European Commission

Draft Assessment Report prepared according to the Commission Regulation (EU) N° 1107/2009

ISOFLUCYPRAM

Volume 3 – B.9 (AS)

Rapporteur Member State : United Kingdom
Co-Rapporteur Member State : France

Version History

When	What
March 2019	Initial DAR

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B.9. ECOTOXICOLOGY DATA

B.9.1. EFFECTS ON BIRDS AND OTHER TERRESTRIAL VERTEBRATES

B.9.1.1. Effects on birds

B.9.1.1.1. Acute oral toxicity to Birds

Previous evaluation:	None; new active substance application.

Report:	KCA 8.1.1.1/01; [redacted] 2015;
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

Material and methods:

Test item: BCS-CN88460 technical, Origin batch No. 2013-006492, Purity 94.2 % w/w.

Test species: Northern Bobwhite quail (16-week-old adults), obtained from the [redacted]. Before test initiation, they were separated by sex and gang-housed to reduce stress. After 19 days, the birds were placed in individual cages to begin the acclimation period in which normal husbandry of the birds occurred with food (Teklad Game Bird Ration) and tap water *ad libitum.* All birds were 22-weeks old and in good health at test initiation.

Test design: Birds were weighed within 28 hours prior to treatment initiation (Day -1). Following 18 hours of fasting, 2000 mg a.s./kg of BCS-CN88460 for each bird were measured on a balance and placed in gelatin capsules based on the dose level and weight of the bird. The capsules were administered to the bird by coating with corn oil and then placing the capsules orally into the bird's crop. No regurgitation was observed for any bird following dosing.

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. All feed and water was provided ad libitum during the length of the test.

Measurements:

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.

The birds were observed at 15-minute intervals for 2 hours immediately following dose administration. The birds were observed three additional times on Day 0 which occurred at approximately three, four, and five hours after dosing. Following Day 0, the birds were observed twice daily (once on weekends and at study termination) during the treatment period for any mortalities and to detect any overt signs of toxicity or other clinical signs.

The study was terminated 14 days following exposure as no mortality occurred during the last three days of the 14-day observation period and no symptoms of toxicity were apparent on Day 14.

At study termination surviving birds were sacrificed by CO2 asphyxiation. All birds were necropsied.

Test conditions: 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). Ventilation: 8 air exchanges per hour.

Statistical analysis: Body weight, growth data, and feed consumption were subjected to statistical analysis. Normality and homogeneity of variance of the data were tested using the Chi-square test ($\alpha = 0.01$) and the F-test ($\alpha = 0.01$), respectively. If the data were normally distributed and the variances were homogenous, the data were subjected to parametric analyses. If the data were not normally distributed and/or variances were not homogenous, then the data were appropriately transformed and subjected to Chi-square and the F-test again. If the transformed data were normally distributed and the variances were homogenous, then the data were subjected to parametric analyses. If the transformed data were not normally distributed and/or variances were not homogenous, then the untransformed data were subjected to non-parametric procedures.

Parametric procedures involved subjecting body weight, growth data, and feed consumption to a standard one-way analysis of variance (ANOVA) followed by a means comparison using a one-tailed t-test ($\alpha = 0.05$), where the means of the dose groups were compared to control means. Non-parametric procedures involved subjecting the data to a Kruskal-Wallis ANOVA test.

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

Findings:

Biological results

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. No signs of toxicity were observed during post-mortem examination. The LD_{50} is presented in Table B.9.1.1.1-1.

Table B.9.1.1.1-1: LD50 of *Colinus virginianus*

Test object	Northern Bobwhite quail (*Colinus virginianus*)
Test substance	BCS-CN88460 technical
LD_{50} [mg a.s./kg bw]	> 2000

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 analysed. 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 (see Table B.9.1.1.1-2 and B.9.1.1.1-3).

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 (see Table B.9.1.1.1-4).

Table B.9.1.1.1-2: Body weight of *Colinus virginianus* during the acute oral study

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

Table B.9.1.1.1-3: Body weight changes of *Colinus virginianus* during the acute oral study.

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

Table B.9.1.1.1-4: Food consumption of *Colinus virginianus* during the acute oral study.

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 % [a)]	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)

[a)] This is also the validity criterion in OECD 223 (2016)

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.

RMS comments

This study was conducted according to EPA OCSPP 850.2100 guidelines and was compliant with GLP. The RMS has assessed the study according to EPA OCSPP 850.2100 and also OECD 223 (2016). All validity criteria were met for the study. There were some deviations from the study guidelines, which are detailed below:

OCSPP 850.2100 guidelines state that the dosage levels should be confirmed by chemical analysis. No analytical verification of the test item was reported in the study report. OECD guidelines do not require the test item to be analysed in this way.

The OSCPP study guidelines state that the test birds should be indistinguishable from wild stock. This information is not given in the study report.

Only the mean temperature in the study was reported. Therefore it was not possible to determine whether the temperature range during the study was within the acceptable limits in OSCPP 850.2100 (15 – 27 °C). Eight, rather than 10-15, air exchanges were performed per hour. However, as the control group mortality was 0% these climatic variations are not thought to have affected the overall endpoint.

This study and the endpoint of LD_{50} >2000 mg/kg bw are both considered to be acceptable by the RMS

Consideration of the appropriate acute endpoint has been conducted in the CP dossier section B.9.1.1.

Previous evaluation:	None; new active substance application.

Report:	KCA 8.1.1.1/02; [redacted] 2016;
Title:	Toxicity of BCS-CN88460 technical during an acute oral LD50 with the canary (*Serinus canaria*)
Report No.:	[redacted]
Document No.:	M-547051-01-1
Guideline(s):	OCSPP 850.2100
Guideline deviation(s):	not specified
GLP/GEP:	yes

Material and Methods:

Test item: BCS-CN88460 technical, Batch No. 2013-006492, Purity 94.2 %

Test species: Adult canaries (*Serinus canaria*) were obtained from [redacted] Canaries were in adult plumage and indistinguishable from wild species. The bird sexes were genetically identified by Avian Biotech Inc. (Tallahassee, Florida). 81 birds were acclimated to the laboratory environment for 14 days prior to being randomized into treatment groups. One bird mortality occurred in the population during the acclimation period. *Ad libitum* feeding and watering occurred during acclimation. All birds were in good health following acclimation. The birds were aged 9 – 11 months at the time of testing. Thirty male and thirty female birds were used for the current study (body weights ranged from 19.0 to 26.0 g; mean = 21.8 ± 1.3 g).

Test design: Birds were weighed within 28 hours prior to treatment initiation (Day -1). Dose amounts of BCS-CN88460 technical for each bird were weighed and placed in gelatin capsules based on the dose level and weight of the bird. Adult canaries were orally dosed with two gelatin capsules between 12:57 and 1:41 pm. Proper dosages of the test material were administered by coating the capsules with corn oil and then delivering the capsules orally by a canula. The doses tested were 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.

Measurements: The birds were observed continuously for two hours following dosing to determine any toxic symptoms and signs of regurgitation. Starting on Day 1, the birds were observed twice daily (once on weekends/holidays and at study termination) for any mortalities and to detect any overt signs of toxicity or other clinical signs. Lethargy (hypo-reactivity to stimuli) was identified by the technician placing a hand in the cage and observing bird movement. The birds were also observed on Day 0 at approximately one, two, and three hours post-dosing. Adult body weights were measured on experimental Day -1, Day 7, and Day 14. Feed consumption measurements and clinical observations occurred daily.

At study termination surviving birds were sacrificed by CO2 asphyxiation. No mortality occurred during the course of the study therefore no birds were subjected to gross necropsy.

Test conditions: 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) and 18 air changes per hour. The birds were housed in commercial metal cages which measured approximately 27 cm (length) x 33 cm (width) x 31 cm (height). The birds were fed with Lab Diet Advanced Protocol Small Avian Maintenance (PMI Nutrition Intl, Brentwood, Missouri). The analysis of the components of the diet are presented in Table B.9.1.1.1-5.

Table B.9.1.1.1-5: Diet composition.

Nutritional component	Recommended range in EPPO OSCPP 850.2100 (%)	Proportion of diet in Lab Diet Advanced Protocol Small Avian Maintenance (used in this study, %)
Crude protein	27 – 29	16
Crude fiber	3.5 – 5.0	2.5
Crude fat	2.5 – 7.0	7.5
Calcium	2.6 – 3.6	0.88
Phosphorus	0.9 – 1.1	0.71

Statistical analysis: Descriptive statistics (mean and standard deviation) for body weights and feed consumption were calculated in Microsoft® Excel. Normality and homogeneity of variance of the data were tested using the Chi-Square test ($\alpha = 0.01$) and the Levene's test ($\alpha = 0.05$), respectively. All data were normally distributed and the variances were homogenous so the data were subjected to parametric analyses.

Parametric procedures involved subjecting mortality, individual body weight, body weight change, and feed consumption data to a one-tailed Dunnett's test or a Bonferroni-adjusted t-test (when sample sizes varied between groups; α=0.05 for both tests), where the means of the dose groups were compared to control means. The statistical analyses on individual body weight, body weight change, and feed consumption data were conducted using TOXSTAT software

Findings:

Biological results

Mortality and clinical observation: No mortalities were observed in any bird in any of the treatments. Lethargy (diminished hypo-reactivity to stimuli) was observed immediately after dosing 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. The LD_{50} is presented in Table B.9.1.1.1-6.

Table B.9.1.1.1-6: LD_{50} of *Serinus canaria*

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

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 (see Table B.9.1.1.1-7 - Table B.9.1.1.1-9)

Table B.9.1.1.1-7: Body weight of *Serinus canaria* during the acute oral study

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.

Table B.9.1.1.1-8: Body weight changes of *Serinus canaria* during the acute oral study

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.

Table B.9.1.1.1-9: Food consumption of *Serinus canaria* during the acute oral study.

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 % [a)]	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

[a)] This is also the validity criterion in OECD 223 (2016)

Conclusion:

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

RMS comments

This study was conducted according to EPA OCSPP 850.2100 guidelines and was compliant with GLP. The RMS has assessed the study according to EPA OCSPP 850.2100 and also OECD 223 (2016). All validity criteria were met for the study. There were some deviations from the study guidelines, which are detailed below:

OCSPP 850.2100 guidelines state that the dosage levels should be confirmed by chemical analysis. No analytical verification of the test item was reported in the study report. OECD guidelines do not require the test item to be analysed in this way.

It is noted that the birds in this study were fasted for 6 hours, rather than the recommended 15 (OCSPP 850.2100). This may have been due to the smaller bird size of a canary. As the test item was administered via capsule directly inserted via a canula, the deviation in fasting time is not thought to affect the exposure of the birds to the test item.

The diet that the birds are fed does not meet the recommended composition in the OSCPP 850.2100 guideline (see Table B.9.1.1.1-5). However, as all birds were healthy by the end of the study, this is not thought to have affected the results of the test.

Only the mean temperature in the study was reported. Therefore it was not possible to determine whether the temperature range during the study was within the acceptable limits in OSCPP 850.2100 (15 – 27 °C). Eighteen, rather than 10-15, air exchanges were performed per hour. However, as the control group mortality was 0% these climatic variations are not thought to have affected the overall endpoint.

The EPA guideline recommends that the birds are dosed in the early morning. However, in this test the birds were dosed between 12:57 – 1:41 pm. This is not a requirement in OECD 223 (2016) so is not thought to be critical to the reliability of the study result.

This study and the endpoint of LD_{50} >2000 mg/kg bw are both considered to be acceptable by the RMS.

Consideration of the appropriate acute endpoint has been conducted in the CP dossier section B.9.1.1.

B.9.1.1.2. Short-term dietary toxicity to birds

The following two short-term dietary toxicity studies have not been evaluated by the RMS as short-term dietary studies are not a data requirement under EU Comissioning Regulation 283/2013. The RMS would consider short-term dietary bird studies if results from mammalian studies indicated a potential for the dietary route to be more toxic than the oral (gavage) route of exposure. In the only acute rat study by gavage, there were no mortalities (LD_{50} >2000 mg/kg bw) and no other adverse effects. There was no acute dietary study for comparison but in the longer term studies there were no findings related to fertility. Therefore, the longer term studies do not indicate a marked difference in toxicity between gavage and dietary administration, however, without an acute dietary study for a direct comparison, it is difficult to say with certainty. As there is no indication of the contrary, and historical anecdote would support gavage as a cause of greater systemic toxicity in acute studies, the RMS has not evaluated the short-term dietary bird studies or relied on them to inform an EU regulatory decision.

Report:	KCA 8.1.1.2/01; [redacted] 2014;
Title:	Toxicity of BCS-CN88460 technical during a dietary LC50 with the mallard duck (*Anas platyrhynchos*)
Report No.:	[redacted]
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

Report:	KCA 8.1.1.2/02; [redacted] 2015;
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

B.9.1.1.3. Sub-chronic toxicity and reproduction to birds

Previous evaluation:	None; new active substance application.

Report:	KCA 8.1.1.3/01; [redacted] 2017;
Title:	Toxicity of BCS-CN88460 on reproduction in the mallard duck (*Anas platyrhynchos*)
Report No.:	[redacted]
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

Material and Methods:

Test item: BCS-CN88460 technical, Origin Batch No. 2013-006492, Purity 94.2 %.

Test species: farm raised mallard ducks (*Anas platyrhynchos*), obtained on 8 July 2015 from [redacted], 21 weeks old at experimental start, The birds were approaching their first breeding season and had not been previously used in testing. Sex of the birds was confirmed by visual examination of the plumage and physical characteristics.

Test design: The birds were observed for three weeks to ensure no disease or infection was present. Ten birds were sent to the Diagnostic Laboratory at the University of Minnesota to be examined for overall health. The test birds were considered to be in good health and tested negative for the following: avian meta-pneumovirus, Newcastle disease virus, *Mycoplasma gallisepticum*, *Mycoplasma synoviae* and avian influenza virus.

The birds were acclimated to laboratory conditions for two weeks prior to experimental start. At the start of acclimation, all birds were apparently healthy and phenotypically indistinguishable from the wild type. Food and water were provided *ad libitum* during the acclimation period. No bird mortality occurred during the acclimation period.

All birds were leg-banded at randomization and were separated into two groups with 72 males and 72 females. The bird pairs were randomized prior to experimental start to treatment level cages based on body weight by a computer randomization program.

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 with 16 pairs of birds at each treatment level. Control diet and each of the treated diets were prepared approximately weekly and presented to the birds each week. Dietary concentrations were adjusted for purity of the test substance and are presented in mg a.s./kg feed (ppm a.s.). The adult diet used for all phases of the study was IUKA Breeder Diet. The test substance was weighed, dissolved in acetone, and mixed with corn oil. The mixture was then mechanically mixed into the adult feed for five minutes using a KitchenAid mixer as a pre-mix. The pre-mix was then mixed

with the appropriate mass of raw feed in a Hobart Mixer for five minutes. Mixed adult feed was placed in labelled containers and stored at approximately -15 ºC.

Observations

The egg-laying phase was monitored for 10 weeks over the course of the study. Eggs were collected twice daily except for weekends and bank holidays in which they were only collected once per day, and stored in an egg cooler. Egg shell thickness was measured in randomly-selected eggs. Eggs were candled weekly to detect cracks and other abnormalities. Abnormal eggs were discarded and the rest were placed into an incubator. Eggs were candled again on Day 14 of incubation to determine embryo viability (fertility) and on day 21 to determine embryo survival. Non-fertile/non-viable eggs were discarded. On day 23 the eggs were placed into a hatcher and allowed to hatch. The hatchlings were removed on day 27/28 and weighed. Hatchlings were observed once daily for signs of toxicity, injuries, illness and mortality. Hatchlings that survived the 14 day observation period were sacrificed by CO_2 asphyxiation, weighed and discarded.

Observation frequency:

- Adult mortality (daily),
- Adult abnormal behavior and signs of toxicity (daily)
- Adult body weight (measured on week 3, 5, 7, 9 and prior to adult termination)
- feed consumption (measured weekly by cage)

Test conditions:

All adult birds and their offspring were given feed and water ad libitum during acclimation and testing. The basal diet fed to adults was IUKA Breeder Diet and offspring were fed Teklad Game Bird Ration. Contaminant analysis of the adult and hatchling feed are presented in Appendix 1 and Appendix 2, respectively. Tap water was provided *ad libitum* during acclimation and throughout the study which was supplied by the Kansas City, Missouri public water supply. Adult birds were provided a constant supply of tap water which was delivered by an automatic watering system. Hatchlings were provided an adequate supply of water in the brooder cages. Additionally, hatchling water was supplemented with Durvet® water-soluble vitamin supplement (1 gram per gallon water) for the first four days following hatch to provide added nutrition.

Adult birds

Adult birds were housed indoors in a single room for the acclimation and the study. The adult mallard cages measured approximately 79 (L) x 61 (W) x 55 (H) cm and were constructed of stainless steel wire grid and stainless steel sheeting. Cage floors were constructed of plastic coated steel wire and slopped to accommodate egg collection. Each cage was equipped with a bin feeder which was filled with sufficient feed on a weekly basis. During the feeding period, additional feed was weighed and added to the bin feeders as needed. Tap water was delivered by an automatic watering system. During the acclimation and study periods, bedding pans were rinsed at least three times per week and the cage racks were changed once.

Photoperiod: 7 hours light: 17 hours dark.

Temperature: 22.5 ºC (mean)

Humidity: 54% (mean)

Light intensity: 175 lux

Room air change: 13 changes per hour.

Hatchlings

Hatchlings were placed in batteries of brooding cages within room L107 of the Ecotoxicology building for the study. Each cage measured 91(L) X 76(W) X 25(H) cm. The external walls and ceilings of each cage were constructed of galvanized wire mesh and galvanized sheeting. The floors were constructed of galvanized wire

mesh. Food (Teklad Starter Ration) and water were available *ad libitum*. The hatchling water was supplemented with Durvet® brand water-soluble vitamin supplement (1 gram per gallon water as recommended by manufacturer) for the first four days in the brooders.

Bedding was changed daily for the study. Thermostats in the brooding compartment of each cage were set to maintain a temperature gradient of approximately 32ºC to 38ºC over the course of the 14-day post hatchling phase. The temperature difference between the brooding compartment and the room provided a temperature gradient for the hatchlings in each brooder unit. The photoperiod for the hatchlings was maintained at 14 hours light per day. The birds received wide spectrum illumination provided by fluorescent lamps.

Temperature: 23.2 ºC (mean)

Humidity: 56% (mean)

Light intensity: 105 lux

Room air change: 14 changes per hour.

Statistical analysis:

Data from treatment groups were compared to controls using the Shapiro-Wilk's test for normality and Bartlett's test of equal variance to determine if dose groups had unequal variances. If assumption of normality ($p \leq 0.01$) and homogeneity of variance ($p \leq 0.05$) were met, then parametric analyses were conducted using analysis of variance (ANOVA) followed by Dunnett's T3 Multiple Comparison test. If variances were unequal, then the non-parametric analyses were conducted using the Jonckheere-Terpstra Step-down Test. Statistical analyses were performed using CETIS statistical software for personal computers with conclusions of statistical significance at the $\alpha = 0.05$ (95% confidence level).

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 (see Table B.9.1.1.3-1). The mixing procedure for BCS-CN88460 was confirmed to be homogenous and stable under test conditions. Treatment levels were therefore based on the mean measured concentrations.

Table B.9.1.1.3-1: Analytical measurement of dietary 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 Table B.9.1.1.3-2.

Table B.9.1.1.3-2: Measured daily dietary dose of BCS-CN99460

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. Other symptoms such as enlarged spleen and changes in liver morphology were observed in the 111 mg a.s./kg bw treatment group, but these observations were not apparent in more than 2 birds and were absent in the 333 and 1000 mg a.s./kg bw groups. Furthermore, there were clinical symptoms observed in control birds that were not observed at any treatment level (solid black mass, discoloured liver, gas-filled intestine).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

No statistical significance or biologically relevant effects occurred at any treatment level for adult bird body weight gain or food consumption. Compared to the control, treatment birds gained more body weight over the course of the test (with the exception of a 4g loss in the top treatment group in female birds). See Table B.9.1.1.1-3 for details. The deviations observed were not biologically-significant because they doesn't cause detrimental effects to the birds.

Table B.9.1.1.1-3: Adult mallard body weights exposed to BCS-CN88460

Male Body Weight (g) by dietary concentration (mg a.s./kg bw) mean (Standard Deviation)				
	Control [a]	**111[a]**	**333[a]**	**1000[a]**
Study initiation	1127 (71)	1132 (70)	1102 (69)	1135 (65)
Week 3	1095 (60)	1095 (74)	1076 (55)	1120 (83)
Week 5	1103 (58)	1124 (92)	1076 (49)	1136 (86)
Week 7	1094 (59)	1112 (80)	1068 (42)	1136 (83)
Week 9	1096 (71)	1126 (84)	1061 (36)	1129 (81)
Termination	1115 (57)	1167 (131)	1155 (71)	1179 (75)
Overall Gain	-13 (74)	36 (129)	52 (50)	44 (66)
Deviation from control (g)	**N/A**	**+ 49**	**+ 65**	**+ 57**
Female Body Weight (g) by dietary concentration (mg a.s./kg bw) mean (Standard Deviation)				
	Control [a]	**111[a]**	**333[a]**	**1000[a]**
Study initiation	1022 (78)	1022 (85)	1023 (84)	1020 (86)
Week 3	989 (87)	1001 (83)	990 (73)	997 (78)
Week 5	999 (83)	1013 (84)	1013 (85)	1004 (75)
Week 7	1005 (84)	999 (83)	1005 (79)	1005 (72)

Week 9	991 (86)	996 (94)	994 (68)	1011 (68)
Termination	1177 (93)	1235 (137)	1221 (79)	1171 (91)
Overall Gain	155 (100)	213 (125)	199 (98)	151 (103)
Deviation from control (g)	**N/A**	**+58**	**+44**	**-4**

[a] Infertile pair data were excluded for all body weight calculations

Egg Reproductive Effects

There were no statistically significant adverse effects for the following egg reproductive endpoints: number of eggs laid, percent of eggs not cracked of eggs laid, and eggshell thickness (see Table B.9.1.1.1-4)

Embryo Reproductive Effects

There were no statistically significant effects from the control for the percent of live embryos of eggs set, percent viable embryos of eggs set and live embryos of viable embryos (see Table B.9.1.1.1-4).

Hatchling Effects

There were no statistically significant effects from the control for the percent number hatched of eggs set, percent hatchling survival of number hatched. (see Table B.9.1.1.1-4)

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 (see Table B.9.1.1.1-4).

Table B.9.1.1.1-4: Reproductive parameters for adult mallards exposed to BCS-CN88460

	Nominal dietary concentrations (mg a.s./kg feed)						
Parameter [b)]	Control	111		333		1000	
		Mean (SD)	% difference (rel. to control)	# (SD)	% difference (rel. to control)	# (SD)	% difference (rel. to control)
Number of laying and fertile pairs	15	13	13	14	6.7	16	-6.7
Average Total Number of Eggs Laid	50.6 (14.7)	58.4 (10.5)	-15.4	59.5 (9.9)	-17.6	56.5 (13.1)	-11.7
Percent eggs not cracked (of eggs laid)	99.6 (0.011)	99.3 (0.014)	0.3	98.6 (0.032)	1.0	99.5 (0.011)	0.1
Eggshell thickness (mm)	0.335 (0.016)	0.343 (0018)	-2.4	0.345 (0.020)	-2.8	0.342 (0.176)	-2.1
Live embryos of eggs set	87.5 (0.200)	91.4 (0.027)	-4.4	91.5 (0.028)	-4.6	94.1 (0.053)	-7.5
Percent viable embryos of eggs set	88.3 (0.200)	92.3 (0.101)	-4.5	92.8 (0.088)	-5.0	95.2 (0.053)	-7.8
Live embryos of viable embryos	99.1 (0.021)	99.1 (0.013)	-0.1	98.5 (0.035)	0.6	98.9 (0.014)	0.2
Percent hatched of eggs set	76.2 (0.219)	75.8 (0.172)	0.5	74.7 (0.147)	1.9	82.0 (0.110)	-7.7
Percent hatchling survival of number hatched	99.2 (0.016)	98.6 (0.024)	0.7	99.1 (0.016)	0.2	99.6 (0.012)	-0.3
Initial Hatching mean body weight (g)	36.1 (2.8)	35.5 (2.2)	1.6	36.4 (2.6)	-1.0	35.2 (1.9)	2.5
Mean 14 day old survival weight	243.4 (12.4)	253.2 (15.5)	-4.1	245.3 (14.3)	-0.8	240.8 (7.2)	1.1

a) a negative number denotes an increase relative to the control.

b) All averages were calculated with outlier data (pairs laying no fertile eggs) removed

- statistically significant compared to the control

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 female 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.

RMS Comments

This study is valid and has been evaluated according to OECD 206 (1984).

OECD 206 (1984) recommends that mallard duck should be 9-12 months old at the beginning of the test. However, the birds in this test were only 21 weeks old, which is younger than recommended in the guideline. This deviation is not considered to have affected the results of the test as the controls met all of the validity criteria.

The initial full validation of the method, which was performed in study [redacted] 2015; M-516743-01-1) has been referred to, but not submitted. An assessment of the validity of the method is therefore based on the concurrent validation performed in the present study which is derived primarily from procedural recovery data. The method is considered to be valid between the concentrations of 111 and 1000 mg/kg, but the proposed LOQ of 50 mg/kg is not supported. The NOEL is within the validated range and is therefore suitable for use in the risk assessment.

NOEL 1000 mg a.s./kg feed, equivalent to 60 mg a.s./kg bw/day.

Previous evaluation:	None; new active substance application.

Report:	KCA 8.1.1.3/02; [redacted] 2018;
Title:	Toxicity of BCS-CN88460 technical in the reproduction of the northern bobwhite quail (*Colinus* virginianus)
Report No.:	[redacted]
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

Material and Methods:

Test item: BCS-CN88460 technical, Origin Batch No. 2013-006492, Purity 94.2 %.

Test species: Adult quail were obtained from the [redacted]) at approximately 17 weeks of age. All birds were from the same hatch (07 September 2015) and were approximately 32 weeks of age upon study start. The birds were approaching their first breeding season and had not been previously used in testing. All birds were phenotypically indistinguishable from a wild population.

Test design: The birds were gang housed by sex upon arrival into the laboratory. This procedure reduces stress to the birds prior to pairing by sex. The birds were observed for three weeks to ensure no disease or infection was present.

The birds were acclimated to laboratory conditions for two weeks prior to experimental start with one reproductive pair of birds housed per cage. Food and water were provided *ad libitum* during the acclimation period.

The bird pairs were wing-banded and randomized into treatment level cages based on body weight. Ninety pairs were used in the study.

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. Control diet and each of the treated diets were prepared approximately weekly and presented to the birds each week. Dietary concentrations were adjusted for purity of the test substance and are presented in mg a.s./kg feed (ppm a.s.). The adult diet used for all phases of the study was IUKA Breeder Diet (27% protein). The test substance was weighed, dissolved in acetone, and mixed with corn oil. The mixture was then mechanically mixed into the adult feed for five minutes using a Hobart mixer for ten minutes. Mixed adult feed was placed in labelled containers and stored at approximately -14 ºC.

Observations

The egg-laying phase was monitored for 10 weeks over the course of the study. Eggs were collected twice daily except for weekends when they were only collected once per day, and stored in an egg cooler. Egg shell thickness was measured in randomly-selected eggs weekly. Eggs were candled weekly to detect cracks and other abnormalities. Abnormal eggs were discarded and the rest were placed into an incubator. Eggs were candled again on Day 11 of incubation to determine embryo viability (fertility) and on day 18 to determine embryo survival. Non-fertile/non-viable eggs were discarded. On day 21 the eggs were placed into a hatcher and allowed to hatch. The hatchlings were removed on day 24/25 and weighed. Hatchlings were observed once daily for signs of toxicity, injuries, illness and mortality. Hatchlings that survived the 14 day observation period were sacrificed by CO_2 asphyxiation, weighed and discarded.

Observation frequency:

- Adult mortality (daily),
- Adult abnormal behavior and signs of toxicity (daily)
- Adult body weight (measured on week 3, 5, 7, 9 and prior to adult termination)
- feed consumption (measured weekly by cage)

Test conditions:

All adult birds and their offspring were given feed and water ad libitum during acclimation and testing. The basal diet fed to adults was IUKA Breeder Diet and offspring were fed IUKA Starter feed. Contaminants in the tap water were analysed by Johnson County, Kansas, potable water supply known as WaterOne. There were no known contaminants in the feed or water supply that would negatively affect the results of the study.

Adult birds were provided a constant supply of tap water. Hatchlings were provided an adequate supply of water in the brooder cages. Additionally, hatchling water was supplemented with Durvet® water-soluble vitamin supplement (approximately 1 gram per gallon of water as recommended by manufacturer) for the first four days following hatch to provide additional nutrition.

Adult birds

Adult birds were housed in cages measured approximately 56 (L) x 28 (W) x 27 (H) cm and were constructed of stainless steel wire grid and stainless steel sheeting. Each cage was equipped with a bin feeder and watering pans and food and water was provided *ad libitum*. During the acclimation and study periods, bedding was changed approximately twice per week and birds were placed in clean cages at approximately nine weeks. Bird beaks were trimmed at least once during the study and then as necessary.

Photoperiod: 7 hours light: 17 hours dark for the first eight weeks. Then 17 hours light: 7 hours dark.

Temperature: 23.5 ºC (mean)

Humidity: 47% (mean)

Light intensity: 125 lux

Room air change: 12.4 changes per hour.

Hatchlings

Hatchlings were placed in batteries of brooding cages, measuring 91(L) X 76(W) X 25(H) cm. The external walls and ceilings of each cage were constructed of galvanized wire mesh and galvanized sheeting. The floors were constructed of galvanized wire mesh. Food (IUKA Starter feed) was available *ad libitum*. The hatchling water was supplemented with Durvet® brand water-soluble vitamin supplement (1 gram per gallon water as recommended by manufacturer) for the first four days in the brooders.

Bedding was changed at least twice weekly. Thermostats in the brooding compartment of each cage were set to maintain a temperature gradient of approximately 32ºC to 38ºC over the course of the 14-day post hatchling phase. The temperature difference between the brooding compartment and the room provided a temperature gradient for the hatchlings in each brooder unit. The photoperiod for the hatchlings was maintained at 14 hours light per day. The birds received wide spectrum illumination provided by fluorescent lamps.

Temperature: 21.3 ºC (mean)

Humidity: 54% (mean)

Light intensity: 50 lux

Room air change: 14.7 changes per hour.

Statistical analysis:

Data from treatment groups were compared to controls using the Shapiro-Wilk's test for normality and Bartlett's test of equal variance to determine if dose groups had unequal variances. If assumption of normality ($p \leq 0.01$) and homogeneity of variance ($p \leq 0.05$) were met, then parametric analyses were conducted using analysis of variance (ANOVA) followed by Dunnett's T3 Multiple Comparison test. If variances were unequal, then the non-parametric analyses were conducted using the Jonckheere-Terpstra Step-down Test. Statistical analyses were performed using CETIS statistical software for personal computers with conclusions of statistical significance at the $\alpha = 0.05$ (95% confidence level).

Findings:

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 Table B.9.1.1.3-3.

Table B.9.1.1.3-3: Concentration Analysis of food and daily dietary dose of test item

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 Mortality & Clinical Observations

Adult mortality occurred during the test with one male bird (band no.148) in the control group having 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 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.

