Document Title

Summary of the Ecotoxicological studies for Isoflucypram

(Code: BCS-CN88460)

Data Requirement(s)

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

Document MCA

Section 8: Ecotoxicological studies

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

Date

2018-04-17

Author(s)

[redacted]

[redacted]

Bayer AG

Crop Science Division

OWNERSHIP STATEMENT

This document, the data contained in it and copyright therein are owned by Bayer AG and/or affiliated entities. No part of the document or any information contained therein may be disclosed to any third party without the prior written authorisation of Bayer AG and/or affiliated entities.

The summaries and evaluations contained in this document are based on unpublished proprietary data submitted for the purpose of the assessment undertaken by the regulatory authority. Other registration authorities should not grant, amend, or renew a registration on the basis of the summaries and evaluation of unpublished proprietary data contained in this document unless they have received the data on which the summaries and evaluation are based, either:

- **from Bayer AG or respective affiliate; or**
- **from other applicants once the period of data protection has expired.**

Version history

Date [yyyy-mm-dd]	Data points containing amendments or additions[1] and brief description	Document identifier and version number
2018-02-06	Original Document MCA 8 Ecotoxicological studies	M-613611-01-1
2018-04-17	Dossier update - CA 8.1.1, CA 8.1.1.3, CA 8.2.2.1, CA 8.2.5.1, CA 8.2.5.2, CA 8.2.5.4, CA 8.2.6.1, CA 8.3.1.2: additional information added - CA 8.8/01: New study M-617426-01-1 added	M-613611-02-1

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

Table of Contents

CA 8 ECOTOXICOLOGICAL STUDIES ON THE ACTIVE SUBSTANCE

INTRODUCTION

Isoflucypram (CAS-No. 1255734-28-1) is a new fungicidal active substance developed by Bayer. This document supports the application for regulatory approval of isoflucypram 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 representative uses, under Regulation (EC) No 1107/2009 in accordance with the requirements laid down in the Commission Regulation (EU) No 283/2013 and under Classification Regulation (EC) No 1272/2008.

Isoflucypram is a novel broad spectrum fungicide of the chemical class of N-cyclopropyl-N-benzyl-pyrazole-carboxamides with an outstanding efficacy against the major economically important fungal diseases of cereal crops (wheat, triticale, rye, barley and oats) and excellent 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 foliar spray at a maximum of 75 g a.s./ha supports an effective anti-resistance management strategy.
Tailor-made and broad spectrum isoflucypram combinations show highly beneficial properties in terms of plant physiology beside the long-lasting and certain curative 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 dossier.

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

The same applies for the metabolite BCS-CN88460-carboxylic acid for which the Bayer Code is BCS-CY26497 and the Bayer-internal short Code M12.

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 long-term effects of isoflucypram on birds is presented in the following chapters.

Some studies were submitted which are not requested for European registration but mandatory for registration by US-EPA. The registration for a US registration includes an LD_{50} test with passerines (canary bird) according to OECD 223, two subacute studies (with quail and mallards) 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 first one 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 8.1.1.3.

CA 8.1.1.1 Acute oral toxicity to birds

Table 8.1.1.1- 1: Acute oral toxicity to birds

Test substance	Test design	Test species	Endpoint		Reference
Isoflucypram	Acute toxicity	Bobwhite quail	LD_{50}	> 2000 mg a.s./kg bw	[redacted]; [redacted] T.; 2015; M-535551-01-1 KCA 8.1.1.1/01
		Wild canary	LD_{50}	> 2000 mg a.s./kg bw	[redacted]; 2016; M-547051-01-1 KCA 8.1.1.1/02

Report: KCA 8.1.1.1/01; [redacted]; [redacted] T.; 2015; M-535551-01-1
Title: Toxicity of BCS-CN88460 technical during an acute oral LD50 with the northern bobwhite quail (*Colinus virginianus*)
Report No.: EBLNN006
Document No.: M-535551-01-1
Guideline(s): OCSPP 850.2100
Guideline deviation(s): not specified
GLP/GEP: **yes**

Objective:
The aim of the study was to determine the acute oral LD_{50} of BCS-CN88460 technical on Northern Bobwhite quail (*Colinus virginianus*).

Material and methods:
BCS-CN88460 technical, 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 level 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 on Day 0 following 18 hours of fasting and monitored for 14 days post-dosing. All feed and water was provided ad libitum.
The birds 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°C) 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 observed in any bird. All birds were normal in 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 body 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 a.s./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 3 to 7, Day 8 to 14) for the 2000 mg a.s./kg treatment group were not significantly different from the control group.

Treatment level [mg a.s./kg bw]	Body weight descriptive statistics							
	d -1		d 3		d 7		d 14	
	Mean [g] ± S.D.	n	Mean [g] ± S.D.	n	Mean [g] ± S.D.	n	Mean [g] ± S.D.	n
Control	209.4 ± 9.4	10	204.4 ± 7.9	10	205.9 ± 7.6	10	208.0 ± 8.0	10
2000	209.5 ± 8.4	10	200.3 ± 7.2	10	205.7 ± 8.6	10	208.8 ± 8.8	10

SD = standard deviation, n = number of surviving birds

Treatment level [mg a.s./kg bw]	Body weight changes					
	Δ d1-d3		Δ d3-d7		Δ d7-d14	
	Mean [g] ± S.D.	n	Mean [g] ± S.D.	n	Mean [g] ± S.D.	n
Control	-4.9 ± 4.2	10	1.4 ± 1.2	10	2.1 ± 2.4	10
2000	-9.2± 4.0*	10	5.5 ± 3.1	10	3.1 ± 2.6	10

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

Treatment level [mg a.s./kg bw]	Food consumption for intervals [g/bird/day]					
	d 1-3		d 4-7		d 8-14	
	Mean [g] ± S.D.	n	Mean [g] ± S.D.	n	Mean [g] ± S.D.	n
Control	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 LD_{50} of BCS-CN88460 technical for Northern Bobwhite quail was > 2000 mg a.s./kg body weight 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; [redacted]; 2016; M-547051-01-1
Title:	Toxicity of BCS-CN88460 technical during an acute oral LD50 with the canary (*Serinus canaria*)
Report No.:	044SRLS14C04
Document No.:	M-547051-01-1
Guideline(s):	OCSPP 850.2100
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

Objective:
The aim of the study was to determine the acute oral LD_{50} of BCS-CN88460 technical on Canary (*Serinus canaria*).

Material and Methods:
BCS-CN88460 technical, Batch No. 2013-006492, Purity 94.2 %
Adult canaries (9 - 11 month old with a body weight range of 19.0 – 26.0 g at study initiation) were orally dosed with BCS-CN88460 based on body weight at dose levels of 0 (control), 125, 250, 500, 1000, and 2000 mg a.s./kg body weight (bw). 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 0 following six 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 72.1°F (22.3°C) at 50 % relative humidity with a photoperiod of 8 hours light: 16 hours dark (227 lux). 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, clinical signs, body weight gain and food consumption. Adult body weights were measured on experimental Day -1, Day 7, and Day 14. Feed consumption measurements and clinical observations occurred daily.
The statistical analyses on body weight were conducted using TOXSTAT.

Findings:
Biological results

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 hypo-reactivity 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 Day 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. No mortality occurred during the course of the study therefore no birds were subjected to gross necropsy.

Body weight and feed consumption

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

Dose level [mg a.s./kg bw]	Body weight descriptive statistics					
	d -1		d 7		d 14	
	Mean [g] ± S.D.	n	Mean [g] ± S.D.	n	Mean [g] ± S.D.	n
Control	22.2 ± 1.7	10	22.4 ± 1.4	10	23.5 ± 1.5	10
125	21.5 ± 1.3	10	21.7 ± 1.4	10	22.4 ± 1.4	10
250	21.7 ± 1.2	10	22.2 ± 1.6	10	23.3 ± 1.7	10
500	21.6 ± 1.1	10	21.7 ± 1.0	10	22.8 ± 1.2	10
1000	21.8 ± 1.2	10	21.7 ± 0.9	10	22.8 ± 1.1	10
2000	22.1 ± 1.6	10	22.3 ± 1.3	10	23.5 ± 1.0	10

SD = standard deviation; n = number of surviving birds.

Dose level [mg a.s./kg bw]	Body weight changes					
	Δ d-1-d7		Δ d7-d14		Δ d-1-d14	
	Mean [g] ± S.D.	n	Mean [g] ± S.D.	n	Mean [g] ± S.D.	n
Control	0.2 ± 0.7	10	1.1 ± 0.5	10	1.4 ± 0.8	10
125	0.2 ± 0.4	10	0.7 ± 0.3	10	0.9 ± 0.4	10
250	0.5 ± 0.8	10	1.1 ± 0.7	10	1.6 ± 0.9	10
500	0.1 ± 0.9	10	1.1 ± 0.6	10	1.2 ± 1.0	10
1000	0.0 ± 0.5	10	1.0 ± 0.4	10	1.0 ± 0.6	10
2000	0.2 ± 0.9	10	1.2 ± 0.6	10	1.4 ± 1.1	10

SD = standard deviation; n = number of surviving birds.

Dose level [mg a.s./kg bw]	Food Consumption Descriptive Statistics [g/bird/day]					
	d 1-7		d 8-14		d 1-14	
	Mean (g) ± S.D.	n	Mean (g) ± S.D.	n	Mean (g) ± S.D.	n
Control	4.1 ± 0.7	10	5.0 ± 0.8	10	4.6 ± 0.7	10
125	4.7 ± 1.2	10	4.8 ± 0.9	10	4.7 ± 1.0	10
250	4.9 ± 0.8	10	5.2 ± 0.8	10	5.0 ± 0.7	10
500	4.3 ± 0.5	10	4.8 ± 0.6	10	4.6 ± 0.5	10
1000	5.0 ± 1.3	10	5.6 ± 1.3	10	5.3 ± 1.3	10
2000	4.7 ± 0.7	10	5.2 ± 0.5	10	5.0 ± 0.5	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	yes

Conclusion:
The LD_{50} of BCS-CN88460 technical for canary is > 2000 mg a.s./kg body weight.

CA 8.1.1.2 Short-term dietary toxicity to birds

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

Test substance	Test design	Test species	Endpoint		Reference
Isoflucypram	Dietary toxicity (short-term)	Mallard duck	LD_{50}	> 1360 mg a.s./kg bw	[redacted] T.; [redacted]; 2014; M-507176-01-1 KCA 8.1.1.2/01
		Bobwhite quail	LC_{50} LD_{50}	> 5087 ppm > 487 mg a.s./kg bw	[redacted]; [redacted] T.; [redacted] M.; 2015; M-516743-01-1 KCA 8.1.1.2/02

Report: KCA 8.1.1.2/01; [redacted] T.; [redacted]; 2014; M-507176-01-1
Title: Toxicity of BCS-CN88460 technical during a dietary LC50 with the mallard duck (*Anas platyrhynchos*)
Report No.: 044SRLS14C06
Document No.: M-507176-01-1
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 dietary LC_{50} of BCS-CN88460 technical on Mallard duck (*Anas platyrhynchos*).

Material and methods:
BCS-CN88460 technical, Batch code BCS-CN88460-01-06, Batch no.: 2013-006492, Purity 94.2 %.
Six day-old mallard hatchlings (*Anas platyrhynchos*) were fed for five days nominal dietary levels of 0 (control), 313, 625, 1250, 2500, and 5000 mg a.s./kg feed. The treatment levels, homogeneity, and stability samples 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) × 76 cm (width) × 25 cm (height).
Feed consumption, mortality and clinical observations for the hatchlings occurred daily. Hatchling body weights were taken on Day 0, Day 5, and Day 8. Post-mortem examinations were conducted on 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.
The average concentration of BCS-CN88460 was measured in feed on study initiation (Day 0) and Day 5.

Findings:

Analytical results
The measured amounts of BCS-CN88460 were determined as control (0), 326, 643, 1323, 2529, and 5554 mg a.s./kg feed representing a recovery range of 101 to 111 % of nominal. The mixing procedure for BCS-CN88460 was confirmed to be homogenous and stable under test conditions.

Nominal dietary concentrations [mg a.s./kg feed)]	Measured dietary concentrations [mg a.s./kg feed]	Overall study
	Mean (SD)	Mean % recovery
Control	ND[a]	ND[a]
313	326 (10)	104%
625	643 (21)	103%
1250	1323 (30)	106%
2500	2529 (42)	101%
5000	5554 (286)	111%

[a] ND = Not Detected (limit of quantitation) = 50 mg a.s./kg feed)

Biological results

Test object	Mallard duck	
Test substance	BCS-CN88460 technical	
	[mg a.s./kg feed] (measured)	[mg a.s./kg bw] (measured)
LC_{50}	> 5554	> 1360
LOEC	> 5554	> 1360
NOEC	5554	1360

Mortality & Clinical Observations
One accidental mortality occurred in the 326 mg a.s./kg 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 hypotheses 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 concentration [mg a.s./kg feed]	**Initiation (Day 0)**		**Day 5**		**Termination Day 8**	
	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	10
326	72.9 ± 4.2	10	170.1 ± 5.5	10	245.5 ± 11.1	9
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	10	241.2 ± 17.8	10
5554	72.6 ± 4.3	10	163.2 ± 9.3	10	235.5 ± 16.7	10

n = number of surviving birds; SD = standard deviation

Body weight changes						
Measured dietary concentration [mg a.s./kg feed]	**Exposure period (Day 0 – Day 5)**		**Recovery period (Day 5 – Day 8)**		**Study period (Day 0 - Day 8)**	
	Mean [g] ± SD	**n**	**Mean [g] ± SD**	**n**	**Mean [g] ± SD**	**n**
Control	92.1 ± 11.6	10	73.0 ± 7.2	10	165.1 ± 14.9	10
326	97.3 ± 7.1	10	75.9 ± 7.1	9	171.9 ± 11.6	9
643	96.6 ± 10.6	10	69.3 ± 10.5	10	165.9 ± 20.6	10
1323	96.2 ± 10.2	10	78.4 ± 7.1	10	174.6 ± 13.8	10
2529	92.4 ± 11.6	10	76.3 ± 10.0	10	168.7 ± 17.9	10
5554	90.6 ± 6.7	10	72.4 ± 9.2	10	163.0 ± 14.4	10

n=number of surviving birds; SD = standard deviation

Feed consumption summary [g/bird/day]			
Measured dietary concentration	**Exposure period Day 0 – Day 5**	**Recovery period Day 5 – Day 8**	**Study period Day 0 - Day 8**
[mg a.s./kg feed]	**(Mean ± SD)**	**(Mean ± SD)**	**(Mean ± SD)**
Control	28.7 ± 8.2	46.0 ± 6.7	35.2 ± 11.4
326	28.8 ± 7.1	48.7 ± 5.9	36.3 ± 12.0
643	28.2 ± 6.9	45.4 ± 5.6	34.6 ± 10.7
1323	30.4 ± 8.4	50.7 ± 5.1	38.0 ± 12.6
2529	28.5 ± 8.7	44.9 ± 3.7	34.7 ± 10.9
5554	28.9 ± 10.8	47.1 ± 4.1	35.7 ± 12.7

Validity criteria:
All validity criteria were met.

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

Conclusion:
The dietary LC_{50} of BCS-CN88460 technical fed to the mallard duck was > 5554 mg a.s./kg feed or > 1360 mg a.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:	KCA 8.1.1.2/02; [redacted]; [redacted] T.; [redacted] M.; 2015; M-516743-01-1
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 dietary LC_{50} of BCS-CN88460 technical on Northern Bobwhite quail (*Colinus virginianus*).

Material and Methods:
BCS-CN88460 technical, Origin Batch No. 2013-006492, Purity 94.2 %.
Ten day-old Northern Bobwhite quail hatchlings were fed for five days nominal dietary levels of 0 (control), 313, 625, 1250, 2500, and 5000 mg a.s. (active ingredient)/kg 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 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 90° to 99 °F (32 to 37°C) with a photoperiod of 14 hours light:10 hours dark. The hatchlings were housed in galvanized steel brooders which measured approximately 91 cm (length) × 76 cm (width) × 25 cm (height).
Feed consumption and clinical observations for the hatchlings occurred daily. Hatchling body weights were taken on Day 0, Day 5, and Day 8. Post-mortem examinations were conducted on all control and high dose level birds and on 40 percent of the other birds sacrificed at study termination.
The statistical analyses on body weight were conducted using TOXSTAT.
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 BCS-CN88460 technical were determined as Control (0), 312, 618, 1235, 2546, and 5087 mg a.s./kg feed representing a recovery range of 98 to 102 % 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.

Nominal dietary concentrations [mg a.s./kg feed]	**Measured dietary concentrations of BCS-CN88460 [mg a.s./kg feed]**	
	Mean measured values (± SD)	**Percent of nominal**
Control	ND[a]	ND[a]
313	312 (8)	100%
625	618 (22)	99%
1250	1235 (24)	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 observed in any bird. Post-mortem examinations revealed no treatment related gross lesions or unusual observations.

Body Weight & Feed Consumption
Body weight was decreased at Day 5 and Day 8 for the 5087 mg a.s./kg feed level as compared to the control group. In addition, body weight change was reduced in the exposure period (Day 0 to Day 5) and the study period (Day 0 to Day 8) for the 5087 mg a.s./kg feed levels as compared to the control group. Feed consumption was also reduced in the 5087 mg a.s./kg feed during the study periods.
Body weight change was significantly reduced from the controls for the 618 mg a.s./kg 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./kg 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.

Body weight descriptive statistics						
Measured dietary concentration [mg a.s./kg feed]	Initiation Day 0		Day 5		Termination Day 8	
	Mean [g] ± SD	n	Mean [g] ± SD	n	Mean [g] ± SD	n
Control	25.1 ± 1.4	10	42.1 ± 2.9	10	54.7 ± 4.0	10
312	25.1 ± 1.4	10	42.1 ± 3.1	10	54.9 ± 4.7	10
618	25.0 ± 1.3	10	41.0 ± 2.0	10	51.9 ± 3.0	10
1235	25.1 ± 1.3	10	40.2 ± 3.1	10	52.1 ± 3.0	10
2546	25.0 ± 1.4	10	40.2 ± 3.5	10	51.7 ± 3.8	10
5087	25.0 ± 1.4	10	38.3 ± 3.6*	10	48.0 ± 3.8*	10

n=number of surviving birds; SD = standard deviation
*Statistically significant difference as compared to the control group by Dunnett's Test.

Body weight descriptive statistics						
Measured dietary concentration [mg a.s./kg feed]	Exposure period (Day 0 – Day 5)		Recovery period (Day 5 – Day 8)		Study period (Day 0 - Day 8)	
	Mean [g] ± SD	n	Mean [g] ± SD	n	Mean [g] ± SD	n
Control	17.1 ± 2.1	10	12.6 ± 1.7	10	29.7 ± 3.3	10
312	17.1 ± 2.2	10	12.7 ± 2.3	10	29.8 ± 4.2	10
618	16.0 ± 1.7	10	10.9 ± 1.4*	10	26.9 ± 2.8	10
1235	15.1 ± 1.9	10	11.9 ± 1.3	10	27.0 ± 2.0	10
2546	15.2 ± 2.7	10	11.5 ± 1.9	10	26.7 ± 2.8	10
5087	13.3 ± 3.2*	10	9.6 ± 1.0*	10	22.9 ± 3.2*	10

n=number of surviving birds; SD = standard deviation
*Statistically significant difference as compared to the control group by Dunnett's Test.

Feed consumption summary			
Measured dietary concentration [mg a.s./kg feed]	**Exposure [g/bird/d] (Mean ± SD)**	**Recovery [g/bird/d] (Mean ± SD)**	**Study period [g/bird/d] (Mean ± SD)**
	Day 0 – Day 5	**Day 5 – Day 8**	**Day 0 - Day 8**
Control	6.3 ± 0.5	7.7 ± 0.5	6.8 ± 0.9
312	6.3 ± 0.6	7.6 ± 0.6	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 ± 0.4	6.6 ± 0.7
5087	5.8 ± 0.5*	6.2 ± 0.9*	6.0 ± 0.7*

*Significantly different from the control.

Validity criteria:
All validity criteria were met.

Validity criteria according to OECD 205 (adopted 04 April 1984)	**Obtained in this study**
Mortality in the controls ≤ 10%	0.0 %
Concentration of the substance being tested should be at least 80 % of the nominal concentration	98 % - 102 %
Lowest treatment level should not result in compound-related mortality or other observable toxic effects	yes

Conclusion:
The dietary LC_{50} 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 5087 mg a.s./kg feed (937 mg a.s./kg body weight).

CA 8.1.1.3 Sub-chronic and reproductive toxicity to birds

Endpoint derivation for the chronic risk assessment in birds

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. This unclear result was a statistically significant 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 lowest level 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 contrary: there were no differences in 14-d chick bodyweight in the second study at any test level, not even in the top concentration of 2500 ppm. Thus, the finding in the initial study can be considered as false positive.

In the table 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 [ppm]	Mean Body-weight [g]	95% LCL	95% UCL	% effect to control	P-value*
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	8.79	0.001
Study M-611653-01-1 (referred as to 'second study')					
0	32.77	31.64	33.9	0.0	-
25	32.63	31.63	33.62	0.43	n.s.
250	31.58	30.27	32.9	3.62	n.s.
2500	32.86	31.68	34.04	-0.28	n.s.

LCL = lower confidence level; UCL = upper confidence level; * according to Dunnett T3 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.

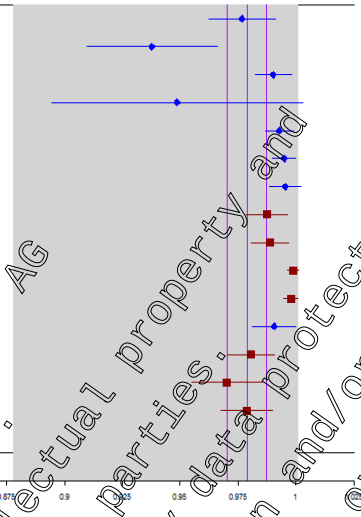
Study M-611590-01-1 (referred as to 'initial study')			
Concentration [ppm]	Mean 14-d survivors [%]	% effect to control	P-value*
0	99.6	0.0	-
37	98.8	0.8	0.9191
111	98.9	0.7	0.9191
333	99.9	-0.3	0.9191
1000	99.8	-0.2	0.9191
Study M-611653-01-1 (referred as to 'second study')			
0	99.06	0.0	-
25	**98.04**	**1.03**	**0.0323**
250	**97.02**	**2.06**	**0.0052**
2500	**97.88**	**1.19**	**0.0271**

* bold: values statistically significant (Jonckheere-Terpstra Step-Down Test p-value)

According to OECD Guideline 206, Table 3, 'Normal values for percent of hatchlings that survive to 14 days' are given as between 75 and 90%. 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):

Study	Treatment	mean	lwr	upr
EBMIL003	Control	0.977	0.962	0.99
07SRLS13C30	Control	0.938	0.91	0.97
07SRLS14C4	Control	0.99	0.982	1
044SRLS14C07	Control	0.949	0.895	1
044SRLS14C13	Control	0.993	0.987	1
044SRUS16C0100	Control	0.995	0.99	1
460 Initial	Control	0.996	0.989	1
460 Initial	37	0.987	0.978	1
460 Initial	111	0.989	0.981	1
460 Initial	333	0.999	0.996	1
460 Initial	1000	0.998	0.995	1
460 New	Control	0.991	0.981	1
460 New	25	0.98	0.97	0.99
460 New	250	0.97	0.955	0.98
460 New	2500	0.979	0.968	0.99
Pooled Control		**0.978**	**0.97**	**0.9887**



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 95% confidence interval. Blue color indicates point and confidence interval estimates from control and dark red 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 all values of the relevant study are well within the confidence interval of the historical control.

Overall assessment of NOEC

In conclusion a NOEC of 2500 ppm is proposed as endpoint for the exposure to quails.

For the mallard duck an overall endpoint of 1000 ppm was defined, representing the highest concentration tested.

Table 8.1.1.3- 1: Reproductive toxicity to birds

Test substance	Test design	Test species	Endpoint	Reference
Isoflucypram	22 weeks feeding chronic, reproduction	Mallard duck	NOEC NOEL	1000 ppm 60 mg a.s./kg bw/d	[redacted] T.; [redacted]; 2017; M-597500-01-1
	23 weeks feeding chronic, reproduction	Bobwhite quail	NOEC NOEL	1000 ppm 64 mg a.s./kg bw/d	[redacted], C.; [redacted], J.; [redacted], J.; [redacted]; 2018; M-611590-01-1 KCA 8.1.1.3/02
	23 weeks feeding chronic, reproduction	Bobwhite quail	NOEC NOEL	2500 ppm 172 mg a.s./kg bw/d	[redacted], J.; [redacted], C.; [redacted]; [redacted], 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** ([redacted] 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 level 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** ([redacted] et al. 2018, M-611653-01-1) 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 hatchling survival rate a significant effect was determined for all concentrations. At the highest test level the effect amounted to 1.19 %. It did not follow a dose response which was therefore not considered substance related.

A **reproduction study with mallards** ([redacted] & [redacted] 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 control and the missing of a dose-response relationship give reason **not to perform an EC10 or an EC20 calculation.**

Report:	KCA 8.1.1.3/01; [redacted] T.; [redacted]; 2017; M-597500-01-1
Title:	Toxicity of BCS-CN88460 on reproduction in the mallard duck (Anas platyrhynchos)
Report No.:	044SRLS14C02
Document No.:	M-597500-01-1
Guideline(s):	EU Directive 91/414/EEC Regulation (EC) No. 1107/2009 OCSPP 850.2300 OECD 206
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

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 Mallard ducks (*Anas platyrhynchos*).

Material and Methods:
BCS-CN88460 technical, Origin 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 feed. 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 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.

Nominal dietary concentrations [mg a.s./kg feed]	Measured dietary concentrations of BCS-CN88460 [mg a.s./kg feed]	
	Mean measured values (± SD)	Percent of nominal
Control	ND[a]	ND[a]
111	106.7 (13.6)	96 %
333	313.0 (16.9)	94 %
1000	923.1 (46.8)	92 %

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

Biological results

Dietary concentration

The nominal amounts of BCS-CN88460 in the dietary feed for the mallard reproduction study were administered at levels of 0 (control), 111, 333, and 1000 mg a.s./kg feed. The average measured amounts of BCS-CN88460 for Week 1, 5, 10, 15, and 20 were determined as 0, 107, 313, and 923 mg a.s./kg feed representing percent nominal values of 96 %, 94 %, and 92 % mg a.s./kg feed, respectively. These values correspond to daily dietary 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-CN88460			
Nominal dietary level ppm [mg a.s./kg feed]	Mean measured dietary level ppm [mg a.s./kg feed]	Percent of nominal (%)	Measured daily dietary dose [mg a.s./kg bw/day]
0 (control)	-	-	-
111	107	96%	8
333	313	94%	21
1000	923	92%	60

Adult Bird Mortality & Clinical Observations

No mortality or significant clinical symptoms were observed during the study in any treatment level. 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 bird was observed with a skin abrasion on the head in the 1000 ppm treatment level. These findings were considered incidental and not treatment related.

Adult Bird Bodyweight & Feed Consumption

The adult body weights in the mallard reproduction 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 consumption 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-filled 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 bird was observed with a skin abrasion on the head in the 1000 ppm treatment level. These findings were considered incidental and not treatment related.

Egg Reproductive Effects
There were no statistically significant adverse effects for the following egg reproductive endpoints: number of eggs laid, percent eggs set of eggs laid, 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 controls for the number of viable embryos and the number of live embryos. No significant difference occurred for the percent viable embryos of eggs set and the percent live embryos of viable embryos.

Hatchling Effects
There were no statistically significant effects from the control for the initial number hatched, 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 at any 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 OECD 206 (adopted 04 April 1984)	**Obtained in this study**
Mortality in the controls ≤ 10%	0.0 %
The average number of 14 day old survivors per hen in the controls should be at least 14 for mallard ducks.	35
The average egg shell thickness for the control group should be at least 0.34 mm for mallard duck.	0.34 mm
Concentration of the substance being tested should be at least 80 % of the nominal concentration	92 % - 96 %

Conclusion:
The No Observed Effect Level (NOEL) for parental toxicity and reproduction endpoints of Mallard duck exposed to BCS-CN88460 technical was 1000 ppm (nominal test level) with a measured concentration of 923 mg a.s./kg feed or the mean achieved dose of 60 mg a.s./kg bw/day. The Lowest Observed Effect Level (LOEL) was > 1000 ppm (nominal test level) equivalent to the measured concentration of 923 ppm or the achieved dose of 60 mg a.s./kg bw/day.

Report: KCA 8.1.1.3/02; [redacted], C.; [redacted], J.; [redacted], J.; [redacted]; 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: **yes**

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 Bobwhite Quail (*Colinus virginianus*).

Material and Methods:
BCS-CN88460 technical, Origin Batch No. 2013-006492, Purity 94.2 %.
Adult bobwhite quail (*Colinus virginianus*) were exposed to BCS-CN88460 technical for approximately 23 weeks to nominal dietary levels of 0 (control), 37, 111, 333, and 1000 ppm (mg a.s./kg feed). Bobwhite quail were approximately 32 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 of the study was conducted from 21 April 2016 to 7 November 2016.

Results:
Dietary Concentration
The nominal amounts of BCS-CN88460 technical in the dietary feed were administered at levels of 0 (control), 37, 111, 333, and 1000 ppm. The average measured amounts of BCS-CN88460 technical for Week 1, 5, 10, 15, and 20 were determined as 0, 37, 106, 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.s./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.s./kg feed]	**Measured dietary level ppm [mg a.s./kg feed]**	**Percent of nominal (%)**	**Measured daily dietary dose [mg a.s./kg bw/day]**
0 (control)	0	-	-
37	37	99	2
111	106	96	7
333	327	98	22
1000	974	97	64

Adult Bird Observations
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 level) 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 had 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 111 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 (band no. 232) had observations resulting from pen mate aggression that resulted in euthanizing both female and male pen mates. At the conclusion of the exposure period, all surviving birds were necropsied. Necropsy of the adult birds showed no apparent treatment-related effects.

Adult Bird Body Weight

The adult body weights in the quail reproduction study were measured prior to dosing 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 Effects

Data for the egg production endpoints; eggs laid, 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 eggs set, and percent live embryos of viable embryos. There were no statistically significant differences for any egg production or 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 weights in the 37, 333, and 1000 ppm treatment levels. The historical control range for the number of 14-day survivor hatchling 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. All treatment level means were within the historical control range 35.2 grams (± 3.1 g). Therefore the NOEC for hatchling effects was determined to be 1000 ppm.

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

[a] Statistically significant difference as compared to control (P <0.05).
[b] The historical control range for the number of 14-day survivor hatchling body weight endpoint is 29.7 grams to 38.5 grams based on studies conducted from 2011 to 2015 (n=6). Therefore, all levels fall within the historical control range and statistical findings are not considered treatment related.

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 a.s./kg bw/day.

Report: KCA 8.1.1.3/03; [redacted] J.; [redacted] C.; [redacted]; [redacted], B.; 2018; M-611653-01-1
Title: BCS-CN88460 Technical: A reproduction study with the northern bobwhite quail (Colinus virginianus)
Report No.: 007SRUS17C006
Document No.: M-611653-01-1
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: yes

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 Bobwhite Quail (*Colinus virginianus*).

Material and Methods:
Adult bobwhite quail (*Colinus virginianus*) were exposed to BCS-CN88460 technical for approximately 23 weeks to nominal dietary levels of 0 (control), 25, 250, and 2500 ppm (mg a.s./kg feed). Bobwhite quail 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 of the study was conducted from 23 March 2017 to 9 October 2017.

Results:

Dietary Concentration

The nominal amounts of BCS-CN88460 technical in the dietary feed were administered at levels of 0 (control), 25, 250, and 2500 ppm. The average measured amounts of BCS-CN88460 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-CN88460			
Nominal Dietary Level ppm (mg a.s./kg feed)	**Measured Dietary Level ppm (mg a.s./kg feed)**	**Percent of Nominal (%)**	**Measured Daily Dietary Dose (mg a.s./kg bw/day)**
0 (control)	0	-	-
25	24	97	2
250	238	95	18
2500	2377	95	174

Adult Bird Observations

Clinical observations of adult birds exhibited no treatment related signs of toxicity. Minor occurrences of feather loss on head/back as well as skin and 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. 726) in the 25 ppm treatment level was unable to maintain normal body position with its head; no observations indicated it was injury related. Adult observation started from 29 June 2017 to 3 July 2017. Abrasions were observed on both feet during daily observation check. However, 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 female bird (band no. 969) in the control group, two birds (female band no. 901 and male band no. 726) in the 25 ppm level, and one female bird (band no. 938) in the 250 ppm level. 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. At the conclusion of the exposure period, all surviving birds were necropsied. Necropsy of the adult birds showed no apparent treatment-related effects. There was one pair (band no. 706 and 906) in the 25 ppm treatment level that mistakenly 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.

