hour
0.024	© 014 O	0,014	65.07	45 3.3	42.7
0.076	0.053	Ø.050 ° (65.3	47.8
0.24	0.20		© 94.3 L	76.6	60.3
0.78	\$ 69.67	~ ,	97@	75.9	57.4
2.50	1.80	1.70	₹ 8 9.8	55.8	47.8
8.00	2,90		© 37.2	17.4	23.2

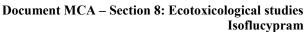
No morphological change in algae was observed in any test conventration. No adherence of cells to the test chambers or floculation or aggregation of cells was observed.

72 hours

Geometric mean measured concentrations after 72 hours (mg a.s.)	Mean cell number (cells/ml 104)*	Inhibition of average specific growth rate (%)
Solvent Control	×359.0	-
0.014	\$\sqrt{362.0}	0
0.053	© 4 344.5	1
0.20	\$ 334.8	2
0.67	333.3	1
1.80	280.8#	4
2.00	221.0#	8

^{*}Mean well number after 32h not presented in study report but calculated on the basis of single replicate values.

[#] Treatment group was significantly reduced (Jonckheere-Terpstra Step Down Trend Test, p < 0.05) when compared to the solvent control mean





Geometric mean measured concentrations (mg a.s./L)	Yield (cells/mL)	Inhibition of yield (%)	
Solvent Control	3580000	-	
0.014	3610000	1 ő	
0.053	3435000	Q4 Q'	
0.20	3337500	© 7	
0.67	3322500	7 0 0	
1.80	2797500# 💍	22 🗸	
2.00	2200000#	2 39 P	

Geometric mean measured concentrations (mg a.s./L)	Yield (cells/mL)	Inhibition of yield (%)
Solvent Control	3580000	-
0.014	3610000	-1
0.053	3435000	4 6 6
0.20	3337500	7
0.67	3322500	7 0 0
1.80	2797500# 🏷	22 27 27 27
2.00	2200000# 💖	390 9 4
% Inhibition: Increase of yield relative t Treatment group was significantly reduced olvent control mean	o the pooled control ced (Jonckheere-Terps Step Down Tro	and Test, p < 0.05 when compared to the
% Inhibition: Increase of yield relative to Treatment group was significantly reduced by the control mean Geometric mean measured concentrations	o the pooled control ced (Jonckheere-Terpsta Step Down Treed (Jonc	The Test, p < 0.05 when compared to the Charles to
% Inhibition: Increase of yield relative to Treatment group was significantly reduced very control mean Geometric mean measured concentrations (mg a.s./L) Solvent Control	o the pooled control and the pooled (Jonckheere-Terpsta Step Down Tro	Test, p < 0.05 when compared to the line of Biomass integral (2)
Solvent Control		The Test, p < 0.05 when compared to the whole when compared
0.014	62310000 5 58278000#	In Test, p < 0.05 when compared to the Compare
% Inhibition: Increase of yield relative to Treatment group was significantly reduced olvent control mean Geometric mean measured concentrations (mg a.s./L) Solvent Control 0.014 0.053 0.20	62310000 5 58278000#	Integral (2)
0.014 0.053	689220000 623100000 58278000#	Lifebition of Biomass integral (2)
0.014 0.053 0.20	62310000 5 58278000# 58352000	Individual of Biomass integral (2)

[#] Treatment group mean was significantly reduced (Dignett's Test, p < 005) when compared to the solvent control mean 96 hours

96 hours

96 nours & &		<i>On</i>
Time weighted mean measured	Mean celf number	Individual
concentrations after hours	$\mathfrak{S}^{\text{ells/mi}} \times 10^4$	specific growth rate (%)
concentrations after oniours (mg a.s./L)		. W
Solvent Comprol	\$ \$\!\^\441 <i>50</i> "	•
0.014	374075	3
	302.0 [#] /	6
0.19	82 6 ° 3 8 3 126°	2
0.63		2
0.050 V V V V V V V V V V V V V V V V V V	393.0	4
2.00	Q, 2349.0°	4
*Mean cell number after 96 la not presented in str	idy report but colculated on the ba	sis of single replicate values.
# Treatment group was significantly reduced (Du	nner Test 0.05) when comp	ared to the solvent control mean
1.70 2.00 *Mean cell number after 96 a not presented in our presented in o		
	* 0	
	Q,	
	V	
	7	



Time weighted mean measured concentrations after 96 hours (mg a.s./L)	Yield (cells/mL)	Inhibition of yield (%)	OU
Solvent Control	4405000	&	
0.014	3695000	1 6	
0.050	3010000#	32	
0.19	3820000	13	
0.63	3845000	13 💆	
1.70	3520000	200 3	
2.00	348000)

2.00	3400000	jy "O	
# Treatment group mean was significantly i	reduced (Dunnett's $p < 0.05$) whe	r compared to the solvent	control mean
Time weighted mean measured	Area under the growth corve	Inhibition & Bior	mass V
concentrations after 96 hours	(bi@mass_i@tegral)	integral (%)	. 4
(mg a.s./L)	<u>. 4 </u>		
Solvent Control	1647420007	A 0 -4,	
0.014	Ø 34Å9979 ® 00 √		
0.050	\$\times \tag{13564\text{\$\tilde{6}\$\tilo		* &
0.19	154242000	\$\text{6.5}	
0.63	£ 2370000 P		
1.70	124476000# 🗸 🔊		/
2.00	√ 10 63 640000 ″∀	370	

[#] Treatment group mean was significantly reduced (Dungert's Test, p < 0.05) when compared to the solvent control mean

The study meets the validity criteria and the 72 hours endpoints based on geometric mean and the 96 hours endpoints based on time weighted mean concentrations are:

E _r C ₅₀ 72 hours (95% C.I.):	> 2.4 mg a.s./L (n.d.)
E _r C ₂₀ 72 hours (95% Cas)	2.0 mg a.s./LQn.d.)
E _r C ₁₀ 72 hours (95% C.I.)	> 20 mg & s./L (n.d.)
LOE _r C 72 hours: O lowest concentration with an ignificant effect	, Ö
lowest concentration with an ignificant effect	S mg a.s./L
compared to the control	
NOE _r C hours: Q Q	
highest concentration without an significant	0.67 mg a.s./L
effect compared to the control	
EbC50 72 hours @5% C.I.):	> 2.0 mg a.s./L
	(n.d.)
LOE _b C 72 hours:	
lowest corcentration with an significant effect	1.8 mg a.s./L
compared to the Control	
NOEC 72 hours:	
highest concentration without an significant	0.67 mg a.s./L
effect compared to the control	
	> 2.0 mg a.s./L
E _y C ₅₀ 72 hours (95% C.I.):	(n.d.)



		•
E _y C ₂₀ 72 hours (95% C.I.):	1.8 mg a.s./L (1.7 to 2.0 mg a.s./L)	
E _y C ₁₀ 72 hours (95% C.I.):	1.7 mg a.s./L (1.4 to 1.9 mg a.s./L)	
LOE _y C 72 hours: lowest concentration with an significant effect compared to the control	1.8 mg a.s./L	
NOE _y C 72 hours: highest concentration without an significant effect compared to the control	0.67 mg a.s./L	
E _r C ₅₀ 96 hours (95% C.I.):	> 20 mg a.s./L (n.d.)	
E _r C ₂₀ 96 hours (95% C.I.)	2.0 mg a.s./(n.d.)	
E _r C ₁₀ 96 hours (95% C.I.)	© > L 0 mg/d.s./L (p/d .)	
LOE _r C 96 hours: lowest concentration with an significant effect compared to the control	2.0 mg a.s./L	
NOE _r C 96 hours: highest concentration without an significant effect compared to the control	3 0 mg 3 7/L	
E _b C ₅₀ 96 hours (95% C.I.):	> 2.0 pg a.s./L(n.d.)	
LOE _b C 96 hours:	1.7 mg a.s./s	
NOE _b C 96 hours: highest concentration without an significant effect compare to the control	0.63 mg a.s./\$\tilde{\psi}	
E _y C ₅₀ 96 hoors (95% C.L)	> 2.0 mg a.s./L (n.d.)	
E_yC_{20} hours (95% $($. $)$:	0.82 mg a s 00.014 to > 2.9 mg a.s./L)	
E _y C ₁₀ 96 hours (95% C.L.)	< 0.014 mor.s./L (n.d.)	
E _y C ₁₀ 96 hours (95% C.I.) LOE _y C 72 hours: lowest concentration with an significant effect compared to the control NOE _y C 72 hours: highest concentration without an significant of the compared to the control	0.82 mg a, s D 0.014 to > 2.9 mg a.s./L) < 0.014 mg a.s./L (n.d.)	
NOE _y C 2 hours: highest concentration without an significant effect compared to the composition	2.0 mg a.s./L	
NOE _y C 2 hours: highest concentration without an significant effect compared to the compol		



CA 8.2.7 Effects on aquatic macrophytes

Material and methods

CA 8.2.7	Effects on aquatic macrophytes
Report: Title:	KCA 8.2.7/01; 2017; M-593965-01-1 Lemna gibba G3 - Growth inhibition test with BCS-CN88460 under semi-static conditions
Report No.: Document No.: Guideline(s):	EBLNN016 M-593965-01-1 EU Directive 91/414/EEC Regulation (EC) Number 1107/2009 US EPA OCSPP 850 4400
Guideline deviation GLP/GEP:	on(s): none yes
Material and n	nethods
Test material	KCA 8.2.7/01; 2017; M-593965-01-1 Lemna gibba G3 - Growth inhibition test with BCS-CN88460 under semi-static conditions EBLNN016 M-593965-01-1 EU Directive 91/414/EEC Regulation (EC) Number 1107/2009 US EPA OCSPP 850.4400 none yes BCS-CN88460 (tech.) Batch ID: 2013-006492 TOX-No.: TOX 20011-01 Specification No. 102000028196 Purity: 94.2 % w/w not specified
Guideline(s) adaptation	not specified by the sp
Test species	Duckweed (Lemna gibba) Strain G3
Acclimation	To ensure that the plants used as inoculum are exponentially growing, an inoculum pre-culture is prepared 7-10 days before the start of the test and cultivated under the same conditions as up the main test.
Culturing	Stock cultures are maintained in glass chishes folled with nutrient medium under illumination of 6500 4000 fox and demograture of 23 – 26°C. Transfers into fresh nutriefit medium are made regularly every 7-10 days.
Test solutions	Nominal concentrations 0.0238 0.0763, 0.244, 0.781, 2.50 and 8.0 mg a.s./L Corresponding peometric mean measured concentrations: 0.0185, 0.0689, 0.249, 0.677 2.09 and 3.02 mg a.s./L Concol: water
A CONTRACTOR OF THE PROPERTY O	Solvent Control DMF (Timethy) formamide) used as solvent (0.1 mL/L test solution) Evidence of undissolved material: If the two highest test concentrations precipitates were observed.
Replication	No of vessels per concentration (replicates): 4 No. of vessels per control (replicates): 4 No. of vessels per solvent control (replicates): 4
Organisms per replicate	No of fronds pervessel: 12
Exposure	Semi-static Total exponre duration: 7 days



Test	Incubation chamber used: not specified
conditions	Vessels: 470 mL glass dishes with 200 mL test solution
	Temperature: 23.6 - 24.3°C
	Photoperiod: permanent light
	Light quality: bank light containing fluorescent lamps
	Light intensity: 6630-6880 lux
	Photoperiod: permanent light Light quality: bank light containing fluorescent lamps Light intensity: 6630-6880 lux pH: 7.8 - 9.0
	Growth medium: 20X AAP
Parameters	Visual observations were made on study days 3, 5 and 7 Counting of fronds and
Measured /	determination of total frond area was done on day 0, 300 and 1.
Observations	Temperature was determined by a continuous measurement in one additional
	incubated glass vessel. pH was measured in all freshly prepared and all aged test
	levels and controls. Light was measured once during the test.
Sampling for	Duplicate samples of freshly prepared media were taken from all test levels and the
chemical	controls on day 0, 3 and 5 and additionally in all aged test levels on day 1, 2, 3, 4, 5,
analysis	6 and 7 of the exposure period.
	Samples were analysed for the actual concentration of BCS CN88460 (tech). The
	water samples were analyzed with HPLC-MS/MS.
Data analysis	ECx values and confidence intervals were calculated with Probit analysis using
	linear maximum likelihood regression. Effect thresholds (e.g. NOECS) were
	determined using Williams multiple sequential t-test procedure.

Results

Validity criteria	, S	~~~		@Required &	V Obtained 4
Doubling time		Å,		2.5 days	2 1.8 days 0
			P //	~ % · ~	

Measured concentrations between day of and day 7 ranged between 14 and 115% and are presented below. Therefore results are passed on geometric mean measured concentrations. No residues of BCS-CN88460 (tech.) were measured in the control and solvent control samples above the lowest standard solution used for determination (\$000626 mg ass./L).

Nommai	©eometric mean	\ \ \ \ \ \ \ \		Q' %	% of \$	ominal	concent	rations			
concentration (mg	concent trations (mg a.s./L)	4	Day 1 Orged	¥ 0	Day 3 Aged	Day 3 New	Day 4 Aged	Day 5 Aged	Day 5 New	Day 6 Aged	Day 7 Aged
0.0238	Ø. Ø185 . A	886	907	103	88	53	61	52	90	87	90
0.0763	0.0689	8 6	91	@ 93	107	77	83	74	103	106	109
0.244	0,249	گ 109 م	r 115	106	84	86	106	81	98	100	84
0.781	≈0°677 .∜	767	108	91	80	72	79	67	95	97	115
250	2.095	3 86	84	79	73	89	82	77	93	68	71
8.00	3.02	58	34	17	14	26	36	16	52	30	43



Biological results:

Observations

No sublethal effects on plants were observed.

				(())*	0/10 4		
Nominal	Geometric mean			Inhibition B &			
concentration (mg a.s./L)	measured test concentration (mg a.s./L)	number on day 7*	Total frond area on day 7* [mm3]	Mean growth rate for frond Humber	Mean growth Tate for frond area		
Control	Control	167	√ ¥425	\$ - \$	Q - 5 ⁷ \$		
Solvent control	Solvent control	170	1434	9 9°- 9	~ - ~		
0.0238	0.0185	183	1563	-3.1			
0.0763	0.0689	184	\$1566\C	\$ 3.3 \$	-0.9		
0.244	0.249	1724	6 14 0 Q	-0.5	O 0.5 O		
0.781	0.677		1465	\$\frac{1}{2} - \P' \times	-1.9		
2.50	2.09	QI54 🛒	12947	J 3.6 J	2.8		
8.00	3.02	√ 1510°	1340	S 1.75	\$.5		

^{*} Mean of 4 replicates

Conclusion

Endpoints were calculated based on geometric mean measured concentrations

Endpoint (0-7 days)	Effect on mean growth tate of frond	Effect mean growth rate of total frond area of plants
E _r C ₅₀ (95% C.I.):	>3,02 mga.s./L	> 3.02 mg a.s./L
E _r C ₁₀ (95% CD):	> 3.00 mg a 5 L	> 3.02 mg a.s./L
E _r C ₂₀ (95° C.I.):	3.02 mg a.s./L	> 3.02 mg a.s./L
LOE _r C: lowest concentration with an significant effect compared to the control	> 3002 mg a.s./L	> 3.02 mg a.s./L
NOE _r C: highest concentration without an significant effect compared to the control	≥ 3.02 mg a.s./L	\geq 3.02 mg a.s./L
CA 8.2.8 Further testing on aqua		
No additional studies were performed.		