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 Bodyweight & Feed Consumption

No statistical significance or biologically relevant effects occurred at any treatment level for adult bird body weight gain or food consumption.

Egg and Embryo 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 (see Table B.9.1.1.3-4). 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.

Table B.9.1.1.3-4: 14-day survivor hatchling body weight

14-Day survivor hatchling body weight			
Nominal treatment (ppm)	Mean [g]	Standard Deviation [g]	Reduction compared to the control (%)
Control	35.9[b]	2.7	-
37	33.2[a]	2.4	7.5
111	33.7	3.0	6.2
333	33.8[a]	2.1	5.8
1000	32.7[a]	2.0	8.9

[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.

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%	5.6 %
The average number of 14 day old survivors per hen in the controls should be at least 12 for bobwhite quail.	45
The average egg shell thickness for the control group should be at least 0.19 mm for bobwhite quail.	0.21
Concentration of the substance being tested should be at least 80 % of the nominal concentration	96 – 99 %

RMS Comments

This study is valid and has been evaluated according to OECD 206 (1984).

The study was terminated prematurely due to unusually poor egg quality in the controls, believed to be the result of a deficiency in the nutritional quality of the basal diet (degradation of Vitamin D3). Therefore it was concluded that meaningful conclusions based on the data collected in this study were not possible. A second attempt at the study was initiated but the RMS does not accept the reasoning to conduct the new study.

It is noted that the birds were approximately 32 weeks old at the start of the test. OECD 206 (1984) recommends an age range of 20-24 weeks for bobwhite quail. The difference in age range between what is recommended and the test birds may account for some of the poor egg quality observed.

The method for the analytical verification of the test item concentration in the diet has been evaluated in B.7 CA. The method was found to be valid over the range 5 to 1200 mg/kg in avian diet. Procedural recoveries performed alongside the study samples were in the range 95 to 120% (n=16). Three of these recovery values fall outside the normally accepted analytical recovery range of 70-110%. Matrix standards were not used in the analysis but neither were they used in the validation where recoveries were all below 103%.

- Some procedural recovery data exceed the normally accepted analytical recovery range of 70-110%.
- Stabilty and homogeneity data are presented in the analytical report.

These deviations have not been assessed for acceptability as this study is not used in the avian risk assessment

It is not considered suitable to use the endpoint in this study for the avian reproductive risk assessment.

Previous evaluation:	None; new active substance application.

The following study was submitted as part of modern data requirements. However, the RMS considers the mallard duck study sufficient to use in the long term bird risk assessment with the below study not indicating a greater sensitivity of this second tested species. Therefore, this study has not been evaluated by the RMS. The endpoints appear below as appeared *verbatim* from the applicant-provided study summary. However, these are not confirmed by the RMS.

Report:	KCA 8.1.1.3/03; [redacted] 2018;
Title:	BCS-CN88460 Technical: A reproduction study with the northern bobwhite quail (Colinus virginianus)
Report No.:	007SRUS17C0061
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**

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.

B.9.1.2. Effects on terrestrial vertebrates other than birds

B.9.1.2.1. Acute oral toxicity to mammals

Study summaries for mammalian toxicity studies considered in relation to the evaluation of isoflucypram are included in Volume 3, Section B.6. (AS).

Table B.9.1.2.1-1 summarises the acute oral toxicity study considered when setting the relevant endpoint for use in risk assessment of isoflucypram.

Table B.9.1.2.1-1: Acute oral toxicity endpoint for use in the mammalian risk assessment

Test Organism	Test system	Endpoint	Reference
Rats	Acute oral (gavage). Up and down procedure; (OECD 425)	LD_{50}: >2000 mg/kg bw	[redacted] (2014a) MamTox reference: B.6.2.1.

B.9.1.2.2. Long-term and reproduction toxicity to mammals

Study summaries for mammalian toxicity studies considered in relation to the renewal of isoflucypram are included in Volume 3, Section B.6 (AS).

Table B.9.1.2.2-1 summarises the long-term toxicity studies considered when setting the relevant endpoint for use in risk assessment of isoflucypram. See Section B.9.1.2. (PPP) for discussion of the appropriate endpoint for use in risk assessment.

Table B.9.1.2.2-1: Information relevant to identifying the ecotoxicologically relevant long-term endpoints for mammals

Endpoint	NOAEL (mg a.s./kg bw.d)	Reference
Body weight change, behavioural effects and systemic toxicity	Rats: 83.3/25.6 mg/kg bw/d in M/F; ↓ body-weight gain & final body weight, ↑ absolute & relative liver weight & ↑ relative thyroid weight *(LOAEL of 22.8 mg/kg bw/d-lowest dose tested-* hyaline droplets in kidneys in male rats, indicative of chronic progressive nephropathy – *a male rat specific effect which can lead to mortality over longer periods)*	Repeated-dose 28-day oral toxicity study in rodents (OECD 407) KCA 5.3.1/.1; [redacted], 2017
	Rats; 18.4/ 21.9 mg/kg bw/d in M/F; ↑ relative liver and thyroid weight (NOAEL of 6.34 mg/kg bw/d hyaline droplets in kidneys in male rats, indicative of chronic progressive nephropathy – *a male rat specific effect which can lead to mortality*	Sub-chronic oral toxicity study-rodent 90-day study; (OECD 408) KCA 5.3.2/01; [redacted], 2006

	over longer periods at the LOAEL of 18.4 mg/kg bw/d)	
	Mice; 32/149 mg/kg bw/d in M/F; Hepatocellular necrotic foci, single cell necrosis, ↑ relative liver weight.	Repeated dose 28-day oral toxicity study in rodents (OECD 407) KCA 5.3.1/02; [redacted] 2012
	Mice; 51/59.8 mg/kg bw/d in M/F; ↑ relative liver weight	Sub-chronic oral toxicity study-rodent 90-day study; (OECD 408); KCA 5.3.2/02; [redacted] 2013
	Rats; 11.27-13.91/11.28-14.62 mg/kg bw/d in M/F; ↑relative liver weight and ↑ relative thyroid weight	Multigeneration study (OECD 416); KCA 5.6.1/01; [redacted] 2018
	Rats; 125 mg/kg bw/d; ↑ abs liver weight (16%), enlarged liver	Developmental toxicity study (OECD 414); KCA 5.6.2/01; [redacted] 2017b
	Rabbits; 70 mg/kg bw/d; ↓ BWG, ↓ FC, 2 does aborted, ↑ abs liver weight	Developmental toxicity study (OECD 414); KCA 5.6.2/92; [redacted] 2017
	Rats; 6.27/**8.54** mg/kg bw/d in M/F; Hair loss (F)↓ body weight gain (F)↑ abs liver weight (F), minimal thyroid histopath changes (M & F)	24-month toxicity and carcinogenicity dietary study, OECD 453, KCA 5.5/01, [redacted] 2018
	Mice; 29/38.1 mg/kg bw/d in M/F; ↑ mortality rate (F), ↓ body weight and body weight gain (M & F), ↑ liver weight and histopathological correlates (M & F), ↑ kidney weight and histopathological correlates (M)	18-month dietary carcinogenicity study, KCA 5.5/02, [redacted] 2017

The endpoint used in the risk assessment is indiated by **bold text.** Further discussion is presented in B.9 (PPP)

B.9.1.3. Active substance bioconcentration in prey of birds and mammals

No studies have been included in the current submission.

B.9.1.4. Other data on effects on terrestrial vertebrate wildlife (birds, mammals, reptiles and amphibians)

No studies have been included in the current submission.

B.9.1.5. Potential for endocrine disruption

On the basis of submitted and available information there is no evidence to support that isoflucypram meets the scientific criteria for endocrine disruption (ED) determination as outlined under Commission Regulation (EU) No. 2018/605. However, with regards to terrestrial non-target organisms it is the conclusion of the RMS that insufficient investigation into such properties have been made, according to the supporting guidance document[1]. The applicant acknowledges this and has proposed a further step-wise data generation and assessment in order to fully support a modern assessment for ED. It is proposed by the RMS that the totality of information be considered once available in order to robustly conclude on the ED potential of isoflucypram to non-target organisms as a whole.

[1] Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009 (EFSA/ECHA, 2018). EFSA Journal, Vol 16, Issue 6, June 2018, e05311 https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2018.5311.

B.9.2. EFFECT ON AQUATIC ORGANISMS

B.9.2.1. Acute toxicity to fish

Previous evaluation:	None; new active substance application.

Report:	KCA 8.2.1/01; [redacted] 2018;
Title:	Amendment no. 1: BCS-CN88460 (tech.) - Acute toxicity to fish (Pimephales promelas) under static conditions
Report No.:	EBLNN356
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
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*) (lot F 05 / 15) used in the study were obtained from a brood stock cultured in the test facility at [redacted]
Acclimation	More than 14 days. During the acclimation period fish were fed daily ad libitum with live brine shrimp (*Artemia salina*) nauplii at minimum twice a day. Additionally, ground flake food (Tetramin®) as supplement was fed on working days once per day. Fish were not fed 48 hours before and during the study. All test fish were held in culture tanks under a 16/8 hour light/dark photoperiod and observed for at least 14 days prior to testing. All unsuitable fish (e.g. injured, deformed, etc.) were eliminated from the test prior to the assignment of test groups. 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 (not corrected for 94.2% purity): 0.0266, 0.0533, 0.107, 0.213 and 0.426 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 Preparation of test solution: 212.6 mg of BCS-CN88460 (tech.) was mixed in 50 mL dimethylformamide and this was serially diluted to create several stock solutions. The test solutions were made by adding 4 mL of the relevant stock solution to 40 L of test water. Evidence of undissolved material: No precipitates during exposure were observed.
Replication	No. of test concentrations: 5 (+ control and solvent control) 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 water	Reconstituted water, prepared by adding salt stock solutions to demineralized water. The water was aerated to reach oxygen saturation. The test water was periodically analysed for impurities. In addition, the suitability for aquatic tests was demonstrated by breeding *Daphnia magna* using deionised water of the same origin.
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.
Test vessels	Glass aquaria (38 x 32 x 36 cm, h x w x d). The test volumes amounted to 40 L each.
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 (according to OECD 203 and 850 - 1075)	Required	Obtained
Mortality in control during test	$\leq$ 10%	0 %
Dissolved oxygen saturation	$\geq$ 60%	94 - 112 %

Analytical results:
Measured concentrations were 81 to 109% of nominal values and were stable throughout the test (see Table B.9.2.1-1). Therefore, the results of this study are based on nominal concentrations.

Table B.9.2.1-1: Measured concentrations of BCS-CN88460 (tech.) in the exposure solutions.

Nominal Concentration (mg a.s./L)	Mean measured / % of nominal concentrations (mg a.s./L)		
	Day 0	Day 2	Day 4
0.0266	0.0272 / 102	0.0243 / 91	0.0232 / 89
0.0533	0.0516 / 103	0.0490 / 98	0.0477 / 95
0.107	0.0980 / 97	0.0814 / 81	0.978 / 97
0.213	0.218 / 108	0.213 / 106	-
0.426	0.413 / 103	0.398 / 99	

Biological results:

Mortality occurred at 0.101, 0.201 and 0.401 mg a.s./L. At 4-hours, several sub-lethal effects were observed in every fish in the two highest treatments (0.201 and 0.401 mg a.s./L). After 24 hours sublethal effects were also observed in the next lower concentration (0.101 mg a.s./L). These are detailed in Table B.9.2.1-2 below (TF denotes dead fish).

Table B.9.2.1-2: Lethal and Sublethal effects of BCS-CN88460 (tech.)

Exposure time (hours)	4	24	48	72	96
Nominal conc. (mg a.s./L)	No affected (%)	No affected (%)	No affected (%)	No affected (%)	No affected (%)
Control	10 N (100%)	10 N (100%)	10 N (100%)	10 N (100%)	10 N (100%)
Solvent Control	10 N (100%)	10 N (100%)	10 N (100%)	10 N (100%)	10 N (100%)
0.0266	10 N (100%)	10 N (100%)	10 N (100%)	10 N (100%)	10 N (100%)
0.0533	10 N (100%)	10 N (100%)	10 N (100%)	10 N (100%)	10 N (100%)
0.107	10 N (100%)	0 N (0%) 1 BO, AT (10%) 4 TS, AT, AK (40%) 3 OB, AT, SR (30%) **2 TF (20%)#**	0 N (0%) 2 OB, AT (20%) 2 BO, AT, AP (20%) **6 TF (60%)#**	0 N (0%) 1 BO, SR, AT (10%) **9 TF (90%)#**	0 N (0%) 1 BO, SR, AT, AP (10%) **9 TF (90%)#**
0.213	0 N (0%) 4 BO, SR, AT (40%) 4 OC, AT, TS, AK (40%) **0 TF (0%)**	**10 TF (100%)#**	-	-	-
0.426	0 N (0%) 4 BO, AT, AP, SR (40%) 4 OB, AT, AP, SR (40%) 2 AT, TS (20%) **0 TF (0%)**	**10 TF (100%)#**	-	-	-

significantly different compared to the control ($p \leq 0.05$)
Abbreviations of behavioral observations:
AK: strongly extended gills
AP: reduced activity; apathy
AT: labored respiration
BO: fish mainly on the bottom
N: no signs of sublethal effects
OB: fish mainly at the water surface
SD: displayed mucous evacuation of the intestine
SR: laid on their sides or backs
TF: dead fish
TS: loss of equilibrium, tumbling during swimming
SR: fish lying on side or back on the bottom
- no observations, all fish dead

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

LC_{50} 96 hours (95% C.I.):	0.0861 mg a.s. / L (0.0721 – 0.0991 mg a.s./L)
LOEC: lowest concentration with a significant effect compared to the control	0.107 mg a.s./L
NOEC: highest concentration without a significant effect compared to the control	0.0533 mg a.s./L

RMS Comments

This study was conducted according to GLP and following the guidelines of OECD 203 (1992) and OPPTS 850.1075 (1996). All validity criteria were met.

According to OECD 203 (1992) only 7 fish per replicate are required to detect effects; 10 fish per replicate were used in this study. However it is noted that according to OCSPP Draft Guideline 850.1075, 10 fish and two replicates are preferred for a more statistically accurate test.

It is noted that the temperature in the test vessels deviated from the recommended 21 – 25 ºC set by OECD 203 (1992) for *P.promelas* as the minimum recorded temperature in this study is 20.5 ºC. However, this temperature deviation was experienced by fish in all control and test groups so any effect would have been relative. Also, the fish in the control vessels showed 0% mortality and no sub-lethal or behavioural issues were noted therefore it is considered that this deviation did not affect the test results.

There is also a slight increase in fish length when compared to the guidelines (OECD 203). This deviation is considered to be minor, especially as fish size appears to have been very consistent in the test.

The analytical confirmation of the media concentrations of the active substance analyses for pure active substance (i.e. the chemical structure of the active substance only). In this study the measured concentrations were within 80-120% of nominal concentrations. Therefore the technical-to-pure correction is unnecessary and the RMS has removed this, presenting the uncorrected concentrations only.

The method is acceptably validated in accordance with SANCO/3029/99 rev.4 and is suitable for the determination of isoflucypram in samples of test water at a LOQ of 0.0625 µg/L (see section B.5.1.2.6.1 of the CA document for further details).

This study is considered valid and the endpoints are confirmed as:
96h LC_{50}: 0.0861 mg a.s. / L (95% C.I : 0.0721 – 0.0991 mg a.s./L)

Previous evaluation:	None; new active substance application.

Report:	KCA 8.2.1/02; [redacted] 2015;
Title:	BCS-CN88460 (tech.) - Acute toxicity to fish (Oncorhynchus mykiss) under static conditions
Report No.:	EBLNN024
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*) (Lot F 4.15) from [redacted]
Acclimation	More than 14 days During the acclimation period fish were fed daily with commercial trout food (e.g. Brutfutter Inicio, BioMar, Denmark). Fish were not fed 48 hours before and during the study. All test fish were held in culture tanks under a 16/8 hour light/dark photoperiod and observed for at least 14 days prior to testing. Less than 5 % mortality was noted during the acclimatization period prior to the test initiation and all unsuitable fish (e.g. injured, deformed, etc.) were eliminated from the test prior to the assignment of test groups. 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 (not corrected for 94.2% purity): 0.0425, 0.0850, 0.170, 0.340 and 0.680 mg a.s./L Corresponding measured concentrations: 0.0394, 0.0753, 0.170, 0.340 and 0.680 mg a.s./L. (the highest three concentrations remained as nominal) Controls: reconstituted water Solvent control: 0.1 ml/L dimethylformamide Preparation of test solution: 340.2 mg of BCS-CN88460 (tech.) was mixed in 50 mL dimethylformamide and this was serially diluted to create several stock solutions. The test solutions were made by adding 25 mL of the relevant stock solution to 50 L of test water. Evidence of undissolved material: No precipitates during exposure were observed.
Replication	No. of test concentrations: 5 (+ water and solvent control) 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.
Test vessels	Glass aquaria (38 x 32 x 36 cm, h x w x d). The test volumes amounted to 40 L each.

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 (according to OECD 203 and 850 -1075	Required	Obtained
Mortality in control during test	≤ 10%	0 %
Dissolved oxygen saturation	≥ 60%	91 - 102 %

Analytical results:

Recoveries were between 76 and 117% of nominal (see Table B.9.2.1-3). The nominal concentrations of 0.0425 and 0.0850 mg a.s./L were not maintained within ±20 of nominal. Therefore, a geometric mean of the three measured concentrations (at day 0, 2 and 4) was taken. The geometric mean concentration was not taken for the nominal concentrations of 0.170, 0.340 and 0.680 mg a.s./L as they were all maintained within ±20% of nominal. Therefore, these concentrations will be expressed as the nominal concentration.

Table B.9.2.1-3: Measured concentrations of BCS-CN88460 (tech.) in the exposure solutions.

Nominal Concentration (mg a.s./L)	Mean measured concentrations (mg a.s./L)[a] (% of nominal)				Geometric mean concentration (mg a.s./L)
	Day 0	Day 1	Day 2	Day 4	
0.0425	0.0462 (109)	-	0.0392 (92)	0.0337 (79)	0.0394
0.0850	0.0893 (105)	-	0.0739 (87)	0.0647 (76)	0.0753
0.170	0.181 (106)	0.199 (117)	-	-	N/A
0.340	0.359 (106)	-	-	-	N/A
0.680	0.726 (107)	-	-	-	N/A

[a] Average of two detections

Biological results:

Table B.9.2.1-4 shows the effects of the test item on mortality and morbidity of the fish. In the controls (negative and solvent) no mortalities or sub-lethal effects were observed within the whole test period. At 0.0.801 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. At all test concentrations > 0.0801 mg a.s./L mortality was 100% by 24 hours.

Table B.9.2.1-4: Lethal and Sublethal effects of BCS-CN88460 (tech.)

Exposure time (hours)	4	24	48	72	96
Concentration. (mg a.s./L)	No affected (%)	No affected (%)	No affected (%)	No affected (%)	No affected (%)
Control	10 N (100%)	10 N (100%)	10 N (100%)	10 N (100%)	10 N (100%)
Solvent Control	10 N (100%)	10 N (100%)	10 N (100%)	10 N (100%)	10 N (100%)
0.0394	10 N (100%)	10 N (100%)	10 N (100%)	10 N (100%)	10 N (100%)
0.0753	0 N (0%) 3 DF, AT (30%) 7 AT (70%)	5 N (50%) 2 BO, AT (20%) 1 TS, AT (10%) 2 AT (20%)	4 N (40%) 3 BO, AT, DF (30%) 1 OB, TS, AT (10%)	5 N (50%) 1 BO, AT, DF (10%) 2 AT (20%) **2 TF (20%)**	3 N (30%) 1 BO, AT, DF (10%) 4 AT (40%) **2 TF (20%)**
0.170	10 N (100%)	**10 TF (100%)**	-	-	-
0.340	**10 TF (100%)**	-	-	-	-
0.680	**10 TF (100%)**	-	-	-	-

Abbreviations of behavioural observations:
AP: Apathy
AT: showed laboured respiration
BO: remained for unusually long periods on the bottom of the aquarium
DF: turned dark in coloration
N: did not show any abnormal signs
OB: remained for unusually long periods at the water surface
SR: laid on their sides or backs
TF: dead
TS: showed loss of equilibrium with lateral deviation from their normal orientation
- no observations as all fish were dead.

Conclusion

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

LC_{50} 96 hours (95% C.I.): [a)]	0.098 mg a.s./L (C.I. 95%: 0.073 – 0.177)

LOEC: lowest concentration with a significant effect compared to the control	0.0753 mg a.s./L
NOEC: highest concentration without a significant effect compared to the control	0.0394 mg a.s./L

a) Following a request for additional information, the applicant replotted the curve according to geometric mean measured concentrations. The RMS also requested that the correction for purity was removed. This changed the LC_{50} value, which is now presented here.

RMS Comments

This study was conducted according to GLP and following the guidelines of OECD 203 (1992) and OPPTS 850.1075 (1996).

All validity criteria were met.

According to OECD 203 (1992) only 7 fish per replicate are required to detect effects; 10 fish per replicate were used in this study. However it is noted that according to OCSPP Draft Guideline 850.1075, 10 fish and two replicates are preferred for a more statistically accurate test.

This study is considered valid and the endpoints are confirmed as:
LC_{50}: 0.098 mg a.s./L (C.I. 95%: 0.073 – 0.177)

The analytical measurements of the media concentrations of the active substance analyses for pure active substance (i.e. the chemical structure of the active substance only). Therefore the technical-to-pure correction is unnecessary and the RMS has removed this, presenting the uncorrected nominal concentrations and the mean measured concentrations only.

The method is acceptably validated in accordance with SANCO/3029/99 rev.4 and is suitable for the determination of isoflucypram in samples of test water at a LOQ of 0.0625 µg/L (see section B.5.1.2.6.1 of the CA document for further details).

Previous evaluation:	New data, submitted for purpose of review

Report:	KCA 8.2.1/03; [redacted] 2015;
Title:	Acute toxicity of BCS-CN88460 technical to the sheepshead minnow (Cyprinodon variegatus) under static conditions
Report No.:	EBLNN023
Guideline(s):	US EPA OCSPP 850.1075 OECD 203
GLP/GEP:	Yes

Material and methods

Test material	BCS-CN88460 techn. Origin batch ID: 2013-006492; Specification number: 10200028196; purity: 94.2% w/w
Test species	Sheepshead Minnow (*Cyprinodon variegatus*) juveniles from [redacted]
Acclimation	More than 14 days. No mortalities during 48 hours prior to testing, no treatments for disease. Fish were fed once daily during holding but not 48 hours prior to testing.
Organism age/size at study initiation	Mean length: 29.6 mm ± 2.1 mm Mean body weight: 0.579 g ± 0.163g
Preparation of test solutions:	A stock solution of 10 g a.s/L was prepared using dimethyformamide (DMF) as solvent; the solution was inverted to mix. Serial dilutions were then made to give the required stock concentrations and an appropriate amount of the solution was added to 30 L of dilution water to give the correct test concentrations in the test vessels. No precipitates during exposure were observed.
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 DMF
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 water:	Synthetic seawater.
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 behavioural 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.
Test vessels:	Glass vessels 38 L volume (49.5 x 25.4 x 30.5 cm) filled with 30 L test solution

Chemical analysis	Samples of each test concentration were taken at 0 and 96 hours 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

Validity criteria according to OECD 203 (1992) and 850-1075 (1996)	Required	Obtained
Mortality in control during test	≤ 10%	0 % in solvent and water control
Dissolved oxygen saturation	≥ 60%	81 – 93%

All validity criteria were met.

Analytical results:

Recoveries were between 61 and 90% (see Table B.9.2.1-5 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.

Table B.9.2.1-5: Measured Concentrations of BCS-CN88460 During the 96-Hour Static Exposure of the Sheepshead Minnow.

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:

Common carp in the control, solvent control, 0.0462 mg a.s./L and 0.0886 mg/L groups showed no sublethal effects. 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 B.9.2.1-6 below).

Table B.9.2.1-6: Cumulative Mortality and Behavioral Observations of the Sheepshead Minnow Exposed to BCS-CN88460 Technical

Exposure time (hours)	**4**		**24**		**48**		**72**		**96**	
Arithmetic mean measured conc. (mg a.s./L)	**No. dead (%)**	**Observ.**	**No dead (%)**	**Observ.**	**No dead (%)**	**Observ.**	**No dead (%)**	**Observ.**	**No dead (%)**	**Observ.**
Control	0 (0)	10N	0 (0)	10N	0 (0)	10N	0 (0)	10N	0 (0)	10N
Solvent Control	0 (0)	10N	0 (0)	10N	0 (0)	10N	0 (0)	10N	0 (0)	10N
0.0462	0 (0)	10N	0 (0)	10N	0 (0)	10N	0 (0)	10N	0 (0)	10N
0.0886	0 (0)	10N	0 (0)	10N	0 (0)	10N	0 (0)	10N	0 (0)	10N
0.196	0 (0)	10N	0 (0)	1DC; 9N	0 (0)	2DC; 8N	0 (0)	2DC; 8N	0 (0)	2DC; 8N
0.395	0 (0)	8DC 2N	0 (0)	8DC; 2N	0 (0)	8DC; 2N	0 (0)	8DC; 2N	1 (10)	1D; 8DC, 1N
0.869	0 (0)	8DC 2N	0 (0)	9DC;1DC, 0B, LR	4 (40)	4D; 1VO, DC, LR; 5DC, LR, E	10 (100)	6D	10 (100)	-

N = Normal ; OB = On bottom ; VO = Vertical orientation ; LR = Laboured respiration ; D = Dead ; DC = Dark colouration ; E = Erratic behaviour

Conclusion
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)**
NOEC: highest concentration without an significant effect compared to the control	0.0886 mg a.s. / L

RMS comments

This study was conducted according to GLP and following test guidelines OECD 203 (1992) and 850.1075 (1996). All validity criteria were met.

The following was noted by the RMS:

According to OECD 203 (1992) it is only necessary to use 7 fish per test concentration and control in order to reduce vertebrate teating; however in this study 10 fish were used. Test guideline 850.1075 (1996) states that 10 fish per replicate should be used for a more statistically accurate result. As the study followed both test guidelines this is considered to be acceptable by the RMS.

The calculated LC50 was based on arithmetic measured concentrations; however geometric mean measured concentrations should have been calculated and the LC50 derived from these values. A request for additional information was made during evaluation for this information, however the applicant stated that as the geometric mean values were very similar to the arithmetic mean values, the impact on the derived endpoint would be minimal. The RMS agrees that this is likely to be the case, however ideally endpoints based on geometric mean measured values would have been provided.

The method is acceptably validated in accordance with SANCO/3029/99 rev.4 and is suitable for the determination of isoflucypram in samples of test water at a LOQ of 5 µg/L (see section B5.1.2.6.1 of the CA document for further details).

This study is considered valid and the endpoints are confirmed as follows:

LC50 96 hours (95% C.I.): 0.544 mg a.s./L (0.472 – 0.626 mg a.s. / L)

NOEC: 0.0886 mg a.s./L

Metabolite - BCS-CN88460-carboxylic-acid (BCS-CY26497)

Report:	KCA 8.2.1/04; [redacted] 2017
Title:	BCS-CN88460-carboxylic-acid (BCS-CY26497) - Acute toxicity to rainbow trout (Oncorhynchus mykiss) under static conditions - Final report
Report No.:	EBLNN193
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
Test species	Rainbow trout (*Oncorhynchus mykiss*) (Lot F 7/15) obtained from and identified [redacted]
Acclimation	At least 14 days, fed daily with commercial trout food Health during acclimation: less than 5% mortality (48 hours prior to the test). All unsuitable fish (injured, deformed) were removed from the test prior to assignment of test groups.

Organism age/size at study initiation	Mean length: 3.9 ± 0.4 cm Mean body weight: 0.5 ± 0.2 g
Preparation of test solutions	A weighed amount of the test item was dissolved in 4.00 mL of dimethylformamide (DMF) before being added to a total volume of 40L of test water to achieve the required test concentrations.
Test solutions	Nominal concentrations: 6.18, 12.4, 24.7, 49.4 and 98.8 mg p.m./L (based on initial range-finder test). Geometric mean measured concentrations: 6.69, 12.9, 25.0, 28.3 and 33.5 mg p.m./L Controls: water and solvent control (DMF) 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 water	Reconstituted water was used during the acclimation period and for the test by adding salt stock solution to demineralised water. Water was aerated to reach oxygen saturation. Test water was periodically analysed for impurities.
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 using commercial measurement devices.
Test vessels	Glass aquaria (38 x 32 x 36 cm) 40 L volume.
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, 48 hours and at test termination.Samples were taken from the centre of the test vessels
Data analysis	Calculation of the geometric mean measured concentrations was performed according to OECD 23 Annex 2 (2000).

p.m. = pure metabolite

Results

Validity criteria (according to OECD 203 and 850-1075)	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 mean recoveries between 31and 108 % of nominal across all test concentrations as shown in Table B.9.2.1-7 below.. Small amounts of undissolved test material were observed at nominally 24.7 mg p.m./L at test initiation. 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 and precipitation of the test item was observed.
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. 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.

Table B.9.2.1-7: Measured concentrations of BCSCY26497 in the exposure solutions

Nominal Concentration (mg p.m./L)	**Measured concentration of BCSCY26497**			**Geometric mean measured concentration (mg p.m./L)**	**% of nominal concentrations***			
	0hr (mg/L)	**48hr (mg/L)**	**96hr (mg/L)**		**0hr**	**48hr**	**96hr**	**Mean**
6.18	6.74	6.69	6.66	6.69	109	108	108	108
12.4	12.8	12.9	12.9	12.87	103	104	104	104
24.7	23.7	25.6	25.3	25.04	96	104	102	102
49.4	15.6**	42.6**	44.3	33.46	32	86	90	76
98.8	10.3**	38.2**	42.8**	28.32	10	39	43	34

* Average of two detections (presented are rounded values, all calculations were done with Microsoft® Excel)
**Mean values of the A and B samples

Biological results:

Observations
In the controls no mortalities or sub-lethal effects were observed within the whole test period. Lethal effects 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. A summary of the results is shown in Table B.9.2.1-8 below.

Table B.9.2.1-8: Summary of mortalities following exposure of *Onchorhynchus mykiss* to BCSCY26497

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
12.87	0	0	0	0	0
25.04	0	0	0	0	0
28.32	0	0	0	0	0
33.46	0	0	1 (10)	1 (10)	1 (10)

Conclusion

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

LC_{50} 96 hours (95% C.I.):	**> 33.46 mg p.m./L (not determined)**
LOEC: lowest concentration with a significant effect compared to the control	33.46 mg p.m./L
NOEC: highest concentration without an significant effect compared to the control	28.32 mg p.m./L

RMS comments

This study was conducted according to GLP and following the guidelines of OECD 203 (1998) and OPPTS 850.1075 (1996).

All validity criteria were met.

The following was noted by the RMS:

According to OECD 203 (1998) only 7 fish per replicate are required to detect effects; 10 fish per replicate were used in this study. However it is noted that according to OCSPP Draft Guideline 850.1075, 10 fish and two replicates are preferred for a more statistically accurate test.

The fish used in the study were slightly below the recommended mean length according to OECD 203 (1998) ; mean values 3.9 ± 0.4 cm in comparison to 5.0 ± 0.4 cm. However the controls performed adequately during the test and the test parameters were within acceptable limits. As such this deviation is considered acceptable.