Adult Bird Body Weight

The adult body weights in the quail reproduction study were measured prior to dosing 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 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 level 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 relative to the control were not observed for any treatment level or endpoint except percent number 14-day survivors of total hatched endpoint. 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 7 November 2016. This statistical finding is not considered to be treatment related, as the treatment hatchling 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, the NOEC is 2500 ppm for hatchling effects.

Percent 14-day survivors of total hatched		
Nominal treatment (ppm)	Mean [%]	Standard Deviation [g]
Control	99.1	0.019
25	98.0[a]	0.020
250	97.1[a]	0.029
2500	97.9[a]	0.023

[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 reproductive endpoints of northern bobwhite quail exposed to BCS-CN88460 technical was 2500 ppm (nominal test level) with a measured concentration of 2377 ppm or 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
Rat	Acute, oral	LD_{50}, male, female	> 2000 mg a.s./kg bw	[redacted], V:; 2014; M-485872-01-1 KCA 5.2.1/01

CA 8.1.2.2 Long-term and reproduction toxicity to mammals

Endpoint derivation for the chronic risk assessment in mammals

This evaluation analyses the toxicity data available for isoflucypram (BCS-CN88460) under the aspect of relevance of findings for wild mammals long-term risk assessment. Especially findings related to the actual survival and the reproductive 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 subchronic rat study and the developmental toxicity studies) were evaluated as well.

The toxicological effects seen with BCS-CN88460 in a 90-day rat study, in a rat reproduction study and in rat and rabbit developmental studies are summarised in the table below.

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

Study type species *dose levels tested*	Overall NOAEL	Findings at Lowest Effect Level	Ecotox NOAEL	Ecotox relevant	Reference
90 day Wistar rat *0 – 100 – 300 - 1000 ppm*	300 ppm	1000 ppm: bw in both sexes ↓, bilirubin concentration in both sexes ↓, liver: organ weight in females ↑ kidney: hyaline droplets in males thyroid gland: minimal follicular cell hypertrophy ↑	1000 ppm (63.5 mg/kg bw/day)	none	[redacted], 2017; M-587478-02-1 KCA 5.3.2/01
2-generation reproduction Wistar rat *0 – 150 – 450 – 1200 ppm*	1200 ppm	1200 ppm: liver: organ weight in both sexes ↑	1200 ppm (**92.9 mg/kg bw/day**)	none	[redacted], R., 2018; M-612750-02-1 KCA 5.6.1/01
developmental rat Sprague Dawley rat *0 – 25 – 125 – 625 mg/kg bw/day*	Maternal and fetal toxicity: 125 mg/kg bw/day	625 mg/kg: mean food consumption between GD 6-8 ↓, bilirubin concentration and mean alkaline phosphatase activity ↓, liver: organ weight ↑ delayed ossification	125 mg/kg bw/day	delayed ossification	[redacted], P.; 2017; M-602126-01-1 KCA 5.6.2/01
developmental rabbit New Zealand White *0 – 10 – 70 – 500 mg/kg bw/day*	Maternal toxicity: 70 mg/kg bw/day development: 500 mg/kg bw/day	500 mg/kg: one abortion, mean bw ↓, food consumption ↓, liver: organ weight ↑	70 mg/kg bw/day	abortion	[redacted], I.; 2017; M-588469-01-1 KCA 5.6.2/02

↓: decrease; ↑: increase;
bw = body weight
Bold: endpoint used in risk assessment

In the 90 d subchronic study in rats some findings were reported at the 1000 ppm dose level, as a decrease in body weight, a lower bilirubin concentration or findings of hyaline droplets in males. Bilirubin concentration and hyaline 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 taken 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 increased 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 low.

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, note that only low effects were found at the highest dose rate of 500 mg/kg bw/day).

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

Substances with a high bioaccumulation potential could theoretically bear a risk of secondary poisoning for birds and mammals if feeding on contaminated prey like fish or earthworms. For organic chemicals, a log P_{OW} 3 is used to trigger an in-depth evaluation of the potential for bioaccumulation. As the log P_{OW} of the active substance isoflucypram 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.

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

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

Amphibians and Reptiles

Currently no test methods are established to assess the population relevant effects of chemicals to amphibians or reptiles. Since isoflucypram is of low toxicity in birds and laboratory rodents, no risk for reptiles and amphibians is to be expected.

CA 8.1.5 Endocrine 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; [redacted], 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 evidence 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 than 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 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 non-mammalian vertebrates.

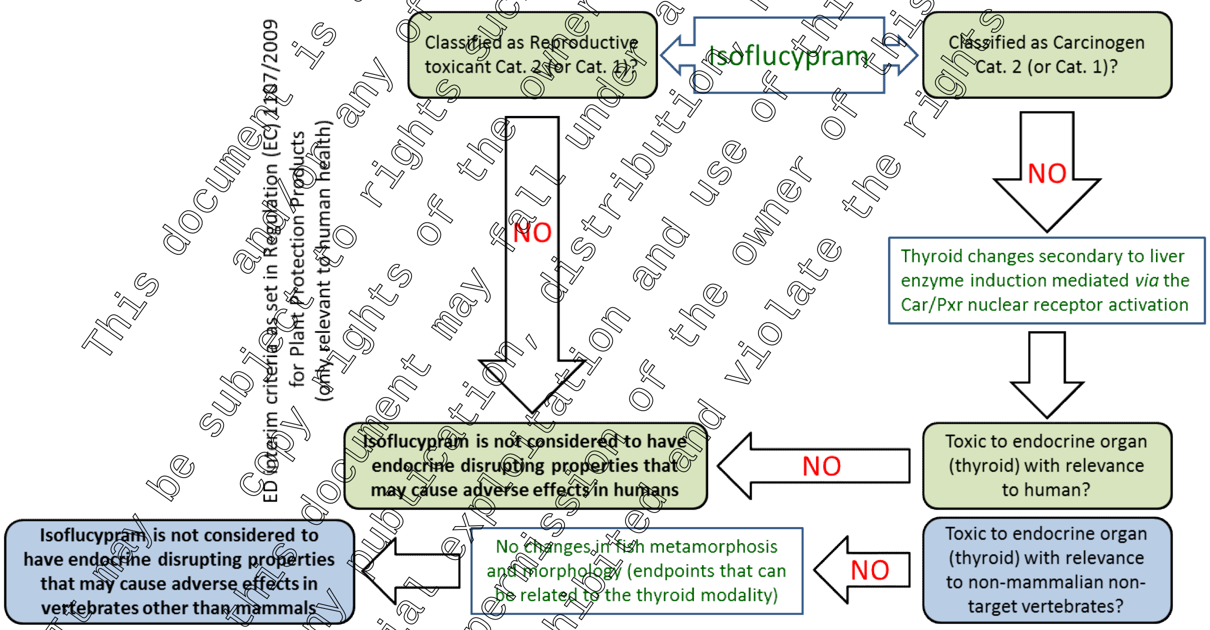


Figure 1: Decision tree applied to the assessment of endocrine-disrupting properties of isoflucypram. The upper part (green cells) is based on the interim criteria as documented by toxicological investigation in mammals; it therefore applies to wild mammals. The lower part (blue cells) concerns non-mammal vertebrates (i.e., fish) where the thyroid modality was investigated. Adapted from the document "*Screening of available evidence on chemical substances for the identification of endocrine disruptors according to different options in the context of an Impact Assessment - Specific Contract SANTE/2015/E3/SI2.706218*" prepared for the European Commission in 2016.

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

Table 8.2- 1: Endpoints used in risk assessment and studies for isoflucypram

Test substance	Test species	Endpoint		Reference
Isoflu-cypram	Fish, acute *Pimephales promelas*	96 h LC_{50}	**0.081 mg a.s./L** (nom)[A]	[redacted]; 2018; M-542897-02-1 KCA 8.2.1/01
	Fish, acute *Oncorhynchus mykiss*	96 h LC_{50}	0.098 mg a.s./L (nom)[A]	[redacted]; 2015; M-543443-01-1 KCA 8.2.1/02
	Fish, acute *Cyprinodon variegatus*	96 h LC_{50}	0.544 mg a.s./L (mm)	[redacted]; [redacted]; 2015; M-537137-01-1 KCA 8.2.1/03
	Fish, acute *Pimephales promelas, Oncorhynchus mykiss, Cyprinodon variegatus*	96 h LC_{50}	**0.1628 mg a.s./L**[B]	Geometric mean acc. to new aquatic guidance document (EFSA Journal 2013;11(7):3290)
	Fish, chronic (ELS) *Pimephales promelas*	33 d NOEC	**0.0156 mg a.s./L** (nom)	[redacted]; [redacted]; 2017; M-580247-01-1 KCA 8.2.2.1/01
	Fish, chronic (ELS) *Cyprinidon variegatus*	35 d NOEC	0.025 mg a.s./L (mm)	[redacted]; [redacted]; 2016; M-575119-01-1 KCA 8.2.2.1/02
	Fish, chronic (ELS) *Pimephales promelas, Cyprinodon variegatus*	35 d NOEC	0.0197 mg a.s./L[B]	Geometric mean acc. to new aquatic guidance document (EFSA Journal 2013;11(7):3290)
	Fish, BCF flow through *Lepomis macrochirus*	BCF	**370** (kinetic BCF lipid normalized and growth corrected)	[redacted]; [redacted], R.; 2017; M-610008-01-1 KCA 8.2.2.3/01
	Invertebrate, acute *Daphnia magna*	48 h EC_{50}	**0.201 mg a.s./L** (gmm)	[redacted]; 2016; M-574184-01-1 KCA 8.2.4.1/01
	Invertebrate, acute *Americamysis bahia*	96 h LC_{50}	0.270 mg a.s./L (mm)	[redacted]; [redacted]; [redacted]; 2016; M-547041-01-1 KCA 8.2.4.2/01
	Invertebrate, acute *Daphnia magna, Americamysis bahia*	EC_{50}	**0.233 mg a.s./L**[B]	Geometric mean acc. to new aquatic guidance document (EFSA Journal 2013;11(7):3290)
	Invertebrate, chronic *Daphnia magna*	21 d EC_{10}	**0.0661 mg a.s./L** (mm)	[redacted]; 2017; M-593961-01-1 KCA 8.2.5.1/01
	Invertebrate, chronic *Americamysis bahia*	28 d NOEC	0.079 mg a.s./L (mm)	[redacted]; [redacted]; [redacted]; [redacted]; 2016; M-567966-01-1 KCA 8.2.5.2/01
	Invertebrate chronic *Leptocheirus plumulosus*	28 d NOEC	11 mg a.s./kg (mm)	[redacted]; 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)	[redacted]; [redacted]; [redacted]; 2016; M-547035-01-1 KCA 8.2.5.2/03
	Invertebrate, chronic *Hyalella azteca*	28 d NOEC 35 d NOEC 42 d NOEC	95 mg a.s./kg (mm) 44 mg a.s./kg (mm) 95 mg a.s./kg (mm)	[redacted]; 2017; M-585874-02-1 KCA 8.2.5.2/04
	Sediment dweller *Chironomus dilutus*	61 d NOEC 61 d LOEC	**85 mg a.s./kg** (mm) > 85 mg a.s./kg (mm)	[redacted]; 2017; M-596883-01-1 KCA 8.2.5.4/01
	Green algae, *Pseudokirchneriella subcapitata*	72h-E_rC_{50} 72h-E_yC_{50}	> 2.02 mg a.s./L (gmm) > 2.02 mg a.s./L (gmm)	[redacted]; 2017; M-586715-01-1 KCA 8.2.6.1/01
	Blue green algae, *Anabaena flos-aquae*	72h-E_rC_{50} 72h-E_yC_{50}	4.8 mg a.s./L (gmm) 3.4 mg a.s./L (gmm)	[redacted], J. R.; [redacted], J. R.; [redacted], K. H.; 2017; M-605074-01-1 KCA 8.2.6.2/01
	Marine diatom, *Skeletonema costatum*	72h-E_rC_{50} 72h-E_bC_{50}	> 3.2 mg a.s./L (gmm) 2.2 mg a.s./L (gmm)	[redacted], J. R.; [redacted]; [redacted], J. R.; [redacted], K. H.; 2017; M-604811-01-1 KCA 8.2.6.2/02
	Freshwater diatom, *Navicula pelliculosa*	72h-E_rC_{50} 72 h-E_yC_{50}	**> 2.0 mg a.s./L** (gmm) **> 2.0 mg a.s./L** (gmm)	[redacted], J. R.; [redacted]; [redacted], J. R.; [redacted], K. H.; 2017; M-604809-01-1 KCA 8.2.6.2/03
	Aquatic macrophyte, *Lemna gibba*	7d-E_rC_{50}	**> 3.02 mg a.s./L** (gmm)	[redacted]; 2017; M-593965-01-1 KCA 8.2.7/01
BCS-CN88460-carboxylic acid (M12)	Fish, acute *Oncorhynchus mykiss*	96 h LC_{50}	**> 33.5 mg p.m./L** (gmm)	[redacted]; 2017; M-587655-01-1 KCA 8.2.1/04
	Invertebrate, acute *Daphnia magna*	48 h EC_{50}	**> 24 mg p.m./L** (nom)	[redacted]; 2016; M-573296-01-1 KCA 8.2.4.1/02
	Green algae, *Pseudokirchneriella subcapitata*	72h-E_rC_{50}	> 35.1 mg p.m./L (gmm)	[redacted]; 2017; M-587659-01-1 KCA 8.2.6.1/02

Bold: endpoints used in risk assessment
Nom = nominal concentrations, mm = mean measured concentration, gmm = geometric mean measured concentration
[A] Endpoint corrected for purity. In study report uncorrected values are cited.
[B] Endpoint based on geometric mean of the given relevant endpoints of acute or chronic studies with the active substance. Detailed information given below under 'Selection of endpoints for Tier 2 risk assessments with the active substance'

Selection of endpoints for Tier 2 risk assessments with the active substance

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

In case of isoflucypram 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 Sensitivity Distribution (SSD).

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 laid 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 fish testing. Data for the different fish species: the rainbow trout (*Oncorhynchus mykiss*), the fathead minnow (*Pimephales promelas*) and the sheepshead minnow (*Cyprinodon variegatus*).
The available data are not sufficient to use tier 2B, the Species Sensitivity Distribution but allow the use of tier 2A, the geomean assessment factor approach.
If the 96h LC_{50} values for the three species, based on the real content of the active ingredient are chosen, the following is observed:

Table 8.2- 2: Geometric mean for fish acute

Species	Species (Scientific name)	96 h LC_{50} [mg a.s./L]	95% confidence limits [mg a.s./L]
Rainbow trout	*Oncorhynchus mykiss*	0.098	0.074 - 0.131
Fathead minnow	*Pimephales promelas*	0.081	0.068 - 0.093
Sheepshead minnow	*Cyprinodon variegatus*	0.544	0.472 - 0.626
Geometric mean:	-	**0.163**	

For the taxonomic group of fish the data requirements (one species) were exceeded. Data for three species are available. Therefore the geomean 96 h LC_{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 waters (EFSA, 2013) an assessment of this has to be made when the difference in sensitivity exceeds 1 or 2 orders of magnitude. In case of fish a factor of 100 should not be exceeded.
The highest and the lowest 96 h LC_{50} derived for fish and isoflucypram differ by a factor of 6.7. Therefore the use of the geomean approach for the fish acute 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 fish, resulting from a Fish Early Life Stage (FELS) test with fathead minnow. The acute RAC based on tier 2A for fish is even a factor of 12.1 below the tier 2a chronic fish value based on the two existing FELS studies for fathead minnow and sheepshead minnow.
This demonstrates that the tier 2A fish acute 96 h LC_{50} 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 to use 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 EC_{50} values (based on the real content of the active ingredient) for the two species are available:

Table 8.2- 3: Geometric mean for crustacean acute

Species	Species (Scientific name)	EC_{50}	95% confidence limits
		[mg a.s. /L]	
Water flea	*Daphnia magna*	0.201 (48h)	176 - 229
Mysid shrimp	*Americamysis bahia*	0.270 (96h)	0.230 - 0.420
Geometric mean:	**-**	**0.233**	

For the taxonomic group of crustaceans the data requirements (one species) were exceeded. Data for two species are available. Therefore the geomean EC_{50} for these two 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 waters (EFSA, 2013) this has to be assessed when the difference in sensitivity exceeds 2 orders of magnitude. In case of crustaceans a factor of 100 should not be exceeded.
The highest and the lowest EC_{50} derived for crustaceans in case of isoflucypram 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 chronic crustacean data can be used to check the appropriateness of the tier 2A related regulatory acceptable concentration (RAC) for acute crustaceans of 0.00233 mg/L. This RAC is a factor of 33.9 below the lowest chronic NOEC for crustaceans which was observed for *Americamysis bahia*.
This demonstrates that the tier 2A for crustaceans acute based on EC_{50} values for *Daphnia magna* and *Americamysis bahia* of 0.233 and the resulting tier 2A RAC of 0.00233 mg/L is protective and can therefore be used within the aquatic risk assessment of isoflucypram.

Fish chronic

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" than it might be appropriate to use tier 2A. In case of isoflucypram 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 (*Pimephales promelas*) and sheepshead 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 (Scientific name)	NOEC [mg a.s. /L]
Fathead minnow	*Pimephales promelas*	0.0156 (33d)
Sheepshead minnow	*Cyprinodon 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 by 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 if 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 chronic tier 2A for fish and isoflucypram 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.

CA 8.2.1 Acute toxicity to fish

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:	KCA 8.2.1/01; [redacted]; 2018; M-542897-02-1
Title:	Amendment no. 1: BCS-CN88460 (tech.) - Acute toxicity to fish (Pimephales promelas) under static conditions
Report No.:	EBLNN356
Document No.:	M-542897-02-1
Guideline(s):	EPA-FIFRA § 72-1/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
GLP/GEP:	yes

Material and methods

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 cm ± 0.2 cm (Mean ± SD) Mean body weight: 0.2 g ± 0.1 g (Mean ± SD)
Test solutions	Nominal concentrations: 0.0251, 0.0502, 0.101, 0.201 and 0.401 mg a.s./L 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, 0.2155 and 0.4055 mg a.s./L Samples were taken from all test chambers 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 concentration (replicates): 1 No. of vessels per control (replicates): 1 No. of vessels per solvent control (replicates): 1
Organisms per replicate	No. of organisms per vessel: 10
Exposure	Static Total exposure duration: 96 hours
Test Vessel Loading	0.050 g fish/L test medium
Feeding during test	None
Test conditions	Temperature: 20.5 – 22.0°C Photoperiod: 16 hours light / 8 hours dark pH: 7.0 – 7.2 Dissolved oxygen saturation: 94 – 112% Hardness: 40 – 60 mg $CaCO_3$/L
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 ToxRat, 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.

Results

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

Analytical results:
Measured concentrations were 81 to 109% of nominal values and were stable throughout the test. Therefore, the results of this study are based on nominal concentrations.

Nominal Concentration (mg a.s./L)*	Arithmetic mean measured concentration (mg a.s./L)	% of nominal concentrations		
		Day 0	Day 2	Day 4
0.0251	0.0249	109	97	93
0.0502	0.0494	103	98	99
0.101	0.0924	97	81	97
0.201	0.2155**	109	106	-
0.401	0.4055**	103	99	-

* Values corrected for purity (94.2%). Nominal values, which were not corrected for purity are stated in study report.
**Mean measured concentration (Day 0, 2)

Biological results:

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

Exposure time (hours)	4	24	48	72	96
Nominal conc. (mg a.s./L)*	No of dead (%)	No of dead (%)	No of dead (%)	No of dead (%)	No of dead (%)
Control	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Solvent Control	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
0.0251	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
0.0502	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
0.101	0 (0)	2 (20)	6 (60)	9 (90)	9 (90)
0.201	0 (0)	10 (100)	10 (100)	10 (100)	10 (100)
0.401	0 (0)	10 (100)	10 (100)	10 (100)	10 (100)

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

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

LC_{50} 96 hours (95% C.I.):	**0.081 mg a.s. / L* (0.068 – 0.093 mg a.s./L)**
LOEC: lowest concentration with an significant effect compared to the control	0.101 mg a.s./L*
NOEC: highest concentration without an significant effect compared to the control	0.0502 mg a.s./L*

* Values corrected for purity (94.2%).Values not stated in study report.

Report:	KCA 8.2.1/02; [redacted]; 2015; M-543443-01-1
Title:	BCS-CN88460 (tech.) - Acute toxicity to fish (Oncorhynchus mykiss) under static conditions
Report No.:	EBLNN024
Document No.:	M-543443-01-1
Guideline(s):	EU Directive 91/414/EEC Regulation (EC) No 1107/2009 (2009) US EPA OCSPP 850.1075 EPA-FIFRA § 72-1/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)1
Guideline deviation(s):	none
GLP/GEP:	**yes**

Material and methods

Test material	BCS-CN88460 techn. BCS batch code: BCS-CN88460-01-06; Origin batch ID.: 2013-006492; Specification number: 10200028196 purity: 94.2% w/w
Guideline(s) adaptation	None specified
Test species	Rainbow trout (*Oncorhynchus mykiss*)
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: 4.2 ± 0.6 cm (Mean ± SD) Mean body weight: 0.8 ± 0.4 g (Mean ± SD)
Test solutions	Nominal concentrations: 0.0400, 0.0801, 0.160, 0.320 and 0.641 mg a.s./L 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 Solvent control: 0.1 ml/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
Test Vessel Loading	0.20 g fish/L test medium
Feeding during test	None
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 – 102% Hardness: 40 – 60 mg $CaCO_3$/L
Parameters Measured / Observations	Observations of mortality and signs of poisoning were made at 4, 24, 48, 72 and 96 hours. Discrete measurements of temperature, dissolved oxygen and pH-value were obtained at test initiation and at 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 ToxRatPro Version 2.10, which estimated the LC_{50} using Weibull analysis.

Results

Validity criteria	Required	Obtained
Mortality in control during test	≤ 10%	0 %
Dissolved oxygen saturation	≥ 60%	91 - 102 %

Analytical results:
Recoveries were between 81 and 127% (see table below). The analytical results confirm a correct dosing and the stability of the test 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 (mg a.s./L)*	Arithmetic mean measured concentrations** (mg a.s./L)	% of nominal concentrations			
		Day 0	Day 1	Day 2	Day 4
0.0400	0.0397	115	-	98	84
0.0801	0.0760	112	-	92	81
0.160	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./L test 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

Exposure time (hours)	**4**	**24**	**48**	**72**	**96**
Nominal conc.* (mg a.s./L)	**No of dead (%)**	**No of dead (%)**	**No of dead (%)**	**No of dead (%)**	**No of dead (%)**
Control	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Solvent Control	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
0.0400	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
0.0801	0 (0)	0 (0)	2 (20)	2 (20)	2 (20)
0.160	0 (0)	10 (100)	10 (100)	10 (100)	10 (100)
0.320	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)
0.641	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)

* Values corrected for purity (94.2%). Values not stated in study report.

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

LC_{50} 96 hours (95% C.I.):	**0.098 mg a.s./L* (0.074 – 0.131 mg a.s./L)***
LOEC: lowest concentration with an significant effect compared to the control	0.0801 mg a.s./L*
NOEC: highest concentration without an significant effect compared to the control	0.0400 mg a.s./L*

* Values corrected for purity (94.2%). Values not stated in study report.

Report: KCA 8.2.1/03; [redacted]; [redacted]; 2015; M-537137-01-1
Title: Acute toxicity of BCS-CN88460 technical to the sheepshead minnow (Cyprinodon variegatus) under static conditions
Report No.: EBLNN023
Document No.: M-537137-01-1
Guideline(s): EU Directive 91/414/EEC
Regulation (EC) No. 1107/2009
US EPA OCSPP 850.1075
Guideline deviation(s): none
GLP/GEP: **yes**

Material and methods

Test material	BCS-CN88460 techn. Origin batch ID: 2013-006492; Specification number: 10200028196; purity: 94.2% w/w
Guideline(s) adaptation	None specified
Test species	Sheepshead Minnow (*Cyprinodon variegatus*)
Acclimation	More than 14 days No mortalities during 48 hours prior to testing, no treatments for disease
Organism age/size at study initiation	Mean length: 29.6 mm ± 2.1 mm Mean body weight: 0.579 g ± 0.163g
Test solutions	Nominal concentrations: 0.0625, 0.125, 0.250, 0.500 and 1.00 mg a.s./L Corresponding mean measured concentrations: 0.0462, 0.0886, 0.196, 0.395 and 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 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
Test Vessel Loading	0.19 g fish/L test medium
Feeding during test	None
Test conditions	Temperature: 21.6 – 22.1°C Photoperiod: 16 hours light / 8 hours dark Light intensity: 751 - 822 lux pH: 7.9 – 8.3 Dissolved oxygen: 6.2 mg/L – 6.8 mg/L (81-93% oxygen saturation) Gentle aeration was added to each test chamber to achieve > 60% saturation throughout the test Salinity: 23 ‰
Parameters Measured / Observations	Observations of mortality, sublethal symptoms and behavioral effects, such as vertical orientation, on bottom, erratic behavior, labored respiration and dark coloration 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	Samples of each test concentration were taken at day 0 and day 4 of the testing period. They were analysed using Liquid Chromatography-Mass Spectrometry/Mass Spectrometry (LC-MS/MS).

Data analysis	The LC_{50} values were calculated using CETIS statistical software and were determined by the characteristics of the data, i.e. the number of concentrations in which survival was between 0 and 100 percent and the 95% confidence intervals. The NOEC and LOEC were empirically determined based upon observation data including lethal and sublethal effects.

Results

Validity criteria	Required	Obtained
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 arithmetic 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./L)	Day 0 % Nominal	Day 4 Measured concentration (mg a.s./L)	Day 4 % Nominal	Arithmetic mean measured concentration (mg a.s./L)	Percent mean measured concentration
Control	< 0.005	NA	< 0.005	NA	< 0.005	NA
Solvent Control	< 0.005	NA	< 0.005	NA	< 0.005	NA
0.0625	0.0513	82%	0.0411	66%	0.0462	74%
0.125	0.101	81%	0.0764	61%	0.0886	71%
0.250	0.207	83%	0.186	74%	0.196	79%
0.500	0.434	87%	0.355	71%	0.395	79%
1.000	0.896	90%	0.841	84%	0.869	87%

Biological results:

Observations
Common carp in the control, solvent control, 0.0462 mg a.s./L and 0.0886 mg/L groups appeared healthy and normal throughout the test. After 96-hours of exposure, two fish in the 0.196 mg a.s./L treatment group showed dark colorations. In the 0.395 mg a.s./L treatment group one fish was dead and eight fish showed dark coloration. After 96 hours. In the 0.869 mg a.s./L treatment group all fish 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 (0)	0 (0)
0.0462	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
0.0886	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
0.196	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
0.395	0 (0)	0 (0)	0 (0)	0 (0)	1 (10)
0.869	0 (0)	0 (0)	4 (40)	10 (100)	10 (100)

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

LC_{50} 96 hours (95% C.I.):	**0.544 mg a.s. / L (0.472 – 0.626 mg a.s. / L)**
LOEC: lowest concentration with an significant effect compared to the control	0.196 mg a.s. / L
NOEC: highest concentration without an significant effect compared to the control	0.0886 mg a.s. / L

Report: KCA 8.2.1/04; [redacted], 2017; M-587655-01-1
Title: BCS-CN88460-carboxylic-acid (BCS-CY26497) - Acute toxicity to rainbow trout (Oncorhynchus mykiss) under static conditions - Final report
Report No.: EBLNN193
Document No.: M-587655-01-1
Guideline(s): EPA-FIFRA § 72-1/SEP-EPA-540/9-85-006 (1982/1985) OCSPP 850.1075 (Public Draft, 1996) OECD No. 203 (rev.1992) JMAFF, 12 Nousan No. 8147 (2000)
Guideline deviation(s): none
GLP/GEP: **yes**

Material and methods

Test material	BCS-CN88460-carboxylic-acid (BCS-CY26497) lot/batch SES 12631-19-9 Tox No. 20054-00 Purity 98.8% w/w
Guideline(s) adaptation	None specified
Test species	Rainbow trout (*Oncorhynchus mykiss*)
Acclimation	At least 14 days, fed daily with commercial trout food Health during acclimation: less than 5% mortality

Organism age/size at study initiation	Mean length: 3.9 ± 0.4 cm Mean body weight: 0.5 ± 0.2 g
Test solutions	Nominal concentrations: 6.18, 12.4, 24.7, 49.4 and 98.8 mg p.m./L Geometric mean measured concentrations: 6.69, 12.9, 25.0, 28.3 and 33.5 mg p.m./L Controls: water and solvent control Evidence of undissolved material: At test start 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 test material was observed over the whole exposure period.
Replication	No. of vessels per concentration (replicates): 1 No. of vessels per control (replicates): 1 No. of vessels per solvent control (replicates): 1
Organisms per replicate	No. of organisms per vessel: 10
Exposure	Static conditions Total exposure duration: 96 hours
Test Vessel Loading	0.13 g fish/L test medium
Feeding during test	No food 48 hours before and during study
Test conditions	Temperature: 13.1 - 13.9°C Photoperiod: 16 hours light / 8 hours dark Light intensity: not specified pH: 6.6 - 7.3 Water hardness: 40 - 60 mg $CaCO_3$/L Dissolved oxygen: 90 - 96% saturation Conductivity: < 10 µS/cm
Parameters Measured / Observations	Fish were observed for mortalities and signs of intoxication for the first four hours after start of exposure and then daily thereafter. Dissolved oxygen, water temperature and pH values were determined daily.
Chemical analysis	The chemical analysis of BCS-CN88460-carboxylic-acid (BCS-CY26497) (in water 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 measured concentrations was performed according to OECD 23.

p.m. = pure metabolite

Results

Validity criteria	Required	Obtained
Mortality in control during test	≤ 10%	0%
Dissolved oxygen saturation	≥ 60%	≥ 90%

Analytical 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 solubility in the medium. The analytical results ranged between 10 % of nominal at test start and 43% of nominal at day 4. 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 BCS-CN88460-carboxylic-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 mg 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./L)	% of nominal concentrations*			
		Day 0	Day 1	Day 2	Day 4
6.18	6.69	109	108	108	108
12.4	12.9	103	104	104	104
24.7	25.0	96	104	104	101
49.4	33.5	32	86	86	69
98.8	28.3	10	39	39	31

* Average of two detections (presented are rounded values, all calculations were done with Microsoft® Excel)

<u>Biological results:</u>

Observations
In the controls no mortalities of sub-lethal 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	24	48	72	96
geometric mean [mg p.m./L]	No. of dead (%)	No. of dead (%)	No. of dead (%)	No. of dead (%)	No. of dead (%)
Control	0	0	0	0	0
Solvent control	0	0	0	0	0
6.69	0	0	0	0	0
12.9	0	0	0	0	0
25.0	0	0	0	0	0
28.3	0	0	0	0	0
33.5	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

CA 8.2.2 Long-term and chronic toxicity to fish

CA 8.2.2.1 Fish early life stage toxicity test

Report: KCA 8.2.2.1/01; [redacted]; 2017; M-580247-01-1
Title: Early-life stage toxicity of BCS-CN88460 (tech.) to fish (Pimephales promelas)
Report No.: EBLNN029
Document No.: M-580247-01-1
Guideline(s): EU Directive 91/414/EEC
Regulation 107/2009 (Europe)
US EPA OCSPP 850.1400
Guideline deviation(s): yes, see report
GLP/GEP: **yes**

Material and methods

Test material:	BCS-CN88460 (tech.) Origin Batch No: NLL 8674-28-2 Batch Code: BCS-CN88460-01-05 purity: 98.0% w/w
Guideline(s) adaptation	None specified
Test species:	Fathead minnow (*Pimephales promelas)*
Organism Age at Experimental Start:	Embryos less than 24 h old
Test solutions	Nominal concentrations: 0.48, 1.53, 4.88, 15.6, 50.0 µg a.s./L Corresponding mean measured concentrations: 0.451 – 1.36 – 4.35 – 14.9 – 48.8 µg a.s./L Controls: water control and solvent control (dimethylformamide 0.1 mL/L) Evidence of undissolved material: Not reported
Replication:	No. of vessels per concentration (replicates): 4 No. of vessels per control (replicates): 4 No. of vessels per solvent control (replicates): 4
Organisms per replicate:	No. of fertilized eggs/embryos per vessel: 35
Exposure:	Flow-through

	Total exposure duration: 33 days (5-day-hatch and 28 d post-hatch)
Test Vessel Loading:	At the end of the test: 0.0054 – 0.0065 g fish/L/day
Feeding during test	Newly hatched larvae were fed live brine shrimp nauplii (*Artemia sp.*) 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:	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
Parameters Measured / Observations	Water temperature was measured and recorded hourly by a data logger in two replicates of the control and in two replicates of the solvent control during the whole test. Dissolved oxygen (in percent saturation), pH and the water temperature were measured in one alternating replicate of all test levels on days 0, 7, 15, 21, 28 and 33. Total hardness was measured in one alternating replicate of four test levels (control, solvent control, lowest and highest test level) on study days 0, 7, 15, 21, 28 and 33. Conductivity (in µS/cm^2) 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 hatched or dead. Hatched larvae were also recorded. During the larval phase observations on mortality were done daily and abnormal behavior and abnormal morphological appearance were recorded at least on working days by visually inspecting each growth chamber. At test termination on study day 33 (post-hatch day 28) the surviving fish were sacrificed. The standard length (mm) was determined by measuring from the tip of the mouth to the tip of the caudal peduncle. Wet weight of control and solvent control fish was recorded for evaluation of test system biomass loading. The dry weights of individual fish 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 pooled. - Shapiro Wilk's test to check the normality of the data set and - Levene's 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 [redacted]

Results

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°C and 25.3°C and did not differ by more than ± 1.5°C between test chambers or between successive days at any time during the test.
Analytical measure of the test concentrations	Compulsory	Compulsory	Done
Hatching success of controls (Control/solvent control)	> 60%	> 70%	83% / 91%
Post-hatch survival of controls	> 70%	> 75%	97% - 100%

*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 measured concentration (µg a.s./L)	Range mean measured concentration [µg a.s./L]	Range % of nominal
0.48	0.451	0.417 – 0.504	87 - 105
1.53	1.36	1.28 – 1.56	84 – 102
4.88	4.35	3.99 – 4.53	82 – 93
15.6	14.9	13.5 – 16.4	87 - 105
50.0	48.8	44.0 – 53.7	88 - 107

Biological results:

Time to hatch and hatching success
The hatching of larvae started on day 4 and lasted until day 5. On post hatch day 0 the mean hatching success (based on the number of inserted eggs) ranged between 77 and 91% in all dose levels. Post hatch day 0 was reached on day 5, when 99% of all fertilised and living embryos in the control and 98% in the solvent control had hatched. The endpoint hatching success on day 5 (post hatch day 0) resulted in a NOEC ≥ 50 µg a.s./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

Larval survival

Mean larval survival at test termination ranged from 15 to 100% in all treatment groups. The endpoint larval survival resulted in a NOEC of 15.6 µg a.s./L and a corresponding LOEC of 50 µg a.s./L.