CA 8.3 Effect on arthropods

CA 8.3.1 Effects on bees

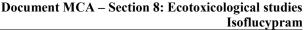
The following studies describing the toxicity to bees have been performed with echnical isoft pypram or the formulation Isoflucypram SC 200 according to current guidelines, guidance documents or the current understanding of the state-of-the-art of testing.

- Acute oral and contact toxicity to honeybees under laboratory conditions,
- Acute oral and contact toxicity to bumble bees under laboratory conditions,
- Chronic 10 day toxicity to adult honeybees under Arboratory conditions,

Chronic 10 day toxicity to additions
 Chronic 22 day toxicity to honeybee larvae under laboratory conditions
 The studies are summarized below and a full list of the relevant ecotoxicological endpoints for isoflucypram is presented in the following table.

Table 8.3.1-1: Toxicity of isoflucypran (technical and formulated product) to been

Test	Test species/	Endpoint S References	
substance	study type		
	Honeybee 48 h	LDS - contact > 100 ustas /bee	
	Honeybee 48 h	KCA 8.3.1.1.2/01 KCA 8.3.1.1.2/01 KCA 8.3.1.1.2/01 KCA 8.3.1.1.2/01 KCA 8.3.1.1.2/02 KCA 8.3.1.1.1/02 KCA 8.3.1.1.1/02	
Isoflucypram tech.	Hones bee lasva,	$NOFO \ge 406 \text{ mg a.s. Kg}$ $NOFO \ge 62.5 \text{ µg a.s. Plarva}$ $M-587515-01-1$ $KCA 8.3.1.3/01$,
EG .	Bumble bee	KCA 8.3.1.3/01 ; 2015; M-542774-01-1 KCA 8.3.1.1.1/03	
	Bumble bee	; 2015; M-509048-01-1 KCA 8.3.1.1.2/03	
Isoflucypyam SC 200	10 day chronic adult feeding	> 89.7 μg a.s./bee/day NOCDD > 89.7 μg a.s./bee/day ≥ 89.7 μg a.s./bee/day KCA 8.3.1.2/01	5;
		KCA 8.3.1.1.1/03	





CA 8.3.1.1 Acute toxicity to bees

CA 8.3.1.1.1 Acute oral toxicity

Report: KCA 8.3.1.1.1/01; ; 2014; M-503824-01-1

Effects of BCS-CN88460 tech. (acute contact and oral) on honey bees (Apis melliform L.) in the laboratory 89641035
M-503824-01-1
OECD 213 and 214 (1998) none yes Title:

Report No.: Document No.:

Guideline(s):

Guideline deviation(s): **GLP/GEP:**

Objective:

The purpose of this study was to determine the acute contact and oral foxicity of BCS technical to the honeybee (A. mellifera L.) In the liboratory. Mortality of the bees was used as the toxic endpoint. Sublethal effects, such as changes in behavior, were also assessed.

Material and methods:

Test item: BCS-CN88460 technical 94.2% w/w/origin batch no.: 2013-006492 Material: BCS-CN88460, technical, Specification No.: 102000028196. Article No.: 80897197 , Certificate of Analysis No: MZ 00809.

Test organism: female worker honeybees (Apis medifera), obtained from a healthy and queen-right colony, bred by IBACON.

Under laboratory conditions Apis mellifera worker bees were exposed for A hours to a single dose of 100.0 μg a.s. per bee, by topical application of 5 μt, in a contact limit test and to a single dose of 106.3 μg a.s. per bee by feeding in arroral limit text (value based on the actual intake of the test item). Furthermore, each test consisted on control, solvent control and a reference trem group. In the contact limit test, tap water containing 0.5 % Adhaesit and our acetone were sed as control and solvent control, respectively. In the oral limit test a 50 % w/v sucross solution containing solvent (5 % acetone) and 50% was sucrose solution were used as solvent control and control, respectively. In both limit tests, Perfekthlon EC (active ingredient 400.9 g/L dimethoate, Batch no.: FRE-000926) was used as reference item. Each treatment group consisted out of replicates (test units) with 10 bees per replicate Test units were stainless steel cages with 0 cm 8.5 cm (length × height × width). The tests were conducted in darkness, temperature was 24 - 25 C and humidity was between 50 and 72 %. Biological observations, including mortality and behavioral changes were recorded 4, 24 and 48 h after application.

The software seed to perform the statistica Quanalysis was PoxRat Professional.

Findings:

Biological findings:

Test item	BCS-	CN88460 tech.
Test object	$\nabla V \sim \nabla V \sim Ap$	is mellifera
Exposure O	Contact	Oral
Exposure	(solution (a acetone)	(sugar/ acetone/water
		solution)
Dose [ug a.s./bee]	100.0	106.3
LD5@Jug a.sabee]	> 100.0	> 106.3
Lizz [µg a a./bee]	> 100.0	> 106.3
LD ₁₀ [µga.s./bee]	> 100.0	> 106.3
NOED [µg a.s./bee]*	≥100.0	≥ 106.3

^{*} The NOED was estimated using Fisher's Exact Test (pairwise comparison, one-sided greater, $\alpha = 0.05$).



Observations

Contact test

At the end of the contact toxicity test (48 hours after application), there was no mortality at 100 fug a.s./bee. Also no mortality occurred in the water control group (water + 0.5 % Adhaesit) and in the solvent control group (acetone). No behavioral abnormalities could be observed during the entire test

	After	r 4 hours	After	24 hours	After	· 48 hours
Dosage [μg a.s./bee]	Mortality	Behavioral abnormalities		Behavioral abnormalities	Mortality	OBehavjoral abnormalities
	mean %	mean %	mean %	mean %	mean %	⊸@ean ‰
Test item 100.0	0.0	0.0	0.0	0.0	08	
Water	0.0	0.0	10 0	0.0	Ø.0 Å	0.0
Solvent	0.0	0.0	₹ 0.0	0.0	0.0	& 0.0 C
Reference item			%			* W
0.30	4.0	22.0	D 98,6		98.0	0 0
0.20	8.0	8.0		Q.0 , U	22.0 O	© 0.0 👋
0.15	0.0	0.0	14.0		© [♥] 20.0€	0.0
0.10	0.0	0.0	≫″ 4.0©″	0.0	× 6.40×	₩ 0.48°

Results are averages from five replicates (ter Quees each) per do age Leontrol

Water = CO₂/water-treated control, solvent CO₂/worker control

Water = CO₂/water-treated control, solvent CO₂/solvent control V

Oral test

In the oral toxicity test, the maximum nominal test level of BCSCN88460 tech. (i.e. 000 μg a.s./bee)

corresponded to an actual intelligible of 100 V

This is a simple of 100 V

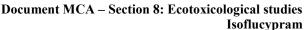
This corresponded to an actual intake of 1063 µg a.s./beo: This dose level led to no mortality after 48 hours. Also no mortality occurred in the water control group (50% w/v sucross solution) and in the solvent control group at the end of test (after 18 hours), respectively. No test item induced behavioral effects were observed at any time in the oral toxicity test.

	🔊 🕻 🐧 fte	r 🏞 🏗 ours 🗸 🔝 💍	y After	24 hours	After	48 hours
Dosage [µg a.s./bee]	Mocrality		Mortality	Bebaviorak abrormalitjes	Mortality	Behavioral abnormalities
<i></i>	Mean %	⊘mean ¾	🦖 mean 🗱	mean%	mean %	mean %
Test item	000		O	© 00	0.0	0.0
106.3	. 0) . 0				0.0	0.0
Water	0.0	∠ 0.0 √	√° 0.0 € ,	$\mathcal{L}^{\geqslant}0.0$	0.0	0.0
Solvent	Ø 0.0 ¥	0.9°Y	\sim 0.00°	0.0	0.0	0.0
Reference item				ZC		
0.32	8 2%0 6	\$\text{QZ.0} \\ \nabla \text{Z}.0 \\ \nabla \text{Z}.0	(, O)O.O	0.0	100.0	0.0
0.16	04.0	38.0	88.0	12.0	94.0	0.0
0.08	0.0	\$\int 0.9\$\int \cdot \tag{\text{\tin}\text{\tint{\tex{\tex	\$ 4,00°	10.0	6.0	0.0
0.05	000	T 090 Z	~Q,0	0.0	0.0	0.0

Results are averages from five replicates (ten bees each) previousage / control

Water water control solvent solven control

Validity criteria.
All validity criteria of the test were inet.





Validity criteria according to OECD 213 and 214	Obtained in this study
Control mortality should not exceed 10 % at test end	Contact test
	Control: 0 %
	Solvent control: 0 %
	Or test
	Control: 0 %
	Solvent control: 0 %
LD ₅₀ of the reference item should be in the specified range (contact	Contact test O' O'
test: 0.10 – 0.30 μg a.s./bee, oral test: 0.10 – 0.35 μg a.s./bee)	£ 0.24 μg a.s. bee
	Oral test
<u>√</u>	0.12 μ@a.s./bee 🗸

Conclusion:

μg a.s./bee in the The LD_{50/20/10} (48 h) were $> 100 \mu g$ a.s./bee in the contact oral toxicity test.

The NOED (48 h) was $\geq 100 \mu g$ a.s./bee toxicity test.

Report:

Effects of BCS CN88460 tech vacute contact and oral) on honey bees Apis mellifera L.) in the laboratory Title:

Report No.: 83991035 Document No.: M-472468-01-1 √ OECD 273 and 214 Guideline(s):

Guideline deviation(s): onot applicable

GLP/GEP:

Objective:

The purpose of this study was to determine the acute contact and oral toxicity of BCS-CN88460 technical to the honeybee (A. welliferal.) in the laboratory. Mortality of the bees was used as the toxic endpoint. Sublethal effects, such as changes in behaviour, were also assessed.

Material and methods:

w. Customer Order No.: TOX 09897-01, batch no.: Test item: BCS-CN88660 technical: NLL 8674-28-

Test organism: female worker honeybees (Appa mell fera), obtained from a healthy and queen-right colony, bred by IBACON.

Under laboratory conditions Apis mellifera worker bees were exposed for 48 hours to a single dose of 100.0 ug a.s. per bee, by topical application of 5pt, in a contact limit test and to a single dose of 109.5 μg a.s. per bee by feeding in an oral limit tost (value based on the actual intake of the test item). Furthermore, each test consisted of a control solvent control and a reference item group. In the contact limit test, tap water containing 0.5% Adhaesit and pure acetone were used as control and solvent control, respectively. In the oral limit test a sugar syrup/water/acetone solution and an untreated sugar syrup/water solution were used as solvent control and control, respectively. In both limit tests, Perfektion Es (active ingredient 411.7 g/L dimethoate, Batch no.: 0001017331) was used as reference item. Each treatment group consisted out of 5 replicates (test units) with 10 bees per replicate. Test units were stainless steel cages with $10 \text{ cm} \times 8.5 \text{ cm} \times 5.5 \text{ cm}$ (length × height × width). The tests were conducted in darkness, temperature was 24 - 25°C and humidity was between 50 and 72%. Biological observations, including mortality and behavioral changes were recorded 4, 24 and 48 h after application.

The software used to perform the statistical analysis was ToxRat Professional.



Findings:

Biological findings:

Findings:		
Biological findings:		Q° %
Diological Infamgs.		CS-CN88460 tech. Apis mellifera
Test item	ВС	CS-CN88460 tech.
Test object		CS-CN88460 tech. Apis mellifera Oral (sugar syrup/acetone/water solution) 100/5
Exposure	Contact	
	(solution in acetone)	(sugar syrup/acetone/water solution) 109.5 109.5 109.5 209.5 209.5 209.5 209.5 209.5 209.5 209.5 209.5 209.5 209.5
		solution)
Dose [µg a.s./bee]	100.0	10 9 /5
LD ₅₀ [μg a.s./bee]	> 100.0	
LD ₂₀ [μg a.s./bee]	> 100.0	2 109.5 ° 4 ° 7
LD ₁₀ [μg a.s./bee]	> 100.0	> 1009.5
NOED [µg a.s./bee]*	> 100 0 \Q	<u> </u>
* The NOED was estimated using Fig.	sher's Exact Test (pairwise o	comparison one-sided greater, $\alpha = 0.05$).
	0, 1	
	A A	
Observations		comparison one-sided greater, α = (05).

^{*} The NOED was estimated using Fisher's Exact Test (pairwise comparison one-sided greater, $\alpha = 0.05$).

Observations

Contact test
At the end of the contact toxicity test (48 hours after application) there was do mortality at 100.0 μg a.s./bee. Also no mortality/occurred in the water control group (water + 0.5% Adhaesit) and in the solvent control group (acetone). Four hours after application 44.0% of the bees showed intensive cleaning at a dose of

100.0 μg a.s./bee. No further test item related behavioral effects occurred anymore.

	Arfte	r 4 hours 🔍 🙎	After	After 24 hours 24 After 48 hours				
Dosage [μg a.s./bee]	Mortality Thean %	Behavioral O abnormalities Onean %	Mortality mean	Behavioral abnormalities Onean %	Mortality mean %	Behavioral abnormalities mean %		
Test item 100.0	, 5'p	44.0			0.0	0.0		
Water	0.0	0.0	0.0	0.0 0	0.0	0.0		
Solvent 🐧	0.0	√ 0.0 4	0.0	0.0	0.0	0.0		
Reference item		54.0						
0.30	6 .0 6 .0	54.0	• 92 .0	₽° ~ \$ 6.0	96.0	0.0		
0.20	0.0	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	√×78.0 €√	△ ″8.0	88.0	6.0		
0.15	0.0 %		© 50.0 °	≥ 20.0	68.0	6.0		
0.10	2 25 0		1,0,0	0.0	16.0	0.0		

Results are averages from Tive regulates (ten bees each) per dosage Control

Water = CO2/water-treated control, solvent = CO2/solvent control

In the oral toxicity text, the maximum nominal text level of BCS-CN88460 tech. (i.e. 100 μg a.s./bee) corresponded to an actual intake of 109.5 vg a.s./bee. This dose level led to no mortality after 48 hours. Also no mortality occurred in the water control group (50 % aqueous sugar syrup solution) and in the solvent control group at the end of test (after 48 hours).

over the state of No test item induced behavioral effects were observed at any time in the oral toxicity test.



	After 4 hours		After 4 hours After 24 hours			After 48 hours		
Dosage [µg a.s./bee]	Mortality mean %	Behavioral abnormalities mean %	Mortality mean %	Behavioral abnormalities mean %	Mortality mean %	Behavioral o abnormalities mean %		
Test item 109.5	0.0	0.0	0.0	0.0		Ø.0 (5)		
Water	0.0	0.0	0.0	0.0	₄ 0.0	© 0.0		
Solvent	0.0	0.0	0.0	0.0	0.0	, O 0.00 «		
Reference item								
0.32	14.0	76.0	94.0 🎝	2.0	96.0%	4.0		
0.16	0.0	40.0	94.0 L 52.00	6.0	96.0 60.0			
0.08	0.0	14.0	1800	4.0	23 .0	0.0		
0.06	0.0	0.0	Q 0.0	0.0	2.0	\$\int_{\sqrt{9}0.0}\$\int_{\sqrt{9}}		

Validity criteria:

	Results are averages from five replicates (ten bees each per dos appel controls,
	Results are averages from five replicates (ten bees each per dosage/control). Water = water control; solvent = solvent control Validity criteria: All validity criteria of the test were metal.
	All validity criteria of the test were met
ĺ	Validity criteria according to OECW 213 and 214 Obtained in this study Control mortality should not exceed 40 % at test end Control morta
	Control mortality should not exceed 60 % as test end
	Control: 0 %
	Solvent control: 0 %
	Solvent control: 0 % Oralliest Control: 0 % Control: 0 %
	Control: 0 %
	SOLVENI CONTROLL U 768
	LD ₅₀ of the reference tem should be in the specified Contact lest range (contact test: 9.10 – 9.30 µg a.s./bec ral test: 9.16 µg a.s./bec
	range (contact test: 0.10 – 0.30 μg a.s./bee oral test: 0.16 μg a.s./bee
	0.10 – 0.35 μg a.g bee) $\sqrt{}$ $\sqrt{}$ $\sqrt{}$ $\sqrt{}$
	O O O O O O O O O O O O O O O O O O O
	© 6 4 5 0 5 μg 4s./bee

Conclusion:

bee in the contact toxicity test and > 109.5 µg a.s./bee in the The $LD_{50/20/10}^{\circ}$ (48 h) oral toxicity test.

contact to city test and \geq 109.5 μg a.s./bee in the oral The NOED (48 lb) toxicity test.