It is noted that for the two highest test concentrations (49.4 and 98.8 mg p.m./L nominal) the test solutions exceeded the solubility limit resulting in low recovery of the test item (76 and 34 % respectively). However geometric mean measured concentrations were calculated and used in the derivation of endpoints ; therefore this is considered acceptable.

The method is acceptably validated in accordance with SANCO/3029/99 rev.4 and is suitable for the determination of BCS-CN88460-carboxylic acid in samples of test water at a LOQ of 0.125 mg/L (see section B5.1.2.6.1 of the CA document for further details).

This study is considered valid and the endpoints are confirmed as:

LC_{50} 96 hours: > 33.46 mg p.m./L
NOEC: 28.32 mg p.m./L

B.9.2.2. Long-term and chronic toxicity to fish

B.9.2.2.1. Fish early life stage toxicity test

Previous evaluation:	None; new active substance application.

Report:	KCA 8.2.2.1/01; [redacted] 2017;
Title:	Early-life stage toxicity of BCS-CN88460 (tech.) to fish (Pimephales promelas)
Report No.:	EBLNN029
Guideline(s):	EU Directive 91/414/EEC Regulation1107/2009 (Europe) US EPA OCSPP 850.1400
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), originating from a brood stock cultured in the test facility at [redacted]. A total of twenty-four breeding groups were used to produce a sufficient number of fertilized eggs for starting the study. One breeding group consisted of 1 male and 2 females. One spawning substrate was used per breeding group (breeding tank (14 L) consisting of a single half tile constructed of stainless steel (100 mm (L) x 75 mm (B)). The parental fish were maintained at the actual test temperature (25 ± 1.5 °C) and in water of the same quality as used in the test and were fed ad libitum with live brine shrimp (Artemia salina) nauplii at minimum twice a day. Additionally, ground flake food (TetraMin®) was fed on working days once per day. Freshly fertilised fathead minnow eggs (< 24 hours old) were exposed to the different test item concentration on study day 0. Eggs were pooled from sixteen breeding pairs to start split I on June 19, 2013 (replicates A and B). One day later on June 20, 2013 split II (replicates C and D) was started with eggs pooled from eight breeding pairs. In total 980 eggs, were randomly introduced in incubation cups at the different dose levels.
Organism Age at Experimental Start:	Embryos less than 24 h old
Test procedure	The exposure phase of the test was split into two events. June 19, 2013 was the biological start (study day 0) for replicates A and B, defined as split I. On June 20, 2013 the biological phase was started for replicates C and D (defined as split II). At the start of each split, two replicate chambers per treatment group were used, each containing 35 impartially selected freshly (< 24 h old) fertilised eggs. The eggs were randomly distributed into incubation cups that are suspended in the test aquaria by adding groups of 5 eggs until each cup contained the desired number of eggs. A total number of 980 eggs were used to start the experiment (490 for split 1, 490 for split 2). The incubation cups had 0.8mm diameter holes in the bottom of the incubation cups, allowing exposure of the eggs to the water control, dimethylformamide (DMF) solvent control or solution of BCS-CN88460 (tech.). Mortality of the eggs was recorded. The

	freshly-hatched larvae were able to swim out of the cups into the corresponding replicate test chamber through the perforated holes. On day 5, larvae were impartially thinned to 15 individuals in each replicate chamber and all test levels.
Test solutions	Nominal concentrations: 0.48, 1.53, 4.88, 15.6, 50.0 µg a.s./L Corresponding mean measured concentrations: 0.45, 1.36, 4.35, 9.48 and 48.7 µg a.s./L Controls: water control and solvent control (DMF 0.1 mL/L) Test solution preparation: For the entire study, five series of stock solutions (SL 1-5) of the test item BCSCN88460 were prepared (Table 14). The stock solutions were prepared by weighing the adequate amount of test item into the solvent dimethylformamide p.a. (DMF) by intensely stirring for at least one hour at room temperature. The solution contained in the mixing chamber constituted the highest nominal test concentration (160 µg a.i./L) and was subsequently diluted (50 %) to provide the remaining nominal exposure concentrations (80,40, 20 and 10 µg a.i./L). Evidence of undissolved material: Not reported
Replication:	No. of vessels per concentration (replicates): 4 (2 from split 1, 2 from split 2) No. of vessels per control (replicates): 4 (2 from split 1, 2 from split 2) No. of vessels per solvent control (replicates): 4 (2 from split 1, 2 from split 2)
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 Test water: Reconstituted water was prepared by adding salt stock solutions prepared of analytical grade salts to demineralized water (conductivity < 0.10 µS/cm). Before use, the reconstituted water was aerated, resulting in oxygen saturations in the test media of > 60 %. Water hardness: 36.7 to 52.9 mg $CaCO_3$/L Dissolved oxygen (% saturation): 84 to 106% Conductivity: 96.9 – 116.1 µS/cm Aquaria: Glass, approximately 15.5 (width) x 16.0 (depth) cm and a water height of approximately 16.0 cm. The test solution volume per replicate aquarium was approximately 4 litres. Flow through: The flow-rate corresponds to approximately 12 exchanges of test solution per day in all replicate test chambers (including water control and solvent control).
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 mortality was recorded daily and abnormal behavior/morphological appearance were recorded 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. The samples taken on study days -2/-1 were analysed for an internal verification of the test concentrations prior to start and were not considered for the evaluation of the analytical results. On days 23/22 and 24/23, respectively, additional samples were taken. On study day 23 (for split I, study day 22 for split II) during the check of the test system an interruption of the stock solution dosing was observed at the test concentration of 15.6 µg a.s./L. The problem was located and corrected immediately. For analytical verifications samples were taken directly after observing the malfunction, a few hours later at day 23/22 and on the following study day 24/23. The results of the additional analytical samples demonstrated, that the observed malfunction was corrected. In addition samples were taken in freshly prepared and aged stock solutions to demonstrate stability of the test item. The sampling dates for the stock solutions were on study day -2/-1, 6/5, 13/12, 20/19, 27/26 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 ToxRat Solutions GmbH, 52477 Alsdorf, Germany.
Test initiation	The exposure phase of the test was split into two events. June 19, 2013 was the biological start (study day 0) for replicates A and B, defined as split I. On June 20, 2013 the biological phase was started for replicates C and D (defined as split II). For both splits, two replicate chambers per treatment group were used, each containing 35 impartially selected freshly (< 24 h old) fertilised eggs. The eggs were randomly distributed into the incubation cups with a plastic pipette by adding groups of 5 eggs until each cup contained the desired number of eggs. A total number of 980 eggs were used to start the experiment (490 for split 1, 490 for split 2).

Results

Validation criteria

The test was judged against OECD 210 (2013). The results of the validity of the study are detailed in the table below.

Validity criteria	Required by OECD 210, 2013	Obtained
Dissolved oxygen concentration throughout the test (% saturation)	≥ 60%	≥ 84 -106%
Water temperature difference between test chambers or between successive days at any time during the test	± 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	Within ± 20 % of the nominal values	**The test item was maintained within ±20% of nominal, except for from day 21-23 when the flow-through system was interrupted in nominal test concentration 15.6 µg a.s./L. Therefore, for this time period, the test concentration was 0.2% of nominal (based on half the LOQ value of 0.05 µg a.s./L)**
Hatching success of controls (Control/solvent control)	> 70%	83% / 91%
Post-hatch survival of controls	> 75%	97% / 100%

Bold denotes where a validity criterion was not met.

Analytical results:

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.

Two samples were taken on each sampling day, therefore arithmetic means of each sampling day were taken and an overall arithmetic mean for each test concentration as recommended for flow-through study designs (see table B.9.2.2.1-1. On days 22/23 and 23/24, respectively, additional samples were taken. On study day 23 (for split I, study day 22 for split II) during the check of the test system an interruption of the stock solution dosing was observed at the test concentration of 15.6 µg a.s./L. The analytical measurement of the immediately taken water sample showed that no test substance was detectable (< LoQ). The problem was located and found directly. The tube of the dosing pump was slipped out of the stock solution. After the air was removed out of the pump and tubes this test level could be restarted again. For analytical verifications samples were taken again a few hours later on study day 23 and 22, respectively, and on the following day. At each sampling time 0.2 and 105 % of nominal concentrations were detected. Therefore the corresponding validity criterion was temporarily not fulfilled. In order to account for the failure of the pump over this time period, a geometric mean was taken of the samples taken on day 23/22 and then an overall arithmetic mean was taken of the remaining concentrations. As the measured concentration on day 23/22 was outside of 80 – 120% of nominal for the test concentration of 15.6 µg a.s./L, biological results are referred to via their mean measured concentrations.

Table B.9.2.2.1-1: Concentrations of BCS-CN88460 measured in exposure solutions using HPLC during the early life-stage exposure with fathead minnow *(Pimephales promelas)*

Nominal Concentration µg a.s./L	0.48	1.53	4.88	15.60	50.00
Day 0	0.43	1.56	3.99	14.40	50.80
Day 0	0.46	1.56	4.42	14.80	48.00
Arithmetic mean of day 0	**0.45**	**1.56**	**4.21**	**14.60**	**49.40**
Day 7	0.45	1.31	4.51	14.90	53.70
Day 7	0.46	1.31	4.47	15.30	49.60
Arithmetic mean of day 7	**0.45**	**1.31**	**4.49**	**15.10**	**51.65**
Day 15	0.42	1.31	4.46	15.40	49.60
Day 15	0.44	1.46	4.34	16.20	47.10
Arithmetic mean of day 15	**0.43**	**1.39**	**4.40**	**15.80**	**48.35**
Day 21	0.45	1.30	4.29	15.60	51.10
Day 21	0.44	1.28	4.30	15.20	44.00
Arithmetic mean of day 21	**0.45**	**1.29**	**4.30**	**15.40**	**47.55**
Day 23/22	-	-	-	0.0337*	-
Day 23/22	-	-	-	13.70	-
Geometric mean of day 23/22	**-**	**-**	**-**	**0.68**	**-**
Day 24/23	-	-	-	**16.40**	-
Day 28	0.45	1.33	4.36	14.70	45.60
Day 28	0.42	1.28	4.25	13.70	47.80
Arithmetic mean of day 28	**0.43**	**1.31**	**4.31**	**14.20**	**46.70**

Day 33	0.48	1.33	4.53	14.60	48.80
Day 33	0.50	1.34	4.29	13.50	49.10
Mean	**0.49**	**1.34**	**4.41**	**14.05**	**48.95**
Overall arithmetic mean of mean daily concentrations	**0.45**	**1.36**	**4.35**	**13.28**	**48.77**
% of nominal	**93.89**	**89.16**	**89.16**	**85.12**	**97.53**

* As < LOQ was detected at sample date 23 (for split I, study day 22 for split II), the value of half of the LOQ of 0.0674 µg a.s./L was used in geometric mean calculations.
**Arithmetic mean calculated based on bold values in the above table.

Biological results:

Due to statistically significant differences between the control and solvent control mean values for dry weight, all the following endpoints were statistically evaluated against the solvent control.

Time to hatch and hatching success
The hatching of larvae started on day 4 and lasted until day 5. 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. 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 (see Table B.9.2.2.1-2 for details). There were no statistically-significant effects of the test item on this parameter. The endpoint hatching success on day 5 (post hatch day 0) resulted in a NOEC ≥ 48.8 µg a.s./L and a LOEC > 48.8 µg a.s./L.

Table B.9.2.2.1-2: Mean cumulative hatching success at day 4 and day 5 (%) after exposure to BCS-CN88460

Mean measured 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
0.45	13	83
1.36	11	84
4.35	22	91
13.28	30	84
48.8	29	77

Larval survival
Mean larval survival at test termination ranged from 15 to 100% in all treatment groups (see Table B.9.2.2.1-3). The endpoint larval survival resulted in a NOEC of 13.28 µg a.s./L and a corresponding LOEC of 48.8 µg a.s./L.

Table B.9.2.2.1-3: Mean larval survival over the length of the test (until day 33) after exposure to BCS-CN88460

Mean measured concentrations (µg a.s./L)	Larval survival [%]
Water control	97
Solvent control	100
0.45	97
1.36	92
4.35	95
13.28	95
48.8	15*

* Statistically-significant difference compared to the solvent control (α=0.05) [Williams-Test]

Growth
The effects of the test item on growth (total length and dry weight) of the fish is found at Table B.9.2.2.1-4. In the highest test concentration (48.8 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 the applicant has not included this concentration in any statistical analysis.

Table B.9.2.2.1-4: Mean total length and dry weight of the fish by the end of the test (until day 33) after exposure to BCS-CN88460

Mean measured concentrations (µg a.s./L)	Mean total length (mm) ± SD	Reduction in comparison to the solvent control (%)	Mean dry weight (mg) ± SD	Reduction in comparison to the solvent control (%)
Water control	17.5 ± 1.26	-	14.3 ± 3.31	-
Solvent control	17.9 ± 1.22	-	15.2 ± 3.18	-
0.45	17.5 ± 1.78	2.2	15.4 ± 4.69	-1.3
1.36	17.6 ± 1.64	1.7	14.9 ± 4.52	2.0
4.35	18.0 ± 1.07	-0.6	15.6 ± 2.64	-2.6
13.28	17.9 ± 1.44	0	15.8 ± 3.73	-3.9
48.8	16.2 ± 2.17	9.4	9.4 ± 4.10*	38.2
48.8	16.2 ± 2.17	9.4	11.5#	24.3#

SD = Standard deviation
* Excluding replicate D as considered by the applicant
# recalculated by RMS to include results from all replicates

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 13.28 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 13.28 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 out of 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. Many of the 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 13.28 µg a.s./L and a LOEC of 48.8 µg a.s./L.

Conclusion
The test fulfilled the validity criteria of OECD 210 (2013), with the exception of the nominal test concentration 15.6 µg a.s./L.

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	> 48.8	48.8	> 13.28	> 13.28	48.8
NOEC [µg a.s./L: highest concentration without an significant effect compared to the control	≥ 48.8	13.28	≥ 13.28	≥ 13.28	12.82
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.

RMS Comments

This test was conducted to GLP, according to OECD guidelines 210 (2013) and is considered valid, despite the deviations discussed below.

OECD 210 (2013) states that test vessels should be large enough to allow proper growth in the control and a 7 L tank volume is suggested for a small fish species. It is noted that 4 L aquaria were used in this test. It is noted that there were several underdeveloped/undernourished fish during study days 6-13 in the solvent control (1 in replicate B on day 6, 1 in replicate C on day 6 and 7 and 2 on day 8). This, however, did not have an effect on larval survival of the solvent control group, as this remained at 100% throughout the test. This is the parameter that the most critical NOEC was taken from. Therefore, the RMS is satisfied that the tank size did not result in a change in the endpoint in this instance.

At day 23/22, the detected concentration of active substance was <LOQ. This was due to the flow-through system becoming detached from the test aquaria of nominal test concentration 15.6 µg a.s./L. The last time the active substance concentration had been analysed in this aquaria was day 21, which was 48 hours before. Therefore, the worst-case assumption is that the test organisms in this group would have been underexposed for 48 hours. Therefore, to estimate exposure of these test organisms, the RMS has applied half LOQ (LOQ being 0.0674 µg a.s./L) as the measured test concentration at day 23/22 and included this value in a geometric mean for the day 23/22 sampling period. This geometric mean valued has then been included in the calculation of an overall arithmetic mean value for the other sampling periods. This resulted in a mean measured concentration of 13.28 µg a.s./L for this test group.

The applicant has excluded replicate D of the highest test concentration (48.7 µg a.s./L) when calculating the mean dry weight of fish at this concentration. The RMS does not agree that this should be the case and has recalculated the value. The values with replicate D excluded has also be added to Table B.9.2.2.1-4 for information.

The applicant explained that the highest test concentration has also been excluded from statistical analyses due to the low number of fish available. The RMS does not agree with this approach and recommends that this test group is included for statistical analysis. However, the lack of statistical analyses on this group does not affect the overall **NOEC of 13.28 µg a.s./L (based on larval survival)**.

The method is acceptably validated in accordance with SANCO/3029/99 rev.4 and is suitable for the determination of isoflucypram in samples of test water at a LOQ of 0.0674 µg/L (see section B5.1.2.6.1 of the CA document for further details).

The endpoint considered valid for use in the risk assessment is NOEC: 13.28 µg a.s./L (based on larval survival)

Previous evaluation:	New data, submitted for purpose of review

Report:	KCA 8.2.2.1/02; [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.:	[redacted]
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: 2013-006492 Batch Code: BCS-CN88460-01-5 Specification No.: 10200028196 purity 94.2% w/w
Test species:	Sheepshead minnow (*Cyprinodon variegatus*) obtained from [redacted]
Organism Age at Experimental Start:	24 - 48 hour old eggs in the nerula stage Sheepshead minnow eggs were received on the morning of the experimental start. The eggs were placed in a stainless steel dish containing dilution water for microscopic observations to determine the stage of development. Eggs that appeared to be in poor health were removed and not used for the study. All healthy eggs were placed in a second stainless steel dish for impartial placement into randomly positioned egg cups.
Test procedure:	On Day 0, 5 eggs were impartially placed into each egg cup until 35 eggs were in each cup; eggs were then placed in test chambers following a randomisation procedure. Observations were made daily for mortality and hatching. When at least 90% of all viable control eggs had hatched, observations were made, and the alevin were impartially thinned to 20 per replicate on day 6 by releasing them from the egg cup to the test vessel in which the egg cup had been suspended. Inadvertently 21 alevin were released for control, replicate B; 2.92 µg a.s./L, replicate B; and 11.3 µg a.s./L, replicate D. Daily observations of abnormal behaviour, physical changes and mortality were made.
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 The stock solutions were prepared by weighing appropriate amounts of compound into volumetric flasks (corrected for percent a.s.). The flasks were brought to volume with Triethylene glycol (TEG). The stock solutions were placed on a magnetic stirrer at room temperature.

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 (21 alevin were mistakenly used in control replicate B of the 2.92 µg a.s./L test group and D of the 11.3 µg a.s./L test group) following thinning after the hatching phase.
Exposure:	Flow-through Total exposure duration: 35 days (6-day-hatch and 29 d post-hatch)
Test item delivery:	Glass 2-L modified Mount-Brungs proportional diluter systemused for intermittent delivery of test solutions to test chambers. Splitter cups were used to equally divide (±10%) test solutions between replicates at each level. Diluter was calibrated for volume delivered to each test chamber to an accuracy of (±10%).
Test Vessel Loading:	0.11 g mean wet weight based on controls. 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; feeding was conducted twice daily on weekends and one to three times daily on weekdays until 24 hours prior to test termination.
Artificial water:	Consisting of artificial sea salts mixed with the process water to produce a salinity of 20 ± 5 parts per thousand.
Test conditions:	Temperature: 24.0 to 24.6°C Light intensity: 615 to 778 lux pH: 8.1 to 8.2 Salinity range: 20 ‰ to 21‰ Dissolved oxygen (% saturation) range: 73% to 91%
Test vessels:	8.4-L glass vessels (21.6 x 12.7 x 30.5 cm)
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

The test was judged against OECD 210 (2013) and OECD 210 (2013). The results of the validity of the study are detailed in the table below.

Validity criteria	**Required by OPPTS 850.1400, 1996**	**Required by OECD 210 (2013)**	**Obtained**
Dissolved oxygen concentration throughout the test (% saturation)	60% - 100%	≥ 60%	> 73%
Water temperature difference between test chambers or between successive days at any time during the test	± 1.5°C max	± 1.5°C max	± 1.0°C max
Concentrations of test substance in solution throughout the test	± 20 % of mean measured values	± 20 % of mean measured values	Yes
Hatching success of controls	> 75%	> 75%	87.9% in water and solvent control
Post-hatch survival of controls	> 80%	> 80%	98.8% in water and solvent control

All validity criteria were met.

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

Table B.9.2.2.1-5: Concentrations of BCS-CN88460 measured in exposure solutions using HPLC during the early life-stage exposure with Sheepshead minnow (*Cyprinodon variegatus*)

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:

Behavioural 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.

Table B.9.2.2.1-5: Mean hatching success at day 6 (%) after exposure to BCS-CN88460

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.

Table B.9.2.2.1-6: Mean alevin survival at day 6 (%) after exposure to BCS-CN88460

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.

Table B.9.2.2.1-7: Mean fry survival at day 35 (%) after exposure to BCS-CN88460

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*

*Statistically significantly different from the control

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.

Table B.9.2.2.1-8: Mean growth at day 35 (%) after exposure to BCS-CN88460

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 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.

RMS comments

This study was conducted according to GLP and following US EPA OCSPP 850.1400 and OECD Guideline 210 (2013). All validity criteria were met.

The following was noted by the RMS:

It is stated that 21 alevin were transferred to one of the replicates of the water control, and test groups 2.92 and 11.3 µg a.s./L rather than the 20 in the other replicates/test groups. As a statistical comparison was made of the % survival and hatching rather than the raw numbers, and the biomass loading and dissolved oxygen concentration was still within acceptable limits at test termination this is considered acceptable.

The method is acceptably validated in accordance with SANCO/3029/99 rev.4 and is suitable for the determination of isoflucypram in samples of test water at a LOQ of 0.25 µg/L (see section B5.1.2.6.1 of the CA document for further details).

This study is valid and the endpoint confirmed for consideration in the risk assessment is as follows:

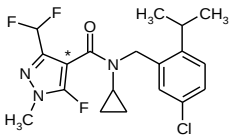
NOEC (fry survival): 25 µg a.s./L

B.9.2.2.2. Bioconcentration in fish

Previous evaluation:	New data, submitted for purpose of review

Report:	KCA 6.2.5/01; [redacted] 2017
Title:	[pyrazole-4-14C] BCS-CN88460 - Aqueous exposure bioconcentration fish test and biotransformation in fish (Lepomis macrochirus)
Report No.:	EBLNN359
Guideline(s):	EU Directive 91/414/EEC; Regulation1107/2009 (Europe); OECD Test Guideline 305; US EPA OCSPP 850.1730
GLP/GEP:	Yes

Material and methods:

Test material	Non-radiolabelled test item: BCS-CN88460 Batch code: BCS-CN88460-01-06 Specification No.: 10200028196 Purity: 94.2% w/w	Radiolabelled test item: BCS-CN88460 [Pyrazole-4-^{14}C] BCS-CN88460  * = denotes the ^{14}C-label position >99% radiochemical and chemical purity
Test species	Bluegill sunfish (*Lepomis macrochirus*) obtained from [redacted], same age, batch, same source and same population.	
Holding conditions :	Flow-through conditions. Reconstituted water was the same as used in the study 16 to 8 hour light/dark period including 30 minutes transient periods. The fish were fed daily with a commercial fish diet (e.g. Brutfutter Inicio 917 1.1 mm BioMar, Denmark) 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. The tanks were cleaned of food debris and faeces, every working day, 30 – 60 minutes after feeding. A prophylactic treatment with oxytetracyclin-hydrochloride (4g / 100L water) was performed immediately after the fish arrived in the laboratory. The fish (F 13/15) were treated from December 17 till December 19, 2015. The fish (F 3/16) were treated from August 04 till August 06, 2016.	
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	Measured one day prior to test in 10 fish: 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 The longest fish was not more than twice the length of the shortest.	
Preparation of test solutions :	The test stock solutions introduced in the study were prepared by dissolving a certain amount of inactive BCSCN88460 and radioactive [pyrazole-4-14C] BCS-CN88460 (dissolved in acetonitrile) in DMF.	
Test concentrations :	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 A dosing system consisting of a ProMinentR mikro g/5a dispenser (for dosing of stock solution) and flow-meters (for water flow control) were used for the introduction of [pyrazole-4-14C] BCS-CN88460 and test water in 2000 mL mixing cells. The mixture was running continuously into the 100 litre test aquaria. Aerated test water was transferred into the glass aquaria at an average rate of approximately 25 L / hour / aquarium during the exposure and the depuration period. This amount was sufficient to replace the approximately 100 L test volume about 6 times in a 24 hour period. The stock solutions with [pyrazole-4-14C] BCS-CN88460 in dimethylformamide were transferred at a rate of 2.5 mL /h. The control aquarium also received an amount of dimethylformamide which was equivalent to the exposure aquaria. The diluter system was calibrated before use and checked daily during the test. Four days prior test start, the dosing system was started to equilibrate the whole flow-through system.
Test Vessel Loading	Biomass loading rate: 0.133 – 0.645 g fish (wet weight) per litre of test medium per day
Test water :	Reconstituted test water according to ISO was used. It was prepared by adding salt stock solutions (analytical grade salts) to demineralized water. The test water was aerated to reach oxygen saturation.
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
Test units :	Glass aquaria with a size of 780 mm (L) x 360 mm (W) x 380 mm (H) were used as test vessels. The aquaria were filled up with water to 360 mm resulting in a water volume of 100 L.
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 was sampled from the test chambers for the determination of test substance concentration before addition of the fish and during the uptake and depuration phases. The water was sampled before feeding. If water and fish samplings were performed at the same day, both samples were taken at the same time. 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 *Radioactivity measurement :* 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. *Lipid content* 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 (see Table 8). In order to determine the lipid content the whole fish were cut into small pieces and transferred into a stainless steel tube. The fish sample were homogenised with a chloroform/methanol mixture using a tissue homogeniser (Ultra Turrax). The amounts of tissue [g], chloroform [mL] and methanol [mL] was in the ratio of 1:1:2. The suspensions were diluted with 1 mL chloroform and 1 mL water for 1 g fish tissue. The mixtures were again homogenised by Ultra Turrax and centrifuged for 20 minutes at 7000 rpm. The supernatants were decanted and after phase partition the aqueous and the organic layers were separated. The organic phases were evaporated to a constant weight. *Biotransformation part* 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.

Evaluation of results:	The bioconcentration factors based on TRR was calculated using two different methods, resulting in the steady-state BCF and the kinetic BCF. Since the concentration of the test substance may be influenced by the lipid content and/or fish growth, further BCF were calculated considering these factors. Additionally, the bioconcentration factors based on parent substance were calculated for the steady-state and the lipid normalized steady-state BCF.
Statistical analysis :	The uptake rate constant (Ku) and depuration rate constant (Kd) and the kinetic biocconcentration factor were determined by using the program Origin 8.6.0 G (64-bit) Sr3b99. This is a non-linear kinetic modelling software which provides optional parameter estimates of rate constants k1 and k2 by utilizing the actual (observed) bioconcentration study data. For the growth correction the statistical evaluation was performed using the package multcomp in the statistical programming environment R (version 3.3.0).

Test procedure:
The study consisted of two phases: the exposure (uptake) and post-exposure (depuration) phases. During the uptake phase, a group of fish (70, randomly assigned to aquaria) of the species *Lepomis macrochirus* was exposed to BCS-CN88460 at the chosen concentrations. Afterwards they were transferred to pure test water without the test substance for the depuration phase. The concentration of BCS-CN88460 in the fish was meausred in both phases of the test. In addition to the exposed treatment groups a control group of fish was held under identical conditions except for the absence of the test substance, to relate possible adverse effects observed in the bioconcentration test to a matching control group and to obtain background concentrations of the test substance. The duration of the uptake phase was 28 days, followed by the depuration phase of 14 days. The bioconcentration factor was calculated also considering the growth dilution of the test substance in growing fish. Additionally, the relationship between the lipid content and the observed bioconcentration was considered by expressing the results as normalized to a fish with a 5.13 % lipid content (based on whole body wet weight). To investigate the metabolism of BCS-CN88460 in fish and test medium, additional fish in a further aquarium were exposed to the test substance. The parent and the metabolites were analysed for different tissues of the fish (edible and viscera part) after 7 and 14 days of exposure and of test media samples from different sampling days.

Results:

Validity criteria

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**
The mortality or other adverse effects/disease in both control and treated fish. .	< 10% at test end	0%

* 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 in Table B.9.2.2.2-1.

Table B.9.2.2.2-1: Measured concentrations of BCS-CN88460 in water samples during the exposure phase

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

Biological results

Fish weightsand lipid content

The edible, non-edible (viscera) and whole fish weights measured during the exposure and depuration periods are presented in Table B.9.2.2.2-2 below.

Table B.9.2.2.2-2: Fish tissue weights during the 28-day exposure phase and 14 day depuration phase

Nominal concentration (µg/L)	Tissue	Fish weights (g)											
		Exposure phase							Depuration phase				
		Day 1	Day 3	Day 7	Day 10	Day 14	Day 21	Day 28	Day 29	Day 31	Day 35	Day 38	Day 42
Control	Edible	0.97	1.53	1.49	1.34	1.70	1.58	1.92	2.24	3.39	2.17	3.70	3.16
	Viscera	0.73	0.99	1.08	1.07	1.41	1.19	1.13	1.41	1.96	1.46	2.29	1.87
	Whole	1.70	2.51	2.56	2.41	3.11	2.77	3.04	3.72	5.34	3.62	5.99	5.03
0.500	Edible	1.18	1.95	1.02	1.71	1.84	2.12	2.10	2.59	2.92	1.76	1.91	4.64
	Viscera	0.81	1.17	0.77	1.14	1.17	1.49	1.21	1.85	1.72	1.24	1.54	2.41
	Whole	1.99	3.11	1.71	2.85	3.02	3.60	3.31	4.44	4.65	3.00	3.45	7.03
5.00	Edible	1.21	1.45	1.51	1.63	1.45	1.71	3.27	1.90	2.10	4.18	2.34	3.85
	Viscera	0.89	0.88	1.04	0.90	1.03	1.13	2.32	1.33	1.35	2.44	1.48	2.12
	Whole	2.10	2.33	2.55	2.54	2.48	2.84	5.59	3.23	3.45	6.60	3.82	5.97

No statistically significant difference between the growth rates in the depuration and uptake phase at different concentrations for both weight and length was observed. Additionally, there were no statistically significant differences found between the different treatment groups and the control.

During the exposure phase, lipid content based on wet weight ranged from 4.70 to 5.54% for the solvent control and 4.31 to 5.34% for the 0.500 µg/L treatment group and 5.50 to 5.58 for the 5.00 µg/L treatment group. After depuration, the lipid content based on wet weight was 6.08% for the solvent control, 7.11 for the 0.500 µg/L

treatment group and 7.03% for the 5.00 µg/L treatment group.

Mean total residues

Mean total residues expressed as mg/kg of [^{14}C]-BCS-CN88460 equivalents in edible tissue, whole fish or viscera parts in the low and high treatment in are given in the following table (Table B.9.2.2.2-3).

Table B.9.2.2.2-3: Mean total residues of [^{14}C]-BCS-CN88460 in edible part, viscera part and whole fish

Study day	**Nom. concentration: 0.5 µg/L (Aquarium B)**			**Nom. concentration: 5.0 µg/L (Aquarium C)**		
	Total residues mg/kg of [^{14}C]-BCS-CN88460 equivalents					
	Edible part	**Whole fish**	**Viscera part**	**Edible part**	**Whole fish**	**Viscera part**
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

Bioconcentration factors (BCF) were determined during the uptake and depuration period by dividing the [^{14}C]-tissue radioactivity by the mean [^{14}C]-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 (Table B.9.2.2.2-4).

Table B.9.2.2.2-4: Bioconcentration factors (BCF) based on TRR

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	354 ± 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 ± 75.0	73.4 ± 30.1	256 ± 160	148 ± 82.1
31	12.0 ± 0.88	27.2 ± 9.56	17.6 ± 2.96	13.5 ± 4.05	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 ± 1.43	8.51 ± 5.73	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 (Table B.9.2.2.2-5 and Table Table B.9.2.2.2-6).