Nominal concentrations (µg a.s./L)	Larval survival [%]
Water control	97
Solvent control	100
0.48	97
1.53	92
4.88	95
15.6	95
50.0	15

Growth

In the highest test concentration (50 µg a.s./L) only a low number of fish was available at the end of the test, due to observed high mortality. The growth of fish is density dependent. Therefore it was decided to exclude the respective concentration from statistical analysis.

Nominal concentrations (µg a.s./L)	Mean total length (mm) ± SD	Mean wet weight (mg) ± SD
Water control	17.5 ± 1.26	14.3 ± 3.31
Solvent control	17.9 ± 1.22	15.2 ± 3.18
0.48	17.5 ± 1.78	15.4 ± 4.69
1.53	17.6 ± 1.64	14.9 ± 4.52
4.88	18.0 ± 1.07	15.6 ± 2.64
15.6	17.9 ± 1.44	15.8 ± 3.73
50.0	16.2 ± 2.17	9.4 ± 4.10

SD = Standard deviation

Morphological and behavioral effects

Between study day 6 and test 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

concentration 15.6 µg 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 larval exposure between study day 6 and 25. In most cases observations of symptoms as "undernourished, 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 in most cases in death or in an obviously retarded development.
The behavioral and morphological observations resulted in a NOEC of 15.6 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 short-term incident (a breakdown of stock solution delivery in test level 15.6 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 (expressed as dry weight and total length), the test revealed the following NOEC, LOEC, MATC and EC_{10} (based on nominal concentrations of BCS-CN88460):

	Hatching success (day 5)	Larval survival (day 33)	Growth (Total Length)	Growth (Dry Weight)	Morphological & Behavioral effects
LOEC [µg a.s./L]: lowest concentration with an significant effect compared to the control	> 50	50	> 15.6	> 15.6	50
NOEC [µg a.s./L]: highest concentration without an significant effect compared to the control	≥ 50	15.6	≥ 15.6	≥ 15.6	15.6
EC_{10} (95%-CL) [µg a.s./L]	n.d.	n.d.	n.d.	n.d.	n.a.

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

For this study EC_{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 µg a.s./L. An EC_{10} or EC_{20} calculation therefore is not possible.

For the endpoint "larval survival" only one test item concentration (50 µg a.s./L) resulted in effects. With only one effect concentration no reasonable EC_{10} or EC_{20} 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 EC_{10} would have been a higher concentration as the presented NOEC.

For the endpoints "length" and "weight" only one effect concentration was observed (50 µg/L): At the NOEC there was no effect detected compared to the controls. It can therefore be stated that an EC_{10} would have been a higher concentration as the presented NOEC. For these reasons no EC_{10} or EC_{20} values were reported.

Report:	KCA 8.2.2.1/02; [redacted]; [redacted]; 2016; M-575119-01-1
Title:	Early life stage toxicity of BCS-CN88460 technical to the sheepshead minnow (Cyprinodon variegatus) under flow-through conditions
Report No.:	044SRLS15C20
Document No.:	M-575119-01-1
Guideline(s):	EU Directive 91/414/EEC Regulation (EC) No. 1107/2009 US EPA OCSPP 850.1400
Guideline deviation(s):	None
GLP/GEP:	**Yes**

Material and methods

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
Guideline(s) adaptation	None specified
Test species:	Sheepshead minnow (*Cyprinodon variegatus*)
Organism Age at Experimental Start:	24-48 hour old eggs in the nerula stage
Test solutions	Nominal concentrations: 3.13, 6.25, 12.5, 25.0, 50.0 µg a.s./L Arithmetic mean measured concentrations: 2.92, 6.13, 11.3, 25.0, 45.8 µg a.s./L Controls: water control and solvent control (triethylene glycol 0.1 mL/L) Evidence of undissolved material: No precipitations 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 fertilized eggs/embryos per vessel: 20
Exposure:	Flow-through Total exposure duration: 35 days (6-day hatch and 29 d post-hatch)
Test Vessel Loading:	At the end of the test: 0.026 g fish/L/day
Feeding during test	Feeding with brine shrimp (*Artemia salina*) starting on Day 5
Test conditions:	Temperature: 24.0 to 24.6 °C Light intensity: 605 to 778 lux pH: 8.1 to 8.2 Salinity range: 20 ‰ to 21‰ Dissolved oxygen (% saturation) range: 73% to 91%
Parameters Measured / Observations	Temperature was measured continuously throughout the exposure in a centrally located test vessel. Dissolved oxygen, pH and salinity were measured at experimental start and at least weekly thereafter. 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	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 or transformed values, then parametric analyses were conducted using analysis of variance (ANOVA) followed by Dunnett's test (p= 0.05). If normality and/or homogeneity of variance were not demonstrated on raw or transformed values, nonparametric procedures were used. ECx values were calculated where applicable using linear interpolation.

Results

Validity criteria	**Required by OPPTS 850.1400, 1996**	**Obtained**
Dissolved oxygen concentration throughout the test (% saturation)	60% - 100%	> 73%
Water temperature difference between test chambers or between successive days at any time during the test	± 1.5°C max	± 1.0°C max
Concentrations of test substance in solution throughout the test	± 20 % of mean measured values	Yes
Hatching success of controls	> 75%	> 75%
Post-hatch survival of controls	> 80%	> 80%

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

Nominal conc. (µg a.s./L)	**Arithmetic mean measured concentration (µg a.s./L)**	**% of nominal concentrations***					
		Day 0	**Day 7**	**Day 14**	**Day 21**	**Day 28**	**Day 35**
3.13	2.92	81	105	94	98	84	97
6.25	6.13	95	110	104	86	94	99
12.5	11.3	87	94	109	74	92	87
25.0	25.0	89	113	109	85	99	103
50.0	45.8	76	103	106	88	85	92

* Mean of 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 if there were 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 87.9 to 92.9%. Statistical analysis indicated that percent hatch was not significantly different from pooled 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	92.1
6.13	92.1
11.3	89.3
25.0	92.9
45.8	90.0

Alevin survival on day 6
Mean percent alevin survival ranged from 87.9 to 92.9%. Statistical analysis indicated that alevin survival was not significantly different from pooled controls in any test level.

Arithmetic mean measured concentration (µg a.s./L)	Mean % survival
Water control	87.9
Solvent control	87.9
2.92	92.1
6.13	92.1
11.3	89.3
25.0	92.9
45.8	90.0

Fry survival on day 35
Mean percent fry survival ranged from 90 to 98.8%. Statistical analysis indicated that fry survival was significantly different from pooled controls in the highest level.

Arithmetic mean measured concentration (µg a.s./L)	Mean % survival
Water control	98.8
Solvent control	98.8
2.92	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 ranged 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 level.

Arithmetic mean measured concentration (µg a.s./L)	Mean total length (mm)	Mean wet weight (mg)
Water control	19.6	107.5
Solvent control	19.2	105.7
2.92	19.1	101.0
6.13	19.4	107.4
11.3	19.4	107.4
25.0	19.5	111.7
45.8	19.9	115.9

Conclusion

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

	% Hatching	% Time to Hatch	Alevin survival	Fry survival	Total length	Wet weight
LOEC [µg a.s./L]: lowest concentration with an significant effect compared to the control	> 45.8	> 45.8	> 45.8	45.8	> 45.8	> 45.8
NOEC [µg a.s./L]: highest concentration without an significant effect compared to the control	45.8	45.8	45.8	25.0	45.8	45.8

For the endpoints "time to hatch" "hatching 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 EC_{10} or EC_{20} calculation therefore is not possible.

For the endpoint "fry survival on day 35" less than 10 % difference to the control at the highest test item concentration of 45.8 µg a.s./L were observed. Thus the number of effect concentrations and the observed effect size were not sufficient for a reasonable EC_{10} and EC_{20} calculation.

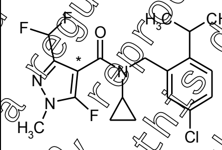
CA 8.2.2.2 Fish full life cycle test

Please refer to Section CA 8.2.2. Based on the triggers state in the EU-directive and the Aquatic Guidance Document, a fish full life cycle (FFLC) study is not required.

CA 8.2.2.3 Bioconcentration in fish

Report:	KCA 6.2.5/01; [redacted]; [redacted], R.; 2017; M-610008-01-1
Title:	[pyrazole-4-14C] BCS-CN88460 - Aqueous exposure bioconcentration fish test and biotransformation in fish (Lepomis macrochirus)
Report No.:	EBLNN359
Document No.:	M-610008-01-1
Guideline(s):	EU Directive 91/414/EEC; Regulation1107/2009 (Europe); OECD Test Guideline 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 hours prior to test initiation. However, due to scheduling issues the TOC content was measured 72 hours and 24 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:	**yes**

Material and methods:

Test material	Non-radiolabelled test item: BCS-CN88460 Batch code: BCS-CN88460-01-06 Specification No.: 102000028196 Purity: 94.2% w/w Radiochemical purity: > 99%	Radiolabelled test item: BCS-CN88460 [Pyrazole-4-^{14}C] BCS-CN88460  * = denotes the ^{14}C-label position
Guideline(s) adaptation	None specified	
Test species	Bluegill sunfish (*Lepomis macrochirus*)	
Acclimation	Fish were acclimated to the test dilution water for ≥ 14 days prior to initiation of testing. No mortality was noted 14 days prior to the test initiation by the fish for the bioconcentration part. The fish for the biotransformation part had a mortality of 0.7% noted in the range of 14 days prior to the test initiation.	
Details on test organisms	Bioconcentration part: - Mean body weight at study initiation: 2.8 g - Length at study initiation: 5.7 cm - Lipid content at test initiation: 4.86 % (w/w) of whole fish Biotransformation part: - Mean body weight at study initiation: 12.9 g - Length at study initiation: 9.1 cm	
Test solutions	Four aquaria (A, B, C, D) were used in the test: <u>Bioconcentration part:</u> Solvent control (Aquarium A): Active substance dissolved in acetonitrile in 0.1 mL/L dimethylformamide Nominal test 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 <u>Biotransformation part:</u> Nominal concentration: 5 µg BCS-CN88460/L Mean measured concentration: 4.88 µg BCS-CN88460/L Evidence of undissolved material: not reported	

Replication	No. of vessels per concentration (replicates): 1 No. of vessels per solvent control (replicates): 1
Organisms per replicate	No. of organisms per vessel: 70
Exposure	Test type: Flow through Route of exposure: aqueous Total exposure duration: 28 days Total depuration duration: 14 days
Test Vessel Loading	Biomass loading rate: 0.133 – 0.645 g fish (wet weight) per litre of test medium per day
Test conditions	Temperature: 21.8 – 22.8°C (continuous measurements), 22.3 – 22.9°C (discrete measurements) Photoperiod: 16:8 hours (30 minute transient period) Light temperature: warm-white fluorescent lamps pH: 7.1-7.8 Water hardness: 2.7 ± 0.3°dH (German hardness) Oxygen saturation: 73 – 97%. The test water was aerated to reach oxygen saturation. TOC: < 2.0 mg/L in dilution water Conductivity: < 10 µS/cm
Feeding during test	Fish were fed daily with a commercial fish diet at a rate of 1 to 2% of body weight. Based on the mean body weight of sampled fish the amount of food was recalculated at regular intervals.
Parameters Measured / Observations	The temperatures in all treatment groups were measured at test start (day 0) and then once a week. Additionally, the temperature was measured continuously in the control aquarium. The pH-values in 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 content was measured prior to test start (test water without test item), at test start (day -3, -1 and 0 before addition of the fish), day 7, 14, 21, 28, 35 and 42. Observations of fish for any signs of abnormalities or mortalities were made 2-4 hours after addition to test vessels, and then daily. On day 0, 28 and 42 four fish were sampled out of aquarium A-C in order to determine the lipid content of the whole fish. The length and weight were also measured from each individual fish.
Sampling for chemical analysis	Stock solution analysis Stock solution samples for the radioactivity measurements were taken at day – 4 (aquaria B, C and D), on Day 14 (aquarium D) and day 28 (aquaria B and C). 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, C). Out of each stock solution (without solvent control) 500 µL were taken. Water analysis Water samples for the radioactivity measurements were taken at day -1, 0, 1, 3, 7, 10, 14, 21, 28, 29, 31, 35, 38 and 42 (aquaria A-C) and on day –1, 0, 1, 3, 7, 10, 14 from aquarium D. 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 aquarium C and on day 7 and 14 from aquarium D. Only the samples of day 0, 1, 28 and 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

	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 viscera / non-edible parts (viscera = head, fins and internal organs) and transferred into pre-weighed 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 co-chromatography with radiolabeled reference compounds.

Results:

Validity criteria	Required (OECD 305, 2012)	Obtained
Water temperature variation over the whole test period	± 2°C	22.3 – 22.9°C
Dissolved oxygen % saturation in all test vessels	> 60%	> 73%
Concentration of test substance in test chambers maintained within required range of the mean of the measured values during the uptake phase	± 20%	82.2 - 118% (Aquarium B: 0.5 µg a.s./L) 90.6 – 119% (Aquarium C: 5 µg a.s./L) 83.8 – 139%* (Aquarium D: 5 µg a.s./L)
The concentration of the test substance is below its limit of solubility in test water	Test concentration < water solubility of test item in test water	Yes**
Mortality or other adverse effects/disease in control and treated fish	< 10%	< 10%

* In test aquarium D the concentration of the test substance was temporary (day 14) above 20% of the mean of the measured values. The exceedance does not influence the outcome 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

Analytical results:

Mean measured water concentrations during the uptake period was 0.478 ± 0.06 µg/L [^{14}C]-BCS-CN88460 equivalents at the low treatment (0.5 µg/L) and 5.05 ± 0.442 µg/L [^{14}C]-BCS-CN88460 equivalents at the high treatment (5.0 µg/L). This represented 96% of the low nominal concentrations and 101% of the high nominal concentration. Water concentrations ranged from 0.393µg/L to 0.568 µg/L in the low treatment and 4.58 µg/L to 6.0 µg/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 1 to day 2 of depuration showed a clear decrease in [^{14}C]-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 [^{14}C]-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)
Uptake	0	0.457	4.75	5.0
	1	0.442	4.58	4.42
	3	0.393	4.79	4.34
	7	0.565	4.82	4.66
	10	0.457	5.15	4.09
	14	0.464	5.17	6.77
	21	0.568	6.00	-
	28	0.481	5.11	-
	Mean	0.478	5.05	4.88

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 day	Nom. concentration: 0.5 µg/L (Aquarium B)			Nom. concentration: 5.0 µg/L (Aquarium C)		
	mg/kg of [14C]-BCS-CN88460 equivalents					
1	0.0758 ± 0.0408	0.1600 ± 0.0076	0.2850 ± 0.0690	0.3580 ± 0.0398	1.4900 ± 0.2170	2.9500 ± 0.1890
3	0.0492 ± 0.0187	0.1590 ± 0.0282	0.3380 ± 0.0450	0.5480 ± 0.0763	1.5000 ± 0.3540	3.0900 ± 1.0000
7	0.0740 ± 0.0149	0.2560 ± 0.5840	0.4920 ± 0.1180	0.531 ± 0.159	1.5400 ± 0.5610	2.9300 ± 1.1500
10	0.0493 ± 0.00955	0.2260 ± 0.1750	0.5370 ± 0.5440	1.0100 ± 0.3970	2.8800 ± 0.8980	1.6900 ± 0.5420
14	0.0608 ± 0.0114	0.1480 ± 0.0387	0.2890 ± 0.1200	0.7890 ± 0.2040	1.7200 ± 0.4250	3.0400 ± 1.1100
21	0.0526 ± 0.0057	0.1360 ± 0.00352	0.2580 ± 0.0180	1.0300 ± 0.5120	3.8800 ± 1.5800	2.2000 ± 0.8800
28	0.0707 ± 0.0215	0.1650 ± 0.0753	0.3240 ± 0.1990	0.6030 ± 0.0859	1.9500 ± 0.7390	3.9400 ± 1.9100
29	0.0231 ± 0.0033	0.0516 ± 0.0359	0.0909 ± 0.0802	0.3700 ± 0.1520	0.7480 ± 0.4150	1.2900 ± 0.8050
31	0.00572 ± 0.000421	0.00843 ± 0.00141	0.0130 ± 0.00457	0.0683 ± 0.0204	0.0927 ± 0.0320	0.1300 ± 0.0499
35	0.00548 ± 0.00123	0.00774 ± 0.00172	0.0108 ± 0.00252	0.04400 ± 0.00772	0.0585 ± 0.0118	0.0816 ± 0.0167
38	0.00443 ± 0.000486	0.00631 ± 0.000682	0.00857 ± 0.00118	0.0430 ± 0.0289	0.0600 ± 0.0231	0.0876 ± 0.0146
42	0.0353 ± 0.0104	0.0437 ± 0.0112	0.0586 ± 0.0154	0.00335 ± 0.00128	0.00428 ± 0.00167	0.00596 ± 0.00224

Bioconcentration factors (BCF) were determined during the uptake and depuration period by dividing the [14C]-tissue 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. concentration: 5.0 µg/L (Aquarium C)		
	BCF edible part (Mean ± SD)	BCF viscera part (Mean ± SD)	BCF whole fish (Mean ± SD)	BCF edible part (Mean ± SD)	BCF viscera part (Mean ± SD)	BCF whole fish (Mean ± SD)
1	169 ± 90.8	634 ±154	356 ± 16.9	76.8 ± 8.54	634 ± 40.5	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	619 ± 244	325 ± 119
10	107 ± 20.6	1161 ± 1175	489 ±379	209 ± 82.4	597 ± 186	350 ± 112
14	131 ± 24.6	623 ± 258	319 ± 83.7	162 ± 41.8	624 ± 228	364 ± 87.2
21	110 ± 11.9	541 ± 37.6	284 ± 7.37	204 ± 102	770 ± 313	438 ± 175
28	148 ± 44.9	678 ± 417	346 ± 157	120 ± 170	781 ± 379	387 ± 147
29	48.3 ± 6.91	190 ± 168	108 ± 73.0	73.4 ± 30.1	256 ± 160	148 ± 82.1
31	12.0 ± 0.88	27.2 ± 9.56	17.6 ± 2.96	13.5 ± 4.08	25.8 ± 9.89	18.4 ± 6.34
35	11.4 ± 0.257	22.7 ± 5.27	16.2 ± 3.61	8.71 ± 1.53	16.2 ± 3.31	11.6 ± 2.33
38	9.27 ± 1.02	17.9 ± 2.47	13.2 ± 1.93	8.51 ± 2.73	17.4 ± 2.90	11.9 ± 4.57
42	7.00 ± 2.68	12.5 ± 4.69	8.95 ± 3.49	7.00 ± 2.07	11.6 ± 3.04	8.66 ± 2.22

To calculate the steady-state bioconcentration factors based on parent substance, the measured TRR concentration was corrected for the amount of BCS-CN88460 found at day 14 in the measurement of the metabolism part of the study. In the edible part 19.7 % and in viscera 9.70 % of TRR, respectively, could be identified as BCS-CN88460. 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 $BCF_{ss\text{-}p}$ for the whole fish was calculated using the adapted values for edible part and viscera 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 (aquarium B)						
sampling day	BCF edible part		BCF viscera part		BCF whole fish	
	mean*	SD	mean*	SD	mean*	SD
1	33.8	18.2	62.6	15.2	45.2	5.1
3	24.4	9.36	83.4	11.11	47.0	9.42
7	25.8	5.21	84.5	20.34	51.3	10.89
10	21.3	4.12	114.2	115.57	54.6	38.75
14	25.8	4.84	60.4	25.01	39.0	7.53
21	18.3	1.98	44.2	3.07	28.7	1.28
28	29.0	8.80	65.4	40.19	42.2	17.87

5.00 µg/L (aquarium C)						
Sampling day	BCF edible part		BCF viscera part		BCF whole fish	
	mean*	SD	mean*	SD	mean*	SD
1	15.4	1.7	62.6	4.0	35.9	4.6
3	22.5	3.14	62.6	20.30	37.5	7.26
7	21.7	6.47	59.0	23.18	37.3	13.25
10	38.6	15.17	54.1	16.90	43.9	14.23
14	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/L			**5.00 µg [pyrazole-4-^{14}C] BCS-CN88460/L**		
C_w Chemical concentration in water considering whole exposure period [µg L^{-1}]	0.478 ± 0.0600			5.05 ± 0.442		
C_w Chemical concentration in water at steady state [µg L^{-1}]	0.504 ± 0.0557			5.43 ± 0.497		
	edible tissue	**viscera tissue**	**whole fish**	**edible tissue**	**viscera tissue**	**whole fish**
C_f Chemical concentration in fish at steady-state [mg kg^{-1}]	0.0614 ± 0.00905	0.290 ± 0.0329	0.150 ± 0.0150	0.807 ± 0.213	3.62 ± 0.502	1.96 ± 0.240
BCF_{SS} Steady-state BCF [L kg^{-1}]	130 ± 18.9	614 ± 68.9	316 ± 31.2	162 ± 42.2	725 ± 87.8	393 ± 42.3
BCF_{SSL} Lipid normalized steady-state BCF [L kg^{-1}]*	127	599	**308**	158	707	**383**
BCF_K Kinetic BCF [L kg^{-1}]	127 ± 13.4	751 ± 75.6	371 ± 33.7	151 ± 14.6	674 ± 39.2	361 ± 20.0
k_1 Overall uptake rate constant [L kg^{-1} day^{-1}]	118 ± 12.5	861 ± 86.8	390 ± 35.5	118 ± 11.5	700 ± 40.8	350 ± 19.4
k_2 Overall depuration rate constant [day^{-1}]	0.932 ± 0.136	1.15 ± 0.0722	1.05 ± 0.0885	0.785 ± 0.0715	1.04 ± 0.0445	0.970 ± 0.0523
BCF_{KL} Lipid-normalized kinetic BCF [L kg^{-1}]*	124	732	362	147	657	352
BCF_{Kg} Growth-corrected kinetic BCF [L kg^{-1}]	129	762	379	155	686	370
k_g Growth rate constant [day^{-1}]**	n.d.	n.d.	0.018	n.d.	n.d.	0.023
k_{2g} Growth-corrected depuration rate constant [day^{-1}]	0.914	1.13	1.03	0.762	1.02	0.947
$t_{1/2g}$	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]						
BCF_{KLG} Lipid-normalized growth-corrected kinetic BCF [L kg^{-1}]*	126	743	**370**	151	669	**361**

n.d. = not determined
*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 viscera tissue

<u>Conclusion</u>
For the whole fish, the lipid normalized steady-state bioconcentration factor (BCF_{SSL}) was calculated to be 308 L/kg and 383 L/kg for the treatment level of 0.5 and 5.0 µg/L, respectively. For the whole fish, the lipid normalized and growth corrected kinetic bioconcentration factor (BCF_{KLG}) was calculated to be 370 L/kg and 361 L/kg for the treatment level of 0.5 and 5.0 µg/L, respectively.

CA 8.2.3 Endocrine 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 8.2.3/01; [redacted], 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 evidence 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 than 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 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 non-mammalian vertebrates.

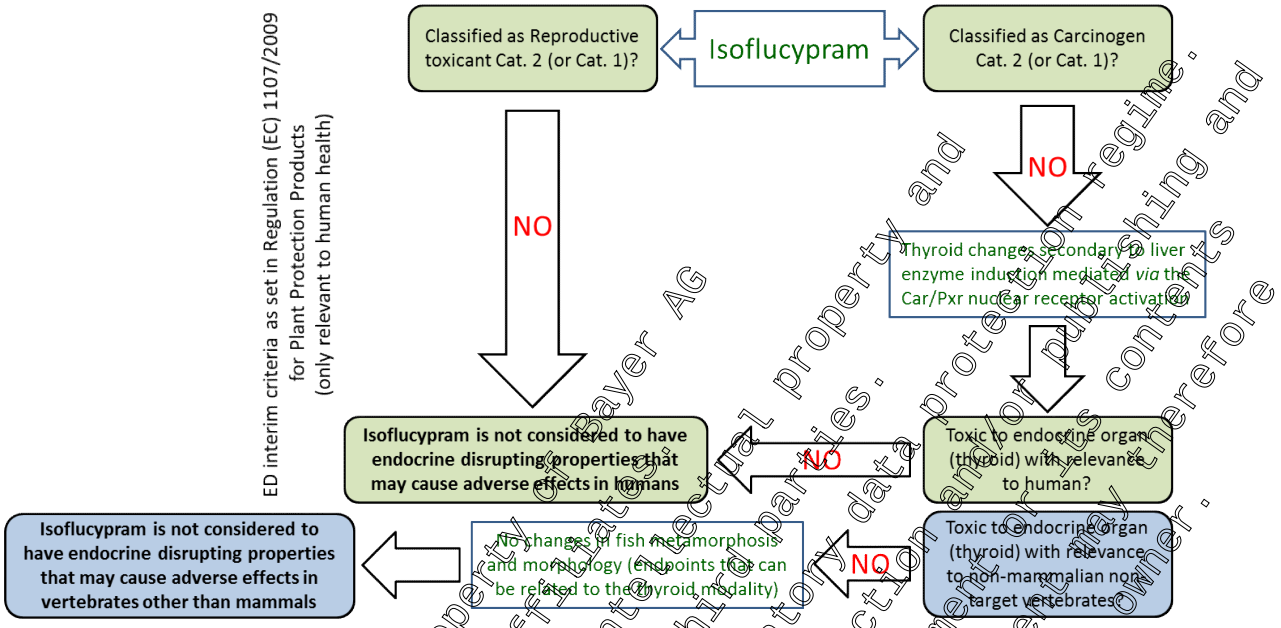


Figure 1: Decision tree applied to the assessment of endocrine-disrupting properties of isoflucypram. The upper part (green cells) is based on the interim criteria as documented by toxicological investigation in mammals, it therefore applies to wild mammals. The lower part (blue cells) concerns non-mammal vertebrates (i.e. fish) where the thyroid modality was investigated. Adapted from the document "*Screening of available evidence on chemical substances for the identification of endocrine disruptors according to different options in the context of an Impact Assessment - Specific Contract SANTE/2015/E3/SI2.706218*" prepared for the European Commission in 2016.

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 8.2.4 Acute toxicity to aquatic invertebrates

CA 8.2.4.1 Acute toxicity to Daphnia magna

Report: KCA 8.2.4.1/01; [redacted]; 2016; M-574184-01-1
Title: Acute toxicity of BCS-CN88460 (tech.) to the waterflea Daphnia magna in a static laboratory test system - Final Report
Report No.: EBLNN033
Document No.: M-574184-01-1
Guideline(s): OECD Guideline No. 235 (Guideline for Testing of Chemicals, Chironomus sp., Acute Immobilisation Test, adopted July 28, 2011)
US EPA OCSPP 850.SUPP
Guideline deviation(s): none
GLP/GEP: **yes**

Material and methods

Test material	BCS-CN88460 tech., Origin batch ID.: 2013-006492 Batch 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/size at study initiation	First instar neonates, less than 24 hours old
Test solutions	Nominal 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: 0.1 ml/L Dimethylformamide Evidence of undissolved material: No remarkable observations, clear media.
Replication	No. of vessels per concentration (replicates): 6 No. of vessels per control (replicates): 6 No. of vessels per solvent control (replicates): 6
Organisms per replicate	No. of organisms per vessel: 5
Exposure	Static Total exposure duration: 48 hours
Feeding during test	None

Test conditions	Temperature: 19.4 – 23.2°C 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 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 h post start and at the end of the test. Oxygen saturation and pH- values were determined at the start and the end of the test.
Chemical analysis	The content of BCS-CN88460 in exposure media was measured for verification of the test item concentrations via HPLC-MS/MS (LOQ = 0.625 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 to the maximum likelihood principle, with the software ToxRat-Professional (Version 2.10)

Results

Validity criteria	Required	Obtained
Mortality in control during test	≤ 10%	0.0 %
Dissolved oxygen concentration at the end of the test	≥ 3 mg/L	≥ 8.6 mg/L

Analytical results:
The accompanying chemical analysis of BCS-CN88460 in the freshly 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 80-120% for nominal range, all results are based on geometric mean-measured concentrations. No contaminations of BCS-CN88460 were detected in samples from untreated water control.