; 2015; M-542774-01-1 Report:

Title:≼ CN88460 tech. Effects (acute oral) on bumble bees (Bombus terrestris 1.) in the

laboratory

Report No.: 97632105

Document No .:

No pecific guidelines available; study design based on OECD 213 (1998), Van der Guideline(s):

Soen (2001) and CPPR non-Apis group (2014)

The purpose of this study was to determine the acute oral toxicity of BCS-CN88460 tech. to the bumble bee (Bombus terrestris L.) in the laboratory. Mortality of the bumble bees was used as the toxic endpoint. Sublethal effects, such as changes in behaviour, were also assessed.



Material and methods:

Test item: BCS-CN88460: 94.2 % w/w (analytical), Origin Batch No.: 2013-006492, Customer Order No.: TOX 20011-00; Material: BCS-CN88460, technical; Specification No.: 102000028196, Article No.: 81782172.

Test organism: female worker bumble bees (B. terrestris), obtained from a healthy and queen-right colony, bred by a commercial bumble bee breeding company (Biobest Belgium N.V.).

Under laboratory conditions Bombus terrestris worker bumble bees were exposed for B how to 30 single dose of 200.2 µg a.s. per bumble bee by feeding in an oral limit test (value based on the actual intake of the test item). Furthermore, the test consisted of a control colvent control and a reference item group. In the oral limit test a 50 % w/v sucrose solution containing solvent (4 % accretion and 1 % C Tween80) and 50% w/v sucrose solution were used Solvent control and control respectively.

BAS 152 11 I EC (active ingredient 420.3 g/L Timethoate, Batch no: FRD-001276) was used as reference item. Each treatment group consisted out of 80 bumble bees with bumble bees per tes cunit (replicate). Bumble bees which did not consume at least 10 mg/bumble bee were excluded from the evaluation of the results so that 37 replicates in the test item group, 45 replicates in the reference item group, 69 replicates in the control group and 72 replicates in the solvent control group were used.

Test units were cylindrical, latticed plastic cages with a length of approximately 2cm and a districter of 2.2 cm at the large and 1.7 cm at the small opening.

The test was conducted in darkness temperature was 25 °C \pm °C and humility was 60 % \pm 10 %. Biological observations, including mortality and sub-lethal effects were recorded 4,24 and 48 h after application.

The software used to perform the statistical analysis was FoxRat for Version 2.40.

Findings:

Biological findings:

Test item S O S BCS CN88460 tech.	
Test object	
Exposure O O O O O O O O O O O O O O O O O O O	
S	ing
(50 % w/v sucrose solution contain maximum 4 % acetone and 1 %)
Tween80)	
Dose [µg a.s./bumble bee] 200.2	
based on recorded sinsumption (considering bumble 0°	
bees with food uptake of 10 me bumble bee)	
LD ₅₀ [μ g a.s./bamble bee] \sim > 200.2	
LD_{20} [µg a.s. Qumble Gee]* \bigcirc > 200.2	
LD ₂₀ [µg as./bumble bee] \sim 200.2 LD ₁₀ [µg as./bumble bee] \sim 200.2	
NOED [\mathscr{C} a.s./bumble \mathscr{C} e]** \mathscr{C} \mathscr{C} $\overset{\sim}{\sim}$ $\overset{\sim}$	

^{*} Since no mortality above 70 and 20% occurred in the test De respective LD_{10/20} values are assumed to be

Observations

Oral test: The maximum nominal test level corresponded to an actual intake of 200.2 ag a sobumble bee. This dose level led to no mortality after 48 hours. No mortality occurred in the control In the solvent control group 1.4 % mortality was found. No test item related behavioral abnormanties or sublethal effects occurred at any time during the test.

> 200. Lag a.s./bumble bee

 ²⁰⁰ sang a.s./pumplexoge
 ** Results obtained from test in treated group were compared to those obtained from the solvent control treated group. The NOED was estimated using Fisher's Exact Test (pairwise comparison, one-sided greater, $\alpha = 0.05$).



	After 4 hours		After 24 hours		After 48 hours	
Treatment Group	Mort- ality	Behavioral abnormalities	Mort- ality	Behavioral abnormalitie s	Mort- ality m@n	Behavioral abnormalities
	mean %	mean %	mean %	mean %		mean %
Test item 200.2 µg a.s./bumble bee	0.0	0.0	0.0	0.0	0.0	\$ 0.0 \$ T
Control	0.0	0.0	0.0	0.0	0.0	W Q ~
Solvent Control	0.0	0.0	0.0	0.0	1.4	9 .0 4
Reference item 3.9 µg dimethoate/bumble bee	33.0	31.1	\$\frac{1}{2}\tau_7.8		97.8 Q	0.6

Mean = mean of all individuals per treatment group

Control = 50 % w/v sucrose solution; solvent control = 50 % w/v sucrose solution containing 4 % a citone 3

Tween80

Considering bumble bees with a food uptake of > 10 mg/bumble bees of treatment group Test Item (n - 77), Control (n = 69), Solvent control (n = 72), Reference Item (n = 48)

Validity criteria:

All validity criteria of the test were met

Validity criteria according	to QECD 247	Obtained in this study
Control mortality should not	t exceed 🐿 % at 🐯 🔏	Control: 0 % * ** **
end		Solvent control: 1,4% &
Mortality of the reference it	em should be ≥ 50 % and	Reference item; 97.8 % considering bumble bees with
test end		Good uptake of 10 mg/bumble bee (in total 45
		bumble bees out of 80)

Conclusion:

The oral LD₅₀ value after 48 bours was $> 200.2 \mu g$ a.s./bomble bee. The oral NQED value was calculated to be $\geq 200.2 \mu g$ s./bumble bee.

CA 8.3.1.1.2 Acute contact toxicat

For acute contact poxicity on howeybees please refer also to Section CA 8.3.1.1.1.

Report: KK A 8.3 1.2/03 (2016), M-509048-01-1

Title: Effects of BCS (N8846) tech, facute contact) on bumblebees (Bombus terrestris L.)

n the Aboratory

Report No.: 90221105

Document No.: M\$09048-01-1

Guideline(s): No specific guidelines available; study design based on OECD 214 (1998) Van der

Steen 2001) and ICPOR non-apis group (2014)

Guideline deviation(s) not applicable

GLP/GEP: S yes S

Objective

The purpose of this study was to determine the acute contact toxicity of BCS-CN88460 tech. to the burnble bee (*Bombus terrestris* L.) in the laboratory. Mortality of the bumble bees was used as the toxic empoint. Sublethal effects, such as changes in behaviour, were also assessed.



Material and methods:

Test item: BCS-CN88460: 94.2 % w/w (analytical), Origin Batch No.: 2013-006492, Customer Order No.: TOX 10421-00; Material: BCS-CN88460, technical; Specification No.: 102000028196, Article No.: 80897197.

Test organism: female worker bumble bees (B. terrestris), obtained from a healthy and queen-right colony, bred by a commercial bumble bee breeding company (Biobest Belgium N.V.).

Under laboratory conditions Bombus terrestris worker bumble bees were exposed to 100 µg a.s. bumble bee by topical application (contact limit test).

Furthermore, the test consisted of a control, solvent control and a reference item group. In the contact limit test tap water with 0.5% Tween80 and aceton were uses a control and solvent control. respectively.

BAS 152 11 I EC (active ingredient 400.9 g/L diggethoate, Batch no.: FRE-000926) was used as reference item. Each treatment group consisted out of 50 bumble bees with 1 bumble bee per test what

Test units were cylindrical, latticed plastic cages with a length of approximately cm and a diameter

of 2.2 cm at the large and 1.7 cm at the small opening. The test was conducted in darkness, temperature was $2 \text{ C} \pm 2 \text{ C}$ and humidity was $60 \text{ M} \pm 10 \text{ M}$. Biological observations, including mortality and sub-lethal effects were recorded 4, 24 and 48 to after application. The NOED was estimated using Fisher Exact Test (pairwise comparison, one-sided greater, $\alpha = 0.05$).

Findings:

Biological findings:

Test item	**** 4	\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\		BCS-CN88460 tech.
Test object	4, 8	y & C		Bombus terrestris , 🖔
Exposure			5 4	J Contact J
	& & .			(solotion in acetone)
Dose [µg a.s./buml	Ĵre bee]O″ _ ″	A, M,		\$ \$\$0 \$
LD ₅₀ [μg a.s./burn	ole bee	¥ . @	Y JY	\$ 100 V
LD ₂₀ [µg a.s./bomb	ole@e]* , O	0 📞		> 106/
LD ₁₀ [μg a / bumb	ole bee]*	Q <u>1</u>		> 100
NOED [ug/a.s./bur	nble bee]**			3 100

is the second s * Since no mortality above (20) and 20% occurred in the test, the respective LD (20) values are assumed to be > 100 μg a.s./bumble bee

Observations @ .

Contact test:

At test termination (48 hours after treatment) no metality occurred at 100 μg BCS-CN88460 tech. a.s. per bumble bee. 6.0 % mortality occurred in the water control group (water + 0.5 % Tween80) and 2.0 % mortality occurred in the solver control group (acetone). No test item related behavioral abnormalities occurred at the solve we contrad abnormalities occurred at any time of the rest.

The NOED was estimated using Fisher's F



	After	After 4 hours		· 24 hours	After 48 hours	
Treatment Group	Mortality	Behavioral abnormalities	Mortality	Behavioral abnormalities	Mortality	abnormatines
	mean %	mean %	mean %	mean %	mean %	mean 🖔 🗸
Test item 100 μg a.s./bumble	0.0	0.0	0.0	0.0	5 0.0	\$0.0 \$
bee				4	10,	
Water control	0.0	0.0	4.0	0.0	6.0	0.00°
Solvent Control	0.0	0.0	2.0 💍	0.0	2.0 🔏	/ ~0.0 F
Reference item 12 μg dimethoate/bumble bee	6.0	58.0	76.0	140	865	Q 1400 0

Validity criteria:

bee		~0° '			. O' &	a y
Mean = mean of 50 indivi	iduals per treatment group					
Water control $=$ tap water	containing 0.5% Twen80					U "
Solvent control = acetone		0",@`			a. A	0
		1		O,	Õ P	, L
Validity criteria:	Ź		Y ~ Y			Ø.
All validity criteria of	the test were metal				<i>)</i>	
All validity Criteria of	the test were men	. L. T)~
				0		
Validity criteria accor	ding to OECD 246		Óbtained in	this study		
Control mortality shoul	d not exceed 10 % of te	estenôd 💍	Control: 6.0	% <u>0</u> C	7	
		~ \$	Solvent contr	rol: 2.9°%	&	
Mortality of the referen	ice item should be ≥ 50	🖔 at test	Reference ite	m@86 %	O´	

Conclusion:

The contact LD50 value after 48 hours was >900 µga.s./bumble bee. The contact NOED while was calculated to be \(\geq \) bo

CA 8.3.1.2

Report:

Chrome oral toxicity test of BCS-CN88460 SC 200 (200 G/L) on the honey bee (Apis Title:

mellifera Ly in the Daboratory

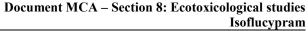
Report No .: Document No.:

and CEB No. 230 with current recommendations of the ring test Guideline(s):

Guideline deviation(s): GLP/GER

Ubjective:

The purpose of this study was to determine the chronic oral toxicity of BCS-CN88460 SC 200 (200 G/L) to the hone bee of meltifera by for a period of ten days.





Material and methods:

Test item: BCS-CN88460 SC 200 (200 G/L): BCS-CN88460: 19 % w/w, 202.3 g/L, Sample Description: TOX10589-00, Batch ID: 2014-005768, Specification No.: 102000027348, density 1.064 g/mL (20°C).

Test organisms were freshly emerged young female worker bees (2 days of at test start) obtained from one healthy and queen-right colony, bred by IBACON. After hatch, the bees were collected and thereafter acclimatized under test conditions for one day. Test units were stainless steel cages with $10 \text{ cm} \times 8.5 \text{ cm} \times 5.5 \text{ cm}$ (length × height × width).

Under laboratory conditions 30 freshly emerged worker bees (Apis mellifera L.) per treatment level, with 3 replicates per treatment, were exposed for 10 days to 5 concentrations (333, 1677, 833, 41%). and 208 mg a.s./kg food) of the test item treated sugar solutions and libitum. These concentrations led to actual mean dose levels of 89.7, 49.9, 28.6, 124 and 6.6 µs a.s./bee per day based on actual daily intake. An untreated control (50% w/v sucrose solution) and a reference item (Perfekthion with 400.9 g/L dimethoate) were included in this study. Bees were kept at 32,34°C, a relative humidity of 50-90% and in darkness. The number of dead bees or any behavioral abnormalities was assessed daily. Actual concentration of the test rem in the feeding solutions was analyzed by liquid chromatography (LOQ = 0.010 mg/kg, LOD = 0.003 mg/kg)

Findings:

<u>Analytical findings:</u>

Analytical findings:

The mean actual concentration of BCS-CN88460 in the feeding solutions was in a range of 88 – 100 % of the nominal concentrations. The measured concentrations of the test from in the feeding solutions were within ±20 % of normal. Therefore the concentrations were confirmed and the endpoints are based on nominal concentrations.

	_
Test item BCS-CN88460 SC 200	
Test object Apisonellifeou Apisonellifeou	
Exposure (50 % % v such see solution)	
Exposure (50 % vv sucrose solution)	
Tested doses [µg a.s. Dee/day] \$9,7,49.9,28.6, 124 and 6.6	
LDD ₅₀ [µg a.s./begatay]	
LDD ₂₀ [µg a.s./bee/day]	
LDD ₁₀ [µg and bee/dacy]*	
NOEDD [16 a.s./bee/day]	

^{*} Since no correlative above wand 20% occurred in the rest, the respective LDD_{10/20} values are assumed to be > 89.7 μg

Observations @

At test end 0 days following start of exposure, 0.0 % mortality occurred in the untreated water control (50% w/s sucrose solution). At 833 mg a.s./kg (corresponding to 28.6 µg a.s./bee/day) 10.0 % mortality occurred, which was not statistically significant (Fisher's Exact Test, $\alpha = 0.05$) and is considered to be not test item related.