Table B.9.2.2.2-5: Steady-state bioconcentration factors based on the parent substance at 0.500 µg/L

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	24.7	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

Table B.9.2.2.2-6: Steady-state bioconcentration factors based on the parent substance at 5.0 µg/L

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

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 the exposure period of day 0 to 28 considering all groups including the control. 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 (Table B.9.2.2.2-7).

Table B.9.2.2.2-7: Summary of bioconcentration factors during the 28 day exposure period and 14 day depuration phase

	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**
Lipid content (wet weight %)	n.a.	n.a.	5.13	n.a.	n.a.	5.13
Lipid normalisation default value (%)	n.a.	n.a.	5.0	n.a.	n.a.	5.0
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	0.932 ± 0.136	1.15 ± 0.0722	1.05 ± 0.0885	0.785 ± 0.0715	1.04 ± 0.0445	0.970 ± 0.0523

	0.500 µg [pyrazole-4-^{14}C] BCS-CN88460/L			5.00 µg [pyrazole-4-^{14}C] BCS-CN88460/L		
constant [day^{-1}]						
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
$t_{1/2g}$ Growth-corrected half-life [day]	0.758	0.613	0.673	0.910	0.680	0.732
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 % across a 28 day exposure period)
**growth rate constants were only calculated for the whole fish but also used for the calculations of edible and viscera tissue

Conclusion

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.

RMS Comments

This study was conducted according to OECD 305 (2012) and OCSPP Draft Guideline 850.1730. The study met the relevant validity criteria.

According to OECD 305 (2012) and the study protocol, the measurement of the total organic carbon (TOC) should 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. As the total organic carbon measured was <2.0 mg/L in the dilution water on both occasions, this is considered acceptable.

OECD 305 states that the natural particle content should not be greater than 5 mg/L. The natural particle content is not reported in the study report, however, the TOC is within the acceptable range according to OECD, therefore this deviation is not considered to have affected the endpoints of this study.

It is noted that pH deviated by >0.5 units during the test, however as all validity criteria were met, this is not considered to invalidate the test.

According to OECD 305 (2012), the variation in depuration and uptake rate constants between concentrations should be <20% to demonstrate that concentration dependence is not evident. The depuration rate constant varies by <20% between concentrations for all tissues; however the uptake rate constant varies by 23% in viscera tissue but not in other tissues measured. Following a request for further information at evaluation, the applicant confirmed that the overall uptake rate constants were <20% and stated that this limit does not apply to viscera. It is not clear to the RMS that this criterion does not apply to viscera, however given that the overall depuration and uptake rate constants were 8 – 11%, the RMS considers this deviation acceptable and does not consider the BCF

value to be concentration-dependent. Furthermore the observed lipid normalized growth corrected BCF values were 370 and 361 at the two different test concentrations of 0.5 and 5.0 µg/L (nominal concentrations) based on total radioactive residues. The respective values based on the parent substance only were presented as 35.7 and 43.6 l/kg. The difference between these two values is ~2.5%. This small difference supports the concentration independency of the BCF value for BCS CN88460.

It is noted that the age of the fish was not reported. OECD 305 states that age of a fish may have a significant effect on BCF values, so all fish should be from the same year-class. According to the study report fish of the same age, batch, same source and same population were used, and therefore effects on BCF values should have been minimised. However as it is not clear whether fish were sexually mature adults, it cannot be confirmed whether the fish were in a spawning state or had recently spawned before or during the test, which may have affected the test results. Following a request for further information during evaluation, the applicant confirmed that the fish used in the study were juveniles. As such, it can be concluded that fish had not recently spawned.

The sex of the fish was not reported; according to OECD 305 this should be stated and if both sexes are used differences in growth and lipid content should be documented to be non-significant before exposure initiation. Following a request for further information at evalution, the applicant stated that the sex of the fish had not been determined as the fish used were juveniles. As fish were not sexually mature and were of a similar length and weight, the RMS considers this acceptable.

According to OECD 305, if lipid analysis was not conducted on all sampled fish, a mean lipid value is used to normalise the BCF. For the steady-state BCF, **t**he mean value recorded at the end of the uptake phase in the treatment group should be used to normalise the BCF. In this study, the mean value of all groups including the control (5.13%) from day 0 to day 28 was used. However, a value of 5.69% should have been used based on the mean of the treatment values at day 28. As using a mean value of 5.13% is more conservative the RMS considers this acceptable.

Lipid content was determined in fish that were not the same as those used to determine the concentration of the test substance. OECD 305 states that where this is the case, the fish should at least be of a similar weight and the same sex. However, the study report does not state the sex of the fish so it is unclear whether the lipid analysis results are representative of the fish population in the tanks. Starting weights for the fish sampled for lipid analysis ranged from 1.26 – 4.27g whereas for the fish used to measure the concentration of the test substance ranges were 0.66 – 1.71g so there was some variability between fish at the start of the test. This adds to uncertainty in the lipid analysis result being representative of those fish sampled to measure active substance concentrations and ascertain the bioconcentration factor. However it is not considered to invalidate the test.

This study is considered valid and the following endpoints are considered acceptable for use in the risk assessment:
Lipid normalized steady-state bioconcentration factor (BCF_{SSL}) = 383 L/kg
Lipid normalized and growth corrected kinetic bioconcentration factor (BCF_{KLG}) = 370 L/kg.

B.9.2.3. Potential for endocrine disruption

On the basis of submitted and available information there is no evidence to support that isoflucypram meets the scientific criteria for endocrine disruption (ED) determination as outlined under Commission Regulation (EU) No. 2018/605. However, with regards to terrestrial non-target organisms it is the conclusion of the RMS that insufficient investigation into such properties have been made, according to the supporting guidance document. The applicant acknowledges this and has proposed a further step-wise data generation and assessment in order to fully support a modern assessment for ED. It is proposed by the RMS that the totality of information be considered once available in order to robustly conclude on the ED potential of isoflucypram to non-target organisms as a whole.

B.9.2.4. Acute toxicity to aquatic invertebrates

Active substance

Previous evaluation:	None; new active substance application.

Report:	KCA 8.2.4.1/01; Kuhl, K.; 2016;
Title:	Acute toxicity of BCS-CN88460 (tech.) to the waterflea Daphnia magna in a static laboratory test system - Final Report -
Report No.:	EBLNN033
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*) Preparation of test organisms: Breeding stock were maintained in a cimate-controlled environment under study conditions and were fed three times per week with living cells of *Desmodesmus subspicatus* in aqueous suspension. There were no males, ephippia or dead animals present in the cultures within 48 hours before the start of the exposure. There was also no disease treatment in the used breeding batch.
Organism age/size at study initiation	First instar neonates, less than 24 hours old, third or later brood of coeval parent daphnids.
Test solutions	Nominal concentrations: 50.0, 100, 200, 400 and 800 µg a.s./L Corresponding geometric mean concentrations: 59.0, 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. Preparation of test solution: the primary stock solution was made by mixing 212.4 mg of technical substance, replenished with Dimethylformamide (DMF) up to 25 mL with artificial test water (Elendt M7). This was stirred with a magnetic stirrer for 26 minutes. Subsequently, a dilution series of secondary DMF stock solutions were prepared using the primary stock solution and DMF. The exposure concentrations were prepared as sub-dilutions of the corresponding secondary stock solution and were stirred for 88 minutes with a magnetic stirrer.
Replication	No. of test concentrations: 5 (+ water and solvent controls) 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: 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	Macroscopic visual counting of mobile daphnids. 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. Verification of sensitivity was performed using acute non-GLP reference testing of $K_2Cr_2O_7$, p.a. grade (test concentrations: 0.56, 0.75, 1.00, 1.33 and 1.78 mg/L). The current 24h EC_{50} of 0.76 mg/L met the range of 0.79 – 0.93 mg/L, as defined by OECD inter-laboratory ringtesting (published with OECD Guideline 202 (2004).
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 (from batch preparation in each treatment and control group) and termination (as composite from all replicates of a treatment group and control group).
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

Validation criteria
The validity criteria were judged against OECD test guidelines 202 (2004)

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

All of these criteria were met.

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 (see Table B.9.2.4-1). 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 the untreated water control.

Table B.9.2.4-1: Measured concentrations of BCS-CN88460 in the exposure solutions

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:
Table Table B.9.2.4-2 shows the effect of the test item on the mobility and morbidity of *D.magna* after 24 and 48 hour exposure to the technical substance.

Table B.9.2.4-2: Effects of Isoflucypram technical on *D.magna*

Exposure time (hours)	Exposed daphnids (=100%)	24	48
Geometric mean measured concentration (µg a.s./L)		No of immobilised daphnids (%)	No of immobilised daphnids (%)
control	30	0 (0)	0 (0)
solvent control	30	0 (0)	0 (0)
59.0	30	0 (0)	0 (0)
116	30	1 (3.3)	2 (6.7)
226	30	4 (13.3)[A]	18 (60.0)[C]
433	30	24 (80.0)[B]	30 (100)
853	30	30 (100)	30 (100)

[A] 5 daphnids showed a clear decrease in frequency of antennae movements (not considered in the % of immobilized daphnids)
[B] 1 daphnid showed a clear decrease in frequency of antennae movements (not considered in the % of immobilized daphnids)
[C] 3 daphnids showed a clear decrease in frequency of antennae movements (not considered in the % of immobilized daphnids)

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)**

RMS Comments

This test was conducted to OECD 202 (2004) and met all relevant validity criteria.

The temperature in the test vessels varied by more than the recommended 1.5 degree maximum specified in OECD 202 (2004) (19.4 – 23.2°C). However, this occurred to the same extent in the control vessel, which was observed to have no mortality and met the validity criteria. Therefore, this deviation is not thought to have had any effect on the test results.

The method is acceptably validated in accordance with SANCO/3029/99 rev.4 and is suitable for the determination of isoflucypram in samples of test water at a LOQ of 0.0625 µg/L (see section B5.1.2.6.1 of the CA document for further details).

The endpoint considered suitable for risk assessment is **48 h EC_{50} (95% CI): 201 µg a.s. / L (176 – 229 µg a.s. /L)**

Metabolite (M12)

Previous evaluation:	None; new active substance application.

Report:	KCA 8.2.4.1/02; Riebschlaeger, T; 2016;
Title:	Acute toxicity of BCS-CN88460-carboxylic-acid (BCS-CY26497) to the waterflea Daphnia magna in a static laboratory test system
Report No.:	EBLNN198
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: SES 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*) Preparation of test organisms: Breeding stock were maintained in a cimate-controlled environment under study conditions and were fed three times per week with living cells of *Desmodesmus subspicatus* in aqueous suspension. There were no males, ephippia or dead animals present in the cultures within 48 hours before the start of the exposure. There was also no disease treatment in the used breeding batch.
Organism age/size at study initiation	First instar neonates, less than 24 hours old, third or later brood of coeval parent daphnids.
Test solutions	Nominal concentrations: 1.5, 3, 6, 12 and 24 mg p.m./L Control: water and solvent control (100 µL dimethylformamide/L) Preparation of test solution: the primary stock solution was made by mixing 2429.2 mg of metabolite M12, replenished with Dimethylformamide (DMF) up to 10 mL. This was stirred with a magnetic stirrer for 10 minutes. Subsequently, a dilution series of secondary DMF stock solutions were prepared using the primary stock solution and DMF. The exposure concentrations were prepared as sub-dilutions of the corresponding secondary stock solution and were stirred for 119 minutes with a magnetic stirrer until the solution was clear and there was no undissolved material visible.
Replication	No. of test concentrations: 5 (+ water and solvent controls) 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	Macroscopic 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 sample) 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. Verification of sensitivity was performed using acute non-GLP reference testing of $K_2Cr_2O_7$, p.a. grade (test concentrations: 0.56, 0.75, 1.00, 1.33 and 1.78 mg/L). The current 24h EC_{50} of 0.76 mg/L met the range of 0.79 – 0.93 mg/L, as defined by OECD inter-laboratory ringtesting (published with OECD Guideline 202 (2004).
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

Validation criteria

The validity criteria were judged against OECD test guidelines 202 (2004)

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

All of these criteria were met

Analytical results:

When the test item was measured on day 0 and day 2, the recoveries were between 108 and 118% of the nominal concentrations (see Table B.9.2.4-3). 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 the untreated water control.

Table B.9.2.4-3: Measured concentrations of BCS-CN88460-carboxylic-acid (BCS-CY26497) in the exposure solutions

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
1.5	1.77	118	1.76	117
3.0	3.42	114	3.47	116
6.0	6.82	114	6.92	115
12.0	13.2	110	13.4	112
24.0	25.9	108	27.2	113

Biological results:

Table B.9.2.4-4 shows the effect of the test item on the mobility of *D.magna* after 24 and 48 hour exposure to the technical substance.

Table B.9.2.4-4: Effects of BCS-CN88460-carboxylic-acid on *D.magna*

Exposure time (hours)	Exposed daphnids (=100%)	24	48
Nominal test concentration (mg p.m./L)		No of immobilized daphnids (%)	No of immobilized daphnids (%)
Control	30	0 (0)	0 (0)
Solvent control	30	0 (0)	0 (0)
1.5	30	0 (0)	0 (0)
3.0	30	0 (0)	0 (0)
6.0	30	0 (0)	1 (3.3)
12.0	30	0 (0)	2 (6.7)
24.0	30	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 0and the EC_{50} (24 and 48 hours) for BCS-CN88460-carboxylic-acid (BCS-CY26497) is:

EC_{50} 24 hours (95% C.I.):	**> 24.0 mg p.m / L**
EC_{50} 48 hours (95% C.I.):	**> 24.0 mg p.m / L**

RMS Comments

This test was conducted to OECD 202 (2004) and met all relevant validity criteria.

The temperature in the control test vessel varied by more than the recommended 1.5 degree maximum specified in OECD 202 (2004) (20.6 – 22.4 °C). However, the control vessel was observed to have no mortality and met the validity criteria. Therefore, this deviation is not thought to have had any effect on the test results.

The method is acceptably validated in accordance with SANCO/3029/99 rev.4 and is suitable for the determination of BCS-CN88460-carboxylic acid in samples of test water at a LOQ of 0.125 mg/L (see section B5.1.2.6.1 of the A document for further details).

The endpoint considered suitable for risk assessment is **48 h EC_{50} (95% CI): > 24.0 mg p.m. / L**

Previous evaluation:	New data, submitted for purpose of review

Report:	KCA 8.2.4.2/01; Brougher, D. S.; Siddiqui, A. I.; Gallagher, S. P.; 2016;
Title:	BCS-CN88460: A 96-hour static-renewal acute toxicity test with the saltwater mysid (Americamysis bahia)
Report No.:	149A-257B
Guideline(s):	US EPA OCSPP 850.1035
GLP/GEP:	Yes

Material and methods

Test material	BCS-CN88460 Batch Code: 2013-006492. Purity 94.2% w/w
Test species	Saltwater mysid (*Americamysis bahia*) from cultures maintained by Wildlife International, Easton, Maryland.
Organism age/size at study initiation	Juvenile mysids, less than 24 hours old
Holding conditions:	Adult mysids were held in water from the same source and at approximately the same temperature as used during the test. No signs of disease or stress were observed. Mysids in the cultures were fed live brine shrimp nauplii (*Artemia sp.*) daily, supplied by Brine Shrimp Direct, Ogden, Utah. The brine shrimp were periodically enriched with a nutrient enrichment (A1 DHA Selco from INVE Aquaculture, Phichit, Thailand) and *Skeletonemna costatum*.
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 Solvent control: 0.1 ml/L dimethylformamide
Preparation of test concentrations:	A primary stock solution was prepared by mixing a calculated amount of test substance into HPLC-grade dimethylformamide (DMF) at a nominal concentration of 9.0 mg a.s/mL, which was sonicated and stirred on a stir plate until no precipitate was visible. Four secondary stock solutions were prepared in DMF at nominal concentrations of 0.56, 1.1, 2.3 and 4.5 mg a.s./mL by proportional dilution of the primary stock. Aliquots (0.3 mL) of the five stock solutions were mixed with 3 L of dilution water to prepare the test solutions in each of two replicate test chambers at nominal concentrations of 0.056, 0.11, 0.23, 0.45 and 0.90 mg a.s./L. Each solution was stirred on a stir plate for approximately 10 minutes.
Dilution water:	Natural seawater from Indian River Inlet, Delaware. The salinity was adjusted with well water to 20‰ and aerated with spray nozzles. Prior to use in the test water was filtered to 0.45µm and passed through a UV steriliser.
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	Live brine shrimp nauplii (*Artemia sp*) once to twice daily. Mysids in the cultures were fed enriched braine shrimp for one of the daily feedings during the test.
Test conditions	Temperature: 23.6 – 25.4 °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; 6.7 – 7.4 mg/L) Salinity: 20 ‰
Test vessels:	2 L glass beakers filled with 1.5 L of seawater randomly placed in a temperature controlled environmental chamber.
Observations:	Observations of mortality were made approximately 3.5, 24, 48, 72 and 96 hours after test initiation. The numbers of individuals exhibiting signs of toxicity or abnormal behavior also were evaluated.

	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
Analytical sampling:	Newly prepared batch solutions were sampled on Day 0 and at 48 hours (±1 hour), and old solutions in the test chambers were sampled at 48 and 96 hours (±1 hour). Samples were collected from mid-depth, placed in glass vials containing 2.0 mL of methanol, and processed immediately for analysis. Sample collection from the 0.90 mg a.s./L treatment group was discontinued after 48 hours due to 100% mortality. Sampled were analysed with HPLC-UV. LOQ was 0.0313 mg a.s./L.
Statistical analyses:	The mortality data were analysed using the computer program of C. E. Stephan.. Based on the mortality pattern in this study, probit analysis was used to calculate the 48 and 72-hour LC50 values. Nonlinear interpolation was used to calculate the 96-hour LC50 value and binominal probability was used to calculate the 95% confidence interval. Due to the method used to calculate the 96-hour LC50 value, the slope of the concentration-response curve could not be calculated. The no-mortality concentration and the no-observed-effect concentration (NOEC) were determined by visual interpretation of the mortality and observation data.

Test procedure

At test initiation, the juvenile mysids (<24 hours old) were collected from the cultures and indiscriminately transferred one and two at a time into transfer chambers until each chamber contained 10 mysids. Each group of mysids then was transferred to an indiscriminately assigned test chamber to initiate the test. All transfers were performed beneath the water surface using wide-bore pipettes.

Mysids were exposed to the test item, control or solvent control for 96 hours; test item solutions were renewed after 48 hours. Obsevations of mortality and abnormal behaviour were made 3.5, 24, 48, 72 and 96 hours after test initiation.

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%

All validity criteria were met.

Analytical results:

Recoveries were between 86.9 and 107% (see Table B.9.2.4-5 below). Biological results are based on arithmetic mean measured concentrations. No residues of BCS-CN88460 above the LOQ were measured in the controls.

Table B.9.2.4-5: Analytical verification of the test item

Nominal Concentration (mg a.s./L)	**Measured concentrations (mg a.s./L)**				**Arithmetic mean measured concentration (mg a.s./L)**	**% of nominal concentrations**	**Range of individual measurements (% of nominal)**
	0hr (new)	**48 h (old)***	**48h (new)**	**96h (old)***			
0.056	0.0581	0.0564	0.0547	0.0597	0.057	102	97.6 - 107
0.11	0.110	0.107	0.110	0.116	0.11	100	96.2 - 105
0.23	0.229	0.217	0.232	0.246	0.23	100	94.2 - 107
0.45	0.416	0.397	0.430	0.445	0.42	93.3	87.9 – 99.3
0.90	0.835	0.787	0.856	**	0.82	91.1	86.9 – 95.2

*Mean of 2 samples calculated by the RMS
**Not measured as all mysids dead

Biological results:

Mortality and behaviour
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. Observations of mortality and abnormal behaviour are presented in the below table (Table B.9.2.4-6):

Table B.9.2.4-6: Cumulative mortality and observations

Exposure time (hours)	**3.5**		**24**		**48**		**72**		**96**	
Nominal conc. (mg a.s./L)	**No. dead**	**Observ.**	**No. dead**	**Observ.**	**No. dead**	**Observ.**	**No. dead**	**Observ.**	**No. dead**	**Observ.**
Control	0	20N	0	20N	0	20N	0	20N	1	20N
Solvent control	0	20N	0	20N	0	20N	0	20N	0	20N
0.057	0	20N	0	20N	0	20N	0	20N	0	20N
0.11	0	20N	0	20N	0	20N	0	20N	0	20N
0.23	0	20N	2	20N	2	20N	3	17N	5	15N
0.42	0	20N	0	20N	15	2C 3N	17	3C	20	-
0.82	0	20N	8	5C 7N	20	-	20	-	20	-

N = Normal behaviour
C = Lethargic

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

RMS comments

This study was conducted according to GLP and following guideline 850.1035 (2016).

It is not clear if any mortality was recorded during the holding period; according to the test guidelines this should be recorded and if >5% mortality or signs of stress are observed mysids should not be used for a test. However, as both controls met the validity criteria and demonstrated ≤5% mortality this is not considered to invalidate the test.

The arithmetic mean measured concentrations were calculated and used for derivation of an LC50 value; ideally geometric mean measured concentrations should have been used for each renewal period and then an overall arithmetic mean. A request for additional information was made during evaluation for this information, however the applicant stated that as the geometric mean values were very similar to the arithmetic mean values, the impact on the derived endpoint would be minimal. The RMS agrees that this is likely to be the case, however ideally endpoints based on geometric mean measured values would have been provided

Due to the method of calculating the LC50 at 96 hours, no slope of the concentration-response curve could be calculated; this adds some uncertainty to the derived value however the confidence intervals are sufficiently narrow and value of 0.23 mg a.s./L appears to be sufficiently conservative given the 25% mortality observed at 0.23 mg a.s./L and 100% mortality observed at 0.42 mg a.s./L.

The method is acceptably validated in accordance with SANCO/3029/99 rev.4 and is suitable for the determination of isoflucypram in samples of test (salt) water at a LOQ of 0.05 mg/L (see section B5.1.2.6.1 of the CA document for further details).

This study is considered valid and acceptable for use in the risk assessment.
The endpoints are confirmed as:

LC50 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

Previous evaluation:	New data, submitted for purpose of review

Report:	KCA 8.2.5.2/03; Brougher, D. S.; Siddiqui, A. I.; Gallagher, S. P.; 2016
Title:	BCS-CN88460: A 96-hour shell deposition test with the eastern oyster (Crassostrea virginica)
Report No.:	149A-258
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 %
Test species	Eastern oyster (*Crassostrea virginica*) from Marinetics, Inc. of Cambridge, Maryland
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. Oysters were fed marine microalgae at a rate of 2.9×10^9 cells/oyster/day during holding.

Organism age/size at study initiation	Initial valve height: 35.8 ± 2.4 mm
Dilution water:	Filtered, ozonated natural seawater obtained at Indian River Inlet, Delaware Salinity diluted to 20‰ with well water. Prior to use in the test water was filtered to 0.45µm and sterilised using UV.
Preparation of test concentrations:	Individual stock solutions were prepared once for each of the five concentrations tested. All test solution concentrations were adjusted to 100% active ingredient during preparation, based on the reported test substance purity (94.2%). 1-L primary stock solution was prepared by mixing a calculated amount of test substance into HPLC-grade dimethylformamide (DMF) at a nominal concentration of 9.0 mg a.s./mL. This solution was sonicated for 30 minutes and appeared clear with no precipitates. Four secondary stock soultions were then prepared in DMF by serial dilution and mixed by inversion. The concentration of DMF in the solvent control and all treatment groups was 0.1 mL/L.
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 DMF
Test solution delivery:	The toxicity test was conducted using a continuous-flow diluter system to provide each concentration of the test substance, a negative control (dilution water only) and a solvent control (0.1 mL/L DMF) to test chambers. Syringe pumps were used to deliver test substance stock solutions to mixing chambers assigned to each treatment group; DMF was delivered to a separate mixing chamber assigned to the solvent control.Test solutions/solvent were mixed with salwater in the mixing chambers prior to delivery to deliver the appropriate nominal concentration. The stock solutions were pumped into the diluter mixing chambers assigned to the treatment groups at a target rate of 35.0 µL/minute and were mixed with dilution water and algal feed suspension in the mixing chambers, delivered at a combined target rate of 350 mL/minute to achieve the required nominal test concentrations. During the test, each test chamber received approximately 9 volume additions of solution every 24 hours. The pumps used to deliver stock solutions or solvent to the mixing chambers, and the rotameters used to control the flow of dilution water to the mixing chambers, were calibrated prior to the test. Delivery of test water to the test chambers was initiated five days prior to the introduction of the test organisms to the test water in order to establish equilibrium concentrations of the test substance.
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 5.8×10^8 cells/oyster/day delivered via peristaltic pumps calibrated prior to and during the test.
Test conditions	Temperature: 18.8 – 19.1ºC 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 (gaping of the shell, excessive mucus production, spawning or abnormal feeding activity) 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 test initiation, and at 48 and 96 hours after test initiation. 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

Test design

Eastern oysters were exposed to a geometric series of five test concentrations, a negative control (dilution water) and a solvent control (0.1 mL/L dimethylformamide) for 96 hours under flow-through conditions.

Delivery of the test substance to the test chambers was initiated five days prior to the introduction of the test organisms to the test water in order to achieve equilibrium of the test substance in the test chambers. Just prior to initiation of the test, recently deposited shell was removed from the oysters. The initial valve height (after grinding) of twenty indiscriminately selected oysters was determined by measuring the longest distance from the umbo to the distal valve edge. Healthy oysters of approximately the same size were impartially assigned to exposure chambers at test initiation. The oysters were oriented with the flat valves facing up (i.e., the left or cupped valve down) and umbos (the hinged narrow end or apex of the shell) facing away from the flow of water.

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, and were used to determine the EC50 value and the no-observed-effect concentration (NOEC). The EC50 is the concentration of test substance in water that is calculated to induce a 50% reduction in shell deposition, relative to the control.

Results:

Validity criteria

Validity criteria according to 850.1025	Required	Obtained
Mortality of the oysters in the control(s)	≤ 10 %	0 % in water and solvent controls
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

All validity criteria were met

Analytical results:

Measured concentrations of the samples ranged from approximately 76 to 127% of nominal (see Table B.9.2.4-7 below). 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).

Table B.9.2.4-7: Measured concentrations of BCS-CN88460 in test solution samples

Nominal Concentration (mg a.s./L)	Measured concentration (mg a.s./L)			Arithmetic mean measured concentration (mg a.s./L)	% of nominal concentrations		
	0hr	48hr	96hr		0hr	48hr	96hr
0.056	0.0501	0.0501	0.0477	0.049	89.4	89.5	85.2
0.11	0.0841	0.0959	0.0925	0.091	76.4	87.2	84.1
0.23	0.220	0.233	0.202	0.22	95.6	101	87.7
0.45	0.357	0.380	0.368	0.37	79.2	84.4	81.8
0.90	1.14	0.735	0.757	0.88	127	81.7	84.1

Biological results:

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.

Table B.9.2.4-8: Cumulative mortality of Oysters exposed to BCS-CN88460

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

Table B.9.2.4-9: Mean shell Deposition and shell growth at 96 hours

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

Endpoints of the study are based on arithmetic mean measured concentrations and are:

EC_{50} 96 hours	**0.170 mg a.s. / L (95% confidence intervals : 0.13 – 0.21 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

RMS comments

This study was conducted according to GLP and following 850.1025 guidelines.

All validity criteria were met.

It is noted that although parameters other than lethality were measured in this test, the guideline followed means that this study should be considered an acute toxicity test.

Arithmetic measured concentrations were calculated; geometric mean measured concentrations should have been calculated. A request for additional information was made during evaluation for this information, however the applicant stated that as the geometric mean values were very similar to the arithmetic mean values, the impact on the derived endpoint would be minimal. The RMS agrees that this is likely to be the case, however ideally endpoints based on geometric mean measured values would have been provided.

The method is acceptably validated in accordance with SANCO/3029/99 rev.4 and is suitable for the determination of isoflucypram in samples of test (salt) water at a LOQ of 0.05 mg/L (see section B5.1.2.6.1 of the CA document for further details).

This study is considered valid and the endpoints are confirmed as:

EC_{50} (96 hours): 0.170 mg a.s. / L (95% confidence intervals : 0.13 – 0.21 mg a.s./L)

NOEC: 0.049 mg a.s. / L

B.9.2.5. Long-term and chronic toxicity to aquatic invertebrates

Previous evaluation:	New data, submitted for purpose of review

Report:	KCA 8.2.5.1/01; Bruns, E.; 2017
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.:	EBLNN031
Guideline(s):	EU Directive 91/414/EEC Regulation1107/2009 (Europe) US EPA OCSPP 850.1300
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
Test species:	Water flea (*Daphnia magna)* Genotype 2. Source: BayerCropScience laboratory breeding stock (in-house cultures)

Organism Age at Experimental Start:	1st instar neonates less than 24 h old ; third or later brood of coeval parent daphnids (20 – 28 days ± 12 hours old). Selected by sieving of a breeding culture of defined age <24h before test initiation.
Breeding conditions	Daphnids were subcultured in glass beakers containing 2000mL of artificial water (Elendt M7, renewed weekly) with 50 – 60 coeval daphnids per container kept under test conditions.
Test solution preparation	A stock solution of the test item was prepared using dimethylformamide (DMF) as a solvent and mixing with a magnetic stirrer. Serial dilutions of the stock solution were then made to achieve the required concentration when 200µL of the solution was added to 2 litres of test water immediately before the start of each exposure interval.
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 DMF/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 (renewal on days 2, 5, 7, 9, 12, 14, 16 and 19) 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*(daily amount 1 x 10^8 cells/L)
Test medium	Artificial water – Elendt M7. Test water was allowed to equilibrate for at least 48 hours under study conditions whilst being artificially aerated.Vitamin components were added immediately before use in a study. Test medium was analysed for contamination twice per year.
Test vessels	250 mL glass beakers covered with transparent glass plates during exposure
Test conditions:	Temperature: 19.7 – 21.7 °C Photoperiod: 16:8 hours light:dark Light intensity: 1000 -1200 lux pH: 7.4 – 8.0 Water hardness: 12 to 14 (°dH, German degrees) Dissolved oxygen: 4.3 - 9.2 mg/L (49.78 - 101% of air saturation) 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, 7, 9, 12, 14, 16 and 19. 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. Sublethal effects including visual comparison of control and treated animals, existence of aborted eggs anf neonate mortality were also recorded.
Sampling for chemical analysis	For verification of the actual test item concentrations during exposure, duplicate water samples were taken as follows: Freshly prepared test media: Sampling on study days 0, 9 and day 19, immediately before distribution to the test vessels, from batch preparation for each treatment and control group. Aged test media: Aged test media: Sampling on study days 2, 12 and 21, immediately after termination of exposure as composite from all replicates of a treatment group and control group 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). Statistically significant differences between the controls were detected using a 2-sided Student t-Test 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. Homogeneity of variance and normality of the data were tested ,and based on the findings either parametric (ANOVA) or non-parametric (Mann-Whitney-Wilcoxon U-test) tests were employed to detect statistically significant differences between control and treated groups. ToxRat-Professional (Version 3.2.1) was used for statistical analyses.