Nominal Concentration (µg a.s./L)	Day 0 Measured Concentration (µg a.s./L)	Day 0 % Nominal	Day 2 Measured Concentration (µg a.s./L)	Day 2 % Nominal	Geometric mean measured concentration (µg a.s./L)
Control	< 0.625	-	< 0.625	-	-
Solvent control	< 0.625	-	< 0.625	-	-
50.0	56.9	114	61.1	122	59.0
100	113	113	120	120	116
200	221	111	231	116	226
400	418	105	448	112	433
800	849	106	856	107	853

Biological results:

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 (0)
50.0	0 (0)	0 (0)
100	1 (3.3)	2 (6.7)
200	4 (13.3)	18 (60.0)
400	24 (80.0)	30 (100)
800	30 (100)	30 (100)

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

EC_{50} 24 hours (95% C.I.):	316 µg a.s. /L (273 – 365 µg a.s. /L)
EC_{50} 48 hours (95% C.I.):	**201 µg a.s. /L (176 – 229 µg a.s. /L)**

Report: KCA 8.2.4.1/02; [redacted] 2016; M-573296-01-1
Title: Acute toxicity of BCS-CN88460-carboxylic-acid (BCS-CY26497) to the waterflea Daphnia magna in a static laboratory test system
Report No.: EBLCN198
Document No.: M-573296-01-1
Guideline(s): EU Directive 91/414/EEC
Regulation1107/2009 (Europe)
US EPA OCSPP 850.1010
Guideline deviation(s): none
GLP/GEP: **yes**

Material and methods

Test material	BCS-CN88460-carboxylic-acid (BCS-CY26497) Origin batch: SLS 12631-19-9 Batch code: BCS-CY26497-01-02 TOX 20054-00 Purity: 98.8%
Guideline(s) adaptation	None specified
Test species	Water flea (*Daphnia magna*)
Organism age/size at study initiation	First instar neonates, less than 24 hours old

Test solutions	Nominal concentrations: 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
Test conditions	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 $CaCO_3$/L Dissolved oxygen: 8.6 - 8.9 mg/L (> 95% saturation) Conductivity: 555 µS/cm Alkalinity: 53 mg $CaCO_3$/L
Parameters Measured / Observations	Counting of mobile daphnids. 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	Freshly prepared 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 HPLC-UV.
Data analysis	Probit analysis, fitted by an iterative weighted linear regression according to the Maximum Likelihood principle. For calculations Tox-Rat-Professional (Version 3.2.1) and Excel 2010 were used.

p.m. = pure metabolite

Results

Validity criteria	Required	Obtained
Mortality in control during test	≤ 10%	0%
Dissolved oxygen concentration at the end of the test	≥ 3 mg/L	≥ 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	1.77	118
3.0	3.42	114	3.47	116	3.45	1.15
6.0	6.82	114	6.92	115	6.87	1.15
12.0	13.2	110	13.4	112	13.3	1.11
24.0	25.9	108	27.2	113	26.6	1.11

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

Biological results:

Observations
No immobilities or other effects on behavior occurred in untreated control within 48 hours of exposure.

Immobility

Exposure time (hours)	24	48
Nominal test concentration (mg p.m./L)	No of immobilized (%)	No of immobilized (%)
Control	0 (0)	0 (0)
Solvent control	0 (0)	0 (0)
1.5	0 (0)	0 (0)
3.0	0 (0)	0 (0)
6.0	0 (0)	1 (3.3)
12.0	0 (0)	2 (6.7)
24.0	0 (0)	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 EC_{50} calculation could not be performed and the EC_{50} (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.

Report:	KCA 8.2.4.2/01; [redacted]; [redacted]; 2016; M-547041-01-1
Title:	BCS-CN88460: A 96-hour static-renewal acute toxicity test with the saltwater mysid (Americamysis bahia)
Report No.:	149A-257B
Document No.:	M-547041-01-1
Guideline(s):	US EPA OCSPP 850.1035
Guideline deviation(s):	none
GLP/GEP:	**yes**

Material and methods

Test material	BCS-CN88460 Batch Code: 2013-006492. Purity 94.2% w/w.
Guideline(s) adaptation	None specified.
Test species	Saltwater mysid (*Americamysis bahia*)
Organism age/size at study initiation	Juvenile mysids, less than 24 hours old
Test solutions	Nominal concentrations: 0.056, 0.11, 0.23, 0.45 and 0.90 mg a.s./L Mean measured concentrations: 0.057, 0.11, 0.23, 0.42 and 0.82 mg a.s./L Controls: natural filtered and aerated seawater obtained at Indian River Inlet, Delaware Solvent control: 0.1 ml/L dimethylformamide Evidence of undissolved material: No precipitates observed.
Replication	No. of vessels per concentration (replicates): 2 No. of vessels per control (replicates): 2 No. of vessels per solvent control (replicates): 2
Organisms per replicate	No. of organisms per beaker: 10
Exposure	Semi-static renewal after 48 hours Total exposure duration: 96 hours
Feeding during test	None
Test conditions	Temperature: 25 ± 2°C Photoperiod: 16 hours light / 8 hours dark at 681 lux pH: 8.0 – 8.2 Dissolved oxygen: ≥ 6.7 mg/L (≥ 91% of the saturation value) 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 chromatography (HPLC) with ultraviolet absorbance detection at a wavelength of 220 nm. LOQ 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 LC_{50} value and the 95% confidence interval by probit analysis, the moving average method, and binomial probability with nonlinear interpolation. Based on the mortality pattern in this study, probit analysis was used to calculate the 48 and 72-hour LC_{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.

Results

Validity criteria according to OPPTS 850.1035	Required	Obtained
Mortality of mysids in controls at test end	≤ 10%	Negative control: 5% Solvent control: 0%
Dissolved oxygen of air-saturation	≥ 60%	≥ 91%

Analytical results:
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 measured concentration (mg a.s./L)	% of nominal concentrations	Range of individual measurements (% of nominal)
0.056	0.057	102	97.6 - 107
0.11	0.11	100	96.2 - 105
0.23	0.23	100	94.2 - 107
0.45	0.42	93.3	87.9 – 99.3
0.90	0.82	91.1	86.9 – 95.2

Biological results:
One dead mysid 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 0.11 mg a.s./L treatment groups also appeared normal throughout the test, with no mortalities or overt signs of toxicity observed.

Mortality

Exposure time (hours)	3.5	24	48	72	96
Nominal conc. (mg a.s./L)	No of dead	No of dead	No of dead	No of dead	No of dead
Control	0	0	0	0	0
Solvent control	0	0	0	0	0
0.057	0	0	0	0	0
0.11	0	0	0	0	0
0.23	0	2	2	3	5
0.42	0	0	15	17	20
0.82	0	8	20	20	20

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

LC_{50} 96 hours (95% C.I.):	**0.27 mg a.s. /L (0.23 mg a.s. /L – 0.42 mg a.s. /L)**
NOEC:	0.11 mg a.s./L

CA 8.2.5 Long-term and chronic toxicity to aquatic invertebrates

Long-term and chronic toxicity studies for aquatic invertebrates are provided under CA 8.2.5.1 and CA 8.2.5.2.

CA 8.2.5.1 Reproductive and development toxicity to Daphnia magna

Report: KCA 8.2.5.1/01; [redacted]; 2017; M-593961-01-1
Title: Effects of BCS-CN88460 (tech.) on development and reproductive output of the waterflea Daphnia magna in a static renewal laboratory test system
Report No.: EBLANN031
Document No.: M-593961-01-1
Guideline(s): EU Directive 91/414/EEC
Regulation1107/2009 (Europe)
US EPA OCSPP 850.1300
Guideline deviation(s): none
GLP/GEP: **yes**

Material and methods:

Test material:	BCS-CN88460 (tech.) BCS batch 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:	1st instar neonates less than 24 h old
Test solutions	Nominal concentrations: 0, 4.5, 9.0, 18, 36, 72 and 144 µg a.s./L Corresponding time weighted average concentrations: 5.33, 10.7, 20.3, 40.4, 80.1 and 162 µg a.s./L Controls: water control and solvent control (0.1 mL dimethylformamide/L test solution) Evidence of undissolved material: No undissolved 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: 1
Exposure:	Static-renewal conditions (approx. 3 water renewals per week) Total exposure duration: 21 days
Test Vessel Loading:	100 mL of test solution in 250 mL glass beakers
Feeding during test	Three times per week with living cells of the green algae *Desmodesmus subspicatus*
Test conditions:	Temperature: 19.7 – 21.7 °C Photoperiod: 16:8 hours light:dark Light intensity: 1000 -1200 lux pH: 7.4 – 7.9 Water hardness: 13 to 14 (°dH, German degrees) Dissolved oxygen: 8.2 - 9.2 mg/L Alkalinity: 3 - 4 (°dH, German degrees, as carbonate hardness)
Parameters Measured / Observations	Measurement of water temperature, total hardness, alkalinity, pH and dissolved oxygen were conducted on day 0, 2, 5, 5, 9, 12, 14, 16 and 19. As endpoints, the total number of living offspring per parental animal, the parental age at 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.
Sampling for chemical analysis	For verification of the actual test item concentrations during exposure, water-samples from start and end of 3 representative exposure-intervals were analysed. BCS-CN88460 was measured by HPLC-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 regression 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 limits for parental immobilisation and total living offspring was calculated by Probit analysis.

Results

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 measured in the controls.

Nominal concentrations (µg a.s./L)	Time weighted mean measured concentrations (µg a.s./L)	% of nominal concentrations	% of nominal concentrations					
			Day 0 New	Day 2 Aged	Day 9 New	Day 12 Aged	Day 19 New	Day 21 Aged
4.5	5.33	118	126	124	118	113	117	116
9.0	10.7	119	126	124	117	112	118	119
18.0	20.3	113	119	120	114	105	113	112
36.0	40.4	112	120	119	112	104	112	110
72.0	80.1	111	120	119	111	102	112	108
144	162	112	119	118	113	106	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 measured concentrations (µg a.s./L)	Length		Dry body weight	
	Mean ± SD (mm)	% Deviation from solvent control	Mean ± SD (g)	% Deviation from pooled controls
Control	3.89 ± 0.2		0.852 ±	-
Solvent control	4.07 ± 0.2	-	0.803 ±	-
Pooled control	3.98 ± 0.2	-	0.828 ±	-
5.33	4.06 ± 0.1	-0.1	0.874 ±	+ 5.6
10.7	3.94 ± 0.2	-3.1	0.935 ±	+ 13.0
20.3	3.91 ± 0.2	-3.9	0.817 ±	- 1.3
40.4	3.93 ± 0.1	-3.0	0.844 ±	+ 2.0
80.1	4.01 ± 0.1	-1.4	0.709 ±	- 14.3
162	3.72 ± 0.1	-8.6*	0.693 ±	- 16.3

* Statistical significant difference 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)
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 measured concentrations (µg a.s./L)	Total offspring per surviving parental female (Mean ± SD)	Average daily offspring per surviving parental female (Mean ± SD)	Age at first offspring emergence (Mean ± SD)
Control	85.5 ± 17.3	6.8 ± 1.2	10.22 ± 0.97
Solvent control	96.5 ± 19.2	7.8 ± 1.6	10.32 ± 1.08
Pooled control	91.0 ± 18.7	7.3 ± 1.5	10.27 ± 1.00
5.33	95.7 ± 7.4	7.3 ± 0.5	9.72 ± 0.32
10.7	91.6 ± 11.5	7.1 ± 0.9	9.92 ± 0.32
20.3	78.3 ± 27.0	6.4 ± 2.3	10.62 ± 1.03
40.4	92.2 ± 13.4	7.3 ± 1.0	10.22 ± 0.70
80.1	92.2 ± 13.8	8.2 ± 1.2	9.92 ± 0.74
162	55.4 ± 20.9	5.4 ± 2.0	12.52 ± 1.49

Conclusion:

For this study the EC_{10} 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 % difference 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 EC_{10} and EC_{20} calculation.

For the endpoint "weight" only the two highest concentrations resulted in a reduced body weight (14.3 and 16.3 % reduced compared to the controls, respectively). The number of effect concentrations and the observed effect size were low. An EC_{10} 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 endpoint "total number of offspring per surviving parental female" an EC_{10} of 97.1 µg/L was calculated.
At the NOEC of 80.1 µg/L 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 emergences	Total offspring per surviving parental female
LOEC [µg a.s./L]: lowest concentration with an significant effect compared to the control	162	162	> 162	162	162
NOEC [µg a.s./L]: highest concentration without an significant effect compared to the control	80.1	80.1	≥ 162	80.1	80.1
EC_{10} [µg a.s./L]	n.a.*	66.1	n.a.*	n.a.*	97.1

*Not applicable due to absence of effects

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

Chronic studies with additional aquatic invertebrate species (*Americamysis bahia*, *Leptocheirus plumulosus*, *Crossostrea virginica and Hyaella azteca*) were conducted for registration outside the EU. These studies are summarised below.

Report: KCA 8.2.5.2/01; [redacted]; 2016; M-567966-01-1

Title: BCS-CN88460: A flow-through life-cycle toxicity test with the saltwater mysid (Americamysis bahia)

Report No.: 149A-256
Document No.: M-567966-01-1
Guideline(s): US EPA OCSPP 850.1350
Guideline deviation(s): none
GLP/GEP: **yes**

Material and methods:

Test material	BCS-CN88460 Batch 2013-006492. Purity 94.2% w/w
Guideline(s) adaptation	None specified
Test species	Saltwater mysid (*Americamysis bahia*)
Organism age/size at study initiation	Juvenile mysids, less than 24 hours old
Test solutions	Nominal concentrations: 22, 44, 88, 175 and 350 µg a.s./L Arithmetic mean measured concentrations: 20, 37, 79, 146 and 299 µg a.s./L Controls: natural ozonated and filtered seawater Solvent control: 0.1 ml/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
Organisms per replicate	Test 1: No. of organisms per compartment: 15 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°C Photoperiod: 16 hours light / 8 hours dark at 115 lux pH: 7.8 – 8.0 Dissolved oxygen: ≥ 5.2 mg/L (≥ 62% of the saturation value) Salinity: 20 ‰
Parameters Measured/ Observations	Observations of mortality and clinical 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 offspring 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.
Chemical analysis	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 beginning of the test, approximately weekly during the test, and at 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. Concentrations of BCS-CN88460 in the samples were determined using an Agilent Model 1100 or 1200 high performance liquid chromatograph equipped with an Agilent Series 1100 or 1200 variable wavelength detector.
Data analysis	Data for survival and the percent of surviving females producing young are considered to be discrete-variable data. Data for the number of young per reproductive day, number of young produced per surviving female and growth are considered to be continuous-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 up) 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. 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	≤ 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 arithmetic 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 measurements (% of nominal)
22	20 ± 1.3	91	80.3 – 97.0
44	37 ± 3.8	84	71.1 – 98.4
88	79 ± 3.2	90	82.6 – 97.2
175	146 ± 7.2	83	78.3 – 88.2
350	299 ± 17	85	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 98.3%, 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 mysid termination on Day 28 in the pooled control group and in the 20, 37, 79, 146 and 299 µg a.s./L treatment 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 ($p \leq 0.05$). 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 per reproductive day in the pooled control group and in the 20, 37, 79, 146 and 299 µg a.s./L treatment groups was 0.699, 0.991, 0.659, 0.555, 0.378 and 0.000 young per day, respectively. Dunnett's 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 mean 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 Survival Initiation to Day 7		Juvenile Survival to Pairing on Day 13		Adult Survival Day 14 to Day 21		Adult Survival Day 14 to Test Termination on 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 survival
Control	60	98.3	60	90.0	49	85.7	49	77.6
Solvent	60	98.3	60	95.0	50	78.0	50	76.0
Pooled	120	98.3	120	92.5	99	81.8	99	76.8
20	60	96.7	60	95.0	48	81.3	48	70.8
37	60	98.3	60	91.7	44	72.7	44	65.9
79	60	98.3	60	96.7	46	71.7	46	54.3*
146	60	98.3	60	95.0	42	78.6	42	66.7
299	60	100	60	98.3	50	44.0*	50	18.0*

* Statistically significant decrease in survival in comparison to the pooled control using Fisher's Exact test ($p \leq 0.05$).
[1] The number alive at pairing may be less than the number surviving to Day 13 due to the fact that extra females that cannot be used to form pairs and any immature mysids are discarded at the time of pairing on Day 13.
[2] While the decrease in survival was statistically significant in comparison to the pooled control it was not considered to be treatment related since it was not dose-responsive.

Reproduction

Arithmetic mean measured conc. (µg a.s./L)	Mean Number of Young Produced Per Reproductive Day ± SD	Percent of Surviving Females Producing Young[1]	Mean Number of Young Per Surviving Female ± SD [1]
Control	0.582 ± 0.270	100	8.8 ± 3.98
Solvent control	0.816 ± 0.132	94.4	12.2 ± 2.04
Pooled control	0.699 ± 0.233	97.3	10.5 ± 3.46
20	0.991 ± 0.173	100	17.3 ± 3.88
37	0.659 ± 0.106	92.9	10.8 ± 1.19
79	0.555 ± 0.166	78.6	9.6 ± 2.98
146	0.378 ± 0.084*[2]	73.3**	6.1 ± 1.64*[2]
299	0.000 ± 0.000*[2]	0.0**	0.0 ± 0.00*[2]

* Statistically significant decrease in mean number of young produced per reproductive day and mean number of young per surviving female in comparison to the pooled control using Dunnett's test ($p \leq 0.05$).
** Statistically significant decrease in percent of surviving females producing young in comparison to the pooled control using Fisher's Exact test ($p \leq 0.05$).
[1] Calculated based on the total number of surviving females present at test termination. Females that died prior to test termination and the young that they produced were excluded from the calculation of the mean percent of females producing young and the mean number of young per female.
[2] 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.i./L treatment groups were excluded from the calculations ($p \leq 0.05$).

Growth parameters after 28 days

Arithmetic mean measured conc. (µg a.s./L)	Mean total length ± SD (mm)		Mean dry weight ± SD (mg)	
	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 ± 0.091	1.17 ± 0.153
79	7.95 ± 0.442	8.16 ± 0.151	1.03 ± 0.109	1.18 ± 0.114
146	7.80 ± 0.141	7.72 ± 0.270	0.96 ± 0.039	1.12 ± 0.144
299	7.47 ± 0.225	7.51 ± 0.365*	0.90 ± 0.173	1.09 ± 0.212[2]

[1] While there was a statistically significant difference found between the negative and solvent control groups in the female total length data (p = 0.0442) the difference between the groups was slight (2.9%) and likely due to the narrow standard deviation within each group of data. Therefore, the control data were pooled for comparison among the treatment groups.
[2] 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 299 µg a.i./L treatment group was excluded from the calculations ($p \leq 0.05$).
* Statistically significant decrease in comparison to the pooled control using the Dunnett's test ($p \leq 0.05$).

Conclusion

The study meets the validity criteria. The most sensitive endpoint was G1 mysid reproduction. The results, based on arithmetic mean measured concentrations, are:

NOEC: highest concentration without an significant effect compared to the control	**79 µg a.s./L**
LOEC: lowest concentration with an significant effect compared to the control	146 µg a.s./L
MATC: maximum Acceptable Toxicant Concentration	107 µg a.s./L
7 - day LC_{50} Survival	> 299 µg a.s./L
13 - day LC_{50} Survival	> 299 µg a.s./L
21 - day LC_{50} Survival	> 299 µg a.s./L
28 - day LC_{50} Survival	> 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 EC_{10} or EC_{20} 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 "juvenile survival, day 7" an ECx calculation was not applicable as no effects were observed. The NOEC was the highest test item concentration at which 100% survival was observed on day 7.

For the endpoint "juvenile 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 (control= 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.

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 for the endpoint mean total length of females. At the highest test item concentration of 299 µg a.s./L the observed percentage difference in length compared to the control was 5.5% only. At the NOEC of 146 µg a.s./L only 2.9% difference to the controls was observed. A potential EC_{10} would numerically exceed therefore a NOEC. The reported NOEC therefore represents a worst case endpoint.

Report:	KCA 8.2.5.2/02; [redacted]; 2017; M-601773-01-1
Title:	BCS-CN88460 - 28-day toxicity test exposing estuarine amphipods (Leptocheirus plumulosus) to a test substance applied to sediment under static-renewal conditions following EPA test methods
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(s):	none
GLP/GEP:	**yes**

Material and methods

Test material	Name of substance: BCS-CN88460 Batch No: 2013-006492 CAS No: 1255734-28-1 Purity: 94.2%
Guideline(s) adaptation	None specified
Test species	*Leptocheirus plumulosus*
Acclimation	Test species were acclimated to test conditions
Organism age/size at study initiation	- Age: 7 to 14 days old at exposure initiation
Test solutions	Nominal concentrations: 3.8, 7.5, 15, 30 and 60 mg a.s./kg sediment dry weight Arithmetic mean measured concentrations - in sediment: 3.1, 5.4, 11, 22 and 43 mg a.s./kg sediment dry weight - in sediment pore water: 0.022, 0.036, 0.086, 0.16 and 0.27 mg a.s./L Controls: Water control, solvent control Evidence of undissolved material: All stock solutions had no visible undissolved test substance following preparation.
Replication	No. of vessels per concentration (replicates): 6 No. of vessels per control (replicates): 6 No. of vessels per solvent control (replicates): 6
Organisms per replicate	No. of organisms per vessel: 20
Exposure	Static-renewal: Total exposure duration: 28 days
Feeding during test	The amphipods were fed a diet consisting of a flaked fish food suspension prepared in natural, filtered sea water.
Test conditions	Temperature: 23-25°C Photoperiod: 16 h light : 8 h darkness Light intensity: 510 – 1000 lux

	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 Measured / Observations	At exposure initiation and termination, dissolved oxygen concentration, salinity, temperature and pH were measured in the overlying water of each remaining replicate vessel of each treatment level and control used for biological monitoring 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 to house the test vessels throughout the study. Ammonia concentration of the overlying water was monitored at exposure initiation and termination in each treatment level and control. Observation of organism mortality and abnormal behavior were made at exposure initiation and at daily intervals thereafter, 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 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 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 treatment level and the controls. The sediment and aqueous samples were analyzed for BCS-CN88460 using liquid chromatography with tandem mass spectrometry detection (LC/MS/MS).
Data analysis	An Equal Variance Two-Sample Test was used to compare the performance of the negative control organisms with that of the solvent control organisms in order to determine if there were any statistically significant positive or negative effects. Shapiro-Wilks' Test for normality (U.S EPA, 2002) was conducted to compare the observed sample distribution with a normal distribution. As a check on the assumption of homogeneity of variance implicit in parametric statistics, data were 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 homogeneity. Consequently, Dunnett's Multiple Comparison Test, a parametric statistical procedure, was used to assess treatment-related effects for all endpoints.

Results:

Validity criteria according to EPA/600/R-01/020	Required	Obtained
Average survival of amphipods in controls Survival in single replicates	≥ 80% > 60%	88% ≥ 70%
Temperature	± 3°C	23-25°C
Salinity	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 (mg a.s./kg)	Arithmetic mean measured sediment concentrations (mg a.s./kg)	% of nominal concentration		
		Day 0	Day 14	Day 28
3.8	3.1	105	81.6	60.5
7.5	5.4	105	73.3	38.7
15	11	100	73.3	50.0
30	22	100	70.0	50.0
60	43	93.3	78.3	41.7

Nominal sediment concentration (mg a.s./kg)	Measured concentration (mg a.s./L)			Arithmetic mean measured concentration (mg a.s./L)
	Day 0	Day 14	Day 28	
	Pore Water			
3.8	0.032	0.021	0.011	0.022
7.5	0.064	0.030	0.014	0.036
15	0.12	0.080	0.053	0.086
30	0.23	0.15	0.092	0.160
60	0.33	0.29	0.190	0.270
	Overlying water			
3.8	0.0025	0.0013	0.00088	0.00156
7.5	0.0086	0.0044	0.0017	0.00490
15	0.0100	0.0120	0.0050	0.00900
30	0.0160	0.0180	0.0074	0.01380
60	0.0230	0.0490	0.0290	0.03366

Biological results:
Mean percent survival, growth rate and number of offspring per surviving female amphipod at exposure termination of the 28-day exposure with BCS-CN88460 and amphipods (*Leptocheirus plumulosus*).

Arithmetic mean measured sediment concentration (mg a.s./kg)	Mean Percent Survival (SD)	Mean Male Growth Rate in mg/day (SD)	Mean Female Growth Rate in mg/day (SD)	Mean number of offspring per surviving female amphipod (SD)
Control	88 (12)	0.092 (0.0051)	0.051 (0.0057)	20 (5.2)
Solvent control	88 (10)	0.098 (0.0068)	0.056 (0.0057)	17 (5.9)
3.1	94 (5)	0.088 (0.0069)	0.053 (0.0059)	15 (2.8)
5.4	83 (9)	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	94 (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 not statistically significant.
Therefore neither for males or females a biologically meaningful EC_{10} could be calculated.

For the endpoint "mean number of offspring per surviving female amphipod" no EC_{10} or EC_{20} calculation was performed. Only the two highest test item concentrations resulted in a reduced number of offspring (12 respectively 10). The observed data do not allow a meaningful EC_{10} calculation. At the reported NOEC of 11 mg a.s./kg for this endpoint no reduction at all was observed.

Endpoints based on arithmetic mean measured concentrations are:

Amphipod percent survival (day 28 endpoint)

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

NA = Not applicable. LC_{50} value was empirically estimated. Therefore, corresponding 95% confidence intervals could not be determined.

Amphipod male growth rate (day 28 endpoint)

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

NA = Not applicable. LC_{50} value was empirically estimated; therefore, corresponding 95% confidence intervals could not be determined.

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	> 0.27
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 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	22	0.16
NOEC: highest concentration without an significant effect compared to the control	11	0.086

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

Report: KCA 8.2.5.2/03; [redacted]; [redacted]; [redacted]; 2016; M-547035-01-1
Title: BCS-CN88460: A 96-hour shell deposition test with the eastern oyster (Crassostrea virginica)
Report No.: 149A-258
Document No: M-547035-01-1
Guideline(s): U.S. EPA OPPTS Number 850.1025
Guideline deviation(s): none
GLP/GEP: **yes**

Material and methods

Test material	BCS-CN88460 Technical Batch code BCS-CN88460-01-06 Origin batch No.: 2013-006492 Purity 94.2%
Guideline(s) adaptation	None specified
Test species	Eastern oyster (*Crassostrea virginica*)

Culturing conditions/ Acclimation	The oysters were held in filtered saltwater from the same source and at approximately the same temperature as used during the test. During the 12-day holding period immediately preceding the test, water temperatures in the culture 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 (≥ 90% of saturation). The salinity of the water on the day of organism receipt was 12 (‰), was raised to 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 age/size at study initiation	Initial valve height: 35.8 ± 2.4 mm
Test solutions	Nominal concentrations: 0.056, 0.11, 0.23, 0.45 and 0.90 mg a.s./L 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 mL/L dimethylformamide
Replication	No. of vessels per concentration (replicates): 1 No. of vessels per control (replicates): 1 No. of vessels per solvent control (replicates): 1
Organisms per replicate	No. of organisms per vessel: 20
Exposure	Flow through Total exposure duration: 96 h
Test Vessel Loading	Test chambers were 54-L glass aquaria filled with approximately 27 L of test water. The depth of the test water in a representative chamber was 14.7 cm.
Feeding during test	Marine microalgae at a target rate of 2.9 × 10^9 cells/oyster/day
Test conditions	Temperature: 18.8 – 19.1°C Photoperiod: 16:8 hours light: darkness Light intensity: 301 lux pH: 7.6 to 8.0 Dissolved oxygen: 7.0 - 8.1 mg/L (≥ 85% of saturation) Salinity: 20‰
Parameters Measured / Observations	Observations of mortality and other signs of toxicity were made approximately 3.5, 24, 48, 72 and 96 hours after test initiation. Measurements of shell deposition for the oysters 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 the beginning of the test, at approximately 24-hour intervals during the test, and at the end of the test. Measurements of pH were made in each test chamber at the beginning of the test, at the approximate mid-point of the test (~ 48 hours), and at the end of the test.
Sampling for chemical analysis	Test concentrations were measured in samples of test water collected from each treatment and control group at the beginning, the approximate mid-point and the end of the test for verification of the aspired exposure concentrations. The analytical method consisted of high performance liquid chromatography (HPLC).
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 EC_{50} 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, an 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 Bonferroni adjustment to identify any significant differences ($p = 0.05$). The no-observed-effect concentration (NOEC) was determined from the statistical analyses of the data and an assessment of the concentration-response pattern. Statistical analyses were conducted using a personal computer with TOXSTAT software.

Results:

Validity criteria	**Required**	**Obtained**
Mortality of the oysters in the control(s)	≤ 10 %	0 %
The dissolved oxygen concentration	≥ 60 %	≥ 85%
Evidence of spawning	No	No
The mean shell growth observed in the control group(s)	≥ 2 mm	Control : 2.5 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 Concentration (mg a.s./L)	**Arithmetic mean measured concentration (mg a.s./L)**	**% of nominal concentrations**		
0.056	0.049	89.4	89.5	85.2
0.11	0.091	76.4	87.2	84.1
0.23	0.22	95.6	101	87.7
0.45	0.37	79.2	84.4	81.8
0.90	0.88	127	81.7	84.1

Biological results:

Observations

No mortalities occurred among oysters in any control or 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 test 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 measured concentration (mg a.s./ L)	Exposed specimen (=100%)	No. dead					Cumulative percent mortality
		~ 3.5 h	24 h	48 h	72 h	96 h	
Negative control	20	0	0	0	0	0	0
Solvent control	20	0	0	0	0	0	0
0.049	20	0	0	0	0	0	0
0.091	20	0	0	0	0	0	0
0.22	20	0	0	0	0	0	0
0.37	20	0	0	0	0	0	0
0.88	20	0	0	0	0	0	0

Shell Deposition and Shell Growth

Arithmetic mean measured concentration (mg a.s./ L)	Mean Shell Deposition ± Standard Deviation (mm)	Shell Growth Inhibition [1,2] (%)
Negative control	2.5 ± 0.80	--
Solvent control	2.8 ± 1.43	--
Pooled control	2.7 ± 1.15	--
0.049	2.5 ± 1.49	8
0.091	2.0 ± 0.97*	24
0.22	0.8 ± 0.82*	69
0.37	0.2 ± 0.46*	93
0.88	0.0 ± 0.00*	100

[1] Shell growth inhibition was calculated relative to the pooled control.
[2] 96-hour EC_{50} (95% confidence interval) = 0.17 mg a.s./L (0.13 – 0.21 mg a.s./L).
* Statistically significant difference ($p \leq 0.05$) from the pooled control using the Wilcoxon's rank sum test (with Bonferroni adjustment, 1 tailed)

Conclusion

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

EC_{50} 96 hours	**0.170 mg a.s. / L**
LOEC: lowest concentration with an significant effect compared to the control	0.091 mg a.s./L
NOEC: highest concentration without an significant effect compared to the control	0.049 mg a.s. / L

This study type is handled within the aquatic risk assessment as an acute invertebrate study. The observed EC_{50} in combination with an assessment factor for acute studies is used to derive a RAC. Therefore it is questionable whether the calculation of an EC_{10} is reasonable.

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

For the shell growth a reasonable dose response relationship is existing. A calculation of an EC_{10} 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; [redacted]; 2017; M-585874-02-1
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 -
Report No.: 13798.6406
Document No.: M-585874-02-1
Guideline(s): US EPA Test Method 100.4
OCSPP 850.1770 (In Preparation)
Guideline deviation(s): none
GLP/GEP: **yes**

Material and methods

Test material	Name of substance: BCS-CN88460 Batch No: 2013-006492 CAS No: 1255734-28-1 Purity: 94.2%
Guideline(s) adaptation	None specified
Test species	*Hyalella azteca*
Organism age/size at study initiation	- Age: 8 days old at exposure initiation
Test solutions	Nominal concentrations: 6.3, 13, 25, 50 and 100 mg a.s./kg sediment dry weight Arithmetic mean measured concentrations - in sediment: 3.8, 11, 22, 44 and 95 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 test substance following preparation.
Replication	No. of vessels per concentration (replicates): 12 No. of vessels per control (replicates): 12 No. of vessels per solvent control (replicates): 12
Organisms per replicate	No. of organisms per vessel: 10
Exposure	Static-renewal. Total exposure duration: 42 days
Feeding during test	During testing, 1.5 mL of flaked fish food suspension (YCT) was added daily to each test vessel, as well as an additional 0.5 mg of ground flake fish food in an aqueous suspension.
Test conditions	Temperature: 22 – 23°C (Daily measurements), 21-24°C (Definitive study) Photoperiod: 16/8 hours, 680 - 940 lux pH: 6.2 – 7.2 Dissolved oxygen: 3.1 – 8.0 (control), 3.1 – 8.1 (solvent control), 2.7 – 8.4 (test concentrations) Ammonia: < 0.10 – 0.45 mg N/L Hardness: 52-96 mg $CaCO_3$/L Alkalinity: 20-44 mg $CaCO_3$/L Conductivity: 640 µS/cm
Parameters Measured / Observations	Dissolved oxygen concentration; temperature, pH, total hardness, alkalinity and conductivity were measured in the overlying water of each replicate vessel of 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 chemical analysis	Dosed sediments were sampled during the mixing/equilibration period prior to the allocation of the sediments into the replicate test vessels. In addition, subsamples of the dosing stock solutions used to dose the sediments were also analyzed for test substance concentration. Results of these pretest analyses were used to confirm that sufficient quantities of BCS-CN88460 had been applied during the dosing process. During the in-life phase of the definitive study, overlying water, pore water, and sediment samples were removed and analyzed for BCS-CN88460 concentration on test days 0 (exposure initiation), day 14 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 Smithers Viscient.
Data analysis	Determination of adverse effects on percent 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 survival, growth and reproduction data to compare the performance of control organisms with that of solvent control organisms in order to determine if there were any statistically significant positive or negative effects. Shapiro-Wilks' Test for normality was conducted to compare the observed sample distribution with a normal distribution for all endpoints. As a check on the assumption of homogeneity of variance implicit in parametric statistics, data were analyzed using Bartlett's Test. Percent survival data (day 28 and 42) and day 42 reproduction did not meet the assumption for normality, therefore, Steel's Many-One Rank Sum Test, a non-parametric statistical procedure, was used to establish treatment effects for these endpoints. Dunnett's Multiple Comparison Test, parametric statistical procedures, was used to establish treatment effects for all remaining endpoints. CETIS™ Version 1.8.7 was used to perform the computations.