In the test item treated groups at 3333, 1667, 417 and 208 mg a.s./kg sugar solution the mortality was 0.0%. No est item related behavioral abnormalities occurred at any time of the test. The reference item (dimethoate) at a concentration of 1 mg dimethoate/kg sugar solution corresponding to 0.02 µg a.s./bee per day caused 100 % mortality at day 5.

s Exact Test (pairwise comparison, one-sided greater, $\alpha = 0.05$). ** The NOEDD was estimated using Fisher



Concentration [mg a.s./kg	Dose level [µg a.s./bee/day]	Mortality at day 10 (% mean)				
sugar solution]						
3333	89.7	0.0	Q° 5			
1667	49.9	0.0				
833	28.6	10.0				
417	12.4	0.0				
208	6.6	0.0				
Water control	0.0	0.0 0.0 10.0 0.0 0.0 0.0 0.0 0.0				
Reference item	0.02	7100*				
Validity criteria: All validity criteria according to OECD 245 Volume 12.4 0.0 0.0 0.0 0.0 100* Validity criteria: All validity criteria were met in this study.						
Validity criteria according to OECD 245						
Reference item mortality > 50 % at the end of the test 100 % 7 0 0 0						
Conclusion:						
In the 10d adult dose-response howeybe laboratory test mortality was on a very low level in the						
control and in all five test tem treatment groups of 6.6, 12.4, 28.6, 49.9 and 89.7 kg a.s./bee/day. Only						
at the medium test level of 28 ong as bee day 10% mortality had been observed, originating from 3						
dead bees being found in 1 out of the 3 replicates. No mortality occurred at any other tested dose and						
also not in the courtol. Therefore LDD ₁₀ and LDD ₂₀ values could not be determined due to						

Validity criteria:

Validity criteria according to Ol	ECD 245		_@ _χ ο	btalned i	in T his stu	Ty &
Control Mortality ≤ 15 %	Ô		\$\big 0';	%		
Reference item mortality $\geq 50 \%$ a	at the end of	the test	10	00 %		

Conclusion:

In the 10d adult dose-response honeybe laboratory test mortality was on a very low level in the control and in all five test from treatment groups of 6.6, 12.4, 28.6, 49.9 and 89.7 fig a.s./bee/day. Only at the medium test level of 28 bug as bee day 10% mortality had been observed, originating from 3 dead bees being found in 1 out of the 3 replicates. No mortality occurred at any other tested dose and also not in the coeffol. Therefore LDD₁₀ and LDD₂₀ values could not be determined due to mathematical reasons an Oare therefore not contained in the kinal report of this study.

The chronic toxicity of BCS-CN88460 SC 2005200 GL) was tested over 10 days. The LC50 value (10 days) was > 3333 mg a.s./kg/feeding solution. The LDD₅₀ value (10 days) was $> 89.7 \mu g$ a.s./bee per day. The NOEC and NOEDD values (10 days) were 3333 mg a.s./kg feeding solution and ≥ 89.7 (gra.s./bee per day, respectively.

Effects of honeybee development and other honeybee life stages CA 8.3.1.3

€017; M-587515-01-1 Report:

Title: BCS-Q 88460 - Honey bee Apis mellifera L.) larval toxicity test (repeated exposure)

Report No.: Document No.: M\$87515-01-1

Regulațion (EC) No 1109/2009 (2009) Guideline(s):

Directive 2005-01 (Conada/PMRA)

USÆPA O©SPP 850.ŠUPP

OSCD Draft Guidance Document on Honey bee (Apis mellifera)

Carval Doxicity Test,

Repeated Exposure (Version dated 20 July 2015)

Guideline deviation(s) Now with impact on the study outcome

yes

Objective:

The objective of this study was to determine the effects of BCS-CN88460 on the emergence of adult honeybees, Apis mellifera L., from repeated feeding exposure in a 22 day laboratory test and to



determine the cumulative mortalities during the larval phase and the pupation phase as well as the adult emergence rate. The Lowest Observed Effect Concentration/Dose (LOEC/LOED), the No Observed Effect Concentration/Dose (NOEC/NOED) as well as the concentrations and doses causing 10, 20 and 50 % reduction of adult emergence (EC₁₀/ED₁₀, EC₂₀/ED₂₀ and the EC₅₀/ED₅₀) were determined for day 22, where possible.

Material and methods:

Test item: BCS-CN88460: 94.2 % w/w (analytical); batch number: 2013-006492.

Honeybees (*Apis mellifera carnica* POLLMANN), synchronized first instar (L1) larvae organizing from three adequately fed, healthy, as far as possible parasite free and queen-right colonies. The test was conducted at the field station of the

Dose response test with a duration of 22 days from grafting on day 1 to the find assessment on day 22; from day 3 until day 6 of the test, five different concentrations of BCS-CN88460 were applied to larvae of the test item groups and one single concentration of the reference item dimethoate was applied to the larvae of the reference item group with diet B or Cothe analysed purity was considered for calculation of the test item and reference item concentrations, the daily feeding volume increased from 20 µL to 50 µL diet per larva over the application period; the cumulative feeding volume from day 3 until day 6 of 140 µL diet per larva and the density of the diet (1 g/cm2) were considered for the calculation of the cumulative doses per larva, a control group and a solvent control group were included in the test and exposed for the same period of time under identical exposure conditions to the untreated artificial diet. Each treatment group consisted of 48 larvae from three different colonies (each colony representing a replicate), assessment of larval mortality during larval phase from day 4 until day 8, assessment of mortality during pupation phase on day 15 and day 22, assessment of adult emergence on day 22; the presence of uncaten food was qualitatively recorded on day 8.

Test concentrations: 1 control exoup, 1 solvest control group, 5 test item groups with 10.4, 26.0, 65.0, 162 and 406 mg BCS CN88460/kg diet, equivalent to camulative doses of 1.60, 4.00, 10.0, 24.9 and 62.5 µg BCS-CN88460/larva per developmental period; 1 dimethoate reference item group with 48.0 mg dimethoate/kg diet, equivalent to a cumulative dose of 7.30 µg dimethoate/larva per developmental period.

Findings: 🧞

Analytical findings:

The measured concentrations of BCS-CN88460 in the larger diet were equivalent to recoveries between 85% and 105% of nominal across all test item groups.

The measured concentrations of the test item in the larval diet were within ± 20 % of nominal. Therefore the concentrations of the test item in the larval diet were confirmed and the endpoints are based on nominal concentrations.

Biological findings:

On day s, larval mortality in the control and solvent control group was 0.0 and 8.3 %, respectively. Larval mortality in the reference from group was 91.7 %.

On day 22, the adult emergence rate in the control and solvent control group was 93.8 and 79.2 %, respectively.

Compared to the solvent control group the adult emergence rate on day 22 was not statistically significantly different in any test item group (Multiple Fisher's exact test with Bonferroni-Holm adjustment, one sided greater $\hat{p} = 0.05$).

The EC and C_{20} values for adult emergence on day 22 were determined by Probit analysis using linear maximum likelihood regression and compensation for solvent control response (20.8 %). The EC could not be determined due to a lack of inhibition above 50 % but can be regarded as > 406 mg BCS-CN 8460/kg diet.

During the assessments of mortality and adult emergence no test item related other observations such as deviating sizes, appearances and malformations of the test organisms were made. On day 8, uneaten food was observed in all treatment groups.



Results for larval mortality until day 8 as well as adult emergence on day 22, including the corresponding endpoints are presented in the following table.

The Effects of BCS-CN88460 on the Larval Mortality and on the Adult Emergence of the Honeybee, Apis mellifera carnica Pollmann. from Repeated Exposure and the Communication of the Honeybee, Apis mellifera carnica Pollmann, from Repeated Exposure and the Corresponding Endpoints

						<u>O</u>	0, A
					Mort	arval ality on	Adult Emergence
Treatment	Co	oncentration		Cumulative Dose	D D	ay 8	on Day 22 a
Group				Ö	\$ (0/)	Corr	700 P
				~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~	(%)	ected (%)	
Control				407	0.0	O (\$ 99.8 °
Solvent control					8.3	Ž :-6	79.2
	10.4		1.60		6.3	*2,2 °	₩ 87, \$
Test Item	26.0	[mg BCS-	4.00	Q DOSCNOSTO/L	\$\frac{1}{2}	\$\frac{4.5}{4.5}	77.1
(BCS-	65.0	CN88460/kg	10.0	μg BCS-CN8&60/la , dev@opmenal perio	1 bc 1 2.5	4.60	\$77.1 L
CN88460)	162	diet] ^b	24.9		4.20	4,5	70.8
	406	,	® 2.5		0° 10.4	§2.3 ×	Ø.5
Reference Item (Dimethoate)	48.0	[mg 6 dimethoate kg diet]	7.3 %	org dimernoate larva per developin o reriod]	ental (*) 91.7	915	\$
Endpoints d							
[mg] BCS-CN88460/kg diet]							
				ECAP	E C20		EC50
	OEC	NOEC	(95%)	confidence (95	% confidence limits)	(95 %	6 confidence
Day 22	406	<u>≥</u> 406 ~		mits)	80 (1 % - 742)	¥	limits) > 406
Day 22 > 406 16@(96.0, 268) 380 (199 - 742) > 406							
μg BGS-CN88460/fagva per Sevelopmental period)							
		P .o. 6		ED ₁₀ (7) (9) (9) (9) (9) (9) (9) (9) (9) (9) (9	ED ₂₀	(0.7.0	ED ₅₀
	OED	NOED	` 1	confidence (95	% confidence	(95 %	6 confidence
Day 22 >	62.5	~/ .	24.6 (111(11)(S) S O			> 62.5
	~ ~		(0°-7 KU - 1	ر ٠٠٠		

statistical evaluation for non-engergence

Based on the analysed purity

Validity criteria: All validity criteria were met in this study				
Validity criteri@according to DECD CD 238	Obtained in this study			
Cumulative loval mortality from day 3 to 8 in control(s): ≤ 15 %	Control: 0.0 %			
	Solvent control: 8.3 %			
Mean adult emergence rate on day 22 in control(s): ≥ 70 %	Control: 93.8 %			
	Solvent control: 79.2 %			
For reference item dimethorte larval mortality at day $8: \ge 50 \%$	91.7 %			

Based on the analysed purity Based on the cumulative feeding volume from day 3 antil day o of 120 µL diet/larva and a density of the diet of 1.1 g/cm³
Lethal concentration/doses (LC, LD_x) apply for day 8, effect concentrations/doses (EC_x/ED_x) apply for day 22



Conclusion:

In a repeated exposure larval toxicity test with BCS-CN88460 the NOEC relating to adult emergence on day 22 was determined as \geq 406 mg a.s./kg diet, equivalent to an NOED of \geq 62.5 μ g a.s./larvaper developmental period.

The EC₅₀ relating to adult emergence on day 22 could not be determined but can be regarded as > 406 mg a.s./kg diet, equivalent to an ED₅₀ of > 62.5 µg a.s./larva per developmental period ℓ

CA 8.3.1.4 Sub-lethal effects

There is no particular study design /test guideline to assess "subjecthal effects" in honeybess However, in each laboratory study as well as in any higher-tier study sub-lethal effects are described and reported.

Effects on non-target arthropods other than bees **CA 8.3.2**

Studies on non-target arthropods have been performed with the representative presented in the respective Document MQP, Section 10.3

CA 8.3.2.1 Effects on Apholius Propalosiphi

ve formulations and are Studies on non-target arthropods have been performed with the presented in the respective Document MCP, Section 30.3.2.

CA 8.3.2.2 Effectson Typhlogromus pyri

Studies on non-target arthropods have been performed with the representative formulations and are presented in the recreation of the respective productions. presented in the respective Document MCP, Section 10.3

CA 8.4 Effects on nonPtarget

CA 8.4.

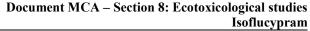
Elevaluated and additional studies on effects on earthworms **Table 8.4-1:**

Test substance	Test/species	Endpoint &	Reference
~Q	Elsenia Getida»	NOE6 ≥ 168 mg a. kg dws*	; 2016; M-548749-01-
Isoflucypram	reproduction C		1
Ø"	56@, mixed	EC not calculable 1)	KCA 8.4.1/01
BCS-CN88460-	Eisenia fetida	NOEC 50 mg m./kg dws* EC160 out calculable ¹⁾	; 2017; M-579263-01-1
carboxylic acid	≪reproduction .	F.C. O Stanlaulabla ¹⁾	, 2017, WI-379203-01-1 KCA 8.4.1/02
(M12)	56 d,@nixed	The calculable	NCA 8.4.1/02

dws = dry weight sol, a.s. = active solustance solute. = fure metabolite

^{*}Endpoint corrected due to lipoplatic substance (log Fow > 2)

1) for details see tudy summary. The State of the S





Report: KCA 8.4.1/01; ; 2016; M-548749-01-1

Title: BCS-CN88460 a.s.: Effects on survival, growth and reproduction on the earthworm

Eisenia fetida tested in artificial soil

Report No.: E 312 4704-1 Document No.: M-548749-01-1

ISO 11268-2: 1998 (E) and OECD 222: April 13, 2004 Guideline(s):

EU Directive 91/414/EEC Regulation (EC) No. 1107/2009 US EPA OCSPP not applicable

Guideline deviation(s): minor deviations

GLP/GEP:

Objective

The purpose of this study was to determine the effects of BCS CN88460 a.s. on survival, growth and reproduction on the earthworm Eisenia fetida tested in Artificial soil. The lest was performed according to the International Standard ISO 16268-261998 and OECD 222 (April 13, 2004).

Material and methods

Test item: BCS-CN88460 a.s.; Batch orde: BCS-CN8846001-06 Certificate no.: No. 1255734-28-1; Spec. no.: 102000028196, analysed content: 94.2 % www.

Test organism: Adult Eisenia fetida, approx. 3 months old synchronised vilture of earthworms); mean bodyweight at the start of the test ranged from 025 to 027 g/vorm.

The test organisms, 8 × 10 animals for each of the control groups (quartz sand treated and solvent on quartz sand treated) and 4 × 10 animals per test concentration of the treatment group, were exposed in artificial soil (with 10% peat content) to the cominal test concentrations of 5.6 × 10, 18, 32, 56, 100, 178 and 326 mg a.s./kg, dry weight artificial soil. The test item was dissolved on organic solvent and afterwards applied and mixed to portion of martz sand. After evaporation of the solvent these portions were mixed into the soot to gain the required concentrations. Non-re-usable plastic boxes (length × width Neight ca. 16.7 cm × 12 cm × 6 cm, area approximately 200 cm²) were used as test vessels. Each test vessel contained in amount of approximately 500 g artificial soil (dry weight) to obtain a depth of approximately 5 cm soil in the test ressels. After 28 days the number of surviving adult earthworms and their weight alteration were defermined. Therefore they were removed from the artificial soil. After further 28 days, the number of offspring was determined. A temperature of 20 ± 2 °C and a light regime of 400-800 lux, 160 light and 8 h dark during the conduct of the study were applied.

The adult earthworms were fed once per week during the test period. The offspring were fed only once at the start of the second 4 weeks exposure period. The surface of the artificial soil was moistened with deionised water once per woek in order to compensate evaporation. As a reference item, Carbendazim (360 g a.s. <u>⚠</u>) was used.

Finding

Toxic reference test:

The most recent reference test (most –GLQ kra-Rg-R-Ref 24/14, July 8, 2014), with the reference substance Cabendazin mixed into the applificial soil, was performed at test concentrations of 1.25, 2.5 and 5.0 mg/a.s./kg/dry woight soil. No mortality of the adult earthworms was observed 28 days after application. The change of body weight of the adult earthworms of the test concentration of 5.0 mg a.s./kg dry weight soft was statistically significant reduced in comparison to the control. The number of juveniles per test vessel of the test concentrations 2.5 and 5.0 mg a.s./kg dry weight soil were statistically significant reduced in comparison to the control.

 EC_{10} , EC_{20} and EC_{50} mean values for reproduction and their 95 % confidence limits were calculated to be 1.474, 1.678 and 2.153 mg a.s./kg dry weight artificial soil, respectively. According to the guideline significant effects should be observed between 1 and 5 mg a.s./kg dry weight artificial soil. Thus the results of this reference test indicated that the test system was sensitive to the reference test item.



Document MCA – Section 8: Ecotoxicological studies Isoflucypram

Experimental conditions:

The pH values measured in the solvent control and the treatments ranged from 5.97 to 6.06 at test start and from 5.96 to 6.14 at test end. The water content during the whole study was between 51,85 and 59.60% of WHC_{max}.