Test procedure

In this 21-day static renewal daphnid-lifecycle study, test vessels containing 100mL of test solution were randomly assigned to consecutively numbered positions in transparent plastic trays. Individual daphnids were randomly assigned to treatment groups and control groups within 30 minutes after the test solutions were added and uniformly distributed to the test vessels.

The test medium was renewed on days 2, 5, 7, 9, 12, 14, 16 and 19; parent animals were transferred to fresh test solution inside a minimal volume of aged test solution.

Observations were made of parental survival, time of brood release, number of offpring per adult per day, parental body length and dry body weight at test termination and sublethal effects including aborted eggs and neonate mortality.

Results

Validity criteria

Validity criteria according to OECD 202/OCSPP 850-1300	Required	Obtained
Mortality of the parent animals in control at the end of the test	≤ 20%	Untreated control: 0% Solvent control: 0%
Mean number of living offspring produced per parent animal surviving in control at the end of the test	> 60	Untreated control: 85.5 Solvent control: 96.5

All validity criteria were met

Analytical results:

No residues of BCS-CN88460 above the LOQ were measured in the untreated and solvent controls. Analytical results are presented in Table B.9.2.5-1 below.

Table B.9.2.5-1: Measured concentrations of BCS-CN88460 in test solution samples

Nominal concentrations (µg a.s./L)	**Mean measured concentrations (µg a.s./L)***						**Mean measured concentrations over the study period (µg a.s./L)**	**% of nominal concentrations**
	Day 0 New	**Day 2 Aged**	**Day 9 New**	**Day 12 Aged**	**Day 19 New**	**Day 21 Aged**		
4.5	5.56 (126)	5.59 (124)	5.31 (118)	5.09 (113)	5.26 (117)	5.20 (116)	5.33	118
9.0	11.4 (126)	11.2 (124)	10.5 (117)	10.0 (112)	10.6 (118)	10.7 (119)	10.7	119
18.0	21.4 (119)	21.5 (120)	20.5 (114)	18.9 (105)	20.4 (113)	20.1 (112)	20.3	113
36.0	43.2 (120)	42.9 (119)	40.5 (112)	37.5 (104)	40.4 (112)	39.4 (110)	40.4	112
72.0	86.5 (120)	86.0 (119)	79.7 (111)	73.4 (102)	80.9 (112)	77.4 (108)	80.1	111
144	172 (119)	169 (118)	163 (113)	153 (106)	160 (111)	160 (111)	162	112

*% of nominal in parentheses

Results are based on nominal test concentrations.

Biological results:

Observations

The statistical comparison of untreated and solvent control demonstrated a statistically significant difference for final body length only. For all other observed endpoints no statistically significant difference was observed. Therefore the results for the endpoint final body length were compared against the solvent control. All other threshold concentrations and ECx calculations were obtained by statistical evaluations performed in comparison to the pooled controls.

Length and body weight measurements at test termination in comparison to the control are presented in the below table (Table B.9.2.5-2)

Table B.9.2.5-2: Length and body weight at test termination in comparison to the control

Nominal 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 ± 0.11	-
Solvent control	4.07 ± 0.2	-	0.803 ± 0.12	-
Pooled control	3.98 ± 0.2	-	0.828 ± 0.12	-
4.5	4.06 ± 0.1	0.25	0.874 ± 0.15	-5.56
9.0	3.94 ± 0.2	3.19	0.935 ± 0.25	-12.92
18.0	3.91 ± 0.2	3.93	0.817 ± 0.17	1.33
36.0	3.95 ± 0.1	2.95	0.844 ± 0.25	-1.93
72.0	4.01 ± 0.1	1.47	0.709 ± 0.14	14.37
144	3.72 ± 0.1	8.60*	0.693 ± 0.16	16.30*

* Statistical significant difference from solvent control (verified by Williams Multiple Sequential t-test Procedure on a 5% level of significance at one-sided smaller or by Step-down Jonckheere-Terpstra test Procedure on a 5% level of significance one-sided smaller)

Negative results denote an increase in comparison to the control

The effect of the test item on the immobility of dapnhids is reported in Table B.9.2.5-3 below.

Table B.9.2.5-3: Effect of isoflucypram on immobility of *Daphnia magna*

Nominal concentrations (µg a.s./L)	No of immobilised daphnids (%)
Control	0 (0)
Solvent control	0 (0)
Pooled control	0 (0)
4.5	0 (0)
9.0	0 (0)
18.0	0 (0)
36.0	0 (0)
72.0	0 (0)
144	0 (0)

The effect of the test item on the reproductive output of daphnids in comparison to the pooled control is reported in Table B.9.2.5-4 below.

Table B.9.2.5-4: Effects of Isoflucypram on reproductive output of *Daphnia magna* in comparison to the control.

Nominal concentrations (µg a.s./L)	Total offspring per surviving parental female (Mean ± SD)	% Deviation from pooled controls#	Average daily offspring per surviving parental female (Mean ± SD)	% Deviation from pooled controls#	Age at first offspring emergence (Mean ± SD)	% Deviation from pooled controls#
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	-
4.5	95.7 ± 7.4	-5.16	7.3 ± 0.5	0.00	9.72 ± 0.32	5.36
9.0	91.6 ± 11.5	-0.66	7.1 ± 0.9	2.74	9.92 ± 0.32	3.41
18.0	78.3 ± 27.0	13.96	6.4 ± 2.3	12.33	10.62 ± 1.03	-3.41
36.0	92.2 ± 13.4	-1.32	7.3 ± 1.0	0.00	10.22 ± 0.70	0.49
72.0	92.2 ± 13.8	-1.32	8.2 ± 1.2	-12.33	9.92 ± 0.74	3.41
144	55.4 ± 20.9	39.12*	5.4 ± 2.0	26.03*	12.52 ± 1.49*	-21.91*

* Statistical significant difference from solvent control (verified by Step-down Jonckheere-Terpstra test Procedure on a 5% level of significance one-sided smaller).
#calculated by the RMS
Negative results denote an increase in comparison to the control

Behavioural effects in neonates were detected only at the highest test concentration of 144 µg a.s./L (9.57% offspring affected).

Conclusion:

The endpoints based on nominal concentrations are:

	Length	Dry body weight	Immobili-sation	Age at first offspring emergences	Total/average offspring per surviving parental female
NOEC [µg a.s./L]: highest concentration without an significant effect compared to the control**	72	72	144	72	72
EC_{10} [µg a.s./L]**	n.a.*	58.4 (95% CI: 29 – 197.5)	n.a.*	n.a.*	112.4 (95% CI: 90.2 – 121.6)

*Not applicable due to absence of effects
**Following a request for additional information during commenting endpoints were recalculated based on nominal concentrations.

RMS comments

This study was conducted according to GLP and following the guidelines of US EPA OCSPP 850.1300 and OECD 202.

The following was noted by the RMS:

It is noted that a decrease of 13.96% in total offspring per surviving female occurred at a test concentration of 20.3 mg a.s./L, which could be considered biologically significant. However this result did not follow a dose response and is not considered to be test item-related.

Although the measured concentrations of the active substance were not within ±20% of nominal concentrations, the RMS notes that each measured concentration is above 100%. Therefore, the RMS considers it appropriate to express the endpoints as nominal concentrations. Following a request for additional information, the applicant re-calculated the EC_{10} values form this study based on nominal test concentrations. An EC_{10} value of 58.4 µg a.s./L was calculated based on parental dry body weight, which is the critical endpoint from this study. The confidence intervals accompanying the derived EC_{10} value are wide (29 – 197.5). However the RMS still considers this value to be protective as the lower confidence interval is acceptable and the derived value of 58.4 µg a.s./L appears to be a reasonable fit to the data and is protective of the statistically-derived NOEC of 72 µg a.s./L, noting a decrease in parental body weight of 14.37% was observed at 72 µg a.s./L and an increase in parental body weight of 1.93% was observed at the next tested concentration of 36 µg a.s./L. Furthermore, the RMS considers it overly conservative to set the NOEC at 36 µg a.s./L, where an increase in parental dry body weight was observed.

The method is acceptably validated in accordance with SANCO/3029/99 rev.4 and is suitable for the determination of isoflucypram in samples of test water at a LOQ of 0.05 µg/L (see section B5.1.2.6.1 of the CA document for further details).

This study is considered valid and the endpoints confirmed as follows:

EC_{10} (dry body weight) = 58.4 µg a.s./L (nominal)

NOEC (dry body weight) = 72 µg a.s./L (nominal)

Previous evaluation:	New data, submitted for purpose of review

Report:	KCA 8.2.5.2/01; Milligan, A. L.; Siddiqui, A. I.; Gallagher, S. P.; Krueger, H. O.; 2016;
Title:	BCS-CN88460: A flow-through life-cycle toxicity test with the saltwater mysid (Americamysis bahia)
Report No.:	149A-256
Guideline(s):	US EPA OCSPP 850.1350
GLP/GEP:	Yes

Material and methods:

Test material	BCS-CN88460 Batch 2013-006492. Purity 94.2% w/w
Test species	Saltwater mysid (*Americamysis bahia*) from cultures maintained by EAG Laboratories-Easton.
Organism age/size at study initiation	Juvenile mysids, less than 24 hours old
Holding period:	Adult mysids in EAG Laboratories-Easton cultures were held in the laboratory for at least 14 days before juveniles were collected for testing, and ranged in age from 25 to 31 days. The cultures were maintained in a flow-through saltwater system using water from the same source and at approximately the same temperature as was used during the test. The mysids in the cultures used for the test showed no signs of disease or stress prior to the test.
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 (TEG)
Test concentration preparation:	Individual stock solutions were prepared for each of the five concentrations tested, and were prepared every two to five days during the test. All test solution concentrations were adjusted to 100% active ingredient during preparation, based on the reported test substance purity (94.2%). During the pretest, a primary stock was prepared at a concentration of 17.5 mg a.s./mL (equating to a solvent concentration of 20 µL TEG/L) and secondary stocks were prepared by proportional dilution of the primary stock at concentrations of 1.10, 2.20, 4.40 and 8.75 mg a.s./mL. Since the analytical results of the samples collected during pretest were lower than anticipated and due to the observation of precipitate in the mixing chambers for the 175 and 350 µg a.s./L treatment groups, the stock solutions were prepared at a higher solvent concentration (100 µL/L) and these stocks were placed on the test system for delivery prior to test initiation. During the study, a primary stock solution was prepared by mixing a calculated amount of test substance into reagent-grade TEG at a nominal concentration of 3.5 mg a.s./mL. The primary stock solution was sonicated for approximately one hour and inverted to mix.
Dilution water:	Natural seawater from Indian River Inlet, Delaware. The salinity was adjusted with well water to 20‰ and aerated with spray nozzles. Prior to use in the test water was filtered to 0.45µm and passed through a UV steriliser.
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	*G1*: No. of organisms per compartment: 15 *G2*: No. of organisms per compartment: 10
Exposure	Flow-through via syringe pumps calibrated prior to the test. The flow of dilution water into each mixing chamber was controlled using rotameters (Juvenile chambers: 5.6 volume additions of test solution/day; Adult test chambers: 6.2 volume additions of test solution/day. Total exposure duration: 28 days
Feeding during test	Mysids in the cultures were fed live brine shrimp nauplii (*Artemia* sp.) ad libitum daily, supplied by Brine Shrimp Direct of Ogden, Utah. The brine shrimp diet was typically enriched once daily with a nutrient enrichment (Easy DHA Selco from INVE Thailand, Ltd.). Mysid food was also supplemented daily with the saltwater alga, *Skeletonema costatum*, cultured by EAG Laboratories-Easton. During the test, the G1 and G2 mysids were fed live brine shrimp nauplii (*Artemia* sp.) up to four times daily, with one of the daily feedings consisting of the enriched diet, and were fed the saltwater alga, *Skeletonema costatum*, once daily. The amount of brine shrimp nauplii fed was adjusted to account for density and growth of the mysids throughout the study. Excess food and waste were siphoned from the test chambers 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 ‰
Test vessels:	*Test chambers:* 15 cm diameter glass petri dishes with sides of nylon mesh screen (425 µm mesh size). The compartments were placed in 19 L glass aquaria containing approximately 8 L of test solution. The depth of the water in a representative test chamber and test compartment was 9.1 and 9.0 cm, respectively *Reproductive compartments:* 10 cm diameter glass petri dishes with sides of nylon mesh screen (425 µm mesh size) placed in 19-L glass aquaria filled with approximately 14.5 L of test solution. Each test chamber contained a self-starting siphoning system to exchange test solution. The depth of the water in a representative test chamber and reproductive compartment was 16.2 and 15.9 cm, respectively.

Parameters Measured/ Observations	*G1 mysids:* Mortality and toxicity recorded daily throughout the exposure period ; at test termination the sex of each surviving first generation was confirmed and total length and dry weight measured. *G2 mysids:* 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 0, 7, 14 and 21, 28, and 32 days after test initiation. 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. Samples were collected from mid-depth, placed in glass vials containing 2.00 mL of methanol and processed immediately for analysis 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 with SAS software.

Test procedure

Saltwater mysids were exposed to a geometric series of five test concentrations, a negative control (dilution water), and a solvent control (0.10 mL/L triethylene glycol) under flow-through conditions for 28 days.

The day prior to initiation of the test, all adult brood tanks were checked for young juvenile mysids and were removed. On the following day, but < 24 hours from the time recorded, the adults were removed from the tank using a separation basket and any young mysids remaining in the tank were considered to be < 24-hour old juveniles for use in the study. These young were impartially distributed one to three at a time into transfer containers until each container held 15 mysids. Each group of mysids then was transferred to an indiscriminately assigned test compartment. All transfers were performed using wide-bore pipettes below the water surface.

Delivery of the test solutions to the test chambers was initiated seven days prior to test initiation in order to achieve equilibrium of the test substance. Four replicate test chambers were maintained in each treatment and control group. The mysids used to initiate the test are referred to as the parental or G1 generation. At test initiation, each replicate contained one compartment with 15 neonate mysids, resulting in a total of 60 mysids in each treatment and control group. After mysids attained sexual maturity and were paired on Day 13 of the test, reproductive pairs were placed in reproductive compartments, one pair per compartment, with up to five compartments placed in each replicate test chamber. Additional compartments were maintained in each test chamber, if necessary, to house additional males. Reproduction of the paired G1 mysids was monitored through termination on Day 28.

Observations for G1 mysid mortality and signs of toxicity were conducted daily throughout the test. At test termination, the total body lengths and dry weights of all surviving first-generation mysids were measured.

Offspring produced by the G1 mysids (the second generation, G2) were exposed to the same nominal test substance concentrations for approximately 96-hours following the release of the G2 mysids from the brood pouch. During the reproductive phase, groups of G2 offspring were collected from the G1 replicate test chambers and were maintained in a separate test compartment within each G1 replicate test chamber, when available. Each G2 test compartment contained up to 10 offspring for a total of up to 40 G2 offspring per treatment and control group, when available. The G2 mysids in each test compartment were observed formortality daily for an approximate 96-hour exposure period.

Results

Validity criteria according to OPPTS 850.1035	**Required**	**Obtained**
Mean survival of the F_0 generation of the control at exposure termination.	≤ 70%	Negative control: 77.6% Solvent control: 76% Pooled control: 76.8%
Females of the first-generation in the controls failed to produce young	≤ 25%	Negative control: 0% Solvent control: 5.6% Pooled control: 2.7%
The average number of young produced by first-generation in the controls	≥ 3	Negative control: 8.8 ± 3.98 Solvent control: 12.2 ± 2.04 Pooled control: 10.5 ± 3.46

All validity criteria were met.

Analytical results:
Recoveries were between 71 and 98% (see Table B.9.2.5-5 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).

Table B.9.2.5-5: Analytical verification of the test item

Nominal Concentration (µg a.s./L)	**Measured concentrations (mg a.s./L)**								**Arithmetic mean measured concentration (µg a.s./L)**	**% of nominal concentrations**	**Range of individual measurements (% of nominal)**
	Day										
	0	**2a ***	**2b ***	**7**	**14**	**21**	**28**	**32**			
22	19.5	-	-	17.7	19.7	21.3	20.4	20.9	20 ± 1.3	91	80.3 – 97.0
44	37.3	33.8	31.3	36.4	36.6	39.9	40.4	43.3	37 ± 3.8	84	71.1 – 98.4
88	73.9	-	-	72.7	80.8	83.7	79.5	85.6	79 ± 5.2	90	82.6 – 97.2
175	154	-	-	137	140	146	142	154	146 ± 7.2	83	78.3 – 88.2
350	289	-	-	271	298	308	312	317	299 ± 17	85	77.3 – 90.5

* Sample collected after a syringe leak was discovered.
**Sample collected after the syringe leak was corrected.

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 ($p \leq 0.05$).

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 ($p \leq 0.05$).

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.

Table B.9.2.5-6: Summary of survival of G1 juvenile saltwater mysids exposed to BCS-CN88460

Mean measured conc. (µg a.s./L)	**Juvenile survival initiation to day 7**			**Juvenile survival to pairing on day 13**		
	No. exposed	**No. surviving**	**% survival**	**No. exposed**	**No. surviving**	**% survival**
Control	60	59	98.3	60	54	90.0
Solvent control	60	59	98.3	60	57	95.0
Pooled control	120	59	98.3	120	55.5	92.5
20	60	58	96.7	60	57	95.0
37	60	59	98.3	60	55	91.7
79	60	59	98.3	60	58	96.7
146	60	59	98.3	60	57	95.0
299	60	60	100	60	59	98.3

* Statistically significant decrease in survival in comparison to the pooled control using Fisher's Exact test ($p \leq 0.05$).

Table B.9.2.5-7: Summary of survival of G1adult saltwater mysids exposed to BCS-CN88460

Mean measured conc. (µg a.s./L	**Adult survival day 14 – 21**				**Adult survival day 14 - 28**			
	No. alive at pairing[1]	**No. surviving**	**% survival**	**% change in survival in comparison to pooled control[2]**	**No. alive at pairing [1]**	**No. surviving**	**% survival**	**% change in survival in comparison to pooled control[2]**
Control	49	42	85.7	-	49	38	77.6	-
Solvent control	50	39	78.0	-	50	38	76.0	-
Pooled control	99	81	81.8	-	99	76	76.8	-
20	48	39	81.3	0.4	48	34	70.8	7.8
37	44	32	72.7	10.9	44	29	65.9	14.2
79	46	33	71.7	12.1	46	25*	54.3*	29.3*
146	42	33	78.6	3.7	42	28	66.7	13.2
299	50	22*	44.0*	46.1*	50	9*	18.0*	76.6*

* 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]In order to compare the reduction in survival of the treatment groups in comparison to the control, due to the different starting numbers % survival in the pooled control was compared to % survival in the treatment groups.

Table B.9.2.5-8: Summary of reproductive output of G1 saltwater mysids exposed to BCS-CN88460

Arithmetic mean measured conc. (µg a.s./L)	Number of Young Produced Per Reproductive Day		Surviving Females Producing Young		Number of Young Per Surviving Female	
	Mean ± SD	% of pooled control	Percent of Surviving Females Producing Young[1]	% of pooled control	Mean ± SD [1]	% of pooled control
Control	0.582 ± 0.270	-	100	102.8	8.8 ± 3.98	-
Solvent control	0.816 ± 0.132	-	94.4	97.0	12.2 ± 2.04	-
Pooled control	0.699 ± 0.233	-	97.3	100.0	10.5 ± 3.46	-
20	0.991 ± 0.173	141.8	100	102.8	17.3 ± 3.88	164.8
37	0.659 ± 0.106	94.3	92.9	95.5	10.8 ± 1.19	102.9
79	0.555 ± 0.166	79.4	78.6	80.8	9.6 ± 2.98	91.4
146	0.378 ± 0.084*[2]	54.1*	73.3**	75.3**	6.1 ± 1.64*	58.1*
299	0.000 ± 0.000*[2]	0.0*	0.0**	0.0**	0.0 ± 0.00*	0.0*

* 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.

Table B.9.2.5-9: Summary of growth parameters of G1 saltwater mysids exposed to BCS-CN88460 after 28 days

Arithmetic mean measured conc. (µg a.s./L)	Mean total length ± SD (mm)				Mean dry weight ± SD (mg)			
	Males	% of pooled control	Females	% of pooled control	Males	% of pooled control	Females	% of pooled control
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	100.9	7.96 ± 0.091	100.1	0.87 ± 0.050	91.6	1.30 ± 0.175	104
37	7.85 ± 0.185	101.9	8.04 ± 0.185	101.1	0.99 ± 0.091	104.2	1.17 ± 0.153	93.6
79	7.95 ± 0.442	103.2	8.16 ± 0.151	102.6	1.03 ± 0.109	108.4	1.18 ± 0.114	94.4
146	7.80 ± 0.141	101.3	7.72 ± 0.270	97.1	0.96 ± 0.039	101.1	1.12 ± 0.144	89.6
299	7.47 ± 0.225	97.0	7.51 ± 0.365*	94.5	0.90 ± 0.173	94.7	1.09 ± 0.212	87.2

[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.

* Statistically significant decrease in comparison to the pooled control using the Dunnett's test ($p \leq 0.05$).

Conclusion

The most sensitive endpoint was adult mortality (14 – 28 days). The results, based on arithmetic mean measured concentrations, are:

NOEC (based on biologically relevant adult mortality from 14 – 28 days):	**20 µg a.s./L**
LOEC: (based on biologically relevant adult mortality from 14 – 28 days): lowest concentration with an significant effect compared to the control	**37 µg a.s./L**
NOEC (based on statistically significant adult mortality from 14 – 28 days):	**37 µg a.s./L**
LOEC: (based on statistically significant adult mortality from 14 – 28 days): lowest concentration with an significant effect compared to the control	**79 µg a.s./L**

RMS comments

This study was conducted following GLP and according to guideline 850.1350 (1996). All validity criteria were met.

The validity criterion of average number of young produced per female in the controls is less than three per day has been found to be incorrect. ASTM E 1191 guideline states that the average number of young per female should be >3, i.e. >3 total and not per day. Therefore this validity criterion is met for this study. Furthermore, all validity criteria are also met for the draft guideline for the Mysid Two-generation Test (2013) adding further support to the validity of this study.

- At least 70% survival of control animals in all test phases over the duration of the chemical exposure;

- At least 75% of the females in the F0 and F1 controls produce young;

- The average total number of young produced per control female in the first two broods is at least eight.

The applicant was requested to calculate EC_{10} values for this study; it was stated that it was not possible to calculate EC_{10} values due to the following reasons:

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.

The RMS does not agree with the above argumentation:

- It is noted that for G1 mysid reproduction a 20.6% reduction in comparison to the pooled control was observed at 79µg a.s/L, which followed a dose-response.
- Furthermore, regarding adult survival at 14 – 21 days, survival was reduced by 10.9 % at 37 µg a.s./L; at day 14 – 28 adult survival was reduced by 14.2 % at 37 µg a.s../L.
- Whilst a clear dose response in adult survival at day 14 – 28 is not observed due to a decrease of 29.3% at 79 µg a.s./L followed by a smaller decrease of 13.2% at 146 µg a.s./L; it is not clear which of these results is anomalous. Furthermore, 14 – 28 day adult survival in replicate A in the 179 µg a.s./L treatment group was 90.9%, whereas in replicates B – D it was 50 – 66.7%, indicating that replicate A was potentially an outlier.
- The following was stated by the applicant with regard to this study following the request for further information:

  The chronic mysid shrimp study was performed according the OPPTS 850.1350 guideline. This type of study is known for a high failure rate due to missed validity criteria and a high variability in general and in the controls. Therefore the results should be handled with care and should not be over-interpreted. So far the studies are performed in North America and there are used for saltwater risk assessments. European laboratories did so far not run this type of study. Meanwhile the first institutes try to establish this type of test in Europe as well.

  Due to the known high variability of historical control data the use of an EC10 in case of this study type is questionable and not foreseen in the underlying guideline.

  This becomes visible when looking at the acceptance criteria for a chronic mysid study as presented in the original report.

  The acceptance criteria give some information about the issues of this study type. Obviously a mortality of up to 30 % is not unusual for control animals. Reproduction does not occur in all animals and up to 25% not reproducing females are still acceptable. The last criterion is related to the average number of young by first generation females which should be equal to or greater than three in controls. This last criterion for example is still met by the second highest concentration of 146 µg a.i.(L with a mean value of 6.1.

The variability in mortality has been taken into account as the increases in mortality are relative to the control mortality, as such these results are considered to be an effect of the test item by the RMS (absolute mortality values of 35.1 and 45.7% at concentrations of 37 and 79 µg a.s../L respectively).

- The RMS requested that LC_{10}/EC_{10} values were calculated from this study where possible. In their response, the applicant was unable to derive LC_{10} values for mortality for statistical reasons; EC_{10} values were derived for number of young produced per reproductive day, surviving females producing young, number of young per surviving female and female mean dry weight (69.1 µg/L, 60.2, 60.2 and 130.2 µg a.s.L respectively). The confidence intervals were wide in each case (8.5 - 98.5; 24.6 – 987.5; 24.6 – 87.5 for number of young produced per reproductive day surviving females producing young and number of young per surviving respectively) and could not be determined for female mean dry weight. The robustness of the calculated EC_{10} is questionable according to the used statistical software for surviving females producing young, number of young per surviving female and female mean dry weight. The RMS agrees that the EC_{10} values derived from this study may not be robust and that it wss not possible to derive LC_{10} values.
- The RMS does not agree with the derived NOEC from this study based on G1 mysid reproduction; whilst not statistically significant, the reductions in adult survival observed at days 14 -21 and days 14 - 28 below the proposed statistical NOEC may be biologically relevant and based on potential biological relevance the NOEC would be 20 µg a.s./L. Furthermore, in the absence of a valid LC_{10} value for mortality, the RMS considers it prudent to be protective of potentially biologically relevant effects on mortality.

The method is acceptably validated in accordance with SANCO/3029/99 rev.4 and is suitable for the determination of isoflucypram in samples of test (salt) water at a LOQ of 0.02 mg/L (see section B5.1.2.6.1 of the CA document for further details).

This study is valid and the endpoint confirmed for use in the risk assessment is as follows:

NOEC (14 – 28 day adult mortality): 20 µg a.s./L

Previous evaluation:	New data, submitted for purpose of review

Report:	KCA 8.2.5.2/02; Bradley, M. J.; 2017
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
Guideline(s):	EPA Test Methods EPA/600/R-01/020 (2001)
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%
Test species	*Leptocheirus plumulosus* obtained from Chesapeake Cultures, Hayes, Virginia,
Acclimation	Test species were acclimated to test conditions for approximately 24 hours prior to testing. During the acclimation period, amphipods were maintained in 2.0-L glass beakers containing approximately 1.8 L of 20‰ seawater. Test conditions during the acclimation period were as follows: Salinity:20‰

	Temperature: 23 - 24 °C Dissolved oxygen: 6.1 - 7.2 mg/L. Feeding: Once with 1 mL of 100 mg/mL flaked fish food suspension to each holding beaker. No mortality was observed in the test population upon receipt or during the subsequent 24 hours prior to exposure initiation.
Organism age/size at study initiation	Age: 7 to 14 days old at exposure initiation A measurement of dry weight was made on three subsets of 20 juvenile amphipods from the same population used to initiate the exposure in order to determine growth rate (mg gained per amphipod per day) at the end of the exposure. The mean value of these three subsets resulted in an initial dry weight of 0.047 mg dry weight per amphipod, which is consistent with the typical initial dry weight range of 0.030 to 0.060 mg per amphipod described in the EPA test method.
Test solutions	Nominal concentrations: 3.8, 7.5, 15, 30 and 60 mg a.s./kg sediment dry weight based on the results of a preliminary range-finding test. 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
Preparation of test solutions :	A 6.0 mg/mL primary stock solution was prepared by dissolving 0.3195 g of BCS-CN88460 (0.3010 g as active ingredient) in 50 mL of acetone. Five individual dosing stock solutions were prepared using the 6.0 mg/mL primary stock solution described above by serial dilution. All stock solutions had no visible undissolved test substance following preparation.
Application of test solutions to the sediment:	A jar-rolling technique was used to apply the test substance to the sediments, and to mix the control sediments . First, a 10-mL volume of each dosing stock solution was applied to 0.050 kg of fine silica sand, which was mixed with a metal spatula for approximately two minutes. The solvent was then allowed to evaporate for 75 minutes. The dry sand, containing the test substance, was then added to the 2.5 kg of wet sediment (0.8183 kg total dry weight based on a percent solid value of 30.73% and including the 0.050 kg of fine silica sand) in individual glass jars. The jars were sealed and positioned horizontally on the rolling mill. Each jar was then rolled for five hours at room temperature at approximately 15 rpm; the jars were stored upright in a dark refrigerator, where they were allowed to equilibrate for a 6-day period. On the fifth day of the equilibration period and again prior to addition into the replicate test vessels, the jars were mixed on the rolling mill for an additional two hours at room temperature to ensure the sediment was homogeneous. The use of a 7-day equilibration was based on the results of a sediment-pore water equilibration trial conducted in association with this testing program. The same process was followed for solvent and negative controls minus the test item. Each vessel contained 120 mL (approximately 2-cm layer) of sediment and 600 mL of overlying water (approximately 10-cm depth). The total overlying water/sediment volume was maintained at approximately 720 mL. The wet weight of the sediment in each test vessel averaged 128 g (39.2 g dry weight). On test day -1, overlying water was gently added to each vessel and then each vessel was randomly placed in the water bath used to house the exposure. A turbulence reducer, consisting of a modified plastic disk, was used to minimize the disruption of the sediment layer during the introduction of overlying water. During this time (approximately 24 hours), the sediment and overlying water were allowed to equilibrate prior to addition of the test organisms.
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: overlying water was renewed by adding two volume additions of water per test vessel per day using an intermittent delivery system in combination with a calibrated water-distribution system,. The water delivery system cycled approximately 12 times per day, providing approximately 1200 mL per vessel every 24 hours (i.e., approximately two overlying volume replacements per vessel per day). Total exposure duration: 28 days
Feeding during test	The amphipods were fed a diet consisting 1.0mL of an appropriate concentration of flaked fish food suspension prepared in natural, filtered sea water once daily: Days 0 - 6: 9.0 mg Days 7 – 14: 14 mg Days 14 – 20: 20 mg Days: 21 -27: 30 mg
Sediment :	Natural marine sediment collected from Sequim Bay, Sequim, Washington passed through a 0.25 mm sieve before use. Sediment characteristics were as follows: Organic carbon: 2.9% Sand: 33% Silt: 47% Clay: 20% pH: 7.8 Water holding capacity: 80.6% Ammonia: 5.0 mg/L (as nitrogen)
Overlying water :	Natural filtered seawater (salinity range of 30 to 32‰ and pH range of 7.8 to 8.0) from Cape Cod Canal, Bourne Massachusetts. Seawater was diluted with on-site laboratory well water to a salinity range of 20 to 21‰, filtered through 20-µm and 5-µm polypropylene core filters, then through 50-µm and 1-µm bag type filters. Prior to use in this study, the pH range of the seawater was measured to be 7.4 to 7.9.
Test conditions	Temperature: 23-26°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 (69.9 – 87.0% ; control), 4.7 – 7.0 mg/L (57.6 – 85.8% ; solvent control), 4.2 – 7.2 mg/L (51.5 – 88.3% ; test concentrations) Salinity: 20-22‰ Ammonia as Nitrogen: 0.10 – 0.59 mg/L Aeration : Constant flow of bubbles provided by a 1mL glass pipette
Test vessels :	1L glass jars with a 12 cm hole for drainage of water during renewal; this was covered with 40-mesh nylon screen to retain any organisms.
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. Negative control and solvent control data were statistically similar for all endpoints. 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. LC/EC50 values were calculated where appropriate. CETISTM Version 1.8 used to perform the statistical analysis.