Results:

Validity criteria	Required	Obtained
Recovery in sediment samples	70 - 120 %	103 ± 3.92 %
Recovery in aqueous sample	80 - 120 %	110 ± 7.29 %

Analytical results:

No BCS-CN88460 residues were measured in the sediment, pore water or overlaying water controls above the minimum detectable limit.

Nominal sediment concentration (mg a.s./kg)	Arithmetic mean measured sediment concentrations	% of nominal concentration		
		Day 0	Day 14	Day 28
6.3	5.8	92	92	92
13	11	88	88	88
25	22	104	76	88
50	44	84	90	92
100	95	97	95	95

Nominal sediment concentration (mg a.s./kg)	Arithmetic mean measured concentration (mg a.s./L)			Arithmetic Mean measured concentration (mg a.s./L)
	Day 0	Day 14	Day 28	
	Pore Water			
6.3	0.18	0.13	0.14	0.15
13	0.33	0.32	0.27	0.31
25	0.84	0.70	0.62	0.72
50	1.2	1.2	1.1	1.1
100	2.1	1.6	1.7	1.8
	Overlying water			
6.3	0.0130	0.0042	0.0037	0.0070
13	0.0360	0.0140	0.0058	0.0186
25	0.0470	0.0340	0.0150	0.0320
50	0.0750	0.0480	0.0350	0.0527
100	0.1900	0.0800	0.0430	0.1043

Biological results:

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

Arithmetic mean measured sediment concentration (mg a.s./kg)	Mean Percent Survival (SD)	Mean Length per Amphipod in mm (SD)
Control	94 (9)	5.55 (0.06)
Solvent control	99 (3)	5.71 (0.16)
5.8	95 (7)	5.59 (0.29)
11	96 (14)	5.49 (0.17)
22	96 (8)	5.44 (0.33)
44	97 (7)	5.75 (0.50)
95	86 (14)	5.50 (0.17)

SD = Standard deviation

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 concentration (mg a.s./kg)	Mean Percent Survival (SD)		Mean Length per Amphipod in mm (SD)		Mean Number of Offspring Released per	
	Day 35	Day 42	Day 35	Day 42	Day 35	Day 42
Control	93 (10)	91 (10)	n.a.	5.95 (0.35)	4.1 (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)	11.8 (7.9)
11	99 (4)	95 (9)	n.a.	6.24 (0.20)	3.8 (3.9)	7.8 (9.3)
22	95 (8)	90 (13)	n.a.	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.a.	6.09 (0.34)	0.74 (1.4)*	3.7 (5.9)

SD = Standard deviation
*Significantly reduced compared to the control, based on Dunnett's Multiple Comparison Test

Conclusion

For the endpoint "mean survival" only at the highest concentration of 95 mg a.s./kg a difference to the controls was observed. At the NOEC of 44 mg a.s./kg no difference to the controls was observed. The number of effect concentrations is not sufficient for a reasonable EC_{10} and EC_{20} calculation.

For the endpoint "length" no dose response was observed. The mean length at the highest test item concentration even exceeds slightly those of controls and solvent controls. Due to absence of effects a reasonable EC_{10} and EC_{20} calculation cannot be performed.

For the endpoints "mean number of offspring day 35 and day 42" 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 EC_{10} and EC_{20} calculation.

Endpoints based on arithmetic mean measured concentrations are:

Amphipod percent survival (day 28 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	> 1.8
NOEC: highest concentration without an significant effect compared to the control	95	1.8

NA = Not applicable. LC_{50} value was empirically estimated. Therefore, corresponding 95% confidence intervals could not be calculated.

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	1.8
NOEC: highest concentration without an significant effect compared to the control	44	1.1

NA = Not applicable; LC_{50} value was empirically estimated. Therefore, corresponding 95% confidence intervals could not be calculated.

Amphipod percent survival (day 42 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	> 1.8
NOEC: highest concentration without an significant effect compared to the control	95	1.8

NA = Not applicable; LC_{50} value was empirically estimated. Therefore, corresponding 95% confidence intervals could not be calculated.

Amphipod growth as length (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.):	> 95 (NA)	> 1.8 (NA)
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

NA = Not applicable; EC_{50} value was empirically estimated. Therefore, corresponding 95% confidence intervals could not be calculated.

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	> 1.8
NOEC: highest concentration without an significant effect compared to the control	95	1.8

NA = Not applicable; LC_{50} value was empirically estimated. Therefore, corresponding 95% confidence intervals could not be calculated.

Amphipod reproduction as offspring per female (day 35 endpoint)

Endpoint	**Arithmetic mean measured sediment (mg a.s./kg)**	**Arithmetic mean measured pore water (mg a.s./L)**
EC_{50} (95% C.I.):	52 (26 – 73)	1.2 (0.31 – 1.5)
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	44	1.1

NA = Not applicable; EC_{50} value was empirically estimated. Therefore, corresponding 95% confidence intervals could not be calculated.

Amphipod reproduction as offspring per female (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.):	ND (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 result was considered unreliable. Consequently, the EC_{50} for 42-day reproduction is not determined.
NA = Not applicable; LC_{50} 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 (*Chironomus dilutes*) was conducted for registration outside the EU. This study is summarised below.

Report:	KCA 8.2.5.4/01; [redacted]; 2017; M-596883-01-1
Title:	Life-cycle toxicity test exposing midges (*Chironomus dilutus*) to BCS-CN88460 technical applied to sediment under static-renewal conditions following EPA test methods
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(s):	not specified
GLP/GEP:	**yes**

Material and methods

Test material:	Name of substance: BCS-CN88460 Batch No.: 2013-006492 Purity 94.2% w/w
Guideline(s) adaptation	None specified
Test species:	Midge *(Chironomus dilutus)*
Organism age:	First instar larvae, three days old at exposure initiation
Preparation of spiked sediment	A 10-mL volume of each dosing stock solution was applied to 0.05 kg of fine silica and placed in glass Petri dishes and the solvent was allowed to evaporate for 90 minutes till dryness. The dry sand, containing the test substance, was added to the 3.0 kg of wet sediment (2.112 kg dry sediment) in individual glass jars. Test vessels contained 100 mL (approximately 4.0 cm layer) of spiked sediment (equivalent to 155 g wet weight per vessel or 107 g dry weight per vessel). Water used during study was laboratory well water (final test water volume per vessel: 0.175 L)
Test solutions	Nominal sediment concentrations: 6.3, 13, 25, 50 and 100 mg a.s./kg Arithmetic mean measured sediment concentrations - in sediment: 5.5, 11, 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 Solvent control: 10 mL acetone per 2.162 kg wet sediment Evidence of undissolved material: Stock solutions were observed to be clear and colorless
Replication:	<u>Vessels to measure biological response:</u> No. of vessels per concentration (replicates): 12 (A-L) No. of vessels per control (replicates): 12 (A-L) <u>Vessels designated for auxiliary male production:</u>

	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)
Organisms per replicate:	No. of organisms per vessel: 12 (Replicates which were established for analytical and pore water quality measurements on test day 0 were not initiated with any larvae)
Exposure:	Static-renewal conditions: Daily renewal of 350 mL water in each test vessel, i.e. seven cycles providing 50 mL of water per cycle. Renewal of 700 mL at test day 9 due to relatively low dissolved oxygen measurements. Total exposure duration: 61 days
Feeding during test	Larvae were fed a diet consisting of a finely ground flaked fish food suspended in laboratory well water (4 mg/mL). During exposure, the food was introduced at a rate of 1.5 mL of flaked fish suspension per test vessel per day.
Test conditions:	Water temperature: 22 to 24°C (daily measurements), 22 to 25°C (continuous measurements) Photoperiod: 16:8 light:dark Light intensity: 500 to 940 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 Conductivity (µS/cm): 490 – 620 Alkalinity ($CaCO_3$): 12 – 28 Ammonia as N (mg/L): 0.37-0.50 (test day 0), ≤ 0.10 (test day 61)
Sediment	Artificial sediment: 3.0 kg sphagnum peat (5%) 12 kg kaolin clay (20%) 45 kg fine sand (75%) 160 g powdered $CaCO_3$ (0.3%)
Parameters Measured / Observations	Dissolved oxygen concentration, temperature, total hardness, alkalinity, conductivity and pH were measured in overlying water of each replicate vessel of each treatment 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 house the test vessels throughout the study. Daily observations of mortality and abnormal behavior were made. Four of twelve replicate test vessels were randomly selected prior to day 16. Midge larval survival and growth measured as ash-free dry weight was assessed. Starting on test day 16 and daily thereafter, the number of male and female midges emerged from each replicate test vessel was observed and recorded. 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. Hatching success was determined by subtracting the number of unhatched eggs 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 on 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 t-Test or Wilcoxon's Rank Sum Two-Sample Test was conducted on all survival, growth, emergence and reproduction data to compare the performance of negative 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 endpoints. 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 or Dunnett's Multiple Comparison Test was used to establish treatment effects for all remaining endpoints. CETIS™ was used to perform the computations.

Results

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

Nominal sediment concentration (mg a.s./kg)	Arithmetic mean measured sediment concentrations (mg a.s./kg)	% of nominal concentration		
		Day 0	Day 16	Day 61
6.3	5.5	94	84	83
13	11	100	92	72
25	21	92	84	76
50	42	86	88	80
100	85	93	81	80

Nominal concentration (mg a.s./kg sediment dry weight)	Measured concentration (mg a.s./L)			
	Day 0	Day 16	Day 61	Arithmetic mean
Overlying water				
6.3	0.018	0.0026	0.00081	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	0.441*
100	0.24	0.029	0.011	0.093*
Sediment pore water				
6.3	0.14	0.090	0.13	0.12
13	0.30	0.23	0.24	0.26
25	0.51	0.47	0.41	0.47
50	0.95	0.93	0.85	0.91
100	1.6	1.2	1.3	1.4

*Mean measured values not given in report; calculated on the basis of concentrations on day 0, day 16 and day 61

Biological results:

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 (i.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 *Chironomus dilutus*.

On test day 16, survival observed among midge exposed to the 5.5, 11, 21, 42, and 85 mg/kg mean measured treatment levels averaged 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 tested compared to the negative control (96%).

On test day 16, growth (ash-free dry weight) among the midge exposed to the 5.5, 11, 21, 42, and 85 mg/kg mean measured treatment levels averaged 1.72, 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 no significant reduction in growth in any of the treatment levels tested compared to the negative control (2.27 mg ash-free dry weight per midge larvae).

Arithmetic mean measured sediment concentration (mg a.s./kg)	Mean percent survival (SD)	Mean Ash-Free Dry Weight Per Larvae in mg (SD)
Negative Control	96 (5)	2.27 (0.56)
Solvent Control	94 (8)	2.68 (0.56)
5.5	96 (5)	1.72 (0.34)
11	92 (12)	1.87 (0.15)
21	88 (14)	2.09 (0.40)
42	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). Mean percent emergence among midges exposed to the 5.5, 11, 21, 42, and 85 mg/kg mean measured treatment levels was 73, 82, 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 no significant reduction in mean emergence rate among male midges exposed to any of the treatment levels tested compared to the negative control (0.0496).

Mean emergence rate among female midges exposed to the 5.5, 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 mean 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 (mg a.s./kg)	Mean Percent Emergence (SD)	Mean Male Emergence Rate (SD)	Mean Female Emergence Rate (SD)
Negative Control	71 (14)	0.0496 (0.0051)	0.0447 (0.0051)
Solvent Control	86 (8)	0.0518 (0.0058)	0.0496 (0.0032)
5.5	73 (12)	0.0544 (0.0054)	0.0473 (0.0037)
11	82 (8)	0.0519 (0.0020)	0.0493 (0.0020)
21	82 (11)	0.0542 (0.0036)	0.0454 (0.0035)
42	81 (10)	0.0538 (0.0041)	0.0478 (0.0056)
85	66 (9)	0.0493 (0.0055)	0.0459 (0.0049)

SD = Standard deviation

Mean days 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 levels tested compared to the negative control (3.3 days).

The mean number of days to death among female midges exposed to the 5.5, 11, 21, 42, and 85 mg/kg mean measured treatment levels was 3.3, 3.2, 3.1, 3.4, and 3.5 days, respectively. Statistical analysis (Steel's Many-One Rank Sum Test) 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).

Arithmetic mean measured sediment concentration (mg a.s./kg)	Mean Male Days to Death (SD)	Mean Female Days to Death (SD)
Negative Control	3.3 (0.93)	3.1 (0.39)
Solvent Control	3.7 (1.0)	3.6 (0.45)
5.5	3.4 (1.14)	3.3 (0.87)
11	3.4 (0.55)	3.2 (0.42)
21	3.2 (1.12)	3.1 (0.29)
42	3.4 (1.03)	3.4 (0.33)
85	3.3 (0.90)	3.5 (0.34)

SD = Standard deviation

Mean number of eggs per mated female, eggs per egg mass, number 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 minimum standard criteria for reproductive endpoints established by EPA based on test method 100.5 and the latest revisions based on discussions with regulatory scientists (≥ 800 eggs per egg mass or ≥ 80% hatch).

The mean number of eggs per egg mass among midges exposed to the 5.5, 11, 21, 42, and 85 mg/kg mean measured treatment levels was 1247, 1077, 1157, 1204, and 1198, respectively. Statistical analysis (Bonferroni's Adjusted t-Test) determined 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, 11, 21, 42, and 85 mg/kg mean measured treatment levels was 91, 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 mean number of egg masses per mated female among midges exposed to the 5.5, 11, 21, 42, and 85 mg/kg mean measured treatment levels was 0.74, 0.95, 0.83, 0.89, and 0.93, respectively. Statistical analysis (Steel's Many-One Rank Sum Test) determined no significant difference in the mean number of egg masses per mated female in any of the treatment levels tested compared to the negative control (0.67).

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 (Bonferroni's Adjusted *t*-Test) determined no significant difference in the mean number of eggs per mated female in any of the treatment levels tested compared to the control (828 eggs per female).

The mean number of days to oviposition was 1.0 and 1.1 among midges in the negative control and solvent control, respectively. The mean number of days to oviposition among midges exposed to the 5.5, 11, 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 (317)	1.0 (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)	911 (155)	1.0 (0.090)
11	1077 (131)	95 (4.1)	0.95 (0.090)	1034 (191)	1.1 (0.16)
21	1157 (130)	95 (3.7)	0.83 (0.19)	961 (271)	1.0 (0.040)
42	1204 (187)	98 (1.2)	0.89 (0.10)	1077 (253)	1.0 (0.060)
85	1198 (116)	93 (8.3)	0.93 (0.10)	1109 (127)	1.1 (0.11)

SD = Standard deviation

Conclusion

No adverse effects up to and including the highest concentration (100 mg a.s./kg) were observed; therefore the calculation of EC_{10} and EC_{20} values is not possible.

Endpoints based on arithmetic mean measured sediment concentrations are:

Endpoint	Arithmetic mean measured sediment concentration (mg a.s./kg)				Arithmetic mean measured pore water concentration (mg a.s./L)			
	NOEC	LOEC	LC_{50} (CI)	EC_{50}	NOEC	LOEC	LC_{50}	EC_{50}
Midge Larval Survival	85	> 85	> 85 (NA)[A]		1.4	> 1.4	> 1.4 (NA)[A]	-
Midge Larval Growth	85	> 85	-	> 85 (NA)[A]	1.4	> 1.4	-	> 1.4 (NA)[A]
Percent emergence	85	> 85	-	> 85 (NA)[A]	1.4	> 1.4	-	> 1.4 (NA)[A]
Male Emergence Rate	85	> 85	-	> 85 (NA)[A]	1.4	> 1.4	-	> 1.4 (NA)[A]
Female Emergence Rate	85	> 85	-	> 85 (NA)[A]	1.4	> 1.4	-	> 1.4 (NA)[A]
Male Days to Death	85	> 85	-	> 85 (NA)[A]	1.4	> 1.4	-	> 1.4 (NA)[A]
Female Days to Death	85	> 85	-	> 85 (NA)[A]	1.4	> 1.4	-	> 1.4 (NA)[A]
Number of Eggs per Egg Mass	85	> 85	-	> 85 (NA)[A]	1.4	> 1.4	-	> 1.4 (NA)[A]
Percent hatch	85	> 85	-	> 85 (NA)[A]	1.4	> 1.4	-	> 1.4 (NA)[A]
Egg masses per mated female	85	> 85	-	> 85[B] (NA)[A]	1.4	> 1.4	-	> 1.4 (NA)[A]
Eggs per Mated Female	85	> 85	-	> 85[B] (NA)[A]	1.4	> 1.4	-	> 1.4 (NA)[A]
Days to Oviposition	85	> 85	-	ND[B] (NA)[A]	1.4	> 1.4	-	ND[B] (NA)[A]

[A] NA = Not applicable. LC/EC_{50} 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; [redacted]; 2017; M-586715-01-1
Title:	Pseudokirchneriella subcapitata growth inhibition test with BCS-CN88460 (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, §122-2, 123-2; OCSPP Guideline 850.4500 (January 2012)
Guideline deviation(s):	According to OCSPP 850.4500 the measured test substance concentration at test 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-CN88460 (tech.) Batch code: BCS-CN88460-01-06 Specification: 102000028196 Purity: 94.2% w/w
Guideline(s) adaptation	According to OCSPP 850.4500 the measured test substance concentration at test initiation is considered appropriate to use for unstable test items. However, in this study the EC_x calculations after 96 hours were performed using the mean measured values to follow the recommendations from OPPTS 850.1000.
Test species	Freshwater green algae (*Pseudokirchneriella subcapitata)* Strain SAG 61.81
Culturing conditions	400 µL of a 7-10 days old stock culture was transferred into a 300 mL cotton plugged Erlenmeyer flask containing about 100 mL of nutrient medium every 7-10 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 age/size at study initiation	Pre cultures were prepared from stock cultures 3 days before the start of the test using OECD medium.
Test solutions	Nominal concentrations: 0.0238, 0.0763, 0.244, 0.781, 2.50 and 8.00 mg a.s./L Corresponding geometric mean measured concentrations (0 – 72 h): 0.020, 0.062, 0.196, 0.598, 1.82 and 2.02 mg a.s./L Corresponding arithmetic mean measured concentrations (0 – 96 h): 0.0201, 0.0633, 0.198, 0.608, 1.61 and 1.82 mg a.s./L 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 found on the surface of the test media over the whole test 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 Total exposure duration: 96 hours
Initial cell density	10^4 cells/mL at test initiation
Test conditions	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 – 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 in 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 72 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-CN88460 present in the test medium of all treatment levels and the controls after 0, 72 and 96 hours.
Data analysis	E_rC_x values (e.g. x = 50) and confidence intervals were calculated for the standard exposure period, using a commercial program (ToxRatPro 3.2.1).

p.m. = pure metabolite

Results:

Validity criteria acc. to OECD TG 201	Required	Obtained
The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period.	16	77
The mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3) in the control cultures must not exceed 35%.	< 35%	14.8%
The coefficient of variation of average specific growth rates during the 72-hour test period in replicate control cultures must not exceed 7%.	< 7%	1.3%

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 arithmetic mean measured concentrations 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 (mg a.s./L)	Geometric mean measured concentrations after 72 hours (mg a.s./L)	Arithmetic mean measured concentrations after 96 hours (mg a.s./L)	% of nominal concentrations*		
			0-hour	72-hour	96-hour
0.0238	0.0200	0.0201	79.8	88.2	84.9
0.0763	0.0620	0.0633	76.1	86.6	86.0
0.244	0.196	0.198	77.5	83.3	82.4
0.781	0.598	0.608	72.6	80.8	80.3
2.50	1.82	1.61	102	52.0	39.3
8.00	2.02	1.82	26.8	23.9	17.8

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

Biological results:
No morphological change in algae was observed in any test concentration.

72 hours

Geometric mean measured concentrations (mg a.s./L)	Mean cell number (cells/mL × 10^4)	Inhibition of average specific growth rate (%)*
Water Control	77.0	-
Solvent Control	77.4	-
0.0200	74.9	0.7
0.0620	76.5	0.2
0.196	75.1	0.6
0.598	67.2	3.2#
1.82	52.2	9.0#
2.02	61.0	5.7#

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

Geometric mean measured concentrations (mg a.s./L)	Yield (cells/mL × 10^4)	Inhibition of yield (%)
Pooled control	76.2	0
0.0200	73.9	3.0
0.0620	75.5	0.9
0.196	74.1	2.8
0.598	66.2	13.2#
1.82	51.2	32.8#
2.02	60.0	21.3#

Significantly (α = 0.05, one-sided smaller) reduced, based on Williams multiple 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	-0.5
0.196	1383.6	3.8
0.598	1272.1	11.5#
1.82	998.5	30.6#
2.02	1138.3	20.8#

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

96 hours

Arithmetic mean measured concentrations (mg a.s./L)	Mean cell number (cells/mL × 10^4)	Inhibition of average specific growth rate (%)*
Water Control	220.0	
Solvent Control	219.4	-
0.0201	209.5	0.9
0.0633	211.8	0.7
0.198	210.6	0.8
0.608	191.4	2.6#
1.61	150.8	7.0#
1.82	140.1	8.3#

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

Arithmetic mean measured concentrations (mg a.s./L)	Yield (cells/mL × 10^4)	Inhibition of yield (%)
Pooled control	218.7	0
0.0201	208.5	4.7
0.0633	210.8	3.6
0.198	209.6	4.1
0.608	190.4	12.9#
1.61	149.8	31.5#
1.82	139.1	36.4#

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

Arithmetic mean measured concentrations (mg a.s./L)	Area under the growth curve (biomass integral)	Inhibition of Biomass integral (%)
Pooled control	4976.5	0
0.0201	4783.6	3.9
0.0633	4881.1	1.9
0.198	4787.9	3.8
0.608	4350.2	12.6#
1.61	3411.0	31.5#
1.82	3526.8	29.1#

Significantly (α = 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_rC_{50} 72 hours (95% C.I.):	**> 2.02 mg a.s./L (n.d.)**
E_rC_{20} 72 hours (95% C.I.)	> 2.02 mg a.s./L (n.d.)
E_rC_{10} 72 hours (95% C.I.)	> 2.02 mg a.s./L (n.d.)
LOE_rC 72 hours: lowest concentration with an significant effect compared to the control	0.598 mg a.s./L
NOE_rC 72 hours: highest concentration without an significant effect compared to the control	0.196 mg a.s./L
E_yC_{50} 72 hours (95% C.I.):	**> 2.02 mg a.s./L (n.d.)**
E_yC_{20} 72 hours (95% C.I.):	1.15 mg a.s./L (0.65 to 1.70 mg a.s./L)
E_yC_{10} 72 hours (95% C.I.):	0.42 mg a.s./L (0.08 to 0.71 mg a.s./L)
LOE_yC 72 hours: lowest concentration with an significant effect compared to the control	0.598 mg a.s./L
NOE_yC 72 hours: highest concentration without an significant effect compared to the control	0.196 mg a.s./L
E_rC_{50} 96 hours (95% C.I.):	**> 1.82 mg a.s./L (n.d.)**
LOE_rC 96 hours: lowest concentration with an significant effect compared to the control	0.608 mg a.s./L
NOE_rC 96 hours: highest concentration without an significant effect compared to the control	0.198 mg a.s./L
E_yC_{50} 96 hours (95% C.I.)	**> 1.82 mg a.s./L (n.d.)**
LOE_yC 96 hours: lowest concentration with an significant effect compared to the control	0.608 mg a.s./L
NOE_yC 96 hours: highest concentration without an significant effect compared to the control	0.198 mg a.s./L
E_bC_{50} 96 hours (95% C.I.)	**> 1.82 mg a.s./L (n.d.)**
LOE_bC 96 hours: lowest concentration with an significant effect compared to the control	0.608 mg a.s./L
NOE_bC 96 hours: highest concentration without an significant effect compared to the control	0.198 mg a.s./L

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

Report:	KCA 8.2.6.1/02; [redacted]; 2017; M-587659-01-1
Title:	Pseudokirchneriella subcapitata growth inhibition test with BCS-CN88460-carboxylic-acid (BCS-CY26497)
Report No.:	EBLNN290
Document No.:	M-587659-01-1
Guideline(s):	OECD Guideline 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test (July 28, 2011) OCSPP Guideline 850.4500: Algal Toxicity (January 2012)
Guideline deviation(s):	According to OCSPP 850.4500 the measured test substance concentration at test 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 850.1000.
GLP/GEP:	**yes**

Material and methods

Test material	BCS-CN88460-carboxylic-acid (BCS-CY26497) Batch code: SES 12631-19-9 Sample description: TOX20054-01 Purity: 98.8% w/w
Guideline(s) adaptation	According to OCSPP 850.4500 the measured test substance concentration at test initiation is considered appropriate to use for unstable test items. However, in this study the EC_x calculations after 96 hours were performed using the mean measured values to follow the recommendations from OPPTS 850.1000.
Test species	Freshwater green algae (*Pseudokirchneriella subcapitata)* Strain SAG 61.81
Culturing conditions	1000 µL of a 7-10 days old stock culture was transferred into a 300 mL cotton plugged Erlenmeyer flask containing about 100 mL of nutrient medium every 7-10 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 age/size at study initiation	Pre-cultures were prepared from stock cultures 3 days before the start of the test using OECD medium.
Test solutions	Nominal concentrations: 3.13, 6.25, 12.5, 25.0 and 50.0 mg p.m./L Corresponding geometric mean measured concentrations (0 – 72 h): 3.46, 6.65, 13.4, 26.3 and 35.1 mg p.m./L Corresponding arithmetic mean measured concentrations (0 – 96 h): 3.43, 6.63, 13.4, 26.2 and 35.3 mg p.m./L 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 No. of vessels per solvent control (replicates): 4
Exposure	Static Total exposure duration: 72 hours with a prolongation to 96 hours
Initial cell density	10,000 cells/mL at test initiation

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 in 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 72 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 commercial program (ToxRatPro 3.2.1)

p.m. = pure metabolite

Results:

Validity criteria acc. to OECD TG 201	Required	Obtained
The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period.	16	72.3
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%	10.6%
The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures must not exceed 7%.	< 7%	1.3%

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 arithmetic mean measured concentrations of BCS-CN88460-carboxylic acid (BCS-CY26497), respectively. No residues of BCS-CN88460-carboxylic acid (BCS-CY26497) 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 (mg p.m./L)	Geometric mean measured concentrations after 72 hours (mg p.m./L)	Arithmetic mean measured concentrations after 96 hours (mg p.m./L)	% of nominal concentrations*		
			0-hour	72-hour	96-hour
3.13	3.46	3.43	1.11	0.99	0.98
6.25	6.65	6.63	1.07	0.99	0.99
12.5	13.4	13.4	1.08	0.99	0.99
25.0	26.3	26.2	1.06	1.00	0.99
50.0	35.1	35.3	0.70	1.01	1.01

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

Biological results:
No morphological change in algae was observed in any test concentration.

72 hours

Geometric mean measured concentrations (mg p.m./L)	Mean cell number after 72 h per mL	Inhibition of average specific growth rate [%]
Water Control	723000	-
Solvent Control	708000	-
3.46	719000	- 0.1
6.65	676000	1.3
13.4	722000	- 0.2
26.3	773000	-1.7
35.1	662000	1.8 #

- % inhibition: Increase in growth relative to the pooled controls.
Significantly (α = 0.05, one-sided smaller) reduced, based on Multiple sequentially-rejective Welsh-t test after Bonferroni Holm

Geometric mean measured concentrations (mg p.m./L)	Yield (cells/mL × 10^4)	Inhibition of yield (%)
Pooled control	706	0
3.46	709	-0.5
6.65	66.6	5.6
13.4	71.2	-0.9
26.3	763	-8.1
35.1	65.2	7.6#

Significantly (α = 0.05, one-sided smaller) reduced, based on Multiple sequentially-rejective Welsh-t test after Bonferroni Holm

Geometric mean measured concentrations (mg p.m./L)	Area under the growth curve (biomass integral)	Inhibition of Biomass integral (%)
Pooled control	1296.6	0
3.46	1267.3	0.2
6.65	1215.1	4.3
13.4	1295.5	-2.1
26.3	1356.1	-6.8
35.1	1189.8	6.3#

Significantly (α = 0.05, one-sided smaller) reduced, based on Multiple sequentially-rejective Welsh-t test after Bonferroni Holm

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	-
Solvent Control	1997000	1.324	-
3.43	2031000	1.328	- 0.2
6.63	1931000	1.316	0.7
13.4	2010000	1.325	0.0
26.2	2014000	1.326	0.0
35.3	1845000	1.304	1.6#

- % inhibition = increase in growth relative to the pooled controls
= Significantly (α = 0.05, one-sided smaller) reduced, based on Multiple sequentially-rejective Welsh-t-test after Bonferroni Holm

Arithmetic mean measured concentrations (mg p.m./L)	Yield (cells/mL × 10^4)	Inhibition of yield (%)
Pooled control	199.6	0
3.43	202.1	-1.3
6.63	192.1	3.8
13.4	200.0	-0.2
26.2	200.4	-0.4
35.3	183.5	8.1#

Significantly (α = 0.05, one-sided smaller) reduced, based on Multiple sequentially-rejective Welsh-t test after Bonferroni Holm

Arithmetic mean measured concentrations (mg p.m./L)	Area under the growth curve (biomass integral)	Inhibition of Biomass integral (%)
Pooled control	4511.6	0
3.43	4543.5	-0.7
6.63	4319.4	4.3
13.4	4550.5	-0.9
26.2	4676.0	-3.6
35.3	4174.2	7.5#

-% inhibition: Increase in growth relative to the pooled control
Significantly (α = 0.05, one-sided smaller) reduced, based on Multiple sequentially-rejective Welsh-t test after Bonferroni Holm

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

E_rC_{50} 72 hours (95% C.I.):	**> 35.1 mg p.m./L (n.d.)**
E_rC_{20} 72 hours (95% C.I.)	> 35.1 mg p.m./L (n.d.)
E_rC_{10} 72 hours (95% C.I.)	> 35.1 mg p.m./L (n.d.)
LOE_rC 72 hours: lowest concentration with an significant effect compared to the control	35.1 mg p.m./L
NOE_rC 72 hours: highest concentration without an significant effect compared to the control	26.3 mg p.m./L
E_yC_{50} 72 hours (95% C.I.):	**n.d.**
E_yC_{20} 72 hours (95% C.I.)	n.d.
E_yC_{10} 72 hours (95% C.I.)	n.d.
LOE_yC 72 hours: lowest concentration with an significant effect compared to the control	35.1 mg p.m./L
NOE_yC 72 hours: highest concentration without an significant effect compared to the control	26.3 mg p.m./L
E_bC_{50} 72 hours (95% C.I.):	**n.d.**
E_bC_{20} 72 hours (95% C.I.)	n.d.
E_bC_{10} 72 hours (95% C.I.)	n.d.
LOE_bC 72 hours: lowest concentration with an significant effect compared to the control	35.1 mg p.m./L
NOE_bC 72 hours: highest concentration without an significant effect compared to the control	26.3 mg p.m./L
E_rC_{50} 96 hours (95% C.I.):	**> 35.3 mg p.m./L (n.d.)**
E_rC_{20} 96 hours (95% C.I.)	n.d.
E_rC_{10} 96 hours (95% C.I.)	n.d.
LOE_rC 96 hours: lowest concentration with an significant effect compared to the control	35.3 mg p.m./L
NOE_rC 96 hours: highest concentration without an significant effect compared to the control	26.2 mg p.m./L
E_yC_{50} 96 hours (95% C.I.):	**> 35.3 mg p.m./L (n.d.)**

E_yC_{20} 96 hours (95% C.I.)	n.d.
E_yC_{10} 96 hours (95% C.I.)	n.d.
LOE_yC 96 hours: lowest concentration with an significant effect compared to the control	35.3 mg p.m./L
NOE_yC 96 hours: highest concentration without an significant effect compared to the control	26.2 mg p.m./L
E_bC_{50} 96 hours (95% C.I.):	**> 35.3 mg p.m./L (n.d.)**
E_bC_{20} 96 hours (95% C.I.)	n.d.
E_bC_{10} 96 hours (95% C.I.)	n.d.
LOE_bC 96 hours: lowest concentration with an significant effect compared to the control	35.3 mg p.m./L
NOE_bC 96 hours: highest concentration without an significant effect compared to the control	26.2 mg p.m./L

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

Only at the highest test item concentration of 35.1 mg p.m./L a growth reduction was observed. The observed effects on growth inhibition were below the 10% level. 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 EC_{10} and EC_{20} calculation.