Biological results:

After 28 days of exposure, no mortality in the control group and 5% in the solvent control group was observed. The statistically significant difference to the solvent control in the treatment group with 10 mg test item/kg dry weight artificial soil is considered not test item related, since in all test item groups with higher concentrations no significant effects on adult mortality were observed. No statistically significant differences concerning the loody weight changes of the adult earthworms in comparison to the solvent control were observed in any test item concentration.

No statistically significant differences concerning the number of juveniles relative to the solvent

No statistically significant differences concerning the number of juveniles relative to the solvent control were observed in any test item concentration up to and including 326 mg a.s./kg dry weight artificial soil. Due to the lack of a clear concentration-response relationship to reliable EC₁₀/EC₂₀ calculation was possible. Therefore no EC₁₀/EC₂₀ value can be reported.

Tost object			4.4		7:800in 6		.0 4			<u> </u>
Test object Test item			Q'	PC	riseviu je S. ČN98	eugu Moor) į	ř "J		
[mg a.s./kg d.w. soil]	Control	Solvent Control	5.6%	100	18	etida 160 a.s.S	36	D 00	178	326
Mortality adults [%] after 28 days	0		Ž.	\$22.5	⊕ 5 °				0	0
Significance (mortality) *	- N	4 - 8				\\ \frac{\pi_{\sigma}}{\pi_{\sigma}}		Ž-	-	-
Mean change b.w. [%] day 0 to 28	©29.3	307	2 3.5	5 23.6	29.1	30.80	29.f	31.2	33.3	36.2
Standard deviation		\$\frac{1}{8}.1	8:4	7.8	89	3 .8	% 1.6	5.1	13.5	8.8
Significan (b.w.) **		<u> </u>	~- ·	Q - (, ć	- W	-	-	-	-
Number ö∰spring per vessel day 56	79 .5	\$ 80.8¢	73.3	7.40,5	73 7.3	\$3.0	79.8	70.8	72.5	67.0
Standard deviation	9.0	44.4 ·	21.8	29.1		15.4	11.6	12.6	21.1	23.8
% of solvent control			907	% .0	3 0.7	102.8	98.8	87.6	89.8	86.5
Coefficient of variance [%]	11%	7.8	29.8 G	37.6	16.9	18.6	14.6	17.9	29.1	35.5
Significance (repro.) ***					-	-	-	-	-	-
	✓ Adru!	lt mørtalit	3	*	Gro	wth		Re	producti	ion
NOEC [mg a.s./kg d.w. soj@		\$326 @			≥ 3	326			≥ 326	
LOEC [mg/ a.s./kg d.w@soil] «		> 326				326		> 326		

^{*} Fisher's Exact Byomina Test, one sided greater, $\alpha = 0.05$), + significant, - not significant

^{**} Dunnets's t-test two-sided, $\alpha = 0.05$, + significant, - not significant; in comparison to solvent control

^{***} Dunnett's t-tost, one stilled smaller, $\alpha = 0.05$, + significant, - not significant; in comparison to solvent control



Validity criteria:

All validity criteria were met in this study.

Validity criteria according to OECD 222 (2004)	Obtained in this study		
Adult mortality in the control ≤ 10 %	0 %		
Rate of reproduction of juveniles (earthworms per control vessel) ≥ 30	≥ 62		
Coefficient of variation of reproduction in the control $\leq 30 \%$	11.3 %		

Conclusion:

Based on the effects observed on mortality, growth and reproduction Qit is concluded, that the overal NOEC for the study is determined to be \geq 326 mg $\frac{1}{2}$ /kg dry weight soil. Thus the overall LOEC determined to be > 326 mg a.s./kg dry weight soil. Due to the lack of a clear concentration-response relationship no reliable EC_{10}/EC_{20} calculation was possible. Therefore no EC_{10}/EC_{20} value can be reported.

Report:

Title: BCS-CN88460-Quboxytic acid @CS-CV264970 Effects on susvival

reproduction of the earthworm Eisenia fetida fested in artificial soil

Report No.: E 312 4705-2 Document No.: M-579263-Q1-1

Guideline(s): EU Directive 91/4/4/EEC; Regulation (EC) No

Applicable

Deviation: on day 10 and 16 the temperature in the stimatic chamber increased for 12 Guideline deviation(s):

and 5 hours up to 25°C Due to technical problems for Zaays no temperature data

were regorded

GLP/GEP:

Objective:

The purpose of this study was to determine the effects of BCSCN88460-carboxylic acid (M12) on survival, growth and teproduction on the earthworm Eisenia fatida tested in artificial soil. The test was performed according to the International Standard 180 11268-2 (2012) and OECD 222 (April 13, 2004).

Material and methods:

Test item: BCS-CN88460-carboxylic acid (M12); batch code: BCS-CY26497-01-02, origin batch code: SES 12631-19-2 analysed confent: 98.8%, Sertificate no.: TOX10705-00 (1st run), TOX20054-01 (2nd run).≪Ö

Test organism: Adult Eisedia fetala, approx. 3 months old (synchronised culture of earthworms) mean bodyweight at the start of the test ranged from 0.26 to 0.35 g/worm in the 1st run and from 0.28 to 0.34 g/worm in the 2nd run.

1st run:

Adult Eisenia setida, approxes months old 8 × 10 earthworms for the control groups (quartz sand treated and so vent on quart sand treated) and the single treatment group were exposed to control and treatment. A nomeral ten concentration of 150 mg pure metabolite/kg dry weight artificial soil was mixed in the antificial soil.

Adult Eisedia fetida, 5-6 months old, 8 × 10 earthworms for the control groups (quartz sand treated and so, whit on quartz sand treated) and 4×10 earthworms per test concentration of the treatment groups, were exposed to control and treatment. Nominal test concentrations of 10, 18, 32, 56 and 100 mg pure metabolite/kg dry weight artificial soil were mixed into the artificial soil.



During the study the earthworms were fed with animal manure. A temperature of 20 ± 2 °C and a light regime of 400 – 800 lux, 16 h light and 8 h dark during the conduct of the study were applied. The artificial soil was prepared according to the guideline with the following constituents (percentage distribution on dry weight basis): 70% fine quartz sand, 10% Sphagnum peat, air dried and finely ground, 20% Kaolin clay.

After 28 days the number of surviving adult earthworms and their weight alteration was determined. Therefore they were removed from the artificial soil. After further 28 days, whe number of offspring was determined.

Findings:

Toxic reference test:

The most recent reference test, with the reference substance Carbendazian mixed into the artificial soil. was performed from August 25 to November 1902015 (kra-Rg/R-Ref 26/15; NON-GLP). Effects on mortality and growth of the adults after an exposure period of 28 days and the number of offspring & 1 after 56 days were determined.

No mortality of the adult earthworms was observed 28 days after application. The change of body weight of the adult earthworms of the test concentration of 5.0 mg a sokg dty weight soil was statistically significant less than the sevent control fresults of a Williams multiple sequentian t-test, two-sided, $\alpha = 0.05$).

The number of juveniles per test dessel of the test concentrations 2 and 60 mg 3.s./kg dry weight soil were statistically significant reduced in comparison to the control (results of Williams multiple sequential t-test, one-sided smaller, $\alpha = 0.05$).

sequential t-test, one-sided smaller, $\alpha > 0.05$). According to the guideline significant effects should be observed between and 5 mg a.s./kg dry weight artificial soil.

Thus the results of this reference test indicated that the test system was sensurive to the reference substance.

Experimental conditions:

 $1^{\rm st}$ run: The pH values measured in the controls and the treatment range from 6.03 to 6.06 at test start and from 6.05 6.14 at test end. The water content during the whole study was between 54.33 % and 57.25 % of WHC_{max}?

2nd run; the pH values measured in the controls and the toatment ranged from 6.04 to 6.83 at test start and from 6.33 to 6.64 at test end. The water content during the whole study was between 53.20 % and 58.73 % of WHC_{max}.

Biological results:

Effects on mortality and wowth of the adults after an exposure period of 28 days and the number of Effects on mortality and wowth of the adults after an exposure period of 28 days and the number of offspring per test vessel after 50 days are shown in the following table and paragraphs (values in this table are sounded values).



Test object	Eisenia fetida BCS-CN88460-carboxylic acid (M12)									
mg pure metabolite/kg		2 nd run						1 st run _{@,} °		
dry weight artificial soil	Con	Solv. con	10	18	32	56	100	Con	Solv.	
Mortality of adult earthworms [%] after 28 days	0	1.25	0	0	2.5	0	6	0	54 . 5	
Significance (mortality)*			-	-	_	- %	J -	%) , j' - ,	Z,
Mean change of body fresh weight of the adults from day 0 to day 28 [%]	63.6	71.4	72.4	72.4	70.4	7Q3	74.1	29 .3	39.1 29.4	}
Standard Deviation	9.2	11.1	7.0	4 2.6	4.3	ී 5.7 _{ලා}	° 10.5%	6.14	8.1 G 6.1	,@ */
Significance (body fresh weight)**			<u>-</u>	- &				\$\frac{1}{2}		
Mean number of offspring per test vessel after 56 days	278.5	255.9	229.0	289.5 289.5) 267.80	293. 6	272.00	795		; °
Standard Deviation (% of control)	50.5	395	25.7	79.4	9 3.5	99.4	~24.5 √	\$9 .0	¥14.4 \$20.5	;
% of solvent control			\$9.5 _~ *	\$126. 4 \$	92.5	1282	94.	<u>-</u> Q	- _© 84.8	3
Coefficient of variance (%)	18.1	¥ 15.4 ^{"(}	13.0	24.3	34,9	201	© 0	10.3	1 5√8 29.9)
Significance (reproduction)***	-0		W.	Z - 1	(V) - (V)	\$ -) - 9) %		
6		ult Mort		0	Growt	th		Repr	oduction	
NOEC [mg pure metabolite/kg dry weight soil]		≥ 150 ⇒ 200			100		J.	₹) <u>≥</u>	≥ 150	
LOEC [mg pure metabolite kg dry weight soil]		>450			© 150			>	> 150	
EC ₁₀ and their 9 % confiden EC ₂₀ and their 95 % confiden	ce limits	(mg test	item/kg	dry weig	ght a rt ific	ial soil)			n.d. n.d.	

1st run: * Fisher's Exact Binominal Test (one sixted greater, $\alpha = 0.05$), + eignificant - not significant

n.d.: could not be determined see observations and conclusion

Mortality A

After 280 days of exposure no mortality in the control groups and 5 % (1st run) and 1.25 % (2nd run) in the solvent control groups, was observed, which is in the range recommended by the guideline. No statistically significant effects up to and including 150 mg pure metabolite/kg dry weight artificial soil (the highest concentration tested) were observed (Fisher's exact binominal test, one-sided greater, $\alpha = 0.05$

NOEC related to mortalito. ≥150 mg pure metabolite/kg dry weight artificial soil LOEC related to nortality: > 150 mg pure metabolite/kg dry weight artificial soil

Effects on growth @

A statistically significant difference for growth relative to the solvent control was observed in the single rest concentration of 150 mg pure metabolite/kg dry weight artificial soil in the 1st run (Studentt test for Homogeneous Variances, two-sided, $\alpha = 0.05$). No statistically significant differences up to and including 100 mg pure metabolite/kg dry weight artificial soil, the highest test concentration were

^{**}Student-t test for Homogeneous Variances (two-sided, $\alpha = 0.05$), + significant, - not significant ** Student-t test for Homogeneous Variances (one sided smaller, $\alpha = 0.05$), + significant, - not significant

 $^{2^{}nd}$ run: * Fisher's Exact Dinominal Test tone-sided greater, $\alpha = 0.05$), + significant, - not significant

^{**} William's test (two-sided a = 0.05), + significant, Onot significant

^{***} Williams t-test cone-sided smaller, $\alpha = 0.05$), + significant - not significant



observed in the 2^{nd} run (William's t-test, two-sided, $\alpha = 0.05$) in comparison to the solvent control group.

NOEC related to growth: 100 mg pure metabolite/kg dry weight artificial soil LOEC related to growth: 150 mg pure metabolite/kg dry weight artificial soil

Effects on reproduction

No statistically significant differences concerning the number of juveniles relative to the solvents control were observed in the 1st run (Student-t test for Homogeneous Variances, one-sided smaller, or = 0.05). No statistically significant effects on reproduction up to and including 100 mg pure metabolite/kg dry weight artificial soil, the highest concentration tested in the 2nd fun were observed of (William's t-test, one-sided smaller, $\alpha = 0.05$) in comparison to the solvent control group.

NOEC related to reproduction: ≥150 mg pure metabolite/kg dry weight artificial soil LOEC related to reproduction: >150 mg pure metabolite/kg dby weight artificial soft

Due to the lack of a clear concentration-response relationship no reliable EG₁₀/EC₂₀ calculation was possible. Therefore no EC₁₀/EC₂₀ value can be reported.

Validity criteria:
All validity criteria were met in this study.

Validity criteria according to SECD 222 (2004) Recommended	Obtained Obtained 15 run 2 2nd run
Adult mortality in the control	\$\int 0 \%\\$\\ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \
Rate of reproduction of juveniles (earthworms per control vessel)	62 to 90 223 to 362
Coefficient of variance of reproduction in the control 30 %	11.3 % 18.1 %

Conclusion: a C

Based on the effects observed on mortality, growth and reproduction it is concluded, that the overall NOEC for the study is determined to be 100 mg p.m. kg dry weight soil. Thus, the overall LOEC is determined to be > 150 mg p.m. kg dry weight soil. Due to the lack of a clear concentration-response determined to be > 150 mg prov/kg day weight soil. Due to the lack of a clear concentration-response relationship no reliable EC. EC.20 calculation was possible. Therefore no EC10/EC20 value can be reported.



CA 8.4.2 Effects on non-target soil mesoand macrofauna (other than earthworms)

Table 8.4.2- 0-1: Ecotoxicological endpoints – Collembola and soil mites reproduction studies with active substance Isoflucypram and its soil metabolite

Test substance	Test species, test design	Ecotoxicological Endpoint	Reference
Collembola, repr	oduction		
Isoflucypram	Folsomia candida reproduction 28 d, mixed	NOEC 49.5 ng a.s./kg dws	522863-04-1 KCA 84-2.1/00
BCS-CN88460- carboxylic acid (M12)	Folsomia candida Reproduction 28 d, mixed	NOEC \$ 9 mg p.m./kg dws* EC ₁₀ 6.7 mg p.m./kg dws* LC ₆₀ B mg p.m./kg dws*	2017; M-4 587760-01-1 CA 8 02.1/02
Soil mites, repro	duction		
Isoflucypram	Hypoaspis aculeifer reproduction 14 d, mixed	NOEC > 495 mg a.s. kg dws*	; Ø 2015; M-528194-01-1 VKCA&4 2.1/03
BCS-CN88460- carboxylic acid (M12)	Hypoaspis aculeifer reproduction 14-d, mixed	NOEC \$\frac{1}{2} 495 \text{ for g p.m. (log dws)} \\ \text{EC}_{10} \qquad \text{not calculable}^1 \end{array}	I.; 2015; M-\$24464401-1 &CA 8.42.1/04

dws = dry weight soil, a.s. = active subsance from = pure metabolite

CA 8.4.2.1 Species level testing

Report: K@A 8.4.25\\01;\\ 2015\\@M-522863-01-1

Title: BOS-CN 88460 a.s.: Influence on the reproduction of the collembolan species

Folsomia candida tested in arthricial soil

Regulation (E©) No. 1107/2009 US EPA OCSPP navapplicable

Guideline deviations: not specified

GLP/GEP: Q res

Objective:

The purpose of this study was to assess the effect of BCS-CN88460 a.s. on survival and reproduction of the collembolan species *Folsomia candida* curing an exposure of 28 days in an artificial soil comparing control and treatment.