Test procedure

The purpose of this study was to determine the chronic effects of BCS-CN88460, applied to sediment on the sediment-dwelling estuarine amphipod *Leptocheirus plumulosus.* The exposure was conducted under static-renewal conditions for 28 days to assess the impact of the test substance on the survival, growth and reproduction of amphipods.

At exposure initiation, amphipods were impartially added to each replicate containing treated/control sediment five at a time until the replicate contained twenty individuals. The exposure was initiated when each respective replicate contained twenty amphipods.

At exposure termination (day 28), the total number of surviving adult and young amphipods was determined in each test vessel by sieving the sediment through a 0.60-mm and 0.25-mm sieve or fine meshed net. According to the test method, adult amphipods recovered at exposure termination were defined as those individuals that were retained on the 0.60 mm sieve. Young amphipods were defined as those individuals that were retained on the 0.25-mm sieve. At exposure termination, adult amphipods were preserved in buffered formalin solution to allow for identification of the gender of each adult at a later date (i.e., one day later). The gender of the surviving adults was determined by the presence or absence of eggs in the brood pouch or further morphological characteristics described in the EPA test method. Growth was determined by pooling the surviving adult amphipods (separated by gender) from each replicate vessel and drying at 62 to 63 ºC for approximately 24 hours in an oven. Amphipods were rinsed gently with deionized water to remove any residual salt deposits prior to being dried in the oven. The

pooled, dry amphipods were then weighed on an analytical balance to the nearest 0.01 mg. Growth rate for both males and female individuals was calculated using the following equation:

mg gain/amphipod/day = (mg/amphipod at termination – mg/amphipod at initiation)/28 days

At exposure termination, the offspring were removed from the 0.25-mm sieve and transferred to a labeled sample jar. Sufficient alcohol (approximately 75 mL of 70% ethanol solution) and 3.0 mL of concentrated Rose Bengal solution (1 mg/mL) was added to the sample jar to preserve and stain the offspring for enumeration at a later date (i.e., one to two days later). Reproduction was determined as the number of young per surviving female amphipod in each replicate vessel.

Results:

Validity criteria

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%
Growth and reproduction of negative control	Measurable growth in all replicates	There was measurable growth and reproduction in all replicates.
Temperature	± 2°C (daily temperature) ± 3°C (instantaneous temperature)	23-25°C (daily) 23-25°C (instantaneous)
Salinity	20‰ ± 2‰ (daily salinity) 20‰ ± 3‰ (instantaneous salinity)	20-22‰ (daily)

All validity criteria were met.

Analytical results:

No BCS-CN88460 residues were measured in the sediment, pore water or overlaying water controls above the limit of quantification (LOQ) as shown in Table B.9.2.5-10.

Table B.9.2.5-10: Analytical verification of BCS-CN88460 in sediment

Nominal sediment concentration (mg a.s./kg)	**Measured sediment concentrations (mg a.s./kg)**				**% of nominal concentration**			
	Day 0	**Day 14**	**Day 28**	**Arithmetic mean**	**Day 0**	**Day 14**	**Day 28**	**Mean**
3.8	4.0	3.1	2.3	3.1	105	81.6	60.5	82.4
7.5	7.9	5.5	2.9	5.4	105	73.3	38.7	72.3
15	15	11	7.5	11	100	73.3	50.0	74.4
30	30	21	15	22	100	70.0	50.0	73.3
60	56	47	25	43	93.3	78.3	41.7	71.1

Table B.9.2.5-11: Analytical verification of BCS-CN88460 in pore water and overlying water

Nominal sediment concentration (mg a.s./kg)	Measured concentration (mg a.s./L)			Arithmetic mean measured concentration
	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*) as shown in Table B.9.2.5-12.

Table B.9.2.5-12: Mean survival, male and female growth rate and number of offspring observed following exposure of *L.plumulosus.* BCS-CN88460 in sediment

Arithmetic mean measured sediment concentration (mg a.s./kg)	Survival		Male growth rate (mg/day)		Female growth rate (mg/day)		No. of offspring per surviving female amphipod	
	Mean% (SD)	Decrease relative to negative control (%)[1]	Mean (SD)	Decrease relative to negative control (%)[1]	Mean (SD)	Decrease relative to negative control (%)[1]	Mean (SD)	Decrease relative to negative control (%)[1]
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)	-6.8	0.088 (0.0069)	4.3	0.053 (0.0059)	-3.9	15 (2.8)	25
5.4	83 (9)	5.7	0.094 (0.0130)	-2.2	0.056 (0.0036)	-9.8	16 (1.6)	20
11	94 (6)	-6.8	0.087 (0.0097)	5.4	0.053 (0.0086)	-3.9	20 (7.4)	0
22	94 (6)	-6.8	0.087 (0.0110)	5.4	0.053 (0.0360)	-3.9	12 (3.6)*	40
43	92 (7)	-4.5	0.089 (0.0071)	3.3	0.052 (0.0037)	-2.0	10 (2.6)*	50

[1] Calculated by the RMS
* Significantly reduced compared to the negative control, based on Dunnett's Multiple Comparison Test
SD = Standard deviation

Conclusion

The endpoints based on arithmetic mean measured concentrations proposed by the applicant are:

Endpoint	**Arithmetic mean measured sediment (mg a.s./kg)**	**Arithmetic mean measured pore water (mg a.s./L)**
LC_{50} (95% C.I.) based on amphipod survival, male and female growth rate. And reproduction :	> 43 (NA)	> 0.27 (NA)
NOEC based on amphipod survival, male and female growth rate :	43	0.27
NOEC based on mean number of psspring per surviving female :	11	0.086

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

RMS comments

This study was conducted according to GLP and following EPA 600/R-01-020 guidelines.

The following was noted by the RMS :

Arithmetic mean measured concentrations were calculated in this study and used to calculate endpoints; however geometric mean measured concentrations should have been calculated and the endpoints based on these values instead.

According to the test guidelines neonates<24hrs old or size selected between 0.25 and 0.6mm should be used in the study. However in this study juveniles 7 – 14 days old were used. It also states in the test guidelines that juveniles passing through the mesh typically have a body weight of 0.03 – 0.06 mg; it is stated in the test report that the average weight of the organisms used was 0.047 mg ; as such the RMS considers this to be acceptable.

The test guidelines state that under static renewal conditions water exchanges should be conducted 3x per week; in this study water exchanges were conducted twice per day. Whilst a deviation from the test guidelines, measurement of water and sediment was conducted at 14 day intervals during the test to confirm that exposure was achieved and exposure is based on measured concentrations. Therefore this is considered acceptable.

According the test guidelines, the following feeding regime should be followed: 20 mg on days 0 – 13 and 40 mg on days 14 – 28 3 times per week after water renewal. However in this study amphipods were fed the following regime daily : Days 0 - 6: 9.0 mg ; Days 7 – 14: 14 mg ; Days 14 – 20: 20 mg ; Days: 21 -27: 30 mg. Whilst a deviation from the test guidelines as all validity criteria were met and controls performed adequately, this is not considered to invalidate the test.

It is noted that for the parameter 'number of offspring per surviving female' a reduction of 25% and 20% was observed for the two lowest test concentrations of 3.1 and 5.4 mg a.s./kg sediment respectively in comparison to the water control. However these reductions were not statistically significant. It is stated in the study report that one of the replicates from the 11 mg a.s./kg sediment treatment group was an outlier and that removal of this outlier allowed the detection of statistically significant effects at 3.1 mg a .s./kg sediment and that this was not considered treatment-related. However, it was not deemed appropriate to remove this replicate in the study report. As statistical significance could not be detected despite a reduction of 20-25%, the RMS considers this study to be insensitive to detecting potentially biologically significant effects. Furthermore, if these effects were to be considered biologically relevant, it would not be possible to set a NOEC from this study. **As such, this study is not considered to be suitable for regulatory use and will not be considered further in the risk assessment**.

Previous evaluation:	New data, submitted for purpose of review

Report:	KCA 8.2.5.2/04; Bradley, M. J.; 2017
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
Guideline(s):	US EPA Test Method 100.4 OCSPP 850.1770 (In Preparation)
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%
Test species	*Hyalella azteca* obtained from laboratory cultures maintained at Smithers Viscient
Organism age/size at study initiation	- Age: 8 days old at exposure initiation
Holding period:	Prior to exposure initiation, the main culture of amphipods was maintained in 38-L glass aquaria containing approximately 31 L of culture water under flow-through conditions. The culture water was from the same source as water used as overlying water during the test. Amphipods (8 days old at exposure initiation) used in the exposure were collected from reproducing adult amphipods removed from the main culture tanks 9 days prior to exposure initiation. The adult amphipods were placed in 9.5-L aquaria (isolation tanks) containing approximately 8 L of water. Neonate amphipods (< 24 hours old) produced by these isolated adults were then removed from the isolation tanks and pipetted into 1-L beakers containing approximately 0.80 L of laboratory well water, where they were held until exposure initiation. During the holding period, dissolved oxygen ranged from 7.2 to 8.1 mg/L and temperature range was maintained at 24 °C. The potential test organisms appeared healthy and no mortality was observed in the test population 48 hours prior to exposure initiation. Amphipods were fed a combination of yeast, cereal leaves and flaked fish food suspension (YCT) daily. In addition, on the first day of holding, *Ankistrodesmus falcatus*, a unicellular green algae and a small amount of 100 mg/mL flaked fish food suspension were also provided as a supplemental food source.
Preparation of test solutions :	A 25 mg/mL primary stock solution was prepared by bringing 1.3454 g of BCS-CN88460 (1.2674 g as active ingredient) to a volume of 50 mL with acetone. Five individual dosing stock solutions were prepared in acetone by serial dilution for application of the test substance to the sediment. No visible undissolved test material was observed during preparation.
Test solutions	Nominal concentrations: 6.3, 13, 25, 50 and 100 mg a.s./kg sediment dry weight following preliminary testing. Arithmetic mean measured concentrations - in sediment: 5.8, 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.
Application of test item to sediment :	A jar-rolling technique was used to apply the test substance to the sediment. A 10-mL volume of each dosing stock solution was applied to 0.050 kg of fine silica sand placed in glass Petri dishes. The solvent was allowed to evaporate for an hour and 45 minutes. The dry sand, containing the test substance, was then added to the 2.5 kg of wet sediment (1.7635 kg total dry weight based on a percent solid value of 68.54% and including the 0.050 kg of silica sand) in individual 4 L glass jars. The jars were sealed and positioned horizontally on the rolling mill. Each jar was then rolled for four hours at room temperature at approximately 15 rpm. Following four hours of rolling, the jars were stored upright at 2 to 8 ºC. The sediments were allowed to equilibrate for a 7-day period in the refrigerator. The use of a 7-day equilibration was based on the results of a sediment-pore water equilibration trial conducted in association with this testing program. Once during the 7-day equilibration period and again prior to distribution of the sediments into the replicate test vessels, the jars were mixed on the rolling mill as previously mentioned for an additional two hours at room temperature to ensure the sediment was homogeneous. Solvent controls were prepared as above but without addition of the test item. Negative controls were prepared as above but without addition of solvent, test substance or 0.050 kg of fine silica sand. Each vessel contained 100 mL (approximately 4.0-cm layer) of sediment (equivalent to 149 g wet weight per vessel or 102 g dry weight per vessel). A turbulence reducer, consisting of a modified plastic disk, was used to minimize the disruption of the sediment layer during the introduction of 175 mL of overlying water. The total overlying water plus sediment volume was maintained at approximately 275 mL. Treated and control sediments were allocated to replicate vessels one day prior to exposure initiation.
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.
Overlying water :	Labotaory well water : Total hardness : 64 – 84 mg/L (as $CaCO_3$) Total alkalinity : 18 – 24 mg/L (as $CaCO_3$) pH : 6.4 – 7.4 Conductivity : 450 – 550 µS/cm
Artificial sediment :	Prepared according to OECD 218 (2004) : 5% spaghnum peat 20% kaolin clay 75% fine sand Organic carbon content : 2.3%

	pH : 7.2 Water holding capacity : 16.9% Prior to being used in the sediment preparation, the peat was soaked for approximately one week in laboratory well water. Over this period, a total of 160 grams of powdered CaCO3 was mixed into the peat suspension in order to increase the pH. The mean pH of the peat suspension increased from 3.8 to 6.2 during the soaking period. The peat was then removed from suspension using a fine-mesh net. All of the sediment components were then blended together in a large-scale laboratory mixer. A total of 10.8 L of laboratory well water was also added to the sediment components during the mixing process.
Test vessels :	300mL glass beakers with a notch cut out which was covered with 40-mesh nylon for drainage.
Renewal of water :	During the exposure, the overlying water was renewed by adding two volume additions of water per test vessel per day using an intermittent delivery system in combination with a calibrated water-distribution system. The water delivery system cycled approximately 7 times per day, providing approximately 350 mL per vessel every 24 hours.
Test conditions	Temperature: 22 - 23°C (daily measurements), 21-24°C (instantaneous measurements) Photoperiod: 16/8 hours, 680 - 940 lux pH: 6.2 – 7.2 Dissolved oxygen (mg/L): 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 were measured in the overlying water of each replicate vessel on days 0, 28, 29 and 42 of the test; dissolved oxygen and temperature were also measured in one alternating replicate of each treatment level of the control and treatment groups during the remianing test days. Total hardness, alkalinity and conductivity were measured on days 0, 28, 29 and 42 from a composite sample . pH and ammonia (as nitrogen) concentration were measured in a pore water sample of the control and the highest treatment level (100 mg/kg) on days 0, 14 and 28. 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. Physical characteristics of the sediment/overlying water were recorded daily. Daily observations of organism behavior (e.g., adverse effects) were made and amphipod survival was recorded at day 28, 35 and 42. Growth (length) was determined at test termination. Reproduction of adult amphipods was measured on days 28, 35 and 42.
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. LC50 and EC50 values were determoned where appropriate using Spearman-Karber and linear interpolation respectively. CETIS™ Version 1.8.7 was used to perform the analyses.

Test procedure

The purpose of this study was to determine the effects of BCS-CN88460, applied to sediment, on the freshwater amphipod, *Hyalella azteca*. The study was performed under static-renewal conditions for a period of 42 days. The first 28-days were conducted as a sediment-water exposure, and the latter 14-days were conducted in clean water without sediment in order to monitor reproduction.

At exposure initiation, amphipods (8 days old) were impartially added to an intermediate set of beakers by adding no more than two amphipods to each beaker until all beakers contained two amphipods. This process was repeated until all intermediate vessels contained ten amphipods. The exposure was initiated when each intermediate beaker of ten amphipods was added to each respective test vessel.

Prior to test day 28, four of the twelve replicate vessels (replicate vessels A, C, D, and F) were randomly selected. Amphipod survival and growth (length) were determined in these test vessels on test day 28 by sieving the sediment through a fine mesh net to remove all surviving amphipods. The adults from replicates A, C, D, and F were preserved in a buffered formalin solution for seven days prior to taking images for length determination. Growth was determined by measuring body length from the base of the first antenna to the tip of the third uropod along the curve of the dorsal surface to the nearest 0.01 mm using a Zeiss AxioCam ICc5 digital camera in combination with Zen lite (2011) imaging software.

The amphipods in the remaining eight replicates were also removed by sieving and survival of these organisms was recorded. Any offspring observed at this time were also enumerated and the total number recorded. The surviving adult amphipods from these replicates were then placed in 300-mL water-only exposure vessels containing a thin layer of fine silica sand (approximately 5 mL) as a substrate and were returned to the exposure system. Offspring present were discarded following enumeration. Reproduction and survival of these adult amphipods was again measured on test days 35 and 42 by removing and counting the adults and offspring in each replicate vessel. On day 35, the contents of each replicate were rinsed into a sorting tray with freshwater. Offspring were enumerated and discarded. Surviving adults were returned to their respective replicate vessels containing clean freshwater and silica sand (approximately 5 mL). At exposure termination (day 42), adult amphipods and any offspring present were again enumerated by rinsing the contents of each vessel into a sorting tray. The adult amphipods were enumerated to assess day 42 survival and preserved in a buffered formalin solution for fourteen days prior to taking images for length determination. The number of adult males and females were determined following preservation. Mature males were identified by the enlarged second gnathopod. Those amphipods not identified as males were recorded as female amphipods. In addition, the number of gravid females (identified by the presence of eggs within the brood pouch) recovered on test day 42 in each replicate was determined following preservation. Reproduction for both day 35 and 42 is expressed as the number of young per adult female amphipod in each replicate based on the number of females present at day 42. The day 42 growth (measured as length) of

surviving, preserved amphipods was determined after exposure termination and measured using the same process to measure length on individuals from day 28.

Results:

Validity criteria

Validity criteria according to EPA 100.4	**Required**	**Obtained**
Mean survival in control	≥80%	94% (control) 99% (solvent control)
Mean length in control	3.2mm	5.55 (control) 5.71 (solvent control)
Mean offspring/female in the control	> 2 offspring/female	9.8 (control) 4.3 (solvent control)
Hardness, alkalinity and ammonia	Should not vary by >50%	**Ammonia and alkalinity varied by >50%** Hardness varied by <50%
Dissolved oxygen	2.5 mg/L	2.7 – 8.4 mg/L
Temperature	23±1°C (mean) 23±3°C (instantaneous)	22 - 23°C (mean) 21 – 24°C (instantaneous)

Validity criteria were met for all parameters with the exception of ammonia and hardness which varied by >50% during the study.

Analytical results:

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

Table B.9.2.5-13: Concentrations of BCS-CN88460 in sediment samples during the chronic exposure of amphipods (*Hyalella azteca*).

Nominal sediment concentration (mg a.s./kg)	**Measured sediment concentrations (mg a.s./kg)**				**% of nominal concentration**		
	Day 0	**Day 14**	**Day 28**	**Arithmetic mean**	**Day 0**	**Day 14**	**Day 28**
6.3	5.8	5.8	5.8	5.8	92	92	92
13	12	12	12	11	88	88	88
25	26	19	22	22	104	76	88
50	42	45	46	44	84	90	92
100	97	95	95	95	97	95	95

Table B.9.2.5-14: Concentrations of BCS-CN88460 in pore and overlying water samples during the chronic exposure of amphipods (*Hyalella azteca*).

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 survival of *H.aquatica* following exposure to BCS-CN88460 after 28, 35 and 42 days is presented in Table B.9.2.5-15 below.

Table B.9.2.5-15: 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)**			**Reduction relative to control (%)**		
	Day 28	**Day 35**	**Day 42**	**Day 28**	**Day 35**	**Day 42**
Control	94 (9)	93 (10)	91 (10)	-	-	-
Solvent control	99 (3)	98 (5)	96 (5)	-	-	-
5.8	95 (7)	91 (8)	89 (8)	-1.1	2.2	2.2
11	96 (14)	99 (4)	95 (9)	-2.1	-6.5	-4.4
22	96 (8)	95 (8)	90 (13)	-2.1	-2.2	1.1
44	97 (7)	96 (7)	95 (8)	-3.2	-3.2	-4.4
95	86 (14)	81 (14)*	81 (14)	8.5	12.9*	11.0

SD = Standard deviation

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

Table B.9.2.5-16: Mean length per amphipod during the chronic exposure of amphipods (*Hyalella azteca*) to BCS-CN88460 on test day 28 and 42.

Arithmetic mean measured sediment concentration (mg a.s./kg)	**Mean Length per Amphipod in mm (SD)**		**Reduction relative to control (%)**	
	Day 28	**Day 42**	**Day 28**	**Day 42**
Control	5.55 (0.06)	5.95 (0.35)	-	-
Solvent control	5.71 (0.16)	5.94 (0.16)	-	-
5.8	5.59 (0.29)	5.88 (0.53)	-0.7	1.2
11	5.49 (0.17)	6.24 (0.20)	1.1	-4.9
22	5.44 (0.33)	5.82 (0.44)	2.0	2.2
44	5.75 (0.50)	6.19 (0.26)	-3.6	-4.0
95	5.50 (0.17)	6.09 (0.34)	0.9	-2.4

SD = Standard deviation

Table B.9.2.5-17: Mean number of offspring per female 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 Number of Offspring Released per Female (SD)**		**Reduction relative to control (%)**	
	Day 35	**Day 42**	**Day 35**	**Day 42**
Control	4.1 (3.2)	9.8 (7.2)	-	-
Solvent control	2.2 (3.1)	4.3 (7.3)	-	-
5.8	5.3 (2.6)	11.8 (7.9)	-29.3	-20.4
11	3.8 (3.9)	7.8 (9.3)	7.3	20.4
22	3.9 (2.4)	9.3 (5.4)	4.9	5.1
44	2.8 (2.5)	9.6 (4.9)	31.7	2.0
95	0.74 (1.4)*	3.7 (5.9)	82.0*	62.2

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

Conclusion
Statistically significant effects on mean survival and mean number of offspring were observed at the highest tested concentration of 95 mg a.s/kg sediment.
Mean length was not statistically significantly affected at any of the tested concentrations.

The endpoints proposed by the applicant based on arithmetic mean measured concentrations and statistical significance 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)
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)
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)
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)
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)
NOEC: highest concentration without a 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)
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 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)
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.

RMS comments

This study was conducted according to GLP and following EPA 600/R-99-064 guidelines.

The following was noted by the RMS :

The test guidelines state that number of adult males and females on day 42 should be determined; however this was not recorded.

Arithmetic mean measured concentrations were calculated in this study and used to calculate endpoints; however geometric mean measured concentrations should have been calculated and the endpoints based on these values instead.

Although there was no statistically significant difference between the solvent and water control, it is noted that a 46% and 56% reduction in mean offpring per female was observed in the solvent control in comparison to the water control.Treatment group results were compared to the water control. As such, it is not possible to exclude effects of the solvent on this endpoint and therefore determine effects that are due to the test item only.

It is stated that EC_{10} and EC_{20} values could not be calculated for any of the derived endpoints and NOECs were provided based on statistical significance. However, it is noted for the 35 day endpoint based on mean offspring per female, a 31.7% reduction was noted at the NOEC of 44 mg a.s./kg sediment based on statistical significance. Furthermore at day 42 a reduction of 62.2% in mean offspring per female was observed in comparison to the control. However this was not statistically significant. This indicates that the test system is not suitable for sensitively detecting potentially biologically significant effects.

Due to the reasons listed above, this study is not considered suitable for regulatory use and will not be considered further in the risk assessment.

Previous evaluation:	New data, submitted for purpose of review

A chronic study with an additional aquatic invertebrate species (*Chironomus dilutus*) was conducted for registration outside the EU. This study is summarised below.

Report:	KCA 8.2.5.4/01; Bradley, M. J.; 2017;
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
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
Reference item:	Potassium chloride (KCL) tested routinely between Sept 2014 – July 2018
Test species:	Midge *(Chironomus dilutus)*
Organism age:	First instar larvae, three days old at exposure initiation reared from egg masses obtained from Smithers Viscient's culture facility
Culturing of test organisms :	Prior to exposure initiation, newly oviposited midge egg masses were placed in 250-mL crystallizing dishes containing approximately 200 mL of laboratory well water at a temperature of approximately 23 °C. The egg masses were observed daily until egg masses hatched. Hatched midge larvae were transferred to a shallow glass bowl containing approximately 1 L of culture water (laboratory well water) and 2.5 mL of *Ankistrodesmus falcatus*, a unicellular green algae (4 × 107 cells/mL), to serve as a substrate. Midge larvae were reared under static conditions in laboratory well water with gentle, oil-free aeration. During the rearing of the midge larvae, the temperature was 23 °C and the dissolved oxygen ranged from 7.1 to 8.0 mg/L. The larvae were reared in the culture bowls for three days after hatching to provide first instar larvae for use during the exposure to BCS-CN88460.
Overlying water :	Laboratory well water : Total hardness : 62 – 78 mg/L ($CaCO_3$) Total alkalinity : 20 – 27 mg/L ($CaCO_3$) pH : 6.8 – 7.2 Conductivity : 420 – 560 µS/cm
Stock solution preparation :	A 25 mg/mL primary stock solution was prepared by placing 1.3178 g of BCS-CN88460 (1.2414 g as active ingredient) in a 50-mL volumetric flask and bringing it to volume with Acetone. Five individual dosing stock solutions were prepared in acetone for application of the test substance to the sediment at concentrations of 1.33, 2.75, 5.28, 10.6 and 21.1 mg a.s./ml.
Preparation of spiked sediment	A 10-mL volume of each dosing stock solution was applied to 0.05 kg of fine silica using a jar-rolling technique 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. The jars were sealed and positioned horizontally on a rolling mill. Each jar was then rolled for

	four hours at room temperature at approximately 15 rpm. Following four hours of rolling, the jars were stored upright in a refrigerator at 2 to 8 ºC in the dark. The sediments were allowed to equilibrate for a 7-day period in the refrigerator. Once during the 7-day equilibration period and again prior to addition into the replicate test vessels, the jars were mixed on the rolling mill for an additional two hours at room temperature. A 7-day equilibration period was deemed acceptable for sediment toxicity testing with BCS-CN88460 based on the results of a sediment-pore water equilibration trial, conducted prior to this study. Solvent controls were prepared in the same manner but with acetone only. 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 vessels :	300 mL glass vessels used in the sediment test. Emergence traps : 3.5 cm tall acrylic tubes with a 10-mesh nylon screen.
Test solutions	*Nominal sediment concentrations*: 6.3, 13, 25, 50 and 100 mg a.s./kg based on results of a previous range-finding test. *Water control*: 2.112 kg dry sediment + 0.05 kg fine silica *Solvent control*: 10 mL acetone per 2.162 kg wet sediment *Reference item*: 1300, 2200, 3600, 6000, 10000 mg KCL/L (historical results provided on request at evalution). 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):4 (M-P) No. of vessels per control (replicates): 4 (M-P) <u>Vessels for chemical analysis:</u> No. of vessels per concentration (replicates): 4 (Q-T) No. of vessels per control (replicates): 4 (Q-T) <u>Vessels for measuring representative pore water characteristics:</u> 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 (the flow rate in the system was doubled so that the water delivery system cycled approximately 14 times per day) at test day 9 due to low dissolved oxygen measurements (30 % of saturation). The calibration of the overlying water renewal system was checked prior to exposure initiation and confirmed at exposure termination. 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) ; ≤ 0.10 - 1.2 (min – max)
Sediment	Artificial sediment prepared according to OECD 218 (2004): 3.0 kg sphagnum peat (5%) 12 kg kaolin clay (20%) 45 kg fine sand (75%) 160 g powdered $CaCO_3$ (0.3%) Organic carbon content : 2.0% pH : 7.4 Water holding capacity : 17.4%
Parameters Measured / Observations	Dissolved oxygen concentration, temperature and pH were measured in overlying water of each replicate vessel of each treatment level and control used for biological monitoring at day 0, day 9, day 16 and day 61. On the remaining test days, dissolved oxygen and temperature were measured in one alternating replicate of each treatment level and control each day. 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. Total hardness, alkalinity, conductivity and ammonia in the overlying water were measured at day 0, 9, 16 and 61 in each treatment group and control solution from a composite sample of all available biological replicates. At exposure initiation, test day 16, and exposure termination, pH, and ammonia (as nitrogen) concentration were measured in a pore water sample of the negative control and 100 mg/kg treatment level. Daily observations of mortality and abnormal behavior were made beginning at exposure initiation.

	Midge larval survival and growth was assessed by sieving the sediment to remove surviving midges. Growth was measured as ash-free dry weight. Any pupae or adult midge observed at or prior to test day 16 were incorporated into the assessment of survival but only larvae were collected for the growth endpoint. 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. The emergence rate of male and female midge in each exposure vessel was determined. Mean development time represents the mean time span between the addition of midge into the replicate test vessels (day 0) and the emergence of the experimental midge. The emergence rate is the reciprocal of the development time (unit: 1/day) and represents that portion of larval development which takes place per day. Reproductive/oviposit chambers for each treatment level and control were checked daily for dead adults and egg masses. Any dead adult midge observed in the reproduction chambers were removed daily. 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. All analyses were conducted using LC/MS/MS.
Data analysis:	LOEC and NOEC values were determined using 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. The negative control and solvent control performance was determined to be statistically similar for all endpoints, with the exception of percent emergence, female emergence rate, and female days to death. 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. LC50 and EC50 values were determined using Spearman Karber or linear interpolation where appropriate. CETIS™ was used to perform the computations.

Test procedure

First instar midge larvae (3 days old) were impartially added to each respective test vessel for the control, solvent control and treatment groups. Midge larvae were exposed to BCS-CN88460 *via* the sediment for 61-days, with twelve replicates of twelve test organisms per treatment and control (i.e. a total of 144 midges per treatment level and control), to determine the biological response and four replicates per treatment and control for chemical analysis. Three additional negative controls were established for measuring pore water quality characteristics,

which did not contain larvae. Four additional replicate vessels were established and designated for auxiliary male production.
Four of twelve replicate test vessels were randomly selected prior to day 16. Midge larval survival and growth was assessed by sieving the sediment to remove surviving midges. Growth was measured as ash-free dry weight by pooling surviving larvae in each replicate and drying in an oven at 59 – 61 °C for 22 hours. Any pupae or adult midge observed at or prior to test day 16 were incorporated into the assessment of survival but only larvae were collected for the growth endpoint.

Starting on test day 16 and daily thereafter, the emerged male and female midges were collected on a replicate basis from the emergence traps and placed in reproductive/oviposit chambers.cOnce the midges were placed in the reproductive/oviposit chambers, approximately 50 mL of laboratory well water was added to the Petri dish. Male and female midges from each treatment level were held individually until sufficient numbers were available to pair male/female midges. Survival of individual midges (male and female) was recorded daily until death. Auxiliary males were used to mate female midges towards the end of the female emergence period as male midges typically start to emerge 5 to 7 days prior to female midges. Each male could be used for mating with females from corresponding treatment levels for up to 5 days and for breeding with more than one new emergent female from corresponding treatment levels. Males from different replicates within the same treatment level were paired with females of replicates where no males had emerged.

The number of eggs produced in each primary egg mass laid by female midges in each treatment level and control were counted the day the egg mass was laid, using the ring method. Five rings of eggs in each egg mass were selected at about equal distances along the length of the egg mass and the number of eggs in these five rings was then counted using a dissecting microscope. The mean number of eggs per ring was then multiplied times the number of rings in the egg mass to estimate the total number of eggs. Egg masses were incubated in cups containing 20 mL of laboratory well water. The number of unhatched eggs was recorded after 6 days of incubation and hatching success determined.

Results

Validity criteria

Validity criteria according to EPA 100.5	Required	Obtained
Average size in the control at 20 d	0.48 mg/larvae AFDW or 0.6 mg/larvae as dry weight	2.27 mg/larvae AFDW at day 16 (negative control) 2.68 mg/larvae AFDW at day 16 (solvent control)
Average survival in control	≥70 % on day 20 ≥65 % at test termination	96% at day 16 (negative control) 94% at day 16 (solvent control) Age at test termination was not stated
Emergence in control	≥ 50 %	71% (negative control) 86% (solvent control)
Time to death after emergence in control	Males < 6.5 days Females < 5.1 days	Males: 3.3 (negative control) Males: 3.7 (solvent control) Females: 3.1 (negative control Females: 3.6 (solvent control)
Mean number of eggs/egg case in control	≥800	1077 (negative control) 1107 (solvent control)
% egg hatch in control	≥ 80 %	**79% (negative control)** 87% (solvent control)
Dissolved oxygen	> 2.5 mg/L	2.5 – 8.8 mg/L
Hardness, alkalinity and ammonia	Should not vary by > 50 % during the test	**Ammonia and alkalinity varied by >50% in the negative control during the course of the test**

Validity criteria in **bold** are not met.