CA 8.2.6.2 Effects on growth of an additional algal species

Report: KCA 8.2.6.2/01; [redacted] J. R.; [redacted]; [redacted], J. R.; [redacted], K. H.; 2017; M-605074-01-1

Title: BCS-CN88460: A 96-hour toxicity test with the cyanobacteria (Anabaena flos-aquae)
Report No.: 149P-111
Document No.: M-605074-01-1
Guideline(s): OECD 201
EU Directive 92/69/EEC, Method C.3.
U.S. EPA OCSPP Number 850.4550
Guideline deviation(s): none
GLP/GEP: **yes**

Material and methods:

Test material	BCS-CN88460 (tech) Batch number: 2013-006492 CAS number: 1255734-28-1 Purity: 94.2% w/w
Guideline(s) adaptation	None specified
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 study initiation	Algal cells for this study were taken from a culture that had been transferred to fresh medium three days prior to test initiation.
Test solutions	Nominal concentrations: 0.024, 0.076, 0.24, 0.78, 2.5 and 8.0 mg a.s./L. 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 (0-96 h): 0.020, 0.072, 0.23, 0.73, 2.1 and 4.8 mg a.s./L Controls: water and solvent control (dimethylformamide at 0.1 µL/mL) Evidence of undissolved material: Visible particulates in 8.0 mg a.s./L test solutions at test initiation and in test chambers throughout the 96 hour 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 Total exposure duration: 96 hours
Initial cell density	10^4 cells/mL at test initiation
Test conditions	Temperature: 23.4 – 23.8°C Photoperiod: continuous light Light intensity: 1940 to 2350 lux pH of controls (0 – 96 hours): 7.3 - 9.5 Growth medium same as culture medium: Yes Type of light: artificial (Cool white fluorescent light)
Parameters Measured / Observations	Temperature was continuously monitored throughout the study. The pH of the medium in each treatment and control group was measured at test initiation and at 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 conducting cell counts 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 adherence of the cells to the test chamber.
Sampling for chemical analysis	Samples were collected from the batches of test solution prepared for each treatment and control group at the beginning of the test, from surrogate replicates included for analytical sampling at 72 hours, and from test solution pooled from the 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 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.

Results:

Validity criteria acc. to OECD TG 201	Required	Obtained
The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period.	16	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%.	< 10%	6.17%*

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

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 mean 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 the limit of quantification (LOQ) = 0.0024 mg a.s./L) which was used as the lowest standard concentration during this study.

Nominal Concentration (mg a.s./L)	Geometric mean measured concentrations after 72 hours (mg a.s./L)	Time weighted mean measured concentrations after 96 hours (mg a.s./L)	% of nominal concentrations		
			0-hour	72-hour	96-hour
0.024	0.021	0.02	90.5	82.6	69.9
0.076	0.075	0.072	106	90.5	81.3
0.24	0.24	0.23	106	91.0	79.1
0.78	0.76	0.73	99.7	94.6	73.3
2.5	2.2	2.1	91.5	81.4	67.7
8.0	5.1	4.8	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 aggregation 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
0.76	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	10
0.24	752000	2
0.76	599500	22
2.2	703000	8
5.1	18500#	98

Treatment group mean was significantly reduced (Dunnett'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	15726000	0
0.021	12270000	22
0.075	13248000	16
0.24	14016000	11
0.76	12222000	22
2.2	13728000	13
5.1	435000#	97

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

96 hours

Time weighted mean measured concentrations after 96 hours (mg a.s./L)	Mean cell number after 96 h (cells/mL × 10^4)*	Inhibition of average specific growth rate (%)
Pooled Control	257.9	-
0.02	246.5	2
0.072	318.9	-4
0.23	258.6	1
0.73	293.0	-2
2.1	255.1	0
4.8	2.15#	89

* Values not given in study report; calculated on the basis of single replicate values given in study report.
- % Inhibition: Increase in growth relative to the pooled control
Treatment group was significantly reduced (Dunnett's Test, $p < 0.05$) when compared to the pooled control mean

Time weighted mean measured concentrations after 96 hours (mg a.s./L)	Yield (cells/mL)	Inhibition of yield (%)
Pooled Control	2569375	-
0.02	2455000	4
0.072	3178750	-24
0.23	2576250	0
0.73	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 (mg a.s./L)	Area under the growth curve (biomass integral)	Inhibition of Biomass integral (%)
Pooled Control	55726500	-
0.02	50334000	10
0.072	59655000	-7
0.23	53955000	3
0.73	54456000	2
2.1	52659000	6
4.8	795000[#]	99

- % 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

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_rC_{50} 72 hours (95% C.I.):	**4.8 mg a.s./L (4.7 to 4.9 mg a.s./L)**
E_rC_{20} 72 hours (95% C.I.):	4.6 mg a.s./L (4.6 to 4.7 mg a.s./L)
E_rC_{10} 72 hours (95% C.I.):	4.3 mg a.s./L (4.3 to 4.4 mg a.s./L)
LOE_yC 72 hours: lowest concentration with an significant effect compared to the control	5.1 mg a.s./L
NOE_rC 72 hours: highest concentration without an significant effect compared to the control	2.2 mg a.s./L
E_bC_{50} 72 hours (95% C.I.):	**3.5 mg a.s./L (1.4 to > 5.1 mg a.s./L)**
LOE_bC 72 hours: lowest concentration with an significant effect compared to the control	5.1 mg a.s./L
NOE_bC 72 hours: highest concentration without an significant effect compared to the control	2.2 mg a.s./L
E_yC_{50} 72 hours (95% C.I.):	**3.4 mg a.s./L (1.5 to > 5.1 mg a.s./L)**
E_yC_{20} 72 hours (95% C.I.):	2.9 mg a.s./L (0.89 to > 5.1 mg a.s./L)
E_yC_{10} 72 hours (95% C.I.):	2.6 mg a.s./L (0.68 to > 5.1 mg a.s./L)
LOE_rC 96 hours: lowest concentration with an significant effect compared to the control	5.1 mg a.s./L
NOE_rC 96 hours: highest concentration without an significant effect compared to the control	2.2 mg a.s./L

E_rC_{50} 96 hours (95% C.I.):	**3.7 mg a.s./L (1.8 to > 4.8 mg a.s./L)**
E_rC_{20} 96 hours (95% C.I.):	3.0 mg a.s./L (0.89 to > 4.8 mg a.s./L)
E_rC_{10} 96 hours (95% C.I.):	2.8 mg a.s./L (0.62 to > 4.8 mg a.s./L)
LOE_rC 96 hours: lowest concentration with an significant effect compared to the control	4.8 mg a.s./L
NOE_rC 96 hours: highest concentration without an significant effect compared to the control	2.1 mg a.s./L
E_bC_{50} 96 hours (95% C.I.):	**3.0 mg a.s./L (2.3 to 4.0 mg a.s./L)**
LOE_bC 96 hours: lowest concentration with an significant effect compared to the control	4.8 mg a.s./L
NOE_bC 96 hours: highest concentration without an significant effect compared to the control	2.1 mg a.s./L
E_yC_{50} 96 hours (95% C.I.)	**2.9 mg a.s./L (2.2 to 3.8 mg a.s./L)**
E_yC_{20} 96 hours (95% C.I.):	2.4 mg a.s./L (1.7 to 3.4 mg a.s./L)
E_yC_{10} 96 hours (95% C.I.):	2.2 mg a.s./L (1.5 to 3.2 mg a.s./L)
LOE_rC 96 hours: lowest concentration with an significant effect compared to the control	4.8 mg a.s./L
NOE_rC 96 hours: highest concentration without an significant effect compared to the control	2.1 mg a.s./L

Growth inhibition was observed only at the highest test item concentration. The number of effect concentrations is not sufficient for a reasonable EC_{15} and EC_{20} calculation.

Report:	KCA 8.2.6.2/02; [redacted], J. R.; [redacted]; [redacted], J. R.; [redacted], K. H.; 2017; M-604811-01-1
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

Test material	BCS-CN88460 (tech.) Batch number: 2013-006492 CAS number: 1255734-28-1 Purity: 94.2% w/w
Guideline(s) adaptation	None specified
Test species	Marine diatom (*Skeletonema costatum)*
Culturing conditions	The algal cells were cultured and tested in saltwater algal 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 study initiation	Algal cells for this study were taken from a culture that had been transferred to fresh medium three days prior to test initiation.
Test solutions	Nominal concentrations: 0.024, 0.076, 0.24, 0.78, 2.5 and 8.0 mg a.s./L Corresponding geometric mean measured concentrations (0 – 72 h): 0.017, 0.060, 0.22, 0.68, 1.8 and 3.2 mg a.s./L Corresponding time-weighted mean measured concentrations (0 – 96 h): 0.016, 0.056, 0.20, 0.65, 1.7, 2.9 mg a.s./L Controls: water and solvent controls (dimethylformamide at 0.1 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 a.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 Total exposure duration: 96 hours
Initial cell density	10^4 cells/mL at test initiation

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	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 nine locations surrounding the test flasks. The pH of the medium in each treatment and control group was measured at test initiation, at approximately 72 hours 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 adherence of the cells to the test chamber.
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 test 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.

Results:

Validity criteria acc. to OECD TG 201	Required	Obtained
The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period.	16	120
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%	23.6%
The coefficient of variation of average specific growth rates during the 72 h test period in replicate control cultures must not exceed 7%.	< 7%	3.4%

<u>Analytical results:</u>

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 (mg a.s./L)	Geometric mean measured concentrations after 72 hours (mg a.s./L)	Time weighted mean measured concentrations after 96 hours (mg a.s./L)	% of nominal concentrations		
			0-hour	72-hour	96-hour
0.024	0.017	0.016	91.4	55.0	37.4
0.076	0.06	0.056	96.4	65.7	41.2
0.24	0.22	0.20	108	76.3	56.6
0.78	0.68	0.65	96.4	79.9	56.0
2.50	1.8	1.7	91.0	59.8	38.0
8.00	3.2	2.9	47.3	33.5	20.1

Biological results:
No morphological change in algae was observed in any test concentration. No adherence of cells to the test chambers or flocculation or aggregation of cells was observed.

72 hours

Geometric mean measured concentrations after 72 hours (mg a.s./L)	Mean cell number (cells/mL × 10^4)*	Inhibition of average specific growth rate (%)
Pooled control	120.3	-
0.017	119.5	2
0.06	119.8	2
0.22	118.0	0
0.68	90.3	6
1.8	50.8	18
3.2	44.7#	20

*Mean cell number after 72 h not presented in study report but calculated on the basis of single replicate values.
Treatment group was significantly reduced (Dunnett's Test, $p < 0.05$) when compared to the pooled control

Geometric mean measured concentrations (mg a.s./L)	Yield (cells/mL)	Inhibition of yield (%)
Pooled control	1192500	-
0.017	1095000	8
0.06	1087500	9
0.22	1170000	2
0.68	892500#	25
1.8	498250#	58
3.2	437250#	63

Treatment group mean was significantly reduced (Dunnett'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
1.8	32250000[#]	44
3.2	28920000[#]	50

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

96 hours

Time weighted mean measured concentrations after 96 hours (mg a.s./L)	Mean cell number (cells/mL × 10^4)	Inhibition of average specific growth rate (%)
Pooled control	161.0	-
0.016	150.5	2
0.056	153.5	1
0.20	158.0	0
0.65	176.0	-2
1.7	124.0	5
2.9	107.0[#]	8

- % Inhibition: Increase in growth relative to the pooled control
[#] Treatment group was significantly reduced (Dunnett's Test, $p < 0.05$) when compared to the pooled control

Time weighted mean measured concentrations after 96 hours (mg a.s./L)	Yield (cells/mL)	Inhibition of yield (%)
Pooled control	1600000	-
0.016	1495000	7
0.056	1525000	5
0.20	1570000	2
0.65	1750000	-9
1.7	1230000	23
2.9	1060000[#]	34

- % 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 (mg a.s./L)	Area under 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
1.7	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

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_rC_{50} 72 hours (95% C.I.):	**> 3.2 mg a.s./L (n.d.)**
E_rC_{20} 72 hours (95% C.I.)	2.8 mg a.s./L (2.3 to 3.5 mg a.s./L)
E_rC_{10} 72 hours (95% C.I.)	1.1 mg a.s./L (0.73 to 1.6 mg a.s./L)
LOE_rC 72 hours: lowest concentration with an significant effect compared to the control	0.68 mg a.s./L
NOE_rC 72 hours: highest concentration without an significant effect compared to the control	0.22 mg a.s./L
E_bC_{50} 72 hours (95% C.I.):	**2.2 mg a.s./L (1.70 to 2.90 mg a.s./L)**
LOE_bC 72 hours: lowest concentration with an significant effect compared to the control	0.68 mg a.s./L
NOE_bC 72 hours: highest concentration without an significant effect compared to the control	0.22 mg a.s./L
E_yC_{50} 72 hours (95% C.I.):	**1.8 mg a.s./L (1.3 to 2.4 mg a.s./L)**
E_yC_{20} 72 hours (95% C.I.):	0.55 mg a.s./L (0.29 to 1.0 mg a.s./L)
E_yC_{10} 72 hours (95% C.I.):	0.30 mg a.s./L (0.13 to 0.72 mg a.s./L)
LOE_yC 72 hours: lowest concentration with an significant effect compared to the control	0.68 mg a.s./L
NOE_yC 72 hours: highest concentration without an significant effect compared to the control	0.22 mg a.s./L
E_rC_{50} 96 hours (95% C.I.):	**> 2.9 mg a.s./L (n.d.)**
E_rC_{20} 96 hours (95% C.I.)	> 2.9 mg a.s./L (n.d.)
E_rC_{10} 96 hours (95% C.I.)	> 2.9 mg a.s./L (n.d.)
LOE_rC 96 hours lowest concentration with an significant effect compared to the control	2.9 mg a.s./L
NOE_rC 96 hours highest concentration without an significant effect compared to the control	1.7 mg a.s./L
E_bC_{50} 96 hours (95% C.I.):	**> 2.9 mg a.s./L (n.d.)**

LOE_bC 96 hours: lowest concentration with an significant effect compared to the control	1.7 mg a.s./L
NOE_bC 96 hours: highest concentration without an significant effect compared to the control	0.65 mg a.s./L
E_yC_{50} 96 hours (95% C.I.)	**> 2.9 mg a.s./L (n.d.)**
E_yC_{20} 96 hours (95% C.I.):	1.6 mg a.s./L (< 0.016 to > 2.0 mg a.s./L)
E_yC_{10} 96 hours (95% C.I.):	1.1 mg a.s./L (< 0.016 to 2.0 mg a.s./L)
LOE_yC 96 hours: lowest concentration with an significant effect compared to the control	2.9 mg a.s./L
NOE_yC 96 hours: highest concentration without an significant effect compared to the control	1.7 mg a.s./L

Report: KCA 8.2.6.2/03; [redacted], J. R.; [redacted]; [redacted], J. R.; [redacted], K. H.; 2017; M-604809-01-1
Title: BCS-CN88460: A 96-hour toxicity test with the freshwater diatom (Navicula pelliculosa)
Report No.: 149P-112A
Document No.: M-604809-01-1
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

Test material	BCS-CN88460 (tech.) Batch number: 2013-006492 CAS number: 1255734-28-1 Purity: 99.1% w/w
Guideline(s) adaptation	None specified
Test species	Freshwater diatom (*Navicula pelliculosa)*
Culturing conditions	The algal cells were cultured and tested in freshwater AAP medium with silica constituents. 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 study initiation	Algal cells for this study were taken from a culture that had been transferred to fresh medium three days prior to test initiation.

Test solutions	Nominal concentrations: 0.024, 0.076, 0.24, 0.78, 2.5 and 8.0 mg a.s./L 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 Controls: water and solvent controls (dimethylformamide at 0.1 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 a.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 Total exposure duration: 96 hours
Initial cell density	10^4 cells/mL at test initiation
Test conditions	Temperature: 24.0 – 24.3°C Photoperiod: Continuous light Light intensity at surface of test vessels: 3870 to 4600 lux pH of controls (0-72 h): 7.5 – 9.9 Growth medium same as culture medium: Yes Type of light: artificial (Cool white fluorescent lamps)
Parameters Measured / Observations	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 nine locations surrounding the test flasks. The pH of the medium in each treatment and control group was measured at test initiation, at approximately 72 hours 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 adherence of the cells to the test chamber.
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 test 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.

Results:

Validity criteria acc. to OECD TG 201	Required	Obtained
The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period.	16	359
The mean coefficient of variation for section-by-section specific growth rates in the control cultures must not exceed 35%.	< 35%	31.5%
The coefficient of variation of average specific growth rates during the 72 h test period in replicate control cultures must not exceed 10%.	< 10%	0.28%

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 (mg a.s./L)	Geometric mean measured concentrations after 72 hours (mg a.s./L)	Time weighted mean measured concentrations after 96 hours (mg a.s./L)	% of nominal concentrations		
			0-hour	72-hour	96-hour
0.024	0.014	0.014	65.0	55.3	42.7
0.076	0.053	0.050	73.7	65.3	47.8
0.24	0.20	0.19	94.3	76.6	60.3
0.78	0.67	0.63	97.7	75.9	57.4
2.50	1.80	1.70	89.8	55.8	47.8
8.00	2.00	2.00	37.2	17.4	23.2

Biological results:
No morphological change in algae was observed in any test concentration. No adherence of cells to the test chambers or flocculation or aggregation of cells was observed.

72 hours

Geometric mean measured concentrations after 72 hours (mg a.s./L)	Mean cell number (cells/mL × 10^4)*	Inhibition of average specific growth rate (%)
Solvent Control	359.0	-
0.014	362.0	0
0.053	344.5	1
0.20	334.8	2
0.67	333.3	1
1.80	280.8#	4
2.00	221.0#	8

*Mean cell number after 72 h 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
0.053	3435000	4
0.20	3337500	7
0.67	3322500	7
1.80	2797500#	22
2.00	2200000#	39

- % Inhibition: Increase of yield relative to the pooled control
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)	Area under the growth curve (biomass integral)	Inhibition of Biomass integral (%)
Solvent Control	68922000	-
0.014	62310000	10
0.053	58278000#	15
0.20	68352000	1
0.67	66360000	4
1.80	48666000#	29
2.00	36204000#	47

Treatment group mean was significantly reduced (Dunnett's Test, $p < 0.05$) when compared to the solvent control mean

96 hours

Time weighted mean measured concentrations after 96 hours (mg a.s./L)	Mean cell number (cells/mL × 10^4)*	Inhibition of average specific growth rate (%)
Solvent Control	441.5	-
0.014	370.5	3
0.050	302.0#	6
0.19	383.0	2
0.63	385.5	2
1.70	353.0	4
2.00	349.0	4

*Mean cell number after 96 h not presented in study report but calculated on the basis of single replicate values.
Treatment group was significantly reduced (Dunnett's Test, $p < 0.05$) when compared to the solvent control mean

Time weighted mean measured concentrations after 96 hours (mg a.s./L)	Yield (cells/mL)	Inhibition of yield (%)
Solvent Control	4405000	-
0.014	3695000	16
0.050	3010000[#]	32
0.19	3820000	13
0.63	3845000	13
1.70	3520000	20
2.00	3480000	21

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

Time weighted mean measured concentrations after 96 hours (mg a.s./L)	Area under the growth curve (biomass integral)	Inhibition of Biomass integral (%)
Solvent Control	164742000	-
0.014	149970000	9
0.050	135618000[#]	18
0.19	154242000	6
0.63	152370000	8
1.70	124476000[#]	24
2.00	104364000[#]	37

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

Conclusion

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_rC_{50} 72 hours (95% C.I.):	**> 2.0 mg a.s./L (n.d.)**
E_rC_{20} 72 hours (95% C.I.)	> 2.0 mg a.s./L (n.d.)
E_rC_{10} 72 hours (95% C.I.)	> 2.0 mg a.s./L (n.d.)
LOE_rC 72 hours: lowest concentration with an significant effect compared to the control	1.8 mg a.s./L
NOE_rC 72 hours: highest concentration without an significant effect compared to the control	0.67 mg a.s./L
E_bC_{50} 72 hours (95% C.I.):	**> 2.0 mg a.s./L (n.d.)**
LOE_bC 72 hours: lowest concentration with an significant effect compared to the control	1.8 mg a.s./L
NOE_bC 72 hours: highest concentration without an significant effect compared to the control	0.67 mg a.s./L
E_yC_{50} 72 hours (95% C.I.):	**> 2.0 mg a.s./L (n.d.)**

E_yC_{20} 72 hours (95% C.I.):	1.8 mg a.s./L (1.7 to 2.0 mg a.s./L)
E_yC_{10} 72 hours (95% C.I.):	1.7 mg a.s./L (1.4 to 1.9 mg a.s./L)
LOE_yC 72 hours: lowest concentration with an significant effect compared to the control	1.8 mg a.s./L
NOE_yC 72 hours: highest concentration without an significant effect compared to the control	0.67 mg a.s./L
E_rC_{50} 96 hours (95% C.I.):	**> 2.0 mg a.s./L (n.d.)**
E_rC_{20} 96 hours (95% C.I.)	2.0 mg a.s./L (n.d.)
E_rC_{10} 96 hours (95% C.I.)	2.0 mg a.s./L (n.d.)
LOE_rC 96 hours: lowest concentration with an significant effect compared to the control	2.0 mg a.s./L
NOE_rC 96 hours: highest concentration without an significant effect compared to the control	2.0 mg a.s./L
E_bC_{50} 96 hours (95% C.I.):	**> 2.0 mg a.s./L (n.d.)**
LOE_bC 96 hours: lowest concentration with an significant effect compared to the control	1.7 mg a.s./L
NOE_bC 96 hours: highest concentration without an significant effect compared to the control	0.63 mg a.s./L
E_yC_{50} 96 hours (95% C.I.)	**> 2.0 mg a.s./L (n.d.)**
E_yC_{20} 96 hours (95% C.I.):	0.82 mg a.s./L (> 0.014 to > 2.0 mg a.s./L)
E_yC_{10} 96 hours (95% C.I.)	< 0.014 mg a.s./L (n.d.)
LOE_yC 72 hours: lowest concentration with an significant effect compared to the control	2.0 mg a.s./L
NOE_yC 72 hours: highest concentration without an significant effect compared to the control	2.0 mg a.s./L

CA 8.2.7 Effects on aquatic macrophytes

Report:	KCA 8.2.7/01; [redacted]; 2017; M-593965-01-1
Title:	Lemna gibba G3 - Growth inhibition test with BCS-CN88460 under semi-static conditions
Report No.:	EBLNN016
Document No.:	M-593965-01-1
Guideline(s):	EU Directive 91/414/EEC Regulation (EC) Number 1107/2009 US EPA OCSPP 850.4400
Guideline deviation(s):	none
GLP/GEP:	**yes**

Material and methods

Test material	BCS-CN88460 (tech.) Batch ID: 2013-006492 TOX-No.: TOX 20011-01 Specification No.: 102000028196 Purity: 94.2 % w/w
Guideline(s) adaptation	not specified
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 in the main test.
Culturing conditions	Stock cultures are maintained in glass dishes filled with nutrient medium under illumination of 6500 - 7000 lux and a temperature of 23 – 26°C. Transfers into fresh nutrient 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 geometric mean measured concentrations: 0.0185, 0.0689, 0.249, 0.677, 2.09 and 3.02 mg a.s./L Control: water Solvent control: DMF (dimethylformamide) used as solvent (0.1 mL/L test solution) Evidence of undissolved material: In 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 per vessel: 12
Exposure	Semi-static Total exposure duration: 7 days

Test conditions	Incubation chamber used: not specified Vessels: 470 mL glass dishes with 200 mL test solution Temperature: 23.6 - 24.3°C 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 Measured / Observations	Visual observations were made on study days 3, 5 and 7. Counting of fronds and determination of total frond area was done on day 0, 3, 5 and 7. 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 chemical analysis	Duplicate samples of freshly prepared media were taken from all test levels and the controls on day 0, 3 and 5 and additionally in all aged test levels on day 1, 2, 3, 4, 5, 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	EC_x 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	Required	Obtained
Doubling time	< 2.5 days	1.8 days

Analytical results:

Measured concentrations between day 0 and day 7 ranged between 14 and 115% and are presented below. Therefore results are based 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 (0.000626 mg a.s./L).

Nominal concentration (mg a.s./L)	Geometric mean concentrations (mg a.s./L)	% of nominal concentrations									
		Day 0 New	Day 1 Aged	Day 2 Aged	Day 3 Aged	Day 3 New	Day 4 Aged	Day 5 Aged	Day 5 New	Day 6 Aged	Day 7 Aged
0.0238	0.0185	88	99	103	88	53	61	52	90	87	90
0.0763	0.0689	86	91	93	107	77	83	74	103	106	109
0.244	0.249	109	115	106	84	86	106	81	98	100	84
0.781	0.677	76	108	91	80	72	79	67	95	97	115
2.50	2.09	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.

Nominal concentration (mg a.s./L)	Geometric mean measured test concentration (mg a.s./L)	Mean frond number on day 7*	Total frond area on day 7* [mm²]	% Inhibition [B]	
				Mean growth rate for frond number	Mean growth rate for frond area
Control	Control	167	1425	-	-
Solvent control	Solvent control	170	1434	-	-
0.0238	0.0185	183	1563	-3.1	-0.8
0.0763	0.0689	184	1566	-3.3	-0.9
0.244	0.249	172	1478	-0.5	-0.5
0.781	0.677	172	1465	-0.5	-1.9
2.50	2.09	154	1294	3.6	2.8
8.00	3.02	151	1340	1.7	3.5

* Mean of 4 replicates

Conclusion
Endpoints were calculated based on geometric mean measured concentrations.

Endpoint (0-7 days)	Effect on mean growth rate of frond	Effect on mean growth rate of total frond area of plants
E_rC_{50} (95% C.I.):	> 3.02 mg a.s./L	> 3.02 mg a.s./L
E_rC_{10} (95% C.I.):	> 3.02 mg a.s./L	> 3.02 mg a.s./L
E_rC_{20} (95% C.I.):	> 3.02 mg a.s./L	> 3.02 mg a.s./L
LOE_rC: lowest concentration with an significant effect compared to the control	> 3.02 mg a.s./L	> 3.02 mg a.s./L
NOE_rC: highest concentration without an significant effect compared to the control	≥ 3.02 mg a.s./L	≥ 3.02 mg a.s./L

CA 8.2.8 Further testing on aquatic organisms

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 technical isoflucypram 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 laboratory conditions,
- 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 isoflucypram (technical and formulated product) to bees

Test substance	Test species/ study type	Endpoint	References
Isoflucypram tech.	Honeybee, 48 h	LD_{50} – oral > 106.3 µg a.s./bee LD_{50} – contact > 100 µg a.s./bee	[redacted]; 2014; M-503824-01-1 KCA 8.3.1.1.1/01 KCA 8.3.1.1.2/01
	Honeybee, 48 h	LD_{50} – oral 109.5 µg a.s./bee LD_{50} – contact 100 µg a.s./bee	[redacted]; [redacted]; 2013; M-472468-01-1 KCA 8.3.1.1.1/02 KCA 8.3.1.1.2/02
	Honeybee larva, 22 days	NOEC ≥ 406 mg a.s./kg NOED ≥ 62.5 µg a.s./larva	[redacted]; 2017; M-587515-01-1 KCA 8.3.1.3/01
	Bumble bee, 48 h	LD_{50} – oral > 200.2 µg a.s./bumble bee	[redacted]; 2015; M-542774-01-1 KCA 8.3.1.1.1/03
	Bumble bee, 48 h	LD_{50} – contact > 100 µg a.s./bumble bee	[redacted]; 2015; M-509048-01-1 KCA 8.3.1.1.2/03
Isoflucypram SC 200	Honeybee, 10 day chronic adult feeding study	LDD_{50} > 89.7 µg a.s./bee/day NOEDD ≥ 89.7 µg a.s./bee/day	[redacted], A.; 2015; M-540173-01-1 KCA 8.3.1.2/01

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; [redacted]; 2014; M-503824-01-1
Title:	Effects of BCS-CN88460 tech. (acute contact and oral) on honey bees (Apis mellifera L.) in the laboratory
Report No.:	89641035
Document No.:	M-503824-01-1
Guideline(s):	OECD 213 and 214 (1998)
Guideline deviation(s):	none
GLP/GEP:	**yes**

Objective:
The purpose of this study was to determine the acute contact and oral toxicity of BCS-CN88460 technical to the honeybee (*A. mellifera* L.) in the laboratory. Mortality of the bees was used as the toxic endpoint. Sublethal effects, such as changes in 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 mellifera*), obtained from a healthy and queen-right colony, bred by IBACON.
Under laboratory conditions *Apis mellifera* worker bees were exposed for 48 hours to a single dose of 100.0 µg a.s. per bee, by topical application of 5 µL, in a contact limit test and to a single dose of 106.3 µg a.s. per bee by feeding in an oral limit test (value based on the actual intake of the test item). Furthermore, each test consisted of a control, solvent control and a reference item group. In the contact limit test, tap water containing 0.5 % Adhaesit and pure acetone were used as control and solvent control, respectively. In the oral limit test a 50 % w/v sucrose solution containing solvent (5 % acetone) and 50 % w/v sucrose solution were used as solvent control and control, respectively. In both limit tests, Perfekthion EC (active ingredient 400.9 g/L dimethoate, Batch no.: FRE-000926) was used as reference item. Each treatment group consisted out of 5 replicates (test units) with 10 bees per replicate. Test units were stainless steel cages with 10 cm × 8.5 cm × 5.5 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 used to perform the statistical analysis was ToxRat Professional.

Findings:

Biological findings:

Test item	**BCS-CN88460 tech.**	
Test object	***Apis mellifera***	
Exposure	**Contact (solution in acetone)**	**Oral (sugar/ acetone/water solution)**
Dose [µg a.s./bee]	100.0	106.3
LD_{50} [µg a.s./bee]	> 100.0	> 106.3
LD_{20} [µg a.s./bee]	> 100.0	> 106.3
LD_{10} [µg a.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, α = 0.05).

Observations

Contact test

At the end of the contact toxicity test (48 hours after application), there was no 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). No behavioral abnormalities could be observed during the entire test.

Dosage [µg a.s./bee]	After 4 hours		After 24 hours		After 48 hours	
	Mortality mean %	Behavioral abnormalities mean %	Mortality mean %	Behavioral abnormalities mean %	Mortality mean %	Behavioral abnormalities mean %
Test item 100.0	0.0	0.0	0.0	0.0	0.0	0.0
Water	0.0	0.0	0.0	0.0	0.0	0.0
Solvent	0.0	0.0	0.0	0.0	0.0	0.0
Reference item						
0.30	4.0	22.0	98.0	0.0	98.0	0.0
0.20	8.0	8.0	18.0	2.0	22.0	0.0
0.15	0.0	0.0	14.0	0.0	20.0	0.0
0.10	0.0	0.0	4.0	0.0	6.0	0.0

Results are averages from five replicates (ten bees each) per dosage / control
Water = CO_2/water-treated control, solvent = CO_2/solvent control

Oral test

In the oral toxicity test, the maximum nominal test level of BCS-CN88460 tech. (i.e. 100 µg a.s./bee) corresponded to an actual intake of 106.3 µg a.s./bee. This dose level led to no mortality after 48 hours. Also no mortality occurred in the water control group (50 % w/v sucrose solution) and in the solvent control group at the end of test (after 48 hours), respectively.
No test item induced behavioral effects were observed at any time in the oral toxicity test.