Material and methods:

Test item: BOS-CNS8460 malytical findings: 94.2 % w/w, origin batch no.: 2013-006492, customer order no.: TOX 10421-020 specification no.: 102000028196, article no.: 81782172.

Test organism: Oulture of the springtails Folsomia candida (Collembolan, Isotomidae), synchronized culture age of 12 days.

10 collemborans (% 12 days old) per replicate (8 replicates for the control group and 4 replicates for each treatment group) were exposed to control (water treated), 99, 176, 313, 556 and 990 mg a.s./kg artificial soil dry weight at 20 ± 2 °C, 400-800 lux, 16 h light: 8 h dark. There was one additional vessel per test item group and control for measurement of pH value and moisture of the artificial soil at the end of the test not loaded with collembolans. Test containers were reusable glass vessels (volume 140 mL, diameter 5 cm). Each test vessel contained 30 ± 1 g wet weight artificial soil.

^{*} Endpoint corrected due to lipophilic substance (log Pow > 2)

¹⁾ for details see study summary



During the study, the test organisms were fed with granulated dry yeast. Directly after the addition of the collembolans, they were fed with granulated dry yeast. Feeding was also done 14 days after test start. Approximately 2-10 mg (one spatula tip) per test vessel was added per feeding date. At test start the pH was measured using the excess artificial soil of control and treatment. At the end of the test the pH was measured again for control and treatment using the additional yessels. Mortalist and reproduction were determined after 28 days.

The software used to perform the statistical analysis was ToxRat Professional

Deviations

500 g artificial soil dry weight was treated instead of 5 g. Therefore the test item concentrations changed. Planned test concentrations 100, 178, 316, 562 and 1000 mg a.s./kg artificial soil dry weight.

Actual test concentrations: 99, 176, 313, 556 and 990 mg a.s./kg artificial soil dry weight.

Findings:

Experimental conditions:

All values were within the range recommended by the guideline.

Test item	р	H Q L	Water co	ntent(%) 🐥	Ű W HÇ i	max ²
concentration 1	Start	Qend	»Start	_ ©End S	Start	🖒 End
Control	6.07	\$\tilde{Q}^{\tilde{y}} 5.6\tilde{\tilde{Q}}	17.89	\$\infty 16.2\$\infty	© 46,110° ×	3 41.17
99	5.74	7, × 5,76° (18042	P) 1 7.6 6 %	47080	45.42
176	5.86	*5 /71	1 8 .51 🐇	5 76.02	Ø8.11 🔊	40.39
313	5.80	& 5.71 °	√18.51 _∞	√√16.49√°	Ø 48.1 <u>1</u>	41.82
556	5.94©	O' 5.73 ³	18,13	17,78	× 46 ₆ 89	45.79
990	5.83	5.80	17,98	/ 16.99 <i>«</i>	#6 :43	43.33

¹ mg a.s./kg soil dry weight≪

Biological results:

EC10 and EC20 Calculations have been performed (ToxRat version 3.2.1). No EC10 and EC20 values could be derived due to the lack of a clear dose-response relationship.

		,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,	, 'O'						
Testitem		BCS-CN8	88460 a.s.						
Test object		🏅 🤝 🦪 Folsomia Çandida 📗							
Exposure 😤		🔍 🞺 🕱 🛣 🕷 rtific	ial soil						
[mg a.s./kg soil	1 1 m Station	Mean number of	Danuaduation (0/						
dry weight]	Adalt mortality	juvenites/test	Reproduction (% of control)	Significance (*)					
(nominal conc.)		vessel ± SD	or control)						
Control	2.9	$2.6 \pm 1.9.3$	-						
995	6 5,60° 0	1346.5 ₹14.3	100.2	-					
₹76	20.0	132.2 206.4	84.3	+					
<i>a</i> ≪ 313	1 0.0 0	$\sqrt[3]{1120} = 213.7$	83.4	+					
₹ 556	7.5	$1.50.5 \pm 88.8$	84.9	+					
990 🛝 🐧	1000 @	$\sqrt{216.0 \pm 83.2}$	90.5	+					
NOEC reproduction mg	a. kg sou dry weight	1	99						
LOEC reproduction [mg	a.s./kg/soil dry weight		176						

Toxic reference test:

Boricacid showed an a non-GLP-test (FRM-Coll-Ref-26/15, March 18, 2015), an EC₅₀ of 77 mg test item Rg artericial soll dryweight (95 % confidence limits from 58 mg to 97 mg Boric acid/kg artificial soil dry weight) for reproduction according Weibull analysis using linear maximum likelihood regression. The result is in the recommended range of the guideline (about 100 mg Boric acid/kg artificial soil dry weight).

water per 100 artificial soil dry weight ² % WHC_{max} = percent of waximum water holding papacity of 47.22 @



and cams
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is determine
erformed
i. The NOEC_{reproduction} was calculated to be < 27 mg Boric acid/kg artificial soil dry weight and accordingly the LOEC_{reproduction} is 27 mg Boric acid/kg artificial soil dry weight according Williams multiple t-test procedure, $\alpha = 0.05$, one-sided smaller. This shows that the test organisms are sufficiently sensitive.

Validity criteria:

All validity criteria were met in this study.

Validity criteria according to OECD 232 (2016)	Obtained in	this study
Mean adult mortality ≤ 20 %	2.9 🐙	W .
Mean number of juveniles/replicate ≥ 100	1343.6	
Coefficient of variation calculated for the number of	\$\vec{\pi}{7}%	\$.
juveniles per replicate ≤ 30 %	., ,,	Y S

Conclusion

Based on the effects observed on reproduction, it is concluded, that the overall SOEC for the study is determined to be 99 mg a.s./kg artificial soil dry weight. Thus, The overall LOEC is Determined to be 176 mg a.s./kg artificial soil dry weight. EC₁₀ and EC₃₀ calculations have Deen performed. No EC₁₀ and EC20 values could be derived due to the lack of a Clear dose-response relationship.

Report:

BCS-CN88460-carbo viic acid (BCS-CY26497): Effects on mortality and Title:

reproduction of the collembolan species Folsomia andida tested to artificial soil 16,10 48,262 S

16,10 48,262 S Report No.: M-587760-01-4 Document No.:

EU Dmective 91/414/EEC Guideline(s):

Regulation EC) No. 1107/2009 (2009)

US EPA OCSPPO ot Applicable

Guideline deviation(s) **GLP/GEP:**

Objective

The purpose of this study was to determine potential effects of the test item on the reproductive output of the collembolar Polsomia candida as a representative of soil micro-arthropods during a test period of 28 days. After 4 weeks the number of of spring (juve files) and surviving parental collembolans were counted.

Material and methods

Test item BCS-CN88460-carboxylic acid M12) Batch code: BCS-CY26497-01-03, Origin Batch No.: NLL 9728-2-9 Customer Order No.: TOX 20233-00, Certificate No.: MZ 01206, LIMS No.: 1624832, analytical findings: 96.8% w/Q

10 Collembola 69-12 days old wer exposed to 18, 32, 56, 100, 178, 316, 562 and 1000 mg pure metabolite/kg dry weight mixed into artificial soil containing 74.7 % quartz sand, 20 % kaolin clay, 5 % sphagnon peat and 0.5 % CaCO₃ & 19.1 - 21.2 °C and a photoperiod: light : dark = 16 h : 8 h(570 lux) and we're fed weekly with granulated dry yeast. Mortality and reproduction were determined after 28 days.

Toxic standard: 44.67, 150 and 225 mg boric acid/kg soil d.w.; control: untreated, solvent control: nome.



Findings:

Biological results										
Test item		В	SCS-CN88460-ca	rboxylic ac	id (M12)					
Test object		Folsomia candida								
Exposure			Artif	icial soil		\ \\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\				
[mg pure						Significance (*)				
metabolite/kg dry	Adult	Significance	Mean num	hor of	Reproduction	Significance				
weight artificial soil]	mortality	Significance	juveniles per t		Kebiomacrion	Significance,				
(nominal	(%)	(**)			(% of control)	(*)***				
concentrations)	(75)	.4	± standard d	leviation						
Control	2.5		738	102						
18	2.5		%755 √ ″	± 160 %		\$\frac{1}{2}\text{\$\frac{1}\text{\$\frac{1}{2}\text{\$\frac{1}{2}\text{\$\frac{1}{2}\text{\$\frac{1}{2}\text{\$\frac{1}{2}\text{\$\frac{1}{2}\text{\$\frac{1}{2}\text{\$\frac{1}{2}\text{\$\frac{1}{2}\text{\$\frac{1}\text{\$\frac{1}\text{\$\frac{1}\text{\$\frac{1}{2}\text{\$\frac{1}\text{\$\frac{1}				
32	17.5		7 417	38	\$ 56\P	+				
56	77.5	× + 5°		± 760	7 - 2 5 - Q	+				
100	92.5	\$\tag{+} \tilde{0}		¥ (99	256	+				
178	92.5	\$ * \$ ~	2 1145	±35	1 5	+				
316	97.5		\$23	± 50	17	+				
56 2	100.0		727	± 15%	10	+				
(1,000	@100,00°	\$\frac{1}{2} + \frac{1}{2} \frac{1}{2}	0 46	± 21	6	+				
D Company)*	Mortality	Reproduction				
NOEC [mg puremetal	odite/kg dry	weight artiticia	I soil		18	18				
LOEC [mg pure metab	oliteÆg dry	Weight actificial	Soil]		32	32				
.4	Ç A				Mortality	Reproduction				
LC ₁₀ ¹ /EC ₁₀ ²⁾ [mg pure i	metaloolite/l	g dry weight ar	tificial soil]		24	13				
95% confidence fimits	<u> </u>				(14 – 40)	(4.9 – 37)				
95% confidence mits	metabolite/k	g dry weight ar	tificial soil]		31	20				
95% confidence mits		<i>y</i>			(20 – 47)	(9.5 – 42)				

The calculation were performed with unrounded values

1) Logit analysis, 2) Probit analysis

⁽Mortiple sequentially-rejective U-test after Bonferroni-Holm, one-sided smaller, α = 0.05, # significant, - = not significant)

^{(**) = (}Multiple sequentially-rejective Fisher Test after Bonferroni-Holm, one-sided greater, $\alpha = 0.05$, + = significant, - = not significant)



Toxic reference test:

In a separate study (BioChem project No. R 16 10 48 003 S, dated 2016-08-08), the EC₅₀ (reproduction) of the reference item boric acid was calculated to be 104 mg/kg soil dry weight. The results of the reference test demonstrate the sensitivity of the test system.

Validity criteria:

All validity criteria were met in this study.

Validity criteria according to OECD 232 (2016)	Obtained in	this study 🔊
Mean adult mortality ≤ 20 %	2.5 % 💇	
Mean number of juveniles per replicate ≥ 100	738 0	Z)
Coefficient of variation (mean number of juveniles per replica ≤ 30 %	13.9 % ©	Ź, Ś

Experimental conditions:

The pH values measured in the solvent control and the treatments ranged from 6.04 to 6.10 at test stort and from 5.82 to 5.92 at test end. The water content during the whole study was between \$7.5 % and 58.0 % of WHC_{max}. All values were within the range commended by the guideline.

Conclusion:

The test item showed statistically significant adverse effects on adult mortality of the collembolan Folsomia candida in artificial soil at concentration including and above 32 mg pure metabolite/kg d.w.. The No-Observed-Effect-Concentration (NOEC) for mortality was determined to be 18 mg pure metabolite/kg soil d.w. The test item caused a significant reduction of reproduction of the collembolan Folsomia Candido in artificial soil at concentration including and above 32 mg pure metabolite/kg d.w. Therefore the No-Observed-Effect-Concentration (NOEC) for reproduction was determined to be 18 mg pure metabolite/kg soil d.w. and the Lowest-Observed-Effect-Concentration (NOEC) for reproduction was determined to be 32 mg pure metabolite/kg soil d.w. and the Lowest-Observed-Effect-Concentration (NOEC) for reproduction was determined to be 32 mg pure metabolite/kg soil d.w. and the Lowest-Observed-Effect-Concentration (NOEC) for reproduction was determined to be 32 mg pure metabolite/kg soil d.w. An EC₁₀ and of 93 mg-pure metabolite was calculated.

Report: ; 2015; M-528194-01-1

Title: BCS-CN88460 as Influence or nortality and reproduction of the soil mite species

Aypoasos aculeifer tested in artificial soil

Report No.: £ 428 7700-55 Document No. M-5 8194-01-1

Guideline(s): EO Directive 91/44/EEC Regulation (EO) No. 1407/2009

US EPA OCSPP not applicable

OEOD 226 from October 1937, 2008: OECD guideline for the Testing of Chemicals - Paradators inite (Propossing (Geolaelaps) aculeifer) reproduction test in soil

Objective:

GLP/GEP:

The purpose of this study was to assess the effect of BCS-CN88460 a.s. (isoflucypram) on mortality and reproduction of the soft mite species *Hypoaspis aculeifer* tested during an exposure of 14 days in artificial soft comparing control and treatment.



Material and methods:

BCS-CN88460 (analytical findings: 94.2 % w/w; batch code: BCS-CN88460-01-06; customer order no.: TOX10421-02; specification no.: 102000028196; material: BCS-CN88460, technical; origin batch no.: 2013-006492; certificate no.: MZ 00994). Ten adult, fertilized, female Hypoaspis aculeife per replicate (8 replicates for the control group and 4 replicates for each treatment group) were exposed to control and treatments. Concentrations of 99, 176, 313, 556 and 990 mg a kg artificial soil by weight were tested. During the test, the *Hypoaspis aculeifer* were fed with nephatodes bred on watered oat flakes. During the study a temperature of 20 ± 2 °C and light regime of 400 - 800 Lys, 16 Night: 8 h dark was applied. The artificial soil was prepared according to the guideline with the following constituents (percentage distribution on dry weight basis 75 % fine quartz sand, 5 % Sphagrum peat, air dried and finely ground, 20 % Kaolin clay. After a period of 14 days, the surviving adults and the o living juveniles were extracted by applying a temperature gradient using a MacFadyen-apparatus Extracted mites were collected in a fixing solution (20% ethylene glycol, 80 % deionised water; 2 g detergent/L fixing solution were added). All Popoaspis aculeifer were counted under a binocular.

Findings:

Experimental conditions:
All values were within the range reconfinence by the guideline.

[mg test item/ kg dry weight	pН	Q,	% water con	itent		% of WH	Ca
artificial soil]	Start	E d d	Start ®	And C	% deviation	Start \$	√End
Control	6.07	₹5.58 ¢	17.8	17.49	2.3 ~ Q	Ø46.16 €	44.88
99	5.74	\$ 5.570°	18 72	18.29	0.7	47.80	47.42
176	5.86	5,33	18.51	1 40 0	6¢A , ""	48,11	44.61
313	5.80	03.55 ×	18. 5] &	18.13	9.1 ×	4 8.11	46.89
556	5 .94	5,640	138913	1830	0.9 ()	46.89	47.42
990	© 5.8 <u>5</u> \	5 63 ₍₄	17.98	18.48	2 V	46.43	48.02

In the control group 8.8% of the adult Flypoaspis aculeifer and which is below the allowed maximum of ≤ 20 mortality.

Concerning the number of liveniles statistical analysis (William's-t test, one-sided smaller, $\alpha = 0.05$) revealed no significant difference between control and any treatment group. Since there were no adverse effects on mortality and reproduction, no EG10/EG3 calculation was possible.