As the current data requirement for testing of a sediment dweller cites OECD 218 (2004) as a suitable study design, the performance of the study has also been checked against the validity criteria in OECD 218 (2004) below:

Validity criteria according to OECD 218/219 (2004)	Required	Obtained
Emergence in controls	≥ 70 % by the end of the test	71% (negative control) 86% (solvent control)
Emergence time to adults in the controls	20 – 65 days	20.2 for males and 22.4 for females (negative control) **19.3 for males** and 20.2 for females (solvent control)
Dissolved oxygen at end of test in all test vessels	≥ 60 %	84 – 102%
pH at end of test in all vessels	6 - 9	6.1 – 7.6
Water temperature	Should not differ by ± 1.0 °C	**Differed by up to 2°C**

Validity criteria in **bold** are not met.

Reference item

Following a request for additional information at evaluation, the RMS confirmed the historical sensitivity range of *C.dilutus* tested with potassium chloride; LC50 values ranged from 5223 – 7102 mg KCL/L.

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.

Table B.9.2.5-18: Mean measured sediment concentration of BCS-CN88460.

Nominal sediment concentration (mg a.s./kg)	**Measured sediment concentration (mg a.s./kg dw)**				**% of nominal concentration**			
	Day 0	**Day 16**	**Day 61**	**Arithmetic mean measured**	**Day 0**	**Day 16**	**Day 61**	**Mean**
6.3	5.9	5.3	5.2	5.5	94	84	83	87
13	13	12	9.4	11	100	92	72	87
25	23	21	19	21	92	84	76	83
50	43	44	40	42	86	88	80	85
100	93	81	80	85	93	81	80	85

Table B.9.2.5-19: Mean measured concentration of BCS-CN88460 in overlying water and pore water.

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):
On test day 16, survival observed among midge exposed to the to 6.3, 13, 25 50 and 100mg a.s./kg sediment (nominal) 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 to 6.3, 13, 25 50 and 100mg a.s./kg sediment (nominal) 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) as shown in Table B.9.2.5-20.

Table B.9.2.5-20: Mean percent survival and mean ash-free dry weight during the life-cycle exposure with BCS-CN88460 and midge (*Chironomus dilutus*).

Nominal Sediment Concentration (mg a.s./kg sediment)	Mean Percent Survival (SD)	Reduction relative to neg. control (%)	Mean Ash-Free Dry Weight Per Larvae in mg (SD)	Reduction relative to neg. control (%)
Negative Control	96 (5)	0.0	2.27 (0.56)	0.00
Solvent Control	94 (8)	2.1	2.68 (0.56)	-18.06
6.3	96 (5)	0.0	1.72 (0.34)	24.23
13	92 (12)	4.2	1.87 (0.15)	17.62
25	88 (14)	8.3	2.09 (0.40)	7.93
50	98 (4)	-2.1	1.85 (0.08)	18.50
100	96 (5)	0.0	2.26 (0.47)	0.44

SD = Standard deviation

Mean percent emergence and mean emergence rate during the life-cycle exposure with BCS-CN88460 and midge (Chironomus dilutus).
Mean percent emergence among midges exposed to 6.3, 13, 25 50 and 100mg a.s./kg sediment (nominal) 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 to 6.3, 13, 25 50 and 100mg a.s./kg sediment (nominal) 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 to 6.3, 13, 25 50 and 100mg a.s./kg sediment (nominal) 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).

Table B.9.2.5-21: Mean percent emergence and emergence rate during the life-cycle exposure with BCS-CN88460 and midge (*Chironomus dilutus*).

Nominal sediment concentration (mg a.s./kg sediment)	**% Emergence**		**Male Emergence Rate**		**Female Emergence Rate**	
	Mean (SD)	**Reduction relative to the neg. control (%)**	**Mean (SD)**	**Reduction relative to the neg. control (%)**	**Mean (SD)**	**Reduction relative to the neg. control (%)**
Negative Control	71 (14)	0	0.0496 (0.0051)	0.00	0.0447 (0.0051)	0.00
Solvent Control	86 (8)	-21.1	0.0518 (0.0058)	-4.44	0.0496 (0.0032)	-11.0
6.3	73 (12)	-2.8	0.0544 (0.0054)	-9.68	0.0473 (0.0037)	-5.8
13	82 (8)	-15.5	0.0519 (0.0020)	-4.64	0.0493 (0.0020)	-10.3
25	82 (11)	-15.5	0.0542 (0.0036)	-9.27	0.0454 (0.0035)	-1.6
50	81 (10)	-14.1	0.0538 (0.0041)	-8.47	0.0478 (0.0056)	-6.9
100	66 (9)	7.0	0.0493 (0.0055)	0.60	0.0459 (0.0049)	-2.7

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 to 6.3, 13, 25 50 and 100mg a.s./kg sediment (nominal) 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 to 6.3, 13, 25 50 and 100mg a.s./kg sediment (nominal) 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).

Table B.9.2.5-22: Mean male and females days to death during the life-cycle exposure with BCS-CN88460 and midge (*Chironomus dilutus*).

Nominal sediment concentration (mg a.s./kg sediment)	Mean Male Days to Death (SD)	Reduction relative to neg. control (%)	Mean Female Days to Death (SD)	Reduction relative to neg. control (%)
Negative Control	3.3 (0.93)	0.0	3.1 (0.39)	0.0
Solvent Control	3.7 (1.0)	-12.1	3.6 (0.45)	-16.1
6.3	3.4 (1.14)	-3.03	3.3 (0.87)	-6.5
13	3.4 (0.55)	-3.03	3.2 (0.42)	-3.2
25	3.2 (1.12)	3.03	3.1 (0.29)	0.0
50	3.4 (1.03)	-3.03	3.4 (0.33)	-9.7
100	3.3 (0.90)	0.00	3.5 (0.34)	-12.9

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. The mean number of eggs per egg mass among midges exposed to the to 6.3, 13, 25 50 and 100mg a.s./kg sediment (nominal) 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 to 6.3, 13, 25 50 and 100mg a.s./kg sediment (nominal) 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 to 6.3, 13, 25 50 and 100mg a.s./kg sediment (nominal) 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 to 6.3, 13, 25 50 and 100mg a.s./kg sediment (nominal) 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 to 6.3, 13, 25 50 and 100mg a.s./kg sediment (nominal) 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).

Table B.9.2.5-23: Mean eggs per egg mass and percent hatch during the life-cycle exposure with BCS-CN88460 and midge (*Chironomus dilutus*).

Nominal sediment concentration (mg a.s./kg sediment)	Mean Eggs per Egg Mass (SD)	Reduction relative to neg. control (%)	Mean Percent Hatch (SD)	Reduction relative to neg. control (%)
Negative Control	1077 (120)	0.00	79 (35.2)	0.00
Solvent Control	1107 (117)	-2.79	87 (14.1)	-34.33
6.3	1247 (264)	-15.78	91 (3.4)	-15.19
13	1077 (131)	0.00	95 (4.1)	-20.25
25	1157 (130)	-7.43	95 (3.7)	-20.25
50	1204 (187)	-11.79	98 (1.2)	-24.05
100	1198 (116)	-11.23	93 (8.3)	-17.72

SD = Standard deviation

Table B.9.2.5-24: Mean egg masses per mated female and days to oviposition during the life-cycle exposure with BCS-CN88460 and midge (*Chironomus dilutus*).

Nominal sediment concentration (mg a.s./kg)	Mean Egg Masses per Mated Female (SD)	Reduction relative to neg. control (%)	Mean Number of Eggs per Mated Female (SD)	Reduction relative to neg. control (%)	Mean Days to Oviposition (SD)	Reduction relative to neg. control (%)
Negative Control	0.67 (0.36)	0.00	828 (313)	0.00	1.0 (0.080)	0.00
Solvent Control	0.90 (0.14)	-2.79	1002 (210)	-21.01	1.1 (0.14)	-10.00
6.3	0.74 (0.060)	-10.45	911 (155)	-10.02	1.0 (0.090)	0.00
13	0.95 (0.090)	-41.79	1034 (191)	-24.88	1.1 (0.16)	-10.00
25	0.83 (0.19)	-23.88	961 (271)	-16.06	1.0 (0.040)	0.00
50	0.89 (0.10)	-32.84	1077 (253)	-30.07	1.0 (0.060)	0.00
100	0.93 (0.10)	-38.81	1109 (127)	-33.94	1.1 (0.11)	-10.00

Conclusion

No statistically significant effects up to and including the highest concentration (100 mg a.s./kg sediment) were observed. The NOEC for all tested endpoints is 100 mg a.s./kg sediment.

RMS comments

This study was conducted following EPA Test Method 100.5 and OECD 218 (2004). The study was conducted according to GLP. The following was noted by the RMS:

EPA 100.5 test guidelines state that larvae < 24 hours old must be used in the test; however the larvae used in this test were 3 days old. Starting a test with substantially older organisms may compromise the emergence and reproductive endpoints, as well as starting organism sensitivity. However according to OECD 218, first instar

larvae 2 – 3 days post-hatching should be used in the test. Therefore this is considered to be acceptable by the RMS for EU regulatory endpoint derivation.

According to EPA 100.5, four replicates should be selected for determination of larval growth and midge survival on day 20, emergence traps should also be set up at this point. However in this study midge survival and larval growth was measured on day 16 and emergence traps were set up on day 16. It is not clear from the study report why this is the case although it is noted that 3 day old larvae were used in the test, which is likely to have affected the time to emergence.

It is noted that for mean percent emergence, female emergence rate, and female days to death a statistically significant difference was observed between solvent and negative control groups. As the solvent control performed better than the negative control in these cases, this is not considered to invalidate the results.

No toxic reference item was included in the test, adding some uncertainty to the sensitivity of the test organisms used in the test. Following a request for additional information at evaluation, the RMS confirmed the historical sensitivity range of *C.dilutus* tested with potassium chloride; LC50 values ranged from 5223 – 7102 mg KCL/L.

Hardness, alkalinity and ammonia in the overlying water typically should not vary by > 50 % during the test according to EPA 100.5. These conditions were met for hardness; however ammonia and alkalinity did vary from a minimum of < 0.10 mg/L to a maximum of 0.80 – 1.2 mg/L for ammonia and 12 – 26 mg/L for alkalinity across concentrations during the test, which is a variation of > 50 %. The validity criterion for % hatch was also not met for the negative control ; 79% hatch was observed rather than the 80% hatch specfied in the test guidelines, although this was met for the solvent control (87%). In addition, age at test termination was not stated, therefore it cannot be confirmed if this validity criterion was met.

Arithmetic mean measured concentrations were calculated as the test item degraded by >20% during the test in some concentrations.This approach is incorrect as it does not take into account the variation in sampling periods during the test. Calculating in this way would give falsely even weighting when clearly the 2nd - 3rd recovery period represents a larger proportion of the exposure period. Therefore the geometric mean measured concentration should have been calculated using the formula provided in OECD 23 (2000). Following a request for additional information, the applicant was requested to recalculate endpoints based on OECD 23; however it was stated that at the test concentration defining the NOEC, recoveries were 80 – 93% of nominal. The RMS agrees with this; however as values were within 20% of nominal, the results should be based on nominal concentrations.

As the current data requirement for testing of a sediment dweller cites OECD 218 (2004) as the only suitable study design, the performance of the study has also been checked against the validity criteria in OECD 218 (2004). It is noted that the dissolved oxygen results were reported as a range during the test and as such it is not clear whether >60% dissolved oxygen was observed as the end of the test as specified in OECD 218 ; it is stated in the study report that on test day 9 dissolved oxygen concentrations among all treatment levels and controls were generally low with some replicates as low as 30% air saturation (2.6 mg/L at 23°C). The renewal rate of the overlying water was subsequently doubled in order to increase dissolved oxygen levels in all replicates. This would indicate that dissolved oxygen levels <60% were observed on test day 9, which was then resolved by increasing the renewal rate of the water; however it is not possible to confirm that this is the case from the study report. Following a request for additional information at evalution, the applicant confirmed that dissolved oxygen concentrations in all test vessels were 84 – 102%; as such this validity criterion is met.

OECD 218 (2004) criteria state that the temperature must not differ by >1.0°C; in this study there was a difference of 2.0°C. In general, the controls met the validity criteria, indicating that this did not have a significant effect on control performance; however the time to emergence of males in the solvent control was 19.3 days, which is below the 20 – 65 days specified in the validity criteria of OECD 218 (2004). However, as there was no statistically significant difference in emergence rate of males between the solvent and negative control for comparison to the test groups, this is not considered to invalidate the test results.

It is noted that >10% effects on mean ash-free dry weight were observed at 5.5, 11 and 42 mg a.s./kg sediment, no clear dose-response is followed and the results were not statistically significantly different from the control. Following a request for additional information at evaluation, the applicant provided historical results that demonstrated that this variability was apparent in other runs of the test. As such, the RMS agrees that reliance on this parameter to set an endpoint from this study is likely to be inaccurate and endpoints should be based on parameters based on reproductive output and emergence instead. In addition, the range of weight measurements are from 1.72 – 2.68 mg, which is a relatively small range. As such, the ability to detect effects due to the test item and the accuracy of this endpoint due to the small values is questionable.

For the sediment analysis, the method is acceptably validated in accordance with SANCO/3029/99 rev.4 and is suitable for the determination of isoflucypram in samples of sediment at levels between 3 and 100 mg/kg. The requirements of SANCO/3029/99 rev.4 have not been met for the water method but the RMS considers that the method is sufficiently accurate and precise as to be considered fit for purpose (see section B5.1.2.6.2 of the CA document for further details).

This study is considered valid and the endpoint confirmed for use in the risk assessment is:

NOEC: 100 mg a.s./kg sediment.

B.9.2.6. Effects on algal growth

B.9.2.6.1. Effects on growth of green algae

Active substance

Previous evaluation:	None; new active substance application.

Report:	KCA 8.2.6.1/01; Kuhl, K.; 2017
Title:	Pseudokirchneriella subcapitata growth inhibition test with BCS-CN88460 (tech.)
Report No.:	EBLNN050
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)
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	Stock cultures of algae were kept at 22 ± 2 °C with 24 hours light (4.50 – 7.00 klux). Test vessels were placed on a tablet rotating 100 rpm to prevent sedimentation of the cells. All operations were conducted under sterile conditions. 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. Pre cultures were prepared from stock cultures. 400 µL of 7-10 days old stock culture was transferred into 300 mL cotton plugged Erlenmeyer flask containing about 100 mL of nutrient medium every 7-10 days.
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: 0.020, 0.062, 0.196, 0.598, 1.82 and 2.02 mg a.s. /L. Controls: water and solvent controls (dimethylformamide at 0.1 µL/mL).

	Prior to the test the stock solution was prepared by solving 848.7 mg of the test substance and 10 mL dimethylformamid (DMF as the solvent) by intense stirring for 5 minutes. An adequate amount of the stock solution was transferred to a dilution series to obtain the concentration levels used in the study. The medium for the pre-culture and the definite test was prepared 5 days prior to the test. Analytical grade salts were dissolved in purified (Milli-Q) water. 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	Number of treatments: 6 (+ water and solvent controls) Number of replicates per group: 4
Exposure	Static Total exposure duration: 96 hours
Initial cell density	104 cells/mL at test initiation
Test conditions	Test units: 300 mL Erlenmeyer flasks sealed with cellulose plugs and placed in a growth incubator. Temperature: 22.2 – 23.4°C Photoperiod: continuous light Light intensity at surface of test vessels: 4520 to 4950 lux. Exposure of flasks to permanent light was made more uniform by randomised repositioning of the individual replicates using a computer-generated random number list after each observation. pH (0 – 96 h): 7.7 – 9.6 (not in same replicate. Max range in same replicate was 7.9 – 9.6) 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. Once each replicate was set up, the measurements of cell biomass and the concentration of BCS-CN88460 were taken at regular intervals. Cell numbers per volume were estimated after 24, 48, 72 and 96 hours using a photometer. Cell morphology (e.g. cell size) was also observed at these time points using a microscope (magnified x 400).
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. The samples were centrifuged for 10 minutes at 11200 g before sampling as the concentrations chosen were above the limit of solubility in the used medium.
Data analysis	ECx values (e.g. x = 50) and confidence intervals were calculated for the standard exposure period, using a commercial program (ToxRatPro 3.2.1). The LOEC was determined using ANOVA procedure and t-tests.

p.m. = pure metabolite

Results:

Validation criteria

The validity criteria were judged against 72 hour results of the pooled controls for OECD 201 (2011)

Test guideline	Criterion	Required Result	Result Obtained
OECD 201 (2011)	Biomass in the control cultures	Increase by a factor of 16	increased by a factor of 207.6 - 232.6
	Mean coefficient of variation for section-by-section specific growth rates in the control cultures	≤ 35%.	14.8%
	Coefficient of variation of average specific growth rates during the whole test period in the control cultures	≤ 7%	1.3%

All of these criteria were met.

Analytical results:

Some recoveries were not within the range of 80 – 120% of nominal (see Table B.9.2.6.1-1). Thus biological results after 72 hours and 96 hours are based on geometric mean measured concentrations of BCS-CN88460. 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.

Table B.9.2.6.1-1: Analytical measurements of BCS-BC88460

Nominal Concentration (mg a.s./L)	Geometric mean measured concentrations (mg a.s./L) [a]	Mean actual value (mg/L) / corresponding % of nominal concentrations		
		0-hour	72-hour	96-hour
0.0238	0.0200	0.0190 / 79.8	0.0210 / 88.2	0.0202 / 84.9
0.0763	0.0620	0.0581 / 76.1	0.0661 / 86.6	0.0656 / 86.0
0.244	0.196	0.189 / 77.5	0.203 / 83.2	0.201 / 82.4
0.781	0.598	0.567 / 72.6	0.631 / 80.8	0.627 / 80.3
2.50	1.82	2.54 / 102	1.30 / 52.0	0.983 / 39.3
8.00	2.02	2.14 / 26.8	1.91 / 23.9	1.42 / 17.8

[a] Geomean was calculated for the time period 0-72 hours as this is the time period over which the endpoints are derived due to the OECD guideline for European evaluation.

Biological results:

Table B.9.2.6.1-2 shows the effect of the test item on growth rate and yield.

Table B.9.2.6.1-2: Growth rate and Yield of *P.subcapitata* after exposure to BCS-CN88460

Measured conc. [mg a.s./L]	Growth rate µ [day-1] and inhibition Ir [%]				Yield y (x 104 cells/mL) and inhibition Iy [%]			
	0-72 hours		0-96 hours		0-72 hours		0-96 hours	
	µ	Ir	µ	Ir	y	Iy	y	Iy
Pooled controla	1.448	N/A	1.348	N/A	76.2	0	218.7	0
0.0200	1.438	0.7	1.336	0.9	73.9	3.0	208.5	4.7
0.0620	1.446	0.2	1.339	0.7	75.5	0.9	210.8	3.6
0.196	1.439	0.6	1.337	0.8	74.1	2.8	209.6	4.1
0.598	1.402	3.2*	1.313	2.6*	66.2	13.2*	190.4	12.9*
1.82	1.317	9.0*	1.253	7.0*	51.2	32.8*	149.8	31.5*
2.02	1.366	5.7*	1.235	8.3*	60.0	21.3*	139.1	36.4*

Results compared to pooled control as there is no statistically-significant difference between the water and the solvent control results.
*significantly (α=0.05, one-sided smaller) reduced, based on Williams multiple sequential t-test procedure

Table B.9.2.6.1-3 shows the effect of the test item on area under the growth curve.

Table B.9.2.6.1-3: Area under the growth curve for *P.subcapitata* after exposure to BCS-CN88460

Mean measured concentration (mg a.s./L)	Area under the growth curve (AUGC) (× 104 cells/ml)							
	24 h		48 h		72 h		96 h	
	biomass	%	biomass	%	biomass	%	biomass	%
Pooled control	51.9	N/A	313.7	N/A	1438.1	N/A	4976.5	N/A
0.0200	51.9	0.0	305.7	2.5	1394.8	3.0	4783.6	3.9
0.0620	56.6	-9.0	326.0	-3.9	1445.4	-0.5	4881.1	1.9
0.196	39.6	23.8*	286.9	8.5*	1383.6	3.8	4787.9	3.8
0.598	38.0	26.7*	277.1	11.7*	1272.1	11.5*	4350.2	12.6*
1.82	32.0	38.3*	224.0	28.6*	998.5	30.6*	3411.0	31.5*
2.02	25.9	50.0*	235.4	25.0*	1138.3	20.8*	3526.8	29.1*

Note; a negative inhibition indicates a growth relative to the pooled control
*significantly (α=0.05, one-sided smaller) reduced, based on Williams multiple sequential t-test procedure

Conclusion

Table B.9.2.6.1-4 shows the endpoints of the study

Table B.9.2.6.1-4: Study endpoints for *P.subcapitata* exposed to BCS-CN88460

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 a significant effect compared to the control	0.598 mg a.s./L
NOE_rC 72 hours: highest concentration without a 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 a significant effect compared to the control	0.598 mg a.s./L
NOE_yC 72 hours: highest concentration without a significant effect compared to the control	0.196 mg a.s./L

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

RMS Comments:

This study was compliant with GLP. The relevant validity criteria are met.

It is noted that the pH (0 – 96 h): deviated by more than 1.5 units in one of the control replicates (the values ranged from 7.9 – 9.6). However, this deviation had not occurred by 72 hours and it does not seem to have affected the growth of the algae as the control meets all validity criteria.

Following a request for additional information, the applicant provided toxicity information on the positive control (potassium dichromate) and stated that the 72-hour EC50 was 1.52 mg test item/L (95% C.I: 1.45 – 1.59). OECD 201 (2011) does not specify a historical toxicity range of potassium dichromate to *P.subcapitata*, but ISO 8692 (2012) states that the range is 0.92 – 1.46 mg/L. This indicates that either the test system or the test species used in this study was not sufficiently sensitive. However, it is noted that for the parameter of growth rate, there were very little effects at the top dose (8.3%), therefore the ErC50 of >2.02 mg a.s./L is considered reliable and appropriate for use in the risk assessment.

The method is acceptably validated in accordance with SANCO/3029/99 rev.4 and is suitable for the determination of isoflucypram in samples of test water at a LOQ of 0.0625 µg/L (see section B5.1.2.6.1 of the CA document for further details).

The endpoint relevant for risk assessment is 72 hour **E_rC_{50}: > 2.02 mg a.s. /L**

Metabolite (M12)

Previous evaluation:	None; new active substance application.

Report:	KCA 8.2.6.1/02; Kuhl, K.; 2017
Title:	Pseudokirchneriella subcapitata growth inhibition test with BCS-CN88460-carboxylic-acid (BCS-CY26497)
Report No.:	EBLNN290
Guideline(s):	OECD Guideline 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test (July 28, 2011) OCSPP Guideline 850.4500: Algal Toxicity (January 2012)
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 ECx 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	Stock cultures of algae were kept at 22 ± 2 °C with 24 hours light (4.50 – 7.00 klux). Test vessels were placed on a tablet rotating 100 rpm to prevent sedimentation of the cells. All operations were conducted under sterile conditions. 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. Pre cultures were prepared from stock cultures. 1000 µL of 7-10 days old stock culture was transferred into 300 mL cotton plugged Erlenmeyer flask containing about 100 mL of nutrient medium every 7-10 days.
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. All concentrations are expressed as nominal except the 50.0 mg p.m/L concentration which is expressed as geometric mean measured concentration of 35.1 mg p.m/L Controls: water and solvent controls (dimethylformamide at 0.1 µL/mL) Prior to the test the stock solution was prepared by solving 1012.4 mg of the test substance and 2.00 mL dimethylformamid (DMF as the solvent) by intense stirring for 20 minutes. An adequate amount of the stock solution was transferred to a dilution series to obtain the concentration levels used in the study. The medium for the pre-culture and the definite test was prepared 3 days prior to the test. Analytical grade salts were dissolved in purified (Milli-Q) water. Evidence of undissolved material: None mentioned
Replication	Number of treatments: 5 (+ water and solvent controls) Number of replicates per group: 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	Test units: 300 mL Erlenmeyer flasks sealed with cellulose plugs and placed in a growth incubator. Temperature: 21.8 – 22.7°C Photoperiod: continuous light Light intensity at surface of test vessels: 4620 to 4940 lux Exposure of flasks to permanent light was made more uniform by randomised repositioning of the individual replicates using

	a computer-generated random number list after each observation. pH (0 – 96 h): 7.5 - 8.2 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	ECx values (e.g. x = 50) and confidence intervals were calculated for the standard exposure period, using a commercial program (ToxRatPro 3.2.1). The LOEC was determined using ANOVA procedure and t-tests.

p.m. = pure metabolite

Results:

Validation criteria
The validity criteria were judged against 72 hour results of the pooled controls for OECD 201 (2011)

Test guideline	**Criterion**	**Required Result**	**Result Obtained**
OECD 201 (2011)	Biomass in the control cultures by 72 hours	Increase by a factor of 16	Increased by a factor of 66.3 – 74.3
	Mean coefficient of variation for section-by-section specific growth rates in the control cultures	≤ 35%.	10.6%
	Coefficient of variation of average specific growth rates during the whole test period in the control cultures	≤ 7%	1.26%

All of these criteria were met.

Analytical results:
Some recoveries were not within the range of 80 – 120% of nominal (see Table B.9.2.6.1-5). Thus biological results after 72 hours and 96 hours are based on geometric 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.

Table B.9.2.6.1-5:Analytical measurements of BCS – CY26497

Nominal Concentration (mg p.m../L)	Geometric mean measured concentrations (mg a.s./L)	Mean actual value (mg/L) / corresponding % of nominal concentrations		
		0-hour	72-hour	96-hour
3.13	N/A[a]	3.47 / 111	3.45 / 110	3.37 / 108
6.25	N/A[a]	6.68 / 107	6.63 / 106	6.59 / 105
12.5	N/A[a]	13.5 / 108	13.4 / 107	13.3 / 106
25.0	N/A[a]	26.4 / 106	26.3 / 105	26.0 / 104
50.0	35.1[b]	35.0 / 70.0	35.3 / 70.6	35.5 / 71

[a] As the % of nominal was within 20% of nominal, geometric mean concentrations have not been calculated and the nominal value is accepted.
[b] Geomean was calculated for the time period 0-72 hours as this is the time period over which the endpoints are derived due to the OECD guideline for European evaluation. It is noted that the % of metabolite did not decrease by any notable amount from 72 to 96 hours.

Biological results:

Table B.9.2.6.1-6 shows the effect of the test item on growth rate and yield.

Table B.9.2.6.1-6: Growth rate and Yield of *P.subcapitata* after exposure to CN88460-carboxylic acid (BCS-CY26497)

Metabolite conc.	Growth rate μ [day^{-1}] and inhibition I_r [%]				Yield y (x 10^4 cells/mL) and inhibition I_y [%]			
	0-72 hours		0-96 hours		0-72 hours		0-96 hours	
[mg p.m./L]	μ	I_r	μ	I_r	y	I_y	y	I_y
Pooled control[a]	1.423	N/A	1.325	N/A	70.6	N/A	199.6	0.0
3.13	1.425	-0.1	1.328	-0.2	70.9	-0.5	202.1	-1.3
6.25	1.405	1.3	1.316	0.7	66.6	5.6	192.1	3.8
12.5	1.426	-0.2	1.325	0.0	71.2	-0.9	200.0	-0.2
25.0	1.447	-1.7	1.326	0.0	76.3	-8.1	200.4	-0.4
35.1	1.398	1.8*	1.304	1.6*	65.2	7.6*	183.5	8.1*

[a)] Results compared to pooled control as there is no statistically-significant difference between the water and the solvent control results.
*Significantly (α = 0.05, one-sided smaller) reduced, based on Multiple sequentially-rejective Welsh-t test after Bonferroni Holm

Table B.9.2.6.1-7 shows the effect of the test item on area under the growth curve.

Table B.9.2.6.1-7: Area under the growth curve for *P.subcapitata* after exposure to CN88460-carboxylic acid (BCS-CY26497)

Metabolite concentration (mg p.m./L)	Area under the growth curve (AUGC) ($\times 10^4$ cells/ml)							
	24 h		48 h		72 h		96 h	
	biomass	%	biomass	%	biomass	%	biomass	%
Pooled control	30.4	0.0	241.7	0.0	1269.6	0.0	4511.6	0.0
3.13	28.9	5.0	237.1	1.9	1267.3	0.2	4543.5	-0.7
6.25	31.9	-5.0	239.8	0.8	1215.1	4.3	4319.4	4.3
12.5	31.2	-2.5	251.7	-4.1	1295.9	-2.1	4550.5	-0.9
25.0	31.2	-2.5	251.6	-4.1	1356.1	-6.8	4676.0	-3.6
35.1	25.9	14.9	229.4	5.1	1189.8	6.3*	4174.2	7.5*

Note; a negative inhibition indicates a growth relative to the pooled control
*Significantly ($\alpha = 0.05$, one-sided smaller) reduced, based on Multiple sequentially-rejective Welsh-t test after Bonferroni Holm

No morphological change in algae was observed in any test concentration.

Conclusion

The study meets the validity criteria and the endpoints 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 a significant effect compared to the control	35.1 mg p.m./L
NOE_rC 72 hours: highest concentration without a 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 a significant effect compared to the control	35.1 mg p.m./L
NOE_yC 72 hours: highest concentration without a significant effect compared to the control	25 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 a significant effect compared to the control	35.1 mg p.m./L
NOE_bC 72 hours: highest concentration without a significant effect compared to the control	25 mg p.m./L

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

At the highest test item concentration of 35.1 mg p.m./L a statistically-significant growth reduction was observed. 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.

RMS Comments:

This study was compliant with GLP. The relevant validity criteria are met. The 72h E_rC_{50} value is recommended rather than the 96 hour value as the test item did not decrease by a notable amount from 72 – 96 hours and the magnitude of effects between these times was minimal.

Following a request for additional information, the applicant provided toxicity information on the positive control (potassium dichromate) and stated that the 72-hour EC50 was 1.52 mg test item/L (95% C.I: 1.45 – 1.59). OECD 201 (2011) does not specify a historical toxicity range of potassium dichromate to *P.subcapitata*, but ISO 8692 (2012) states that the range is 0.92 – 1.46 mg/L. This indicates that either the test system or the test species used in this study was not sufficiently sensitive. However, it is noted that for the parameter of growth rate, there were very little effects at the top dose (<2%), therefore the ErC50 of >35.1 mg p.m./L is considered reliable and appropriate for use in the risk assessment.

The method is acceptably validated in accordance with SANCO/3029/99 rev.4 and is suitable for the determination of BCS-CN88460-carboxylic-acid in samples of test water at a LOQ of 0.125 mg/L (see section B5.1.2.6.1 of the CA document for further details).

The endpoint relevant for risk assessment is the 72 hour E_rC_{50} > 35.1 mg p.m / L

B.9.2.6.2. Effects on growth of an additional algal species

Previous evaluation:	None; new active substance application.