Dosage [µg a.s./bee]	After 4 hours		After 24 hours		After 48 hours	
	Mortality mean %	Behavioral abnormalities mean %	Mortality mean %	Behavioral abnormalities mean %	Mortality mean %	Behavioral abnormalities mean %
Test item 106.3	0.0	0.0	0.0	0.0	0.0	0.0
Water	0.0	0.0	0.0	0.0	0.0	0.0
Solvent	0.0	0.0	0.0	0.0	0.0	0.0
Reference item						
0.32	82.0	12.0	100.0	0.0	100.0	0.0
0.16	4.0	38.0	88.0	12.0	94.0	0.0
0.08	0.0	0.0	4.0	10.0	6.0	0.0
0.05	0.0	0.0	0.0	0.0	0.0	0.0

Results are averages from five replicates (ten bees each) per dosage / control
Water = water control, solvent = solvent control

Validity criteria

All validity criteria of the test were met.

Validity criteria 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 % Oral test Control: 0 % Solvent control: 0 %
LD_{50} of the reference item should be in the specified range (contact test: 0.10 – 0.30 µg a.s./bee, oral test: 0.10 – 0.35 µg a.s./bee)	Contact test 0.24 µg a.s./bee Oral test 0.12 µg a.s./bee

Conclusion:

The $LD_{50/20/10}$ (48 h) were > 100 µg a.s./bee in the contact toxicity test and > 106.3 µg a.s./bee in the oral toxicity test.

The NOED (48 h) was ≥ 100 µg a.s./bee in the contact toxicity test and ≥ 106.3 µg a.s./bee in the oral toxicity test.

Report:	KCA 8.3.1.1.1/02; [redacted]; [redacted]; 2013; M-472468-01-1
Title:	Effects of BCS-CN88460 tech. (acute contact and oral) on honey bees (Apis mellifera L.) in the laboratory
Report No.:	83991035
Document No.:	M-472468-01-1
Guideline(s):	OECD 213 and 214 (1998)
Guideline deviation(s):	not applicable
GLP/GEP:	**yes**

Objective:

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

Material and methods:

Test item: BCS-CN88460 technical: 98.0 % w/w, Customer Order No.: TOX 09897-01, batch no.: NLL 8674-28-2.

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

Under laboratory conditions *Apis mellifera* worker bees were exposed for 48 hours to a single dose of 100.0 µg a.s. per bee, by topical application of 5µL, in a contact limit test and to a single dose of 109.5 µg a.s. per bee by feeding in an oral limit test (value based on the actual intake of the test item). Furthermore, each test consisted of a control, solvent control and a reference item group. In the contact limit test, tap water containing 0.5% Adhaesit and pure acetone were used as control and solvent control, respectively. In the oral limit test a sugar syrup/water/acetone solution and an untreated sugar syrup/water solution were used as solvent control and control, respectively. In both limit tests, Perfekthion EC (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 cm × 8.5 cm × 5.5 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 used to perform the statistical analysis was ToxRat Professional.

Findings:

Biological findings:

Test item	BCS-CN88460 tech.	
Test object	*Apis mellifera*	
Exposure	Contact (solution in acetone)	Oral (sugar syrup/acetone/water solution)
Dose [µg a.s./bee]	100.0	109.5
LD_{50} [µg a.s./bee]	> 100.0	> 109.5
LD_{20} [µg a.s./bee]	> 100.0	> 109.5
LD_{10} [µg a.s./bee]	> 100.0	> 109.5
NOED [µg a.s./bee]*	≥ 100.0	≥ 109.5

* 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 100.0 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.

Dosage [µg a.s./bee]	After 4 hours		After 24 hours		After 48 hours	
	Mortality mean %	Behavioral abnormalities mean %	Mortality mean %	Behavioral abnormalities mean %	Mortality mean %	Behavioral abnormalities mean %
Test item 100.0	0.0	44.0	0.0	0.0	0.0	0.0
Water	0.0	0.0	0.0	0.0	0.0	0.0
Solvent	0.0	0.0	0.0	0.0	0.0	0.0
Reference item						
0.30	6.0	54.0	92.0	6.0	96.0	0.0
0.20	0.0	36.0	78.0	8.0	88.0	6.0
0.15	0.0	14.0	50.0	20.0	68.0	6.0
0.10	0.0	0.0	10.0	0.0	16.0	0.0

Results are averages from five replicates (ten bees each) per dosage / control
Water = CO_2/water-treated control, solvent = CO_2/solvent control

Oral test

In the oral toxicity test, the maximum nominal test level of BCS-CN88460 tech. (i.e. 100 µg a.s./bee) corresponded to an actual intake of 109.5 µg 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).

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

Dosage [µg a.s./bee]	After 4 hours		After 24 hours		After 48 hours	
	Mortality mean %	Behavioral abnormalities mean %	Mortality mean %	Behavioral abnormalities mean %	Mortality mean %	Behavioral abnormalities mean %
Test item 109.5	0.0	0.0	0.0	0.0	0.0	0.0
Water	0.0	0.0	0.0	0.0	0.0	0.0
Solvent	0.0	0.0	0.0	0.0	0.0	0.0
Reference item						
0.32	14.0	76.0	94.0	2.0	96.0	4.0
0.16	0.0	40.0	52.0	6.0	60.0	0.0
0.08	0.0	14.0	18.0	4.0	24.0	0.0
0.06	0.0	0.0	0.0	0.0	2.0	0.0

Results are averages from five replicates (ten bees each) per dosage/control;
Water = water control; solvent = solvent control

Validity criteria:
All validity criteria of the test were met.

Validity criteria 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 % Oral test Control: 0 % Solvent control: 0 %
LD_{50} of the reference item should be in the specified range (contact test: 0.10 – 0.30 µg a.s./bee, oral test: 0.10 – 0.35 µg a.s./bee)	Contact test 0.16 µg a.s./bee Oral test 0.15 µg a.s./bee

Conclusion:
The $LD_{50/20/10}$ (48 h) were > 100 µg a.s./bee in the contact toxicity test and > 109.5 µg a.s./bee in the oral toxicity test.
The NOED (48 h) was ≥ 100 µg a.s./bee in the contact toxicity test and ≥ 109.5 µg a.s./bee in the oral toxicity test.

Report: KCA 8.3.1.1.1/03; [redacted]; 2015; M-542774-01-1
Title: BCS-CN88460 tech. Effects (acute oral) on bumble bees (Bombus terrestris l.) in the laboratory
Report No.: 97632105
Document No.: M-542774-01-1
Guideline(s): No specific guidelines available; study design based on OECD 213 (1998), Van der Steen (2001) and ICPPR non-Apis group (2014)
Guideline deviation(s): none
GLP/GEP: **yes**

Objective:
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 48 hours to a 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, solvent control and a reference item group. In the oral limit test a 50 % w/v sucrose solution containing solvent (4 % acetone and 1 % Tween80) and 50% w/v sucrose solution were used as solvent control and control, respectively. BAS 152 11 I EC (active ingredient 420.3 g/L dimethoate, Batch no.: FRE-001226) was used as reference item. Each treatment group consisted out of 80 bumble bees with 1 bumble bee per test unit (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 7 cm and a diameter of 2.2 cm at the large and 1.7 cm at the small opening.

The test was conducted in darkness, temperature was 25°C ± 2°C and humidity was 60 % ± 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 ToxRat Pro Version 2.10.

Findings:

Biological findings:

Test item	**BCS-CN88460 tech.**
Test object	***Bombus terrestris***
Exposure	**Oral (50 % w/v sucrose solution containing maximum 4 % acetone and 1 % Tween80)**
Dose [µg a.s./bumble bee] based on recorded consumption (considering bumble bees with food uptake of ≥ 10 mg/bumble bee)	200.2
LD_{50} [µg a.s./bumble bee]	> 200.2
LD_{20} [µg a.s./bumble bee]*	> 200.2
LD_{10} [µg a.s./bumble bee]*	> 200.2
NOED [µg a.s./bumble bee]**	≥ 200.2

* Since no mortality above 10 and 20% occurred in the test, the respective $LD_{10/20}$ values are assumed to be > 200.2 µg a.s./bumble bee

** Results obtained from test item 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, $\alpha = 0.05$).

Observations

Oral test:

In the oral toxicity test the maximum nominal test level corresponded to an actual intake of 200.2 µg a.s./bumble 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 abnormalities or sublethal effects occurred at any time during the test.

Treatment Group	After 4 hours		After 24 hours		After 48 hours	
	Mort-ality mean %	Behavioral abnormalities mean %	Mort-ality mean %	Behavioral abnormalities mean %	Mort-ality mean %	Behavioral abnormalities mean %
Test item 200.2 µg a.s./bumble bee	0.0	0.0	0.0	0.0	0.0	0.0
Control	0.0	0.0	0.0	0.0	0.0	0.0
Solvent Control	0.0	0.0	0.0	0.0	1.4	0.0
Reference item 3.9 µg dimethoate/bumble bee	33.0	31.1	97.8	0.0	97.8	0.0

Mean = mean of all individuals per treatment group
Control = 50 % w/v sucrose solution; solvent control = 50 % w/v sucrose solution containing 4 % acetone + 1 % Tween80
Considering bumble bees with a food uptake of > 10 mg/bumble bee per treatment group Test Item (n = 37), Control (n = 69), Solvent control (n = 72), Reference Item (n = 45)

Validity criteria:
All validity criteria of the test were met.

Validity criteria according to OECD 247	Obtained in this study
Control mortality should not exceed 10 % at test end	Control: 0 % Solvent control: 1.4 %
Mortality of the reference item should be ≥ 50 % at test end	Reference item: 97.8 % considering bumble bees with food uptake of > 10 mg/bumble bee (in total 45 bumble bees out of 80)

Conclusion:
The oral LD_{50} value after 48 hours was > 200.2 µg a.s./bumble bee.
The oral NOED value was calculated to be ≥ 200.2 µg a.s./bumble bee.

CA 8.3.1.1.2 Acute contact toxicity

For acute contact toxicity on honeybees please refer also to Section CA 8.3.1.1.1.

Report: KCA 8.3.1.1.2/03; [redacted]; 2015; M-509048-01-1
Title: Effects of BCS-CN88460 tech. (acute contact) on bumblebees (*Bombus terrestris* L.) in the laboratory
Report No.: 90281105
Document No.: M-509048-01-1
Guideline(s): No specific guidelines available; study design based on OECD 214 (1998) Van der Steen (2001) and ICPBR non-apis group (2014)
Guideline deviation(s): not applicable
GLP/GEP: **yes**

Objective:
The purpose of this study was to determine the acute contact 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 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. per bumble bee by topical application (contact limit test).
Furthermore, the test consisted of a control, solvent control and a reference item group. In the contact limit test tap water with 0.5% Tween80 and acetone were uses as control and solvent control, respectively.
BAS 152 11 I EC (active ingredient 400.9 g/L dimethoate, Batch no.: FRE-000926) was used as reference item. Each treatment group consisted out of 50 bumble bees with 1 bumble bee per test unit (replicate).
Test units were cylindrical, latticed plastic cages with a length of approximately 7 cm and a diameter of 2.2 cm at the large and 1.7 cm at the small opening.
The test was conducted in darkness, temperature was 25 °C ± 2 °C and humidity was 60 % ± 10 %. Biological observations, including mortality and sub-lethal effects were recorded 4, 24 and 48 h after application. The NOED was estimated using Fisher Exact Test (pairwise comparison, one-sided greater, $\alpha = 0.05$).

Findings:

Biological findings:

Test item	**BCS-CN88460 tech.**
Test object	***Bombus terrestris***
Exposure	**Contact (solution in acetone)**
Dose [µg a.s./bumble bee]	100
LD_{50} [µg a.s./bumble bee]	> 100
LD_{20} [µg a.s./bumble bee]*	> 100
LD_{10} [µg a.s./bumble bee]*	> 100
NOED [µg a.s./bumble bee]**	100

* Since no mortality above 10 and 20% occurred in the test, the respective LD_{10} and LD_{20} values are assumed to be > 100 µg a.s./bumble bee
** The NOED was estimated using Fisher's Exact Test (pairwise comparison with control, one-sided greater, $\alpha = 0.05$).

Observations

Contact test:
At test termination (48 hours after treatment) no mortality occurred at 100 µg 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 solvent control group (acetone). No test item related behavioral abnormalities occurred at any time of the test.

Treatment Group	After 4 hours		After 24 hours		After 48 hours	
	Mortality mean %	Behavioral abnormalities mean %	Mortality mean %	Behavioral abnormalities mean %	Mortality mean %	Behavioral abnormalities mean %
Test item 100 µg a.s./bumble bee	0.0	0.0	0.0	0.0	0.0	0.0
Water control	0.0	0.0	4.0	0.0	6.0	0.0
Solvent Control	0.0	0.0	2.0	0.0	2.0	0.0
Reference item 12 µg dimethoate/bumble bee	6.0	58.0	70.0	14.0	86.0	14.0

Mean = mean of 50 individuals per treatment group
Water control = tap water containing 0.5% Twen80
Solvent control = acetone

Validity criteria:
All validity criteria of the test were met.

Validity criteria according to OECD 246	Obtained in this study
Control mortality should not exceed 10 % at test end	Control: 6.0 % Solvent control: 2.0 %
Mortality of the reference item should be ≥ 50% at test	Reference item: 86 %

Conclusion:
The contact LD_{50} value after 48 hours was > 100 µg a.s./bumble bee.
The contact NOED value was calculated to be ≥ 100 µg a.s./bumble bee.

CA 8.3.1.2 Chronic toxicity to bees

Report: KCA 8.3.1.2/01, [redacted], A., 2015, M-540173-01-1
Title: Chronic oral toxicity test of BCS-CN88460 SC 200 (200 G/L) on the honey bee (Apis mellifera L.) in the laboratory
Report No.: 93851136
Document No.: M-540173-01-1
Guideline(s): OECD 213 (1998) and CEB No. 230 with current recommendations of the ring test group (2014)
Guideline deviation(s): none
GLP/GEP: yes

Objective:
The purpose of this study was to determine the chronic oral toxicity of BCS-CN88460 SC 200 (200 G/L) to the honeybee (*A. mellifera L.*) 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 old 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 cm × 8.5 cm × 5.5 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 (3333, 1667, 833, 417 and 208 mg a.s./kg food) of the test item treated sugar solutions *ad libitum*. These concentrations led to actual mean dose levels of 89.7, 49.9, 28.6, 12.4 and 6.6 µg 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 item in the feeding solutions was analyzed by liquid chromatography (LOQ = 0.010 mg/kg, LOD = 0.003 mg/kg).

Findings:

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 item in the feeding solutions were within ± 20 % of nominal. Therefore the concentrations were confirmed and the endpoints are based on nominal concentrations.

Biological findings:

Test item	BCS-CN88460 SC 200
Test object	***Apis mellifera***
Exposure	**Oral (50 % w/v sucrose solution)**
Tested doses [µg a.s./bee/day]	89.7, 49.9, 28.6, 12.4 and 6.6
LDD_{50} [µg a.s./bee/day]	> 89.7
LDD_{20} [µg a.s./bee/day]*	> 89.7
LDD_{10} [µg a.s./bee/day]*	> 89.7
NOEDD [µg a.s./bee/day]**	≥ 89.7

* Since no mortality above 10 and 20% occurred in the test, the respective $LDD_{10/20}$ values are assumed to be > 89.7 µg a.s./bee/day

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

Observations

At test end, 10 days following start of exposure, 0.0 % mortality occurred in the untreated water control (50 % w/v 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, α = 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 test 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.

Concentration [mg a.s./kg sugar solution]	Dose level [µg a.s./bee/day]	Mortality at day 10 (% mean)
3333	89.7	0.0
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
Reference item	0.02	100*

* = statistically significant difference compared to the control (Fisher`s Exact Test, pairwise comparison, one-sided greater, α = 0.05)

Validity criteria:
All validity criteria were met in this study.

Validity criteria according to OECD 245	Obtained in this study
Control Mortality ≤ 15 %	0.0 %
Reference item mortality ≥ 50 % at the end of the test	100 %

Conclusion:
In the 10d adult dose-response honeybee laboratory test mortality was on a very low level in the control and in all five test item treatment groups of 6.6, 12.4, 28.6, 49.9 and 89.7 µg a.s./bee/day. Only at the medium test level of 28.6 µg a.s./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 control. Therefore LDD_{10} and LDD_{20} values could not be determined due to mathematical reasons and are therefore not contained in the final report of this study.

The chronic toxicity of BCS-CN88460 SC 200 (200 G/L) was tested over 10 days. The LC_{50} value (10 days) was > 3333 mg a.s./kg feeding solution. The LDD_{50} value (10 days) was > 89.7 µg a.s./bee per day. The NOEC and NOEDD values (10 days) were 3333 mg a.s./kg feeding solution and ≥ 89.7 µg a.s./bee per day, respectively.

CA 8.3.1.3 Effects on honeybee development and other honeybee life stages

Report: KCA 8.3.1.3/01; [redacted]; 2017; M-587515-01-1
Title: BCS-CN88460 - Honey bee (Apis mellifera L.) larval toxicity test (repeated exposure)
Report No.: S16-00461
Document No.: M-587515-01-1
Guideline(s): Regulation (EC) No 1107/2009 (2009)
Directive 2003-01 (Canada/PMRA)
US EPA OCSPP 850.SUPP
OECD Draft Guidance Document on Honey bee (Apis mellifera) Larval Toxicity Test,
Repeated Exposure (Version dated 20 July 2015)
Guideline deviation(s): None with impact on the study outcome
GLP/GEP: 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 (EC_{10}/ED_{10}, EC_{20}/ED_{20} and the EC_{50}/ED_{50}) 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 originating 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 [redacted].
Dose response test with a duration of 22 days from grafting on day 1 to the final assessment on day 22; from day 3 until day 6 of the test, five different concentrations of 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 C; the 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 (1.1 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 uneaten food was qualitatively recorded on day 8.
Test concentrations: 1 control group, 1 solvent control group; 5 test item groups with 10.4, 26.0, 65.0, 162 and 406 mg BCS-CN88460/kg diet, equivalent to cumulative 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 7.39 µg dimethoate/larva per developmental period.

Findings:

Analytical findings:
The measured concentrations of BCS-CN88460 in the larval 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 8, larval mortality in the control and solvent control group was 0.0 and 8.3 %, respectively. Larval mortality in the reference item 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, $\alpha = 0.05$).
The EC_{10} and EC_{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_{50} could not be determined due to a lack of inhibition above 50 % but can be regarded as > 406 mg BCS-CN88460/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 Corresponding Endpoints

Treatment Group	Concentration		Cumulative Dose		Larval Mortality on Day 8 (%)	Larval Mortality on Day 8 Corr-ected (%)	Adult Emergence on Day 22 [a] (%)
Control	---	---	---	---	0.0	---	93.8
Solvent control	---		---		8.3	---	79.2
Test Item (BCS-CN88460)	10.4	[mg BCS-CN88460/kg diet] [b]	1.60	[µg BCS-CN88460/larva per developmental period] [b c]	6.3	-2.2	87.5
	26.0		4.00		4.2	-4.5	77.1
	65.0		10.0		12.5	4.6	77.1
	162		24.9		4.2	-4.5	70.8
	406		62.5		10.4	2.3	62.5
Reference Item (Dimethoate)	48.0	[mg dimethoate/kg diet] [b]	7.39	[µg dimethoate/larva per developmental period] [b c]	91.7	91.7	---

Endpoints [d]

[mg BCS-CN88460/kg diet] [b]

	LOEC	NOEC	EC_{10} (95 % confidence limits)	EC_{20} (95 % confidence limits)	EC_{50} (95 % confidence limits)
Day 22	> 406	≥ 406	160 (96.0 - 268)	380 (194 - 742)	> 406

[µg BCS-CN88460/larva per developmental period] [b c]

	LOED	NOED	ED_{10} (95 % confidence limits)	ED_{20} (95 % confidence limits)	ED_{50} (95 % confidence limits)
Day 22	> 62.5	≥ 62.5	24.6 (14.8 - 41.3)	58.5 (29.9 - 114)	> 62.5

[a] statistical evaluation for non-emergence
[b] Based on the analysed purity
[c] Based on the cumulative feeding volume from day 3 until day 6 of 140 µL diet/larva and a density of the diet of 1.1 g/cm^3
[d] Lethal concentration/doses (LC_x/LD_x) apply for day 8, effect concentrations/doses (EC_x/ED_x) apply for day 22

<u>Validity criteria:</u>
All validity criteria were met in this study.

Validity criteria according to OECD GD 239	Obtained in this study
Cumulative larval 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 dimethoate larval mortality at day 8: ≥ 50 %	91.7 %

Conclusion:
In a repeated exposure larval toxicity test with BCS-CN88460 the NOEC relating to adult emergence on day 22 was determined as ≥ 406 mg a.s./kg diet, equivalent to an NOED of ≥ 62.5 µg a.s./larva per developmental period.
The EC_{50} 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 ED_{50} of > 62.5 µg a.s./larva per developmental period.

CA 8.3.1.4 Sub-lethal effects

There is no particular study design /test guideline to assess "sub-lethal effects" in honeybees. However, in each laboratory study as well as in any higher-tier study, sub-lethal effects, if occurring, are described and reported.

CA 8.3.2 Effects on non-target arthropods other than bees

Studies on non-target arthropods have been performed with the representative formulations and are presented in the respective Document MCP, Section 10.3.2.

CA 8.3.2.1 Effects on Aphidius rhopalosiphi

Studies on non-target arthropods have been performed with the representative formulations and are presented in the respective Document MCP, Section 10.3.2.

CA 8.3.2.2 Effects on Typhlodromus pyri

Studies on non-target arthropods have been performed with the representative formulations and are presented in the respective Document MCP, Section 10.3.2.

CA 8.4 Effects on non-target soil meso and macrofauna

CA 8.4.1 Earthworm, sub-lethal effects

Table 8.4- 1: EU evaluated and additional studies on effects on earthworms

Test substance	Test species	Endpoint	Reference
Isoflucypram	*Eisenia fetida* reproduction 56 d, mixed	NOEC ≥ 162 mg a.s./kg dws* $EC_{10/20}$ not calculable[1)]	[redacted]; 2016; M-548749-01-1 KCA 8.4.1/01
BCS-CN88460-carboxylic acid (M12)	*Eisenia fetida* reproduction 56 d, mixed	NOEC 50 mg p.m./kg dws* $EC_{10/20}$ not calculable[1)]	[redacted]; 2017; M-579263-01-1 KCA 8.4.1/02

dws = dry weight soil, a.s. = active substance, p.m. = pure metabolite
*Endpoint corrected due to lipophilic substance (log P_{OW} > 2)
1) for details see study summary

Report:	KCA 8.4.1/01; [redacted]; 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
Guideline(s):	ISO 11268-2: 1998 (E) and OECD 222: April 13, 2004 EU Directive 91/414/EEC Regulation (EC) No. 1107/2009 US EPA OCSPP not applicable
Guideline deviation(s):	minor deviations
GLP/GEP:	**yes**

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 test was performed according to the International Standard ISO 11268-2 (1998) and OECD 222 (April 13, 2004).

Material and methods

Test item: BCS-CN88460 a.s.; Batch code: BCS-CN88460-01-06; Certificate no.: MZ-00994; CAS No. 1255734-28-1; Spec. no.: 102000028196; analysed content: 94.2 % w/w.
Test organism: Adult *Eisenia fetida*, approx. 3 months old (synchronised culture of earthworms); mean bodyweight at the start of the test ranged from 0.25 to 0.47 g/worm.

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 nominal 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 in organic solvent and afterwards applied and mixed to a portion of quartz sand. After evaporation of the solvent these portions were mixed into the soil to gain the required concentrations. Non-re-usable plastic boxes (length × width × height ca. 16.5 cm × 12 cm × 6 cm, area approximately 200 cm^2) were used as test vessels. Each test vessel contained an amount of approximately 500 g artificial soil (dry weight) to obtain a depth of approximately 5 cm soil in the test vessels. After 28 days the number of surviving adult earthworms and their weight alteration were determined. 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, 16 h 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 week in order to compensate evaporation. As a reference item, Carbendazim (360 g a.s./L) was used.

Findings:

Toxic reference test:

The most recent reference test (non –GLP kra-Rg-R-Ref 24/14, July 8, 2014), with the reference substance Carbendazim mixed into the artificial soil, was performed at test concentrations of 1.25, 2.5 and 5.0 mg a.s./kg dry weight 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 soil 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.

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 51.81 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 body 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 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 no reliable EC_{10}/EC_{20} calculation was possible. Therefore no EC_{10}/EC_{20} value can be reported.

Test object	***Eisenia fetida***									
Test item	**BCS-CN88460 a.s.**									
[mg a.s./kg d.w. soil]	**Control**	**Solvent control**	**5.6**	**10**	**18**	**32**	**56**	**100**	**178**	**326**
Mortality adults [%] after 28 days	0	5	0	22.5	5	0	0	0	0	0
Significance (mortality) *	-	-	-	+	-	-	-	-	-	-
Mean change b.w. [%] day 0 to 28	29.3	37.1	23.5	23.6	29.1	30.8	29.1	31.2	33.3	36.2
Standard deviation	6.1	8.1	8.4	7.8	8.9	3.8	11.6	5.1	13.5	8.8
Significance (b.w.) **	-	-	-	-	-	-	-	-	-	-
Number offspring per vessel day 56	79.5	80.8	73.3	77.5	73.3	83.0	79.8	70.8	72.5	67.0
Standard deviation	9.0	14.4	21.8	29.1	12.4	15.4	11.6	12.6	21.1	23.8
% of solvent control	-	-	90.7	96.0	90.7	102.8	98.8	87.6	89.8	86.5
Coefficient of variance [%]	11.3	17.8	29.8	37.6	16.9	18.6	14.6	17.9	29.1	35.5
Significance (repro.) ***	-	-	-	+	-	-	-	-	-	-
	Adult mortality				**Growth**			**Reproduction**		
NOEC [mg a.s./kg d.w. soil]	326				≥ 326			≥ 326		
LOEC [mg a.s./kg d.w. soil]	> 326				> 326			> 326		

* Fisher´s Exact Binominal Test, one-sided greater, α = 0.05), + significant, - not significant
** Dunnett´s t-test, two-sided, α = 0.05, + significant, - not significant; in comparison to solvent control
*** Dunnett´s t-test, one-sided smaller, α = 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 ≤ 30 %	11.3 %

Conclusion:
Based on the effects observed on mortality, growth and reproduction, it is concluded, that the overall NOEC for the study is determined to be ≥ 326 mg a.s./kg dry weight soil. Thus, the overall LOEC is 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:	KCA 8.4.1/02; [redacted]; 2017; M-579263-01-1
Title:	BCS-CN88460-carboxylic acid (BCS-CY26497): Effects on survival, growth and reproduction of the earthworm Eisenia fetida tested in artificial soil
Report No.:	E 312 4705-2
Document No.:	M-579263-01-1
Guideline(s):	EU Directive 91/414/EEC; Regulation (EC) No. 1107/2009; US EPA OCSPP Not Applicable
Guideline deviation(s):	Deviation: on day 15 and 16 the temperature in the climatic chamber increased for 12 and 15 hours up to 25°C. Due to technical problems for 2 days no temperature data were recorded
GLP/GEP:	**yes**

Objective:
The purpose of this study was to determine the effects of BCS-CN88460-carboxylic acid (M12) on survival, growth and reproduction on the earthworm *Eisenia fetida* tested in artificial soil. The test was performed according to the International Standard ISO 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 12631-19-9, analysed content: 98.8%, Certificate no.: TOX10705-00 (1st run), TOX20054-01 (2nd run).
Test organism: Adult *Eisenia fetida*, 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 fetida*, approx. 3 months old, 8 × 10 earthworms for the control groups (quartz sand treated and solvent on quartz sand treated) and the single treatment group were exposed to control and treatment. A nominal test concentration of 150 mg pure metabolite/kg dry weight artificial soil was mixed into the artificial soil.

2nd run:
Adult *Eisenia fetida*, 5-6 months old, 8 × 10 earthworms for the control groups (quartz sand treated and solvent 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, the number of offspring was determined.

Findings:

Toxic reference test:
The most recent reference test, with the reference substance Carbendazim mixed into the artificial soil, was performed from August 25 to November 19, 2015 (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 after 56 days were determined.
No mortality of the adult earthworms was observed 28 days after application. The change of body weight of the adult earthworms of the test concentration of 5.0 mg a.s./kg dry weight soil was statistically significant less than the solvent control (results of a Williams multiple sequential t-test, two-sided, $\alpha = 0.05$).
The number of juveniles per test vessel of the test concentrations 2.5 and 5.0 mg a.s./kg dry weight soil were statistically significant reduced in comparison to the control (results of a Williams multiple sequential t-test, one-sided smaller, $\alpha = 0.05$).
According to the guideline significant effects should be observed between 1 and 5 mg a.s./kg dry weight artificial soil.
Thus the results of this reference test indicated that the test system was sensitive to the reference substance.

Experimental conditions:
1st run: The pH values measured in the controls and the treatment ranged 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 treatment 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 growth of the adults after an exposure period of 28 days and the number of offspring per test vessel after 56 days are shown in the following table and paragraphs (values in this table are rounded values).

Test object	***Eisenia fetida*** **BCS-CN88460-carboxylic acid (M12)**									
mg pure metabolite/kg dry weight artificial soil	**2nd run**							**1st run**		
	Con	**Solv. con**	**10**	**18**	**32**	**56**	**100**	**Con**	**Solv. con**	**150**
Mortality of adult earthworms [%] after 28 days	0	1.25	0	0	2.5	0	0	0	5	0
Significance (mortality)*	--	--	-	-	-	-	-	--	--	-
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	74.3	74.1	29.3	37.1	26.4
Standard Deviation	9.2	11.1	7.0	2.6	4.3	5.7	10.5	6.1	8.1	6.1
Significance (body fresh weight)**	--	--	-	-	-	-	-	--	--	+
Mean number of offspring per test vessel after 56 days	278.5	255.9	229.0	289.5	267.8	293.5	272.0	79.5	80.8	68.5
Standard Deviation (% of control)	50.5	39.3	29.7	70.4	93.5	79.4	24.5	9.0	14.4	20.5
% of solvent control	--	--	89.5	126.4	92.5	128.2	94.0	-	-	84.8
Coefficient of variance (%)	18.1	15.4	13.0	24.3	34.9	27.1	9.0	11.3	17.8	29.9
Significance (reproduction)***	--	--	-	-	-	-	-	--	--	-

	Adult Mortality	**Growth**	**Reproduction**
NOEC [mg pure metabolite/kg dry weight soil]	**≥ 150**	**100**	**≥ 150**
LOEC [mg pure metabolite/kg dry weight soil]	**> 150**	**150**	**> 150**
EC_{10} and their 95 % confidence limits (mg test item/kg dry weight artificial soil)			**n.d.**
EC_{20} and their 95 % confidence limits (mg test item/kg dry weight artificial soil)			**n.d.**

1st run: * Fisher`s Exact Binominal Test (one sided greater, α = 0.05), + significant, - not significant
** Student-t test for Homogeneous Variances (two-sided, α = 0.05), + significant, - not significant
*** Student-t test for Homogeneous Variances (one-sided smaller, α = 0.05), + significant, - not significant
2nd run: * Fisher`s Exact Binominal Test (one-sided greater, α = 0.05), + significant, - not significant
** William's t-test (two-sided, α = 0.05), + significant, - not significant
*** William's t-test (one-sided smaller, α = 0.05), + significant, - not significant
n.d.: could not be determined, see observations and conclusion

Mortality

After 28 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,. α = 0.05).

NOEC related to mortality: ≥150 mg pure metabolite/kg dry weight artificial soil
LOEC related to mortality: >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 test concentration of 150 mg pure metabolite/kg dry weight artificial soil in the 1st run (Student-t test for Homogeneous Variances, two-sided, α = 0.05). No statistically significant differences up to and including 100 mg pure metabolite/kg dry weight artificial soil, the highest test concentration were

observed in the 2nd run (William's t-test, two-sided, α = 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 solvent control were observed in the 1st run (Student-t test for Homogeneous Variances, one-sided smaller, α = 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 run were observed (William's t-test, one-sided smaller, α = 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 dry weight artificial 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.

Validity criteria:
All validity criteria were met in this study.