	. () 								
Test item		Y Q BCS-CN984	160 a.s.						
Test Officet									
Exposure	~~~ Q	Artificial Soil							
	% mortality	Mean number of	Reproduction	Significance					
dry weight	(admits)	juveniles per test	(% of control)	(*)					
artificial soil		vessel ± standard dev.							
Control &	^ \ 8.8 Ø′ → 1000	240.9 ± 18.6							
99	y" 1 00 "	202.8 ± 18.5	113.2	-					
176	P.5 💸	250.8 ± 10.4	104.1	-					
176	2.5	286.8 ± 21.0	119.0	-					
₹ 56 €	5.05	286.5 ± 9.3	111.5	-					
\$\sqrt{990}\tilde{\ti}}}}}}}}}}}}}}}}}}}}}}}}}}}}}}}}}}}}	5 .9	265.8 ± 27.2 ght artificial soil] ≥ 990	110.3	-					
NOECreptoaction [mg	a.s./kg dry weig	tht artificial soil] ≥ 990							
LOEC Production [mg	a.s./kg dry weig	ht artificial soil] > 990		_					

Calculations were done with un-rounded values.

^{(*)=}William's-t.-test one sided smaller; α =0.05; "-": non-significant; "+": significant



Validity criteria:

All validity criteria were met.

•		Ø) ° 🛼
Validity criteria according to OECD 226 (2008)	Obtained in this study	
Mean adult mortality should not exceed 20 % at the end of test	the 8.8 % \$\infty\$	
Mean number of juveniles per replicate should be at least 5 (with 10 mites introduced)	240.9	
Coefficient of variation calculated for the number of juveniles per replicate should not be higher than	30%	
Toxic reference test:	LAR/HR-Q-16/12 January 05	
In a separate study (LAR/HR >(.)-16/1⊘4 ° Ianıí‱v 05 ©	2015)@mertor@bed

Toxic reference test:

LAR/HR Q-16/12, January 05, 2015) performed In a separate study (with the reference item dimethoate at test concentrations 1.0, F.8, 3.2, 5.6, and 10 0 mg dimethoate/kg dry weight artificial soil, the LC₅₀ was calculated to be 2.54 mg as /kg 65 % confidence limits from 0.85 mg a. s./kg to 3.30 mg a. s./kg) for mortality The NOEC was calculated to be 30 mg acs./kg and the LOEC was 5.6 mg a.s./kg. Since variances of the data were homogenous Williams-t test $\alpha = 0.05$, one-sided smaller. Dimethoate EC 400 G showed an EC of 547 mg a. s./kg (95% confidence limits from 4.09 mg a. s./kg to 7.30 mg a. s./kg) for reproduction. This is in the recommended range of the guideline and demonstrates the consitivity of the test system.

Conclusion:

The NOEC reproduction and LOEC reproduction of BCS CN88460 were determined to be \$\geq 990\$ mg a.s./kg artificial soil dry weight and > 990 mg at./kg aftificial soil dry weight, respectively. Since there were no adverse effects on mortality and reproduction, no 2 C₁₀/FC₂₀ calculation was possible.

Report: I.; 20¥5; M∗524464-01-1

BCS@N88460-cartoxylic acid (B@S-CY26497): Influence on mortality and Title:

reproduction of the soil mite species Hypoaspis aculeifer tested in artificial soil

£428 4£999-2 Report No% Document No.: M-524464-014

EU Directive 91/4 PEEC; Regulation (EC) No. 1107/2009; US EPA OCSPP: not Guideline(s):

applicable OECD 226 from October 03, 2008: OECD guideline for the Testing of Chemicals - Predatory to te (Hypoaspi Geolaelaps) aculeifer) reproduction test in soil

Guideline deviation(s) not specified

GLP/GEP:

Objective

The aim of this study was to determine the effect of BCS-CN88460-carboxylic acid (M12) on mortality and reproduction of the soil mite species *Hypoaspis aculeifer* tested during an exposure of 14 days in artificial soil comparing Control and treatment.

Material and methods.

Test item BCS N88460-carboxylic acid (M12) (analytical findings: 98.8 % w/w; batch code: BCS-CY26497-01 62; customer order no.: TOX10705-00; Origin batch no. SES 12631-19-9; material no.: BCS Y26 7, teomical certificate no.: MZ00984).

Test organisms: Female Hypoaspis aculeifer (Acari: Laelapidae); mites from a synchronized culture at an age of 30 days after start of egg laying.

Ten adult, fertilized, female Hypoaspis aculeifer per replicate (8 replicates for the control group and 8 replicates for the single treatment group) were exposed to control and treatment. A single



concentration of 990 mg pure metabolite/kg artificial soil dry weight was tested. Test containers were reusable glass vessels (volume 140 mL, diameter 5 cm at the bottom, height 7 cm). Each test vessel contained 20 g dry weight artificial soil (height of artificial soil layer approximately 1.5 cm). The artificial soil was prepared according to the guideline with the following constituents (percentage distribution on dry weight basis): 75% fine quartz sand, 5% Sphagnum peat, air dried and finely ground, 20% Kaolin clay. During the test, the *Hypoaspis aculeifer* were fed with nematode bred on oat flakes. During the study a temperature of 20 ± 2 °C and light regime of 400–800 Lux, 16 h light: 8 h dark were applied.

After a period of 14 days, the surviving adults and the living juveniles, were extracted by applying temperature gradient using a MacFadyen-apparatus. Extracted mites were collected in a fixing solution (20% ethylene glycol, 80% deionised water; 2 g detergent/L from solution were added All Hypoaspis aculeifer were counted under a binocular. The statistical analysis was performed using ToxRat Professional.

Findings:

Biological findings:

For reproduction, no significant difference between control and treatment group detected

Test item Test object Exposure	BCS-CN88460-carbaxylic aold (MAZ) Hypoaspis aculeifer Artificial soil
[mg pure metabolite/kg dry weight artificial soil]	% meriality (adults) Mean number of Juveniles per test vesser ± SD Significance (*)
Control	8.8 <u>2</u> 246.9 ± 18.6 V
990	(2.5 ∑ , ? 282.6 ± 2.7 √ 1 7.3 € √ -
NOEC reproduction [mg py	re metabolite kg dry weight artificial soil] Soil Soi
LOEC _{reproduction} [mgg]u	re metabolite kg dry weight artificial soil] \(\big \text{\(\mathcal{Q} \) \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \

Calculations were done with un-rounded values.

The study was performed as limit test and on adverse effects on mortality and reproduction were observed Therefore

All values were within the range recommended by the guideline.

[mg test	p B		% Wat	er conte	nt _	% of W	VHC _{max}
item/kg dry weight	Start	Engl	Start	End	© % deviation	Start	End
weight 🎻 " artificial soil]		Q _	> .	End		2001	2.14
Control	6.97	5 .\$58 _ @	7.89 @	77.49	2.3	46.16	44.88
990>	5.93	آھے 5.62	18.63∜	17.63	5.5	48.48	45.40

Reference test

The most recent non-60 P-test (LAROHR-O-16/14, January 05, 2015) with the reference item dimethoa@ was@erformed at test concentrations 1.0, 1.8, 3.2, 5.6 and 10.0 mg dimethoate/kg dry weight artificial soil Dimetroate showed a LC₅₀ of 2.51 mg a.s./kg (95% confidence limits from 0.85 mg a.s. (kg) for mortality of the adult mites according Probit analysis using maximum rkelihood regression.

The repoduction of the soil mites was not significantly reduced in comparison to the control up to 3.2 mg a.s./kg dry weight artificial soil. Therefore the NOEC is calculated to be 3.2 mg a.s./kg and accordingly the LOEC is 5.6 mg a.s./kg. Since variances of the data were homogenous Williams-t test $\alpha = 0.05$, one-sided smaller was used. Dimethoate EC 400E G showed an EC₅₀ of 5.47 mg a.s./kg

non-significant; "+": significant (*) = Student-t-testone side smaller; $\alpha = 0.05$,



(95% confidence limits from 4.09 mg a.s./kg to 7.30 mg a.s./kg) for reproduction according Probit analysis using maximum likelihood regression.

This is in the recommended range of the guideline, indicating that an EC₅₀ based on the number of juveniles of 3.0-7.0 mg a.s./kg dry weight artificial soil shows that the test organisms are sufficiently sensitive.

Validity criteria:

All validity criteria were met in this study.

Validity criteria according to OECD 226 (2008)	Obtained i	n this stody
Mean adult mortality ≤ 20 %	8.8.%	"OA
Mean number of juveniles per replicate ≥ 50	2 40.9	Q
Coefficient of variation (juveniles/replicate) ≤ 30 %	∂7.7 %	

Conclusion:

Based on the results observed for reproduction, it is concluded, that the overall NOEC for the study is determined to be \geq 990 mg pure metabolite/kg dry weight artificial soil. Thus, the overall LOFC is determined to be > 990 mg pure metabolite/kg/dry worght attificial soil. The study was performed as limit test and no adverse effects on Portality and reproduction were observed. Therefore, no EC 10/20 calculation was possible.

CA 8.5

Effects of Isofficypram on soil nitrogen transformation **Table 8.5 - 1:**

		,		V
Test substance	Test species, test design	S.	Ecotoxicological Endowint	Reference
N-transformatio	n 💸 🧴			
	Study duration 28		application rate \$375 ca.s./ha (633 mga.s./kg@vil)	; 2015; M- 532055-01-1 KCA 8.5/01
BCS-CN88460-			no tinacceptable effects at an	; 2015;
carboxylic acid	Study duration 28	da/ys"	application rate 6403 g p.m./ha	M-538059-01-1
(M12)	ľ Q' Š		(0.54 mg, m./kg soil)	KCA 8.5/02

a.s. = active substance; p.m. = pure netaboli

26¥5; M-532055-01-1 Report;

60 a Effects on the activity of soil microflora (Nitrogen transformation Title.

Report No .: Document No. @©CD 2⊀6; 2006POECD Guideline(s):

Guideline deviation (s)



Document MCA – Section 8: Ecotoxicological studies Isoflucypram

Objective:

The purpose of this study was to determine the effects of BCS-CN88460 a.s. on the activity of soil microflora with regard to nitrogen transformation in a laboratory test. The test was performed in accordance with OECD guideline 216 (2000) by measuring the nitrogen turnover.

Material and methods:

Test item: BCS-CN88460 a.s., Batch code: BCS-CN88460-01-06, Origin Batch No.: 2013-00 LIMS No.: 1442835, Customer order No.: TOX 10421 02, Specification No.: 102000028196, C No.: 1255734-28-1, Article No.: 81782172, Certificate No.: MZ 00994 analysed purity: 942 % w/w

A loamy sand soil (DIN 4220) was exposed for 8 days to 0,11 mg/test item/kg/soil dry weight, 0.53 mg test item/kg soil dry weight and a control. Each treatment consisted of 3 replicates. Application rates were equivalent to 0.080 kg test flem/ha corresponding to 0.075 kg a.s./ha) and 0.398 kg test item/ha (corresponding to 0.375 kg a s/ha). The nittogen transformation was determined in soil enriched with lucerne meal (concentration in soil 0.5 %). NH₄-nitroger NO₃- and NO₂-nitrogen were determined by an autoanalyzer of different sampling intervals (0, 7, 14 and 28 days after treatment). A reference item is not required by the guideline. Nevertheless Dinglerb is sested routinely as reference item in a separate study to verity the sensitivity of the test system.

The test conditions were: 19.2 to 21.5 % in a climatic and dark room, 4403 to 46.56 % of WHC_{max} and pH values of 5.6 to 5.9 in the soil. The water content of the soil in each test ressel was determined at test start (after application) and adjusted once a week to the required range of 40-50 % of WHC_{max}. The pH-values in the soil used in the test were weasured at test start (after application) and at the final sampling on day 28.

A statistical evaluation of the test results was performed by means of a 2 sided Student-t-test (for homogeneous variances at 5% significances level).

Findings:

The test item BCS-CN88460 as caused temporary mhibitions of the daily nitrate rate at the tested concentrations of 0.11 mg test item/kg soil dry weight at time interval 7-14 days after application.

No adverse effects of BCS CN8 60 a.s. on not ogen transformation in soil could be observed at both tested concentrations at the end of the test, 28 days after application (time interval 14-28). Differences from the control of +0.0.2 % (test concernation 0.11 mg test item/kg soil dry weight) and + 7.9 %

concentration 0.53 mg test item bg soil dr were measured at the end of the 28-day incubation period (time interval 1428).

Time interval	~ @	Sontro		weight	equiv	st stem/ alent to item/ha	kg soil dry 0.080 kg test		nt equiva		kg soil dry 0.398 kg test
(days)	Ni	trate-	N I	Nitrat	e V	% 0	lifference to control	Nitra	nte-N 1		lifference to control
0-7	5.72	±	$\odot_{0.95}$	[≫] 6.16	±	0.92	+ 7.7 n.s.	6.04	±	0.94	+5.6 n.s.
7-14	00%		1.85	- 0.98	±	1.56	- 374.7 ^{n.s.}	- 1.51	±	1.04	- 524.0 ^{n.s.}
4 4-28	2 3.39	±	20 .16	3.74	±	0.30	+ 10.2 n.s.	3.66	±	0.94	+ 7.9 n.s.

The calculations were performed with unrounded values.

¹ Rate: Nurate-N in mg/kg soil dry weight/time interval/day, mean of 3 replicates and standard deviation

n.s. No statistically significant difference to the control (Student-t-test for homogeneous variances, 2-sided, $p \le 0.05$)



In a separate study the reference item Dinoterb caused an effect of + 39.1 %, + 62.5 % and + 112.0 % (required ≥ 25 %) on the nitrogen transformation in a field soil at the tested concentrations of 6.80, 16.00 and 27.00 mg Dinoterb per kg soil dry weight, respectively, 28 days after application (time interval 14-28) and thus demonstrates the sensitivity of the test system.

Validity criteria:

Validity criteria: All validity criteria were met in this study.	
Validity criteria according to OECD 216 (2000)	Obtained in this study
The coefficient of variation in the control for NO_3 - $N \le 15$	8% V V V
Effect of toxic standard ≥ 25 %	\geq 39.1 % (separate study), \vee
Conclusion:	

Conclusion:

BCS-CN88460 a.s. caused no adverse effects (deference to control 25 %, OECO 216) on the soil nitrogen transformation (expressed as NO₃-N-production rate) at the end of the 28-day increasion period. The study was performed in a field soil at concentration up to \$0.53 mg test item/kg/soil dry weight, which are equivalent to application rates up to 0.398 kg test item/ha (corresponding to 0.375 kg a.s./ha).

Report:

BCS-C 88460-carboxylic acid BCS-C 2649Q): Effects on the activity of soil Title:

microtora (nitrogen stansformation test)

15 10 48 03 N Report No.: M-\$38059-01-1 Document No.:

OECD 216 (2000); OFOD 216 adopted January 21, 2000, OFOD Guideline for the Guideline(s):

Testing of Chemicals, Soil Microorganisms. Witrogen Transformation

Guideline deviation(s) **GLP/GEP:**

Objective:

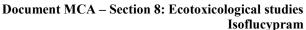
The aim of his study was to determine the effects of the test item on the activity of soil microflora with regard to nitrogen transformation in a laboratory test. The test was performed in accordance with OECD guideline 216 2000) by measuring the nitrogen turnover.

Material and methods

Test item: BCS-CN88460-carboxylic acid (M12), BCS-code: BCS-CY26497, Batch code.: BCS-CY26497-01 92, Origin Batch No.: SES 12631, 19-9, LIMS No.: 1441413, Customer order No.: TOX 10705-00, Certificate No.: MZ 10984, Analysed purit 98.8 % w/w.