Validity criteria according to OECD 222 (2004)	Recommended	Obtained	
		1st run	2nd run
Adult mortality in the control	≤ 10 %	0 %	0 %
Rate of reproduction of juveniles (earthworms per control vessel)	≥ 30	62 to 90	223 to 362
Coefficient of variance of reproduction in the control	≤ 30 %	11.3 %	18.1 %

Conclusion:
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 relationship no reliable EC_{10}/EC_{20} calculation was possible. Therefore no EC_{10}/EC_{20} 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, reproduction			
Isoflucypram	*Folsomia candida* reproduction 28 d, mixed	NOEC 49.5 mg a.s./kg dws* EC_{10} not calculable[1]	[redacted]; 2015; M-522863-01-1 KCA 8.4.2.1/01
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_{50} 12 mg p.m./kg dws*	[redacted]; 2017; M-587760-01-1 KCA 8.4.2.1/02
Soil mites, reproduction			
Isoflucypram	*Hypoaspis aculeifer* reproduction 14 d, mixed	NOEC ≥ 495 mg a.s./kg dws* EC_{10} not calculable[1]	[redacted]; 2015; M-528194-01-1 KCA 8.4.2.1/03
BCS-CN88460-carboxylic acid (M12)	*Hypoaspis aculeifer* reproduction 14-d, mixed	NOEC ≥ 495 mg p.m./kg dws* EC_{10} not calculable[1]	[redacted] I.; 2015; M-524464-01-1 KCA 8.4.2.1/04

dws = dry weight soil, a.s. = active substance, p.m. = pure metabolite
* Endpoint corrected due to lipophilic substance (log P_{OW} > 2)
[1] for details see study summary

CA 8.4.2.1 Species level testing

Report:	KCA 8.4.2.1/01; [redacted]; 2015; M-522863-01-1
Title:	BCS-CN88460 a.s.: Influence on the reproduction of the collembolan species Folsomia candida tested in artificial soil
Report No.:	E 314 4697-4
Document No.:	M-522863-01-1
Guideline(s):	EU Directive 91/414/EEC Regulation (EC) No. 1107/2009 US EPA OCSPP not applicable
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

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* during an exposure of 28 days in an artificial soil comparing control and treatment.

Material and methods:

Test item: BCS-CN88460, analytical findings: 94.2 % w/w, origin batch no.: 2013-006492, customer order no.: TOX 10421-02, specification no.: 102000028196, article no.: 81782172.

Test organism: Culture of the springtails *Folsomia candida* (Collembolan, Isotomidae), synchronized culture, age of 9-12 days.

10 collembolans (9-12 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.

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 vessels. Mortality 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 495 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 concentration [1]	pH		Water content (%)		WHCmax [2]	
	Start	End	Start	End	Start	End
Control	6.07	5.68	17.89	16.28	46.16	41.17
99	5.74	5.70	18.42	17.66	47.80	45.42
176	5.86	5.71	18.51	16.02	48.11	40.39
313	5.80	5.71	18.51	16.49	48.11	41.82
556	5.94	5.73	18.13	17.78	46.89	45.79
990	5.85	5.80	17.98	16.99	46.43	43.33

[1] mg a.s./kg soil dry weight
[2] % WHC_{max} = percent of maximum water holding capacity of 47.22 g water per 100 g artificial soil dry weight

Biological results:
EC_{10} and EC_{20} calculations have been performed (ToxRat version 3.2.1). No EC_{10} and EC_{20} values could be derived due to the lack of a clear dose-response relationship.

Test item Test object Exposure	BCS-CN88460 a.s. *Folsomia candida* Artificial soil			
[mg a.s./kg soil dry weight] (nominal conc.)	Adult mortality (%)	Mean number of juveniles/test vessel ± SD	Reproduction (% of control)	Significance (*)
Control	2.9	1343.6 ± 117.3	-	
99	5.0	1346.5 ± 14.3	100.2	-
176	20.0	1132.3 ± 206.4	84.3	+
313	10.0	1120.0 ± 213.7	83.4	+
556	7.5	1140.5 ± 88.8	84.9	+
990	10.0	1216.0 ± 83.2	90.5	+
$NOEC_{reproduction}$ [mg a.s./kg soil dry weight]			99	
$LOEC_{reproduction}$ [mg a.s./kg soil dry weight]			176	

Toxic reference test:
Boric acid showed, in a non-GLP-test (FRM-Coll-Ref-26/15, March 18, 2015), an EC_{50} of 77 mg test item/kg artificial soil dry weight (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).

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, α = 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 %
Mean number of juveniles/replicate ≥ 100	1343.6
Coefficient of variation calculated for the number of juveniles per replicate ≤ 30 %	8.7 %

Conclusion
Based on the effects observed on reproduction, it is concluded, that the overall NOEC 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. EC_{10} and EC_{20} calculations have been performed. No EC_{10} and EC_{20} values could be derived due to the lack of a clear dose-response relationship.

Report: KCA 8.4.2.1/02; [redacted] 2017; M-587760-01-1
Title: BCS-CN88460-carboxylic acid (BCS-CY26497): Effects on mortality and reproduction of the collembolan species Folsomia candida tested in artificial soil
Report No.: 16 10 48 262 S
Document No.: M-587760-01-1
Guideline(s): EU Directive 91/414/EEC
Regulation (EC) No 1107/2009 (2009)
US EPA OCSPP Not Applicable
Guideline deviation(s): none
GLP/GEP: **yes**

Objective
The purpose of this study was to determine potential effects of the test item on the reproductive output of the collembolan *Folsomia candida* as a representative of soil micro-arthropods during a test period of 28 days. After 4 weeks the number of offspring (juveniles) 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/w.
10 Collembola (9-12 days old) were 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 % sphagnum peat and 0.3 % $CaCO_3$, at 19.1 – 21.2 °C and a photoperiod: light : dark = 16 h : 8 h (570 lux) and were fed weekly with granulated dry yeast. Mortality and reproduction were determined after 28 days.
Toxic standard: 44, 67, 100, 150 and 225 mg boric acid/kg soil d.w.; control: untreated, solvent control: none.

Findings:

Biological results

Test item	BCS-CN88460-carboxylic acid (M12)							
Test object	*Folsomia candida*							
Exposure	Artificial soil							
[mg pure metabolite/kg dry weight artificial soil] (nominal concentrations)	Adult mortality (%)	Significance (**)	Mean number of juveniles per test vessel ± standard deviation			Reproduction (% of control)	Significance (*)	
Control	2.5		738	±	102	-		
18	2.5	-	755	±	160	102	-	
32	17.5	+	417	±	38	56	+	
56	77.5	+	183	±	60	25	+	
100	92.5	+	181	±	99	25	+	
178	92.5	+	114	±	35	15	+	
316	97.5	+	123	±	50	17	+	
562	100.0	+	72	±	15	10	+	
1000	100.0	+	46	±	21	6	+	

	Mortality	Reproduction
NOEC [mg pure metabolite/kg dry weight artificial soil]	18	18
LOEC [mg pure metabolite/kg dry weight artificial soil]	32	32

	Mortality	Reproduction
LC_{10}[1)]/EC_{10}[2)] [mg pure metabolite/kg dry weight artificial soil]	24	13
95% confidence limits	(14 – 40)	(4.9 – 37)
LC_{20}[1)]/EC_{20}[2)] [mg pure metabolite/kg dry weight artificial soil]	31	20
95% confidence limits	(20 – 47)	(9.5 – 42)

The calculations were performed with unrounded values
[1)] Logit analysis , [2)] Probit analysis
(*) = (Multiple sequentially-rejective U-test after Bonferroni-Holm, one-sided smaller, $\alpha = 0.05$, + = significant, - = not significant)
(**) = (Multiple sequentially-rejective Fisher Test after Bonferroni-Holm, one-sided greater, $\alpha = 0.05$, + = significant, - = not significant)

Toxic reference test:
In a separate study (BioChem project No. R 16 10 48 003 S, dated 2016-08-08), the EC_{50} (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
Coefficient of variation (mean number of juveniles per replicate) ≤ 30 %	13.9 %

Experimental conditions:
The pH values measured in the solvent control and the treatments ranged from 6.04 to 6.10 at test start and from 5.82 to 5.92 at test end. The water content during the whole study was between 57.5 % and 58.0 % of WHC_{max}. All values were within the range recommended by the guideline.

Conclusion:
The test item showed statistically significant adverse effects on adult mortality of the collembolan *Folsomia candida* in artificial soil at concentrations 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. and the Lowest-Observed-Effect-Concentration (LOEC) for mortality was determined to be 32 mg pure metabolite/kg soil d.w. The test item caused a significant reduction of reproduction of the collembolan *Folsomia candida* in artificial soil at concentrations 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 (LOEC) for reproduction was determined to be 32 mg pure metabolite/kg soil d.w. An EC_{10} value of 13 mg pure metabolite was calculated.

Report:	KCA 8.4.2.1/03; [redacted]; 2015; M-528194-01-1
Title:	BCS-CN88460 a.s.: Influence on mortality and reproduction of the soil mite species Hypoaspis aculeifer tested in artificial soil
Report No.:	E 428 4700-5
Document No.:	M-528194-01-1
Guideline(s):	EU Directive 91/414/EEC Regulation (EC) No. 1107/2009 US EPA OCSPP not applicable OECD 226 from October 03, 2008: OECD guideline for the Testing of Chemicals - Predatory mite (Hypoaspis (Geolaelaps) aculeifer) reproduction test in soil
Guideline deviation(s):	none
GLP/GEP:	**yes**

Objective:
The purpose of this study was to assess the effect of BCS-CN88460 a.s. (isoflucypram) 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:

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 aculeifer* 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.s./kg artificial soil dry weight were tested. During the test, the *Hypoaspis aculeifer* were fed with nematodes bred on watered oat flakes. During the study a temperature of 20 ± 2 °C and light regime of 400 – 800 Lux, 16 h light : 8 h dark was applied. The artificial soil was prepared according to the guideline with the following constituents (percentage distribution on dry weight basis): 75 % fine quartz sand, 5 % Sphagnum peat, air dried and finely ground, 20 % Kaolin clay. After a period of 14 days, the surviving adults and the living juveniles were extracted by applying a temperature gradient using a MacFadyen-apparatus. Extracted mites were collected in a fixing solution (20% ethylene glycol, 80 % deionised water; 2 g detergent/L fixing solution were added). All *Hypoaspis aculeifer* were counted under a binocular.

Findings:

Experimental conditions:

All values were within the range recommended by the guideline.

[mg test item/ kg dry weight artificial soil]	pH		% water content			% of WHC$_{max}$	
	Start	End	Start	End	% deviation	Start	End
Control	6.07	5.58	17.89	17.49	2.3	46.16	44.88
99	5.74	5.57	18.42	18.29	0.7	47.80	47.42
176	5.86	5.53	18.51	17.40	6.4	48.11	44.61
313	5.80	5.55	18.51	18.13	2.1	48.11	46.89
556	5.94	5.64	18.13	18.30	0.9	46.89	47.42
990	5.85	5.63	17.98	18.48	2.7	46.43	48.02

Biological results:

In the control group 8.8 % of the adult *Hypoaspis aculeifer* died which is below the allowed maximum of ≤ 20 % mortality.

Concerning the number of juveniles statistical analysis (William's-t test, one-sided smaller, α = 0.05) revealed no significant difference between control and any treatment group. Since there were no adverse effects on mortality and reproduction, no EC_{10}/EC_{20} calculation was possible.

Test item	BCS-CN88460 a.s.			
Test Object	*Hypoaspis aculeifer*			
Exposure	Artificial Soil			
[mg test a.s./kg dry weight artificial soil]	% mortality (adults)	Mean number of juveniles per test vessel ± standard dev.	Reproduction (% of control)	Significance (*)
Control	8.8	240.9 ± 18.6		
99	10.0	272.8 ± 18.5	113.2	-
176	7.5	250.8 ± 10.4	104.1	-
313	2.5	286.8 ± 21.0	119.0	-
556	5.0	286.5 ± 9.3	111.5	-
990	5.0	265.8 ± 27.2	110.3	-
$NOEC_{reproduction}$ [mg a.s./kg dry weight artificial soil] ≥ 990				
$LOEC_{reproduction}$ [mg a.s./kg dry weight 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.

Validity criteria according to OECD 226 (2008)	Obtained in this study
Mean adult mortality should not exceed 20 % at the end of the test	8.8 %
Mean number of juveniles per replicate should be at least 50 (with 10 mites introduced)	240.9
Coefficient of variation calculated for the number of juveniles per replicate should not be higher than 30 %	7.7 %

Toxic reference test:
In a separate study ([redacted], LAR/HR-O-16/14, January 05, 2015) performed with the reference item dimethoate at test concentrations 1.0, 1.8, 3.2, 5.6 and 10.0 mg dimethoate/kg dry weight artificial soil, the LC_{50} was calculated to be 2.51 mg a.s./kg (95 % confidence limits from 0.85 mg a. s./kg to 3.30 mg a. s./kg) for mortality. The NOEC was calculated to be 3.2 mg a.s./kg and the LOEC was 5.6 mg a.s./kg. Since variances of the data were homogenous Williams-t test $\alpha = 0.05$, one-sided smaller. Dimethoate EC 400E G showed an EC_{50} of 5.47 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 sensitivity of the test system.

Conclusion:
The $NOEC_{reproduction}$ and $LOEC_{reproduction}$ of BCS-CN88460 were determined to be ≥ 990 mg a.s./kg artificial soil dry weight and > 990 mg a.s./kg artificial soil dry weight, respectively. Since there were no adverse effects on mortality and reproduction, no EC_{10}/EC_{20} calculation was possible.

Report: KCA 8.4.2.1/04; [redacted] I.; 2015; M-524464-01-1
Title: BCS-CN88460-carboxylic acid (BCS-CY26497): Influence on mortality and reproduction of the soil mite species Hypoaspis aculeifer tested in artificial soil
Report No.: E 428 4699-2
Document No.: M-524464-01-1
Guideline(s): EU Directive 91/414/EEC; Regulation (EC) No. 1107/2009; US EPA OCSPP: not applicable; OECD 226 from October 03, 2008: OECD guideline for the Testing of Chemicals - Predatory mite (Hypoaspis (Geolaelaps) aculeifer) reproduction test in soil
Guideline deviation(s): not specified
GLP/GEP: **yes**

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-CN88460-carboxylic acid (M12) (analytical findings: 98.8 % w/w ; batch code: BCS-CY26497-01-02; customer order no.: TOX10705- 00; Origin batch no. SES 12631-19-9; material no.: BCS-CY26497, technical 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 nematodes 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 a temperature gradient using a MacFadyen-apparatus. Extracted mites were collected in a fixing solution (20% ethylene glycol, 80% deionised water; 2 g detergent/L fixing solution were added). All *Hypoaspis aculeifer* were counted under a binocular. The statistical analysis was performed using ToxRat Professional.

Findings:

<u>Biological findings:</u>
For reproduction, no significant difference between control and treatment group detected.

Test item **Test object** **Exposure**	**BCS-CN88460-carboxylic acid (M12)** ***Hypoaspis aculeifer*** **Artificial soil**			
[mg pure metabolite/kg dry weight artificial soil]	**% mortality (adults)**	**Mean number of juveniles per test vessel ± SD**	**Reproduction (% of control)**	**Significance (*)**
Control	8.8	240.9 ± 18.6		
990	2.5	282.6 ± 32.7	117.3	-
NOEC$_{reproduction}$ [mg pure metabolite/kg dry weight artificial soil] **LOEC**$_{reproduction}$ [mg pure metabolite/kg dry weight artificial soil]				≥ 990 > 990

Calculations were done with un-rounded values.
(*) = Student-t-test one sided smaller; α = 0.05; "-": non-significant; "+": significant

The study was performed as limit test and no adverse effects on mortality and reproduction were observed. Therefore, no $EC_{10/20}$ calculation was possible.

<u>Experimental conditions:</u>
All values were within the range recommended by the guideline.

[mg test item/kg dry weight artificial soil]	**pH**		**% Water content**			**% of WHC$_{max}$**	
	Start	**End**	**Start**	**End**	**% deviation**	**Start**	**End**
Control	6.07	5.58	17.89	17.49	2.3	46.16	44.88
990	5.93	5.62	18.63	17.65	5.5	48.48	45.40

<u>Reference test:</u>
The most recent non-GLP-test (LAR/HR-O-16/14, January 05, 2015) with the reference item dimethoate was performed at test concentrations 1.0, 1.8, 3.2, 5.6 and 10.0 mg dimethoate/kg dry weight artificial soil. Dimethoate showed a LC_{50} of 2.51 mg a.s./kg (95% confidence limits from 0.85 mg a.s./kg to 3.30 mg a.s./kg) for mortality of the adult mites according Probit analysis using maximum likelihood regression.
The reproduction 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 α = 0.05, one-sided smaller was used. Dimethoate EC 400E G showed an EC_{50} of 5.47 mg a.s./kg

(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 EC_{50} based on the number of juveniles of 3.0–7.0 mg a.s./kg dry weight artificial soil shows that the test organisms are sufficiently sensitive.

Validity criteria:
All validity criteria were met in this study.

Validity criteria according to OECD 226 (2008)	Obtained in this study
Mean adult mortality ≤ 20 %	8.8 %
Mean number of juveniles per replicate ≥ 50	240.9
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 ≥ 990 mg pure metabolite/kg dry weight artificial soil. Thus, the overall LOEC is determined to be > 990 mg pure metabolite/kg dry weight artificial soil. The study was performed as limit test and no adverse effects on mortality and reproduction were observed. Therefore, no $EC_{10/20}$ calculation was possible.

CA 8.5 Effects on nitrogen transformation

Table 8.5 - 1: Effects of Isoflucypram on soil nitrogen transformation

Test substance	Test species, test design	Ecotoxicological Endpoint	Reference
N-transformation			
Isoflucypram	Study duration, 28 days	no unacceptable effects at an application rate of 375 g a.s./ha (0.53 mg a.s./kg soil)	[redacted]; 2015; M-532055-01-1 KCA 8.5/01
BCS-CN88460-carboxylic acid (M12)	Study duration, 28 days	no unacceptable effects at an application rate of 403 g p.m./ha (0.54 mg p.m./kg soil)	[redacted]; 2015; M-538059-01-1 KCA 8.5/02

a.s. = active substance; p.m. = pure metabolite,

Report; KCA 8.5/01; [redacted]; 2015; M-532055-01-1
Title: BCS-CN88460 a.s.: Effects on the activity of soil microflora (Nitrogen transformation test)
Report No.: 15 10148 032 N
Document No: M-532055-01-1
Guideline(s): OECD 216; 2000, OECD
Guideline deviation(s): none
GLP/GLP: **yes**

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.: 2013-006492, LIMS No.: 1442835, Customer order No.: TOX 10421402, Specification No.: 102000028196, CAS No.: 1255734-28-1, Article No.: 81782172, Certificate No.: MZ 00994, analysed purity: 94.2 % w/w.

A loamy sand soil (DIN 4220) was exposed for 28 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 item/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 nitrogen transformation was determined in soil enriched with lucerne meal (concentration in soil 0.5 %). NH_4-nitrogen, NO_3- and NO_2-nitrogen were determined by an autoanalyzer at different sampling intervals (0, 7, 14 and 28 days after treatment). A reference item is not required by the guideline. Nevertheless Dinoterb 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, 44.53 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 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 a.s. caused temporary inhibitions of the daily nitrate rate at the tested concentrations of 0.11 mg test item/kg and 0.53 mg test item/kg soil dry weight at time interval 7-14 days after application.
No adverse effects of BCS-CN88460 a.s. on nitrogen transformation in soil could be observed at both tested concentrations at the end of the test, 28 days after application (time interval 14-28). Differences from the control of + 10.2 % (test concentration 0.11 mg test item/kg soil dry weight) and + 7.9 % (test
concentration 0.53 mg test item/kg soil dry weight) were measured at the end of the 28-day incubation period (time interval 14-28).

Time interval (days)	Control			0.11 mg test item/kg soil dry weight equivalent to 0.080 kg test item/ha				0.53 mg test item/kg soil dry weight equivalent to 0.398 kg test item/ha			
	Nitrate-N [1]			Nitrate-N [1]		% difference to control		Nitrate-N [1]		% difference to control	
0-7	5.72	±	0.95	6.16	±	0.92	+ 7.7 [n.s.]	6.04	±	0.94	+ 5.6 [n.s.]
7-14	0.36	±	1.85	- 0.98	±	1.56	- 374.7 [n.s.]	- 1.51	±	1.04	- 524.0 [n.s.]
14-28	3.39	±	0.16	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: Nitrate-N in mg/kg soil dry weight/time interval/day, mean of 3 replicates and standard deviation
[n.s.] No statistically significant difference to the control (Student-t-test for homogeneous variances, 2-sided, $p \leq 0.05$)

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:
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 ≤ 15 %	8 %
Effect of toxic standard ≥ 25 %	≥ 39.1 % (separate study)

Conclusion:
BCS-CN88460 a.s. caused no adverse effects (difference to control < 25 %, OECD 216) on the soil nitrogen transformation (expressed as NO_3-N-production rate) at the end of the 28-day incubation period. The study was performed in a field soil at concentrations up to 0.53 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:	KCA 8.5/02; [redacted], 2015; M-538059-01-1
Title:	BCS-CN88460-carboxylic acid (BCS-CY26497): Effects on the activity of soil microflora (nitrogen transformation test)
Report No.:	15 10 48 033 N
Document No.:	M-538059-01-1
Guideline(s):	OECD 216 (2000); OECD 216, adopted January 21, 2000, OECD Guideline for the Testing of Chemicals, Soil Microorganisms: Nitrogen Transformation
Guideline deviation(s):	none
GLP/GEP:	yes

Objective:
The aim of this 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-CY26497-01-02, Origin Batch No.: SES 12631-19-9, LIMS No.: 1441413, Customer order No.: TOX 10705-00, Certificate No.: MZ 00984, analysed purity: 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/ha) and 0.408 kg test item/ha (corresponding to 0.403 kg pure metabolite/ha), respectively. The nitrogen transformation was determined in soil enriched with lucerne meal (concentration in soil 0.5%). NH_4-nitrogen, NO_3- and NO_2-nitrogen were determined by an autoanalyzer at different sampling intervals (0, 7, 14 and 28 days after treatment). A reference item is not required by the guideline. Nevertheless Dinoterb 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 dry weight at time interval 7-14 days after application.
However, no adverse effects of BCS-CN88460-carboxylic acid (M12) on nitrogen transformation in soil could be observed at both tested concentrations at the end of the test, 28 days after application (time interval 14-28). Differences from the control of +17.0 % (test concentration 0.11 mg test item/kg soil dry weight) and -7.4 % (test concentration 0.54 mg test item/kg soil dry weight) were measured at the end of the 28-day incubation period (time interval 14-28).

Time interval [days]	Control			0.11 mg test item/kg soil dry weight equivalent to 0.082 kg test item/ha				0.54 mg test item/kg soil dry weight equivalent to 0.408 kg test item/ha			
	Nitrate-N [1]			Nitrate-N [1]			% difference to control	Nitrate-N [1]			% difference to control
0-7	5.72	±	0.95	5.63	±	0.36	- 1.5 n.s.	6.00	±	1.05	+ 4.9 n.s.
7-14	0.36	±	1.85	1.85	±	0.81	+ 481.7 n.s.	0.89	±	0.47	+ 148.0 n.s.
14-28	3.39	±	0.16	3.97	±		+ 17.0 n.s.	3.14	±	0.25	- 7.4 n.s.

The calculations were performed with unrounded values.
[1] Rate: Nitrate-N in mg/kg soil dry weight/time interval/day, mean of 3 replicates and standard deviation
n.s. = No statistically significant difference to the control (Student-t-test for homogeneous variances, 2-sided, $p \leq 0.05$)

In a separate study the reference item Dinoterb caused a stimulation of nitrogen transformation of +39.1 %, +62.5 % and +112.0 % at 6.80 mg, 16.00 mg and 27.00 mg Dinoterb per kg soil dry weight, respectively, determined 28 days after application (time interval 14-28).
Measured pH- values for all treatment groups were 5.9 at test start and 5.7 at the final sampling day on day 28.

<u>Validity criteria:</u>
All validity criteria were met in this study.

Validity criteria according to OECD 216 (2000)	Obtained in this study
Coefficient of variation in the control ≤ 15 %	≤ 8.0 %
Effect of toxic standard ≥ 25 %	≥ 39.1 %

Conclusion:
BCS-CN88460-carboxylic acid (M12) caused no adverse effects (difference to control < 25 %, OECD 216) on the soil nitrogen transformation (expressed as NO_3-N-production) at the end of the 28-day incubation period. The study was performed in a field soil at concentrations up to 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).

CA 8.6 Effects on terrestrial non-target higher plants

CA 8.6.1 Summary of screening data

Not necessary as guideline GLP studies conducted with the representative formulation for isoflucypram for terrestrial non-target plants are available (see Point KCP 10.6.2).

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.

CA 8.7 Effects on other terrestrial organisms (flora and fauna)

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 Summary Section 7, Point CA 7.2.1 and 7.2.2, no further studies are deemed necessary.~~

Report:	KCA 8.8/01; [redacted], A.; 2018; M-617426-01-1
Title:	Activated sludge respiration inhibition test with isoflucypram technical
Report No.:	2018/0009/01
Document No.:	M-617426-01-1
Guideline(s):	EU method C.11 (2008); OECD TG 209 (2010)
Guideline deviation(s):	none
GLP/GEP:	**no**

Objective:
The study was performed to assess the toxicity of isoflucypram (BCS-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 Inhibition Test" (2008).

Materials and Methods:
Test item: Isoflucypram 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 mixed population of aquatic microorganisms) was exposed to isoflucypram technical at different concentrations (10, 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 consumption, 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 glass tube at 50-100 L/ha with clean oil-free air. For the measurement, the content of the Erlenmeyer flasks was completely transferred to 250 mL BOD bottles and oxygen content was measured with an oxygen meter (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 (N-allylthiourea), 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 reaction 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 tested 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.

EC_x values for the test item and the reference substance were calculated from the respiration rates at different test item concentrations using the statistics programme ToxRatPro Version 2.10 (release 2010-09-10). The No Observed Effect Concentration was calculated according to Dunnett'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, 2018 – March 15, 2018

Results:

Validity Criteria
All validity criteria were met.

Validity criteria according to OECD 209 (adopted 22 July 2010)	Obtained in this study
Oxygen uptake of blank controls per one gram of activated sludge (dry weight of suspended soils) in an hour ≥ 20	25.960 mg oxygen/gram (without ATU) 25.632 mg oxygen/gram (with ATU)
Coefficient of variation of oxygen uptake in the control replicates at the end of the test should be ≤ 30%	7.7% (without ATU) 10.3% with ATU)
EC_{50} of reference compound 3,5-Dichlorophenol should be in the range of 2-25 mg/L for total respiration and 5-40 mg/L for heterotrophic respiration.	15.265 (total respiration) 20.959 mg/L (heterotrophic respiration)

Analytical Findings:
The test item and reference compound concentrations were not confirmed by analytical methods, they were based on nominal concentrations.

Biological Findings:
Isoflucypram technical showed 11.9 % respiration inhibition of activated sludge at a test item 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.

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	--
Control 2	31.774	19.8	8.1	--
Control 3	31.090	19.3	8.1	--
Control 4	31.487	19.1	8.3	--
Control 5	27.061	18.7	8.3	--
Control 6	30.907	19.5	8.3	--
Control, mean	**31.152**	--	--	--
10 mg/L test item	28.976	19.1	8.1	6.983
10 mg/L test item	27.553	18.4	8.2	11.553
10 mg/L test item	27.571	18.5	8.2	11.496
10 mg/L test item, mean	**28.033**	--	--	**10.010**
32 mg/L test item	28.744	19.0	8.2	7.729
32 mg/L test item	28.753	18.8	8.2	7.701
32 mg/L test item	28.138	18.7	8.2	9.674
32 mg/L test item, mean	**28.545**	--	--	**8.368**
100 mg/L test item	27.007	18.8	8.3	13.306
100 mg/L test item	30.114	19.0	8.3	3.331
100 mg/L test item	28.422	18.9	8.3	8.763
100 mg/L test item, mean	**28.514**	--	--	**8.467**
320 mg/L test item	27.188	19.0	8.3	12.725
320 mg/L test item	30.058	19.1	8.3	3.511
320 mg/L test item	27.785	18.8	8.3	10.807
320 mg/L test item, mean	**28.344**	--	--	**9.014**
1000 mg/L test item	27.435	19.1	8.4	11.932
1000 mg/L test item	28.373	19.1	8.3	8.919
1000 mg/L test item	26.542	19.1	8.3	14.797
1000 mg/L test item, mean	**27.450**	--	--	**11.883**
Physico-chemical oxygen consumption control - 1000 mg/L	0.284	19.1	7.3	--
2.5 mg/L reference compound	25.992	19.3	8.2	16.563
5 mg/L reference compound	28.375	19.1	8.1	8.913
10 mg/L reference compound	20.469	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)

Treatment [mg/L]	Respiration rate [mg/L × h]	Mean Temp. [°C]	pH-value	Inhibition [%]
Control 1	33.790	20.7	8.4	--
Control 2	33.017	20.2	8.4	--
Control 3	33.456	20.1	8.4	--
Control 4	29.846	19.1	8.5	--
Control 5	25.956	19.0	8.5	--
Control 6	28.485	19.6	8.5	--
Control, mean	**30.758**	**--**	**--**	**--**
10 mg/L test item	27.310	19.0	8.5	11.212
10 mg/L test item	25.979	18.6	8.5	15.538
10 mg/L test item	25.811	18.4	8.5	16.084
10 mg/L test item, mean	**26.367**	**--**	**--**	**14.278**
32 mg/L test item	25.528	18.9	8.5	17.005
32 mg/L test item	26.985	18.7	8.5	12.268
32 mg/L test item	25.600	18.5	8.5	16.771
32 mg/L test item, mean	**26.038**	**--**	**--**	**15.348**
100 mg/L test item	26.170	18.9	8.5	14.915
100 mg/L test item	26.486	18.5	8.5	13.890
100 mg/L test item	25.939	18.7	8.5	15.667
100 mg/L test item, mean	**26.199**	**--**	**--**	**14.824**
320 mg/L test item	25.729	18.8	8.4	16.350
320 mg/L test item	28.390	19.0	8.5	7.700
320 mg/L test item	25.734	18.6	8.5	16.337
320 mg/L test item, mean	**26.618**	**--**	**--**	**13.462**
1000 mg/L test item	25.418	19.0	8.5	17.361
1000 mg/L test item	26.337	18.7	8.5	14.375
1000 mg/L test item	25.231	18.8	8.5	17.971
1000 mg/L test item, mean	**25.662**	**--**	**--**	**16.569**
Physico-chemical oxygen consumption control - 1000 mg/L	0.206	19.1	7.3	-
2.5 mg/L reference compound	29.917	19.6	8.5	2.737
5 mg/L reference compound	27.525	19.4	8.3	10.514
10 mg/L reference compound	23.967	19.5	8.4	22.080
20 mg/L reference compound	14.563	19.3	8.4	52.655
40 mg/L reference compound	9.369	18.9	8.5	69.541

Results of the test item isoflucypram (Calculated nitrification respiration: total respiration minus heterotrophic respiration)

Treatment [mg/L]	Mean resp. rate (Total resp.) [mg/L × h]	Mean resp. rate (Heterotrophic resp.) [mg/L × h]	Mean resp. rate (Nitrification resp.) [mg/L × h]	Inhibition Nitrification [%]
Control	31.152	30.758	0.394	
10 mg/L test item	28.033	26.367	1.666	-322.843
32 mg/L test item	28.545	26.038	2.507	-536.294
100 mg/L test item	28.514	26.199	2.315	-487.563
320 mg/L test item	28.344	26.618	1.726	-338.074
1000 mg/L test item	27.450	25.662	1.788	-353.807
2.5 mg/L reference comp.	25.992	29.917	-3.925	1096.193
5 mg/L reference comp.	28.375	27.525	0.850	-115.736
10 mg/L reference comp.	20.469	23.967	-3.498	987.817
20 mg/L reference comp.	11.088	14.563	-3.475	981.980
40 mg/L reference comp.	7.148	9.369	-2.221	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 heterotrophic respiration. This in particular 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:

Test substance	Isoflucypram technical
Test	Activated sludge, respiration inhibition
Total respiration	
EC_{50}	> 1000 mg/L
EC_{10}	n.d.*
NOEC	320**
Heterotrophic respiration	
EC_{50}	> 1000 mg/L
EC_{10}	n.d.*
NOEC	< 10 mg/L
Nitrification respiration	
EC_{50}	> 1000 mg/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 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 was not reasonable. As the EC_{50} 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 nominal concentration, since no analytical monitoring was performed.

CA 8.9 Monitoring data

No ecological monitoring studies were conducted. For monitoring of isoflucypram in the environment please refer to the Summary MCA Section 7, Point 7.5.