A loamy sand soil (DIN 4220) was exposed for 28 days to 0.11 mg test item/kg soil dry weight and 0.54 mg test item/kg soil dry weight. These application rates were equivalent to 0.082 kg test item/ha (corresponding to 0.081 kg pure metabolite/ba) and 0.408 kg test item/ha (corresponding to 0.403 kg pure metabolite ha), respectively. We nitrogen transformation was determined in soil enriched with lucerne meal@concentration in soil 0.5\% NH₄-nitrogen, NO₃- and NO₂-nitrogen were determined by an autoanalyzer at different sampling intervals (0, 7, 14 and 28 days after treatment). A reference item is not required by the guideline. Nevertheless Dinoterb is tested routinely as reference item in a separate study to verify the sensitivity of the test system.

The test conditions were: 19.2 to 21.5 °C in a climatic and dark room, 42.94 to 46.34 % of WHC_{max} and pH values of 5.7 to 5.9 in the soil. The water content of the soil in each test vessel was determined at test start (after application) and adjusted once a week to the required range of 40-50 % of WHC_{max}. The pH-values in the soil used in the test were measured at test start (after application) and at the final sampling on day 28.





A statistical evaluation of the test results was performed by means of a 2-sided Student-t-test (for homogeneous variances at 5 % significance level).

Findings:

The test item BCS-CN88460-carboxylic acid (M12) caused a temporary stimulation of the daily nitrate rate at the tested concentrations of 0.11 mg test item/kg and 0.54 mg test item/kg soil oby weight at time interval 7-14 days after application.

However, no adverse effects of BCS-CN88460-carboxylic acid (M12) or nitrogen transformation in soil could be observed at both tested concentrations at the end of the test, 28 days after application (time interval 14-28). Differences from the control of +170 % (test concentration 0.1) mg test itemæg soil dry weight) and -7.4 % (test concentration 0.54 mg test item/kg soil dry weight) were measured at 6 the end of the 28-day incubation period (time interval 4-28).

Time interval	C	ont	rol		ht eq		kg soll dry to 0.082 kg to	xveigh	t ¢oyuiva	em/kg soil dry left to 0.408 &m/ha
[days]	Nit	rate	e-N ¹	Ni	trate	-N 1	difference Lo control	Nitra	te-N	difference to control
0-7	5.72	±	0.95	5.63	±	@36 _%	y - 1,5 ys	6.00	¥ 1.05€	+ 45 n.s.
7-14	0.36	\pm	1.85	1.85	±,(0.81	+ 481.7 n.s.«	$\sqrt{0.89}$	0,47	+348.0 n
14-28	3.39	Ħ	0.16	3.97	Ą,	Ĉ	±17.0 ₺	3.14	± _ © 25	~ 7.4 °

The calculations were performed with parounded values?

In a separate study the reference item Dinogerb can sed a stimulation of nitrogen transformation of +39.1 %, +62.5 % and +112.0 % at 680 mg 16.0 mg and 27.0 mg Dinoter ber kg soil dry weight, respectively, determined 28 days after application time interval 14-280

Measured pH- values for all treatment groups Sat test start and 5.7 at the final sampling day on day 28.

Validity criteria

All validity criteria were met in this stage

Validity criteria according to OE D 216 Q000) Obtained in this study
Coefficient of variation in the control $\leq 15\%$ $\leq 8.0\%$
Effect of toxic standard 25 % S S S S S S S S S S S S S S S S S S

Conclusion:

BCS-CN \$460-carbox dic acid (M120 caused no adverse effects (difference to control < 25 %, OECD 216) on the soil nitrogen transformation (expressed as NO₃-N-production) at the end of the 28-day incubation period. The study was performed in a field soil at concentrations up to 0.54 mg test item/kg soil dry weight, which are equivalent to application rates up to 0.408 kg test item/ha (corresponding to 0.403 kg pure metabolite/ha)

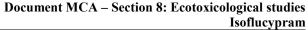
Effects on terrestrial non-target higher plants

Summary of screening data

Not necessary as guideline GLP studies conducted with the representative formulation for isofluc pram for terrestrial non-target plants are available (see Point KCP 10.6.2).

Rate: Nitrate-N in mg/kg soil dry weight/time/interval/day, mean of 3 keplicates and standard deviation

 $^{^{}n.s.}$ = No statistically significant difference to the control (Student-t-test for hortogeneous variances, 2-sided, $p \le 0.05$)





CA 8.6.2 Testing on non-target plants

Studies on terrestrial non-target plants (seedling emergence and vegetative vigour) conducted with the representative formulation for isoflucypram are presented under Point KCP 10.6.2.

In view of the results presented in the Summary MCP Section 10, Point 10.6.2, no further studies are deemed necessary.

CA 8.8 Effects on biological methods for sewage treatment.

In view of the results presented in the MCA Summar studies are deemed necessary.

Report: KCA 8.8/01;

Activated sludge respiration inhibition test with soft 2018/0009/01 Title:

Report No.: M-617426-0\(\frac{1}{2}\)1 Document No.:

EU method ©.11 (2008); (DECD) Guideline(s):

Guideline deviation(s): **GLP/GEP:**

Objective:

The study was performed to assess the toxicity of isothucyprom (BOS-CN88460) to bacteria. The study was conducted in accordance with OECD Guideline 209 Activated Sludge, Respiration Inhibition Test (Carbon and Ammonium Oxidation) (adopted: 22 July 2010) and considered the Question-and-Answer Document by the German Federal Environment Agency (Version 2012-03-02). This test method is in most essential parts equal to Council Regulation (EC) No 440/2008, Method C.11 "Biodegradation: Activated Sludge Respiration Philipition Test" (2008).

Materials and Methods:

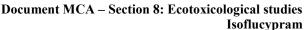
Test item: Isofluçoram technical; Batch code: BCS-CN88460-01-06; Batch No: 2013-006492; Specification No. 102000028196; Customer Order No.: TOX 20011-05; Certificate No.: MZ 01373; purity 94.2% w/w.

The activated sludge (a prixed copulation of quatic microorganisms) was exposed to isoflucypram technical or different concentrations (00, 32, 100, 320 and 1000 mg test item/L). The respiration rate of each mixture was determined after 3 hours with permanent aeration. The activated sludge was daily fed with a standard amount synthetic sewage.

To measure the oxygen consemption, 250 mL of sludge with the test item (or control or reference compound) was incubated for 3 h in 300 mL closed Erlenmeyer flasks (with air inlet and outlet) and aerated through a stass tube at 50-100 Pha with clean oil-free air. For the measurement, the content of the Erlenwieyer Wasks was completely transferred to 250 mL BOD bottles and oxygen content was measured with an oxygen niger (redox electrode).

Six controls without the test item were included in the test design, three at the start and the others at the end of the test series.

The test was performed with different test item concentrations with 3 replicates. Each batch of activated sludge was checked using 5 concentrations in the range of 2.5 - 40 mg/l of 3,5-Dichlorophenol as a reference compound.





The respiration rate is classified into two processes of oxidation. The oxidation of organic carbon and the ammonium oxidation (nitrification). The use of the specific nitrification inhibitor, ATU Nallylthiourea), enables the direct assessment of the inhibitory effects of test substances on heterotrophic oxidation, and by subtracting the oxygen uptake rate in the presence of ATU from the total uptake rate, the effects on the rate of nitrification may be calculated Two sets of mixtures were prepared, one without ATU and one with ATU.

Since some substances may consume oxygen by chemical reactivity, a physico-chemical oxygen consumption control was carried out additionally for both sets. In order to be able to differentiate between physico-chemical oxygen consumption and biological oxygen consumption (respiration), at least the maximum concentration of the test item was dested without activated sludge.

The respiration rate for each concentration was determined from the linear part of the curve of the oxygen content versus time. The inhibitory effect of the test item at a particular concentration is expressed as a percentage of the mean of the respiration rates of the six controls.

ECx values for the test item and the reference substance were calculated from the respiration rates at different test item concentrations using the ratistics programme fox ParPro Fersion 2.10 Felease different test item concentrations using the statistics programme for RatPro Sersion 2.10 (release 2010-09-10). The No Observed Effect Concentration was calculated according to Durnett's Multiple t-test Procedure using the same statistics programme mentioned above.

The test temperature during exposure was 20 ± 2°C.

Dates of experimental work: February 19, 2048 – March 15, 2018

Results:

Validity Criteria
All validity criteria were met

Validity exteria according to OPCD 209	Obtained in this study
Oxygen uptake of blank controls per one gran of activated.	2\daggeq 960 mg oxygen/gram (without ATU)
sludge (dry weight of suspended soils) in an hour 20	25.632 mg oxygen/gram (with ATU)
replicates at the end of the test should be \(\le 30\)	7.7% (without ATU) 10.3% with ATU)
EC50 of reference compound 3,5-Dichloroptional should be in	15.265 (total respiration)
the range 12-25 mg/L for total respiration and 5-40 mg/L for	20.959 mg/L (heterotrophic respiration)
heterotrophic respiration.	

Analytical Findings:

The test item and reference composind concentrations were not confirmed by analytical methods, they were based of nominal consentrations.

Isoflucypram technical showed 11.9 % respiration inhibition of activated sludge at a test item concentration of 1000 me/L for total respiration and 16.6 % respiration inhibition at a test item concentration of 1000 mg/L for heterotrophic respiration.



Results of the test item isoflucypram without ATU (total respiration)

Treatment [mg/L]	Respiration rate [mg/L × h]	Mean Temp. [°C]	pH-value	Inhibition [%]
Control 1	34.592	20.3	8.0	\T
Control 2	31.774	19.8	<mark>8</mark> P	
Control 3	31.090	19.3	8.1	Z
Control 4	31.487	19.1	€ ³ 8.3	
Control 5	27.061	18.7	8.3	7 20
Control 6	30.907	19.5	8.3	2 5 Q
Control, mean	31.152	Q g		y <mark>o</mark> y
10 mg/L test item	28.976 ©	191	8.1 N	8.983
10 mg/L test item	27.5 5 %	78.4	8.2	11.553
10 mg/L test item	27.571	18.5	8.2 8,2	1/0496
10 mg/L test item, mean	28.033		~ ~ ×	10.010 10 10 10 10 10 10 10 10 10 10 10 10
32 mg/L test item	28,744 ×	27 <mark>19.0</mark> 0	% 820°	7.7 <mark>29</mark>
32 mg/L test item	280.753 × 3	18.8	8.2 \$	7.701
32 mg/L test item	28.13 2	98.7 0 0	8.20	⁹ .674
32 mg/L test item, mean	28.545	~ 0~ 0		8.368
100 mg/L test item	₹ <mark>27.007</mark>	18.8	³ ∕8.3 √2	13.306
100 mg/L test item	30.144	19.0	8. 3	3.331
100 mg/L test item 100 mg/L test item	28 ,422	18.9	<mark>\$</mark> .3	<mark>8.763</mark>
100 mg/L test item 100 mg/L test item nean	28.514	~		8.467
	4 27 88 4	19. 0	8.3	12.725
320 mg/L test item 320 mg/L test item	30.058 ©	7 1 <u>9 1</u>	8.3	3.511
320 mg/L test item 320 mg/L test item, mem	27.783	0 018.8 0	8.3	10.807
320 mg/L test item, mean	28,344 _. 0			<mark>9.014</mark>
1000 mg/L test item	27.435	19.1	8.4	11.932
1000 mg/L test item 1000 mg/L test item	28. 3 73	19.1	8.3	<mark>8.919</mark>
	20.542 V	19.1	8.3	14.797
1000 mg/Latest item, mean	27.450	2		11.883
Physicochemical oxygen consumption control 1000 mg/L		19.1	<mark>7.3</mark>	
2.5 m/g/L reference composited	25.99	19.3	8.2	16.563
5 mg/L reference compound	28 <mark>375</mark>	19.1	8.1	8.913
10 mg/L reference compound	2 0.469	19.3	8.0	34.292
20 mg/L reference compound	11.088	19.2	8.1	64.408
40 mg/L reference compound	7.148	18.6	8.2	77.054
		-		

The physico-chemical oxygen consumption has been determined at a test item concentration of 1000 mg/L. As nearly no physico-chemical oxygen consumption was observed at that test item concentration this observation also holds true for the lower test item concentrations.



Results of the test item isoflucypram with ATU (heterotrophic respiration)

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Results of the test item isoflucypram (Calculated nitrification respiration: total respiration minus heterotrophic respiration)

Treatment [mg/L]	<mark>Mean resp.</mark>	Mean resp. rate	Mean resp. rate	Inhibition
	<mark>rate</mark>	(Heterotrophic resp.)		Nitriffeation
	(Total resp.)	[mg/L × h]	[mg/L >	
	[mg/L × h]		Ö	
Control	31.152	30.758	√ <mark>∮394</mark>	
10 mg/L test item	28.033	<mark>26.367</mark> 🕲	1.666 ×	\$22 .843
32 mg/L test item	28.545	26.038	2.507 v	\$536,294
100 mg/L test item	28.514	26.499	2.315	-486.563 @
320 mg/L test item	28.344	2 <mark>6.618</mark>	<u>" Ø 1.72</u> 6″ 💜	338.07
1000 mg/L test item	<mark>27.450</mark>	% 25.662 S	₹ <mark>788</mark> 🔊 🔭	√ -353.807
2.5 mg/L reference comp.	<mark>25.992</mark>	29 .917	7 3.925	10 9 6.193
5 mg/L reference comp.	28.375 ×	27.523 ~	0.850 4,	15.786°
10 mg/L reference comp.	20.469 V	23.967 ,	Ø 3.498 €	9875817
20 mg/L reference comp.	11.088	4.563	-3.455 Z	281.980
40 mg/L reference comp.	<mark>7.1⁴8</mark> ⊗	9.369	20221	° 663.706

resp. = respiration, comp. = compound

No nitrification respiration inhibition could be determined for the test item concentrations and reference compound. The seemingly high nitrification inhibitions (percent inhibition) are only due to minor differences between total and reference respiration. This imparticular in the light that there was no concentration response with the test item and that there have been similar inhibitions for all test item concentrations.

After an incubation period of 3 hours, analysis of the respiration rates gave the following values:

20°	0 2			"A" "A			
Test substance		Isoflucypram	technica				
Test S	Activat	<mark>d sludge, resp</mark>	ration i	hibition			
Q		d sludge, resp	,				
Total respiration			, O	O ^y			
EC_{50}		y 2000 pg	en e	ď			
EC ₁₀		n.d.*	W.C.				
NOEC 🛇	y	~ Z ?0**					
	<i>₹</i>						
Heterotrophic respiration C							
EC ₅₀	ION O	> 10 Q 0 m	g/L				
EC_{10}	~	©n.d.*					
NOEC &		[∕] < 10 mg	<mark>/L</mark>				
Nitrification respiration .							
ECSO TO TO	Ž,	> 1000 m	g/L				
EC ₁₀		n.d.*					
NOEC		n.d.*					

^{*} n.d. = not determined (due to mathematical reasons or inappropriate data)

^{**} Determined by expert judgement (details on page 35 of the study report)



Conclusion:

Isoflucypram technical showed 11.9 % respiration inhibition of activated sludge at a test item pragran the service of the service o concentration of 1000 mg/L for total respiration and 16.6 % respiration inhibition at a test item concentration of 1000 mg/L for heterotrophic respiration. The calculation of nitrification respiration CA 8.9 Monitoring data

No ecological monitoring studies were conducted. For monitoring of softucypramen the envelopment of the Summary MCA Section 7, Point 3.5. was not reasonable. As the EC₅₀ for total and heterotrophic respiration was > 1000 mg/L the EC_{50} for nitrification respiration is equally > 1000 mg/L. The effect value relates to a forminal concentration, The state of the s the environment of the state of since no analytical monitoring was performed.