Report:	KCA 8.2.6.2/01; Arnie, J. R.; Siddiqui, A. I.; Porch, J. R.; Martin, K. H.; 2017;
Title:	BCS-CN88460: A 96-hour toxicity test with the cyanobacteria (Anabaena flos-aquae)
Report No.:	149P-111
Guideline(s):	OECD 201 EU Directive 92/69/EEC, Method C.3. U.S. EPA OCSPP Number 850.4550
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	Algal cells for this study were taken from a culture that had been transferred to fresh

age/size at study initiation	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) Stock solutions were prepared by dissolving the active substance in N,N-dimethylformamide (DMF). Each stock solution was prepared independently rather than by serial dilution to minimise any solubility issues. Each test solution was prepared by diluting 100 µL of each respective stock in 1000 mL of freshwater AAP medium. All test solutions were sonicated for 2 hours and inverted 20 times to mix. A solvent control solution contained 0.1mL DMF/L, which was equivalent to the solvent concentration in all of the treatment groups. The negative control solution consisted of freshwater AAP medium without test substance. 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 treatments: 5 (+ water and solvent control) No. of replicates per group: 4 No. of vessels per control (replicates): 4
Exposure	Static Total exposure duration: 96 hours
Initial cell density	10^4 cells/mL at test initiation
Test conditions	Test units were sterile, 250-mL Erlenmeyer flasks plugged with foam stoppers, and contained 100 mL of test or control medium. The test flasks were labeled with the project number, test concentration and replicate, and were indiscriminately positioned daily on mechanical shakers in an environmental chamber Temperature: 23.4 – 23.8°C Photoperiod: continuous light Light intensity: 1940 to 2350 lux pH (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 test to determine concentrations of the test substance. The samples were centrifuged at 872 RTF (g) for 10 minutes and diluted in 50: 50 (v/v) methanol: HPLC-grade water. The samples were analysed by LC/MS/MS.
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:

Validation criteria
The validity criteria were judged against 72 and 96 hour results of the pooled controls for OECD 201 (2011)

Test guideline	Criterion	Required Result	Result Obtained
OECD 201 (2011)	Biomass in the control cultures by 72 hours	Increase by a factor of 16	72h: 51.6 -107.8 96h: 209 – 353.5
	Mean coefficient of variation for section-by-section specific growth rates in the control cultures	≤ 35%.	72h: 33.6% 96h: 32.8%
	Coefficient of variation of average specific growth rates during the whole test period in the control cultures	≤ 7%	72h: 6.1% 96h: 3.2%

Analytical results:
Some recoveries were not within the range of 80 – 120% of nominal (see Table B.9.2.6.2-1). 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. The below results are from the biotic samples for each test concentration.

Table B.9.2.6.2-2:Analytical measurements of BCS – CY26497

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

The time weighted mean measured concentrations for 96 hours (calculated as per OECD 23: Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures, 2000) were used to define endpoints as the 96 h endpoint was more critical than the 72 hour endpoint.

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.

Table B.9.2.6.2-2 shows the effect of the test item on growth rate and yield.

Table B.9.2.6.2-2: Growth rate and Yield of *A.flos-aquae* after exposure to CN88460 (BCS-CN88460)

Measured conc. [mg a.s./L]	Growth rate μ [day^{-1}] and inhibition I_r [%]				Yield y (x 10^4 cells/mL) and inhibition I_y [%]			
	0-72 hours		0-96 hours		0-72 hours		0-96 hours	
	μ	I_r	μ	I_r	y	I_y	y	I_y
Pooled control[a]	1.44	N/A	1.38	N/A	76.4	N/A	256.9375	N/A
0.02	1.42	1.4	1.36	1.4	71.7	6	245.5	4
0.072	1.39	3.5	1.44	-4.3	68.85	10	317.8750	-24
0.23	1.41	2.1	1.38	0	75.2	2	257.6250	0
0.73	1.37	4.9	1.42	-2.9	59.95	22	292.000	-14
2.1	1.42	1.4	1.38	0	70.3	8	254.1250	1
4.8	0.29	79.9	0.006	99.6*	1.85	98	1.4750	99

Results compared to pooled control as there is no statistically-significant difference between the water and the solvent control results.
Note; a negative inhibition value indicates an increase in growth rate or yield compared to the pooled control.

Table B.9.2.6.2-3 shows the effect of the test item on area under the growth curve.

Table B.9.2.6.2-3: Area under the growth curve for *A.flos-aquae* after exposure to BCS-CN88460

Geometric mean measured concentrations (mg a.s./L)	72 h		96 h	
	Area under the growth curve (biomass integral)	Inhibition of biomass integral (%)	Area under the growth curve (biomass integral)	Inhibition of biomass integral (%)
Pooled control	15726000	0	55726500	-
0.02	12270000	22	50334000	10
0.072	13248000	16	59655000	-7
0.23	14016000	11	53955000	3
0.73	12222000	22	54456000	2
2.1	13728000	13	52659000	6
4.8	435000[#]	97	795000[*]	99

[*] 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 a significant effect compared to the control	5.1 mg a.s./L
NOE_rC 72 hours: highest concentration without a 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 a significant effect compared to the control	5.1 mg a.s./L
NOE_bC 72 hours: highest concentration without a 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 a significant effect compared to the control	5.1 mg a.s./L
NOE_rC 96 hours: highest concentration without a 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 a significant effect compared to the control	4.8 mg a.s./L
NOE_rC 96 hours: highest concentration without a 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 a significant effect compared to the control	4.8 mg a.s./L
NOE_bC 96 hours: highest concentration without a 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 a significant effect compared to the control	4.8 mg a.s./L
NOE_rC 96 hours: highest concentration without a 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_{10} and EC_{20} calculation.

RMS Comments

This study was compliant with GLP. The relevant validity criteria are met.

The pH of all control and test groups (excluding the nominal 8.0 mg a.s.L group) increased by more than the recommended 1.5 units over 96 hours in OECD 201 (2011). As this deviation occurred in both test and control groups, it is not considered to have affected the comparative inhibition results of the study and therefore the study endpoints.

It is noted that there is no reference to a positive control in this study (toxic reference). OECD 201 (2011) recommends that a reference substance is tested at least twice a year as a means of checking the sensitivity of the test organism and the test procedure, although it is noted that there is no historic reference range for *A.flos-aquae*. Afte a request for additional information, the applicant confirmed that *A.flos-aquae* had not been tested with a positive control. **Therefore, the study and its endpoint should be considered with caution.**

The method is acceptably validated in accordance with SANCO/3029/99 rev.4 and is suitable for the determination of isoflucypram in samples of test water at a LOQ of 0.0024 mg/L (see section B5.1.2.6.1 of the CA document for further details).

Note, as the 96h E_rC_{50} value is lower, it is recommended in preference over the corresponding 72h value.

96h ErC50: **3.7 mg a.s./L**

Previous evaluation:	None; new active substance application.

Report:	KCA 8.2.6.2/02; Arnie, J. R.; Siddiqui, A. I.; Porch, J. R.; Martin, K. H.; 2017;
Title:	BCS-CN88460: A 96-hour toxicity test with the marine diatom (*Skeletonema costatum)*
Report No.:	149P-113
Guideline(s):	OECD 201 EU Directive 92/69/EEC, Method C.3. U.S. EPA OCSPP Number 850.4500
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	Algal cells for this study were taken from a culture that had been transferred to fresh medium three days prior to test initiation.

study 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, 0.048, 0.186, 0.589, 1.478 and 2.538 mg a.s./L Corresponding time-weighted mean measured concentrations (0 – 96 h): 0.015, 0.054, 0.20, 0.64, 1.7, 2.9 mg a.s./L Controls: water and solvent controls (dimethylformamide at 0.1 µL/mL) Stock solutions were prepared by dissolving the active substance in N,N-dimethylformamide (DMF). Each stock solution was prepared independently rather than by serial dilution to minimise any solubility issues. Each test solution was prepared by diluting 100 µL of each respective stock in 1000 mL of saltwater algal medium. All test solutions were sonicated for 2 hours and inverted 20 times to mix. A solvent control solution contained 0.1mL DMF/L, which was equivalent to the solvent concentration in all of the treatment groups. The negative control solution consisted of saltwater algal medium without test substance. 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 treatments: 5 (+ water and solvent control) No. of replicates per group: 4 No. of vessels per control (replicates): 4
Exposure	Static Total exposure duration: 96 hours
Initial cell density	10^4 cells/mL at test initiation
Test conditions	Test units were sterile, 250-mL Erlenmeyer flasks plugged with foam stoppers, and contained 100 mL of test or control medium. The test flasks were labeled with the project number, test concentration and replicate, and were indiscriminately positioned daily on mechanical shakers in an environmental chamber. Flasks were shaken continuously at 100 rpm. 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. All analytical samples were centrifuged to remove any potential precipitates as well as algal cells prior to analysis.

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. Shapiro-Wilk's test for normality, Levene's Test for Equality of Variance, Analysis of Variance and Dunnett's Test, $p<0.05$ were used to determine statistical significance of the growth rate and yield between the treatment groups and the controls.

Results:

Validation criteria
The validity criteria were judged against 72 hour results of the pooled controls for OECD 201 (2011)

Test guideline	Criterion	Required Result	Result Obtained
OECD 201 (2011)	Biomass in the control cultures by 72 hours	Increase by a factor of 16	Increased by a factor of 99 - 163
	Mean coefficient of variation for section-by-section specific growth rates in the control cultures	≤ 35%.	23.9%
	Coefficient of variation of average specific growth rates during the whole test period in the control cultures	≤ 7%	3.4%

Analytical results:
Some recoveries were not within the range of 80 – 120% of nominal (see Table B.9.2.6.2-4). 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.

The geometric mean measured concentration was calculated with the values from the biotic samples at 0 and 96 hours as the 96 hour valies are more sensitive.

Table B.9.2.6.2-4: Analysed concentrations of BCS-CN88460 in *S.costatum* toxicity test

Nominal Concentration (mg a.s./L)	Measured concentration (mg a.s./L)		Geometric mean measured concentrations after 72 hours (mg a.s./L)	Geometric mean measured concentrations after 96 hours (mg a.s./L)	% of nominal concentrations		
					0-hour	72-hour	96-hour
0.024	0h	0.0219	0.017	0.014	91	71	57
	72h	0.0132					
	96h	0.00897					
0.076	0h	0.0730	0.060	0.048	96	79	64
	72h	0.0499					
	96h	0.0313					
0.24	0h	0.259	0.218	0.186	108	91	78
	72h	0.183					
	96h	0.136					
0.78	0h	0.752	0.684	0.589	96	88	76
	72h	0.623					
	96h	0.437					
2.50	0h	2.28	1.843	1.478	91	74	59
	72h	1.49					

	96h	0.950					
8.00	0h	3.79	3.187	2.538	47	40	32
	72h	2.68					
	96h	1.61					

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.
Table Table B.9.2.6.2-5 shows the effect of the test item on growth rate and yield.

Table B.9.2.6.2-5: Growth rate and Yield of *S.costatum* after exposure to CN88460 (BCS-CN88460)

Geometric mean measured conc. [mg a.s./L]	Growth rate μ [day^{-1}] and inhibition I_r [%]				Mean yield y (cells/mL) and inhibition I_y [%]	
	0-72 hours		0-96 hours		0-72 hours	
	μ	I_r	μ	I_r	y	I_y
Pooled control	1.59	N/A	1.27	N/A	1192500	N/A
0.014	1.57	1.3	1.24	2	1095000	8
0.048	1.56	1.9	1.25	2	1087500	9
0.186	1.59	0	1.26	1	1170000	2
0.589	1.50	6	1.29	-2	892500	25*
1.478	1.31	18	1.20	6	498250	58*
2.538	1.27	20*	1.16	9*	437250	63*

Note; a negative inhibition value indicates an increase in growth rate or yield compared to the pooled control.
[#] Treatment group mean was significantly reduced (Dunnett's Test, $p < 0.05$) when compared to the pooled control

Table B.9.2.6.2-6 shows the effect of the test item on area under the growth curve.

Table B.9.2.6.2-6: Area under the growth curve for *S.costatum* after exposure to BCS-CN88460

Geometric mean measured concentrations (mg a.s./L)	72h		96h	
	Area under the growth curve (biomass integral)	Inhibition of Biomass integral (%)	Area under the growth curve (biomass integral)	Inhibition of Biomass integral (%)
Pooled control	57519000	N/A	57519000	N/A
0.014	53436000	7	53436000	7
0.048	55950000	3	55950000	3
0.186	56436000	2	56436000	2
0.589	50220000	13	50220000	13
1.478	32250000[*]	44	32250000[*]	44
2.538	28920000[*]	50	28920000[*]	50

[*] Treatment group mean was significantly reduced (Dunnett's Test, $p < 0.05$) when compared to the pooled control mean
Note; a negative inhibition value indicates an increase of biomass integral relative to the pooled control.

Conclusion
The study meets the validity criteria and the 96 hour endpoints based on geometric mean are:

E_rC_{50} 96 hours (95% C.I.):	**> 2.538 mg a.s./L (n.d.)**
E_rC_{20} 96 hours (95% C.I.) [a)]	2.3 mg a.s./L
E_rC_{10} 96 hours (95% C.I.) [a)]	0.91 mg a.s./L

LOE_rC 96 hours: lowest concentration with a significant effect compared to the control	2.538 mg a.s./L
NOE_rC 96 hours: highest concentration without a significant effect compared to the control	1.478 mg a.s./L
E_bC_{50} 96 hours (95% C.I.):	**2.538 mg a.s./L (1.70 to 2.90 mg a.s./L)**
LOE_bC 96 hours: lowest concentration with a significant effect compared to the control	1.478 mg a.s./L
NOE_bC 96 hours: highest concentration without a significant effect compared to the control	0.589 mg a.s./L
E_yC_{50} 96 hours (95% C.I.) [a)]	**1.5 mg a.s./L**
E_yC_{20} 96 hours (95% C.I.) [a)]	0.48 mg a.s./L
E_yC_{10} 96 hours (95% C.I.) [a)]	0.27 mg a.s./L
LOE_yC 96 hours: lowest concentration with a significant effect compared to the control	0.589 mg a.s./L
NOE_yC 96 hours: highest concentration without a significant effect compared to the control	0.186 mg a.s./L

a) endpoints recalculated by the applicant, according to geometric mean measured concentrations after a request for additional information

RMS Comments

This study was compliant with GLP. The relevant validity criteria are met. The 96h E_rC_{50} value is recommended rather than the 72 hour value as the test item decreased by a notable amount from 72 – 96 hours and the 96 hour endpoints are more sensitive.

It is noted that there is no reference to a positive control in this study (toxic reference). OECD 201 (2011) recommends that a reference substance is tested at least twice a year as a means of checking the sensitivity of the test organism and the test procedure. Following a request for additional information the applicant provided historical data for *S.costatum* exposed to potassium dichromate, resulting in an E_rC_{50} of >0.71 mg/L. This is outside the normal range in ISO 8692 (2012) states that the range is 0.92 – 1.46 mg/L, noting that the E_rC_{50} is a > value and therefore could be within the reference range. As this indicates that the test organisms may be of increased sensitivity the RMS does not consider this to invalidate the test.

The method is acceptably validated in accordance with SANCO/3029/99 rev.4 and is suitable for the determination of isoflucypram in samples of test water at a LOQ of 0.0024 mg/L (see section B5.1.2.6.1 of the CA document for further details).

The 96 hr E_rC_{50} to use in the risk assessment is > 2.538 mg a.s./L

Previous evaluation:	None; new active substance application.

Report: KCA 8.2.6.2/03; Arnie, J. R.; Siddiqui, A. I.; Porch, J. R.; Martin, K. H.; 2017;
Title: BCS-CN88460: A 96-hour toxicity test with the freshwater diatom (Navicula pelliculosa)

Report No.:	149P-112A
Guideline(s):	OECD 201 EU Directive 92/69/EEC, Method C.3. U.S. EPA OCSPP Number 850.4500
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	OECD 201 EU Directive 92/69/EEC, Method C.3. U.S. EPA OCSPP Number 850.4500
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 Controls: water and solvent controls (dimethylformamide at 0.1 µL/mL) Prior to the test the stock solution was prepared by dissolving calculated masses of BCS-CN8860 technical in N,N-dimethylformamid (DMF). The stocks were sonicated for 1 minuteand inverted at least 20 times to mix. Each stock solution was prepared independently rather than by serial dilution to avoid any solubility issues. 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 treatments: 6 (+ negative (water) and solvent control) 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	Test units were sterile, 250-mL Erlenmeyer flasks plugged with foam stoppers, and contained 100 mL of test or control medium. The test flasks were labeled with the project number, test concentration and replicate, and were indiscriminately positioned daily on mechanical shakers in an environmental chamber. The test flasks were shaken continuously at 100 rpm. Temperature: 24.1 – 24.3°C Photoperiod: Continuous light Light intensity at surface of test vessels: 3870 to 4600 lux pH (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. All samples were centrifuged at 872 RCF (g) for 10 minutes to remove and potential precipitates and diluted in 50: 50 (v/v) methanol: HPLC-grade water. The samples were analysed by LC/MS/MS.
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. The negative and solvent control were compared (t-test) and were significantly different for cell density and growth rate at 72h. Therefore, comparison of these parameters to a pooled control is inappropriate. Therefore, results for both controls are presented and also checked for validity. Inhibition of growth rate, yield and area under the growth curve is calculated by comparison with the solvent control as the negative control results are not valid (see validity criteria section below).

Results:

Validation criteria
The validity criteria were judged against 72 & 96 hour results of the negative and solvent controls for OECD 201 (2011).

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	Negative control: 346 Solvent control: 348
The mean coefficient of variation for section-by-section specific growth rates in the control cultures must not exceed 35%.	< 35%	72h Negative control: **43%** 96h Negative control: **77%** 72h Solvent control: 31.5% 96h Solvent control: **67.6%**
The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures must not exceed 10%.	< 10%	72h negative control: 0.34% 96h negative control: 3.2% 72h solvent control: 0.28% 96h solvent control: 3.8%

Note: Results in bold do not meet the validity criteria

The 96 hour results are not valid, nor are the negative control results. Therefore the 72 hour results as compared to the solvent control will be used to define the biological endpoints.

Analytical results:
Some recoveries were not within the range of 80 – 120% of nominal (see Table B.9.2.6.2-7 below). Thus biological results after 72 hours are based on geometric mean measured concentrations of BCS-CN88460. 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.

Table B.9.2.6.2-7:Analytical measurements of CN88460 (BCS-CN88460)

Nominal Concentration (mg a.s./L)	Measured concentration (mg a.s./L)		Geometric mean measured concentrations after 72 hours (mg a.s./L)	% of nominal concentrations		
				0-hour	72-hour	96-hour
0.024	0h	0.0156	0.014	65	60	54
	72h	0.0133				
	96h	0.0103				
0.076	0h	0.056	0.053	74	69	61
	72h	0.0496				
	96h	0.0364				
0.24	0h	0.226	0.20	94	85	76
	72h	0.184				
	96h	0.145				
0.78	0h	0.762	0.67	98	86	75
	72h	0.592				
	96h	0.448				
2.50	0h	2.24	1.80	90	71	62
	72h	1.39				
	96h	1.2				
8.00	0h	2.97	2.00	37	25	25
	72h	1.39				
	96h	1.85				

[a] Geomean was calculated for the time period 0-72 hours as this is the time period over which the endpoints are derived due to the OECD guideline for European evaluation. It is noted that the % of active substance did not decrease by any notable amount from 72 to 96 hours.

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.

Table B.9.2.6.2-8 shows the effect of the test item on growth rate and yield. The inhibition was calculated by comparing the results to the solvent control as this is the worst-case comparison, and the negative control results are not valid (see Validation Criteria above). The 96 hour results are presented but are not used to set endpoints as the 96 hour control growth rates were not valid, nor is a 96 h endpoint required for European authorisation.

Table B.9.2.6.2-8: Growth rate and Yield of *N.pelliculosa* after exposure to CN88460 (BCS-CN88460)

Measured conc. [mg a.s./L]	Growth rate μ [day^{-1}] and inhibition I_r [%]				Mean yield y (cells/mL) and inhibition I_y [%]			
	0-72 hours		0-96 hours		0-72 hours		0-96 hours	
	μ	I_r	μ	I_r	y	I_y	y	I_y
Negative control	1.95	N/A	1.49	N/A	3445000	N/A	3935000	N/A
Solvent control	1.96	N/A	1.52	N/A	3580000	N/A	4405000	N/A
0.014	1.96	0	1.48	3	3610000	-1	3695000	16
0.053	1.95	1	1.43[#]	6	3435000	4	3010000	32
0.20	1.92	2	1.49	2	3337500	7	3820000	13
0.67	1.93	1	1.49	2	3322500	7	3845000	13
1.80	1.88 [*]	4	1.46	4	2797500*	22	3520000	20
2.00	1.80 [*]	8	1.46	4	2200000*	39	3480000	21

Note : a negative inhibition indicates a higher yiled compared to the solvent control.
[#] Treatment group mean was significantly reduced (Dunnett's Test, $p < 0.05$) when compared to the the solvent control mean
[*] Treatment group mean was significantly reduced (Jonckheere-Terpstra Step-Down Trend Test, $p < 0.05$) when compared to the solvent control mean.

Table B.9.2.6.2-9 shows the effect of the test item on area under the growth curve.

Table B.9.2.6.2-9: Area under the growth curve for *N.pelliculosa* after exposure to BCS-CN88460

Geometric mean measured concentrations (mg a.s./L)	72h		96h	
	Area under the growth curve (biomass integral)	Inhibition of Biomass integral (%)	Area under the growth curve (biomass integral)	Inhibition of Biomass integral (%)
Solvent Control	68922000	-	164742000	-
0.014	62310000	10	149970000	9
0.053	58278000*	15	135618000*	18
0.20	68352000	1	154242000	6
0.67	66360000	4	152370000	8
1.80	48666000*	29	124476000*	24
2.00	36204000*	47	104364000*	37

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

Conclusion

The 72 hour endpoints based on geometric means (compared to solvent control) 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 a significant effect compared to the control	1.8 mg a.s./L
NOE_rC 72 hours: highest concentration without a 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 a significant effect compared to the control	1.8 mg a.s./L
NOE_bC 72 hours: highest concentration without a 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 a significant effect compared to the control	1.8 mg a.s./L

NOE_yC 72 hours: highest concentration without a significant effect compared to the control	0.67 mg a.s./L
LOE_yC 72 hours: lowest concentration with a significant effect compared to the control	> 2.0 mg a.s./L
NOE_yC 72 hours: highest concentration without a significant effect compared to the control	2.0 mg a.s./L

RMS comments

This study was compliant with GLP. The validity criteria are met for solvent control (72 hr results).

The pH of the solvent control and several test groups increased by more than the recommended 1.5 units over 96 hours in OECD 201 (2011). As this deviation occurred in both test and control groups, it is not considered to have affected the comparative inhibition results of the study and therefore the study endpoints.

The light intensity at surface of test vessels deviated from the recommended 4440 - 8880 lux (OECD 201) as the minimum light intensity was 3870 lux. The RMS is satisfied that any effects of this lower light intensity were experienced by all test organisms as all vessels were rotated frequently. Additionally, it is noticed that the minimum growth rate for the solvent control (validity criterion) was met. Therefore, the lower light intensity is not considered to have had an effect on the endpoints of the study.

It is noted that there is no reference to a positive control in this study (toxic reference). OECD 201 (2011) recommends that a reference substance is tested at least twice a year as a means of checking the sensitivity of the test organism and the test procedure.

Following a request for additional information the applicant provided historical data for *N.pelliculosa* exposed to potassium dichromate, resulting in an E_rC_{50} of >0.16 mg/L. This is below the range in ISO 8692 (2012) of 0.92 – 1.46 mg/L. As the E_rC_{50} is a greater-than value, this indicates that the toxic reference was tested at a rate that was lower than the range in the guidelines. As no further details regarding the level of effect at this top dose of -0.16 mg/L, the RMS cannot use this data to confirm the sensitivity of the test system. Therefore the following endpoint should be treated with caution.

It should be noted that the validity criterion for coefficient of variation was not met for the negative controls at 72 and 96h, however as results were compared to the solvent control this is not considered to invalidate the test. The coefficient of variation was also exceeded for the solvent control at 96h; as such the endpoint is based on growth rate at 72 hours.

The method is acceptably validated in accordance with SANCO/3029/99 rev.4 and is suitable for the determination of isoflucypram in samples of test water at a LOQ of 0.0024 mg/L (see section B5.1.2.6.1 of the CA document for further details).

72 h E_rC_{50} of > 2.0 mg a.s./L

B.9.2.7. Effects on aquatic macrophytes

Previous evaluation:	None; new active substance application.

Report:	KCA 8.2.7/01; Kuhl, K.; 2017;
Title:	Lemna gibba G3 - Growth inhibition test with BCS-CN88460 under semi-static conditions
Report No.:	EBLNN016
Guideline(s):	EU Directive 91/414/EEC Regulation (EC) Number 1107/2009

	US EPA OCSPP 850.4400
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	OECD Guideline 221 (2006), US EPA OSCPP 850.4400
Test species	Duckweed (*Lemna gibba*) Strain G3 from Eurofins Agroscience Services EcoChem GmbH Eutinger Straße 24, 75223 Niefern-Öschelbronn, Germany
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 Control: water Solvent control: DMF (dimethylformamide) used as solvent (0.1 mL/L test solution) Preparation of the test solution: Prior to test start and at every test medium renewal a stock solution was prepared by solving ca. 170 mg of the test substance ad 2.00 ml DMF by intense stirring for 5 minutes and ultrasonic treatment of the test solutions for 30 minutes. An adequate amount of the stock solution was transferred to a dilution series to obtain the concentration levels used in the study. Evidence of undissolved material: In the two highest test concentrations precipitates were observed
Nutrient medium	The 20X AAP medium was freshly prepared 1-2 days before test start, sterilized by membrane filtration and stored at 0 – 10 ºC. The nutrient medium used in the study was prepared by adding 20 mL/L of each stock solution to deionised water. The pH was adjusted to 7.5 ±0.1 with HCl
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, the test item was applied into freshly prepared test medium on day 0, 3 and 5. Total exposure duration: 7 days
Test conditions	Vessels: 470 mL glass dishes with 200 mL test solution Temperature: 23.6 - 24.3°C Photoperiod: permanent light Light quality: The vessels were exposed to permanent light and repositioned in randomised order after each observation. The uniform overhead illumination was provided by a bank light containing fluorescent lamps attached to the ceiling. 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. Total frond area was carried out using the LemnaTec Scanalyzer machine. 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 in 9 places within the test area.

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.). In order to remove any precipitated test substance to give an accurate presentation of the dissolved test substance, the media were centrifuged for 10 minutes at 11200 g before analytical samples were taken. 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

Validation criteria

The test results were judged against the validity criteria set out in OECD 221 (2006).

Validity criteria	Required	Obtained
Doubling time	< 2.5 days	1.8 days

The validity criterion was met.

Analytical results:

Measured concentrations between day 0 and day 7 ranged between 14 and 115% and are presented below in Table B.9.2.7-1. Therefore results are based on geometric mean measured concentrations. Further clarification of the geometric mean concentration is discussed in the RMS Comments section of this study summary. 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).

Table B.9.2.7-1: Analytical measurements of CN88460 (BCS-CN88460)

Nominal concen-tration (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	88	99	103	88	53	61	52	90	87	90
0.0763	86	91	93	107	77	83	74	103	106	109
0.244	109	115	106	84	86	106	81	98	100	84
0.781	76	108	91	80	72	79	67	95	97	115
2.50	86	84	79	73	89	82	77	93	68	71
8.00	58	34	17	14	26	36	16	52	30	43

Biological results:

Observations

There was no significant difference between the results of the control and the solvent control. Therefore the % inhibition values are given relative to the pooled controls. No statistically significant effects on plants were observed.

Table B.9.2.7-2: Biological results for *Lemna gibba* following exposure to Isoflucypram

Nominal concentration (mg a.s./L)	**Mean frond number on day 7***	**Total frond area on day 7* [mm^2]**	**% Inhibition**	
			Mean growth rate for frond number	**Mean growth rate for frond area**
Control	167	1425	-	-
Solvent control	170	1434	-	-
0.0238	183	1563	-3.1	-0.8
0.0763	184	1566	-3.3	-0.9
0.244	172	1478	-0.5	-0.5
0.781	172	1465	-0.5	-1.9
2.50	154	1294	3.6	2.8
8.00	151	1340	1.7	3.5

* Mean of 4 replicates

RMS Comments

This study was compliant with GLP and conducted according to OECD 221 (2006). The relevant validity criteria are met.

As *Lemna* spp. float on the surface, they may be exposed to substances that collect at the water-air interface. It is noted that the test item was observed at the water surface for much of the time during the test at the highest tested concentration. Under this circumstance, exposure will result from material other than in solution. However, there were no biological effects of the test item on *L.gibba* up to and including the highest tested concentration. Therefore, this deviation from the study guidelines is not thought to have affected the results of the test.

The method is acceptably validated in accordance with SANCO/3029/99 rev.4 and is suitable for the determination of isoflucypram in samples of test water at a LOQ of 0.0625 µg/L (see section B5.1.2.6.1 of the CA document for further details).

The test concentrations were measured in aged solutions each day of the test and also on renewal days (test media was renewed on day 3 and 5. This resulted in three separate test media exposure ‘events’. The RMS approached the applicant to account for the uneven time weighting of the three ‘exposure events’ (i.e. the first event was 3 days in length, from day 0 to 3, whereas the other two were 2 days in length, from day 3 to 5 and 5 to 7) in their calculation of the geometric mean concentrations.

The applicant responded with the following:

“The following results were obtained for the highest test concentration by the chemical analysis:

Table 4: (contd)

day	Nominal concentration [mg/L]	Actual concentration of BCS-CN88460 1. Value [mg/L]	2. Value [mg/L]	Mean value [mg/L]	% of nominal
0 new	8.00	4.77	4.55	4.66	58.3
1 aged		2.70	2.72	2.71	33.9
2 aged		1.37	1.33	1.35	16.9
3 aged		1.13	1.12	1.12	14.0
3 new		2.13	2.09	2.11	26.4
4 aged		2.83	2.84	2.84	35.5
5 d aged		1.26	1.26	1.26	15.8
5 new		4.11	4.24	4.18	52.3
6 aged		2.36	2.40	2.38	29.8
7 aged		3.45	3.48	3.47	43.4

LOQ of this study: 0.000626 mg/L

As daily measurements were performed and in addition 2 samples were investigated on the days on which the test solutions were renewed data for every day of the testing period exist. The time intervals for measurements were daily and do not vary between the measurements with the exception of day 3 and 5 where two values were observed by measuring the aged and the new test solutions.

To consider the fact that on day 3 and 5 two values exist the geomean out of the two measurements (new and aged test solution) can be taken. By this procedure daily values exist and the standard formula for calculation of geometric mean calculations can be used.

The geomean for day 3 is: 1.537

The geomean for day 5 is : 2.295

Using these two values the following measured concentrations are observed for day 0 until day 7.

Day	***Measured test concentration [mg/L]***
0	*4.66*
1	*2.71*
2	*1.35*
3 (geomean of new and aged test solution)	***1.54***
4	*2.89*
5 (geomean of new and aged test solution)	***2.29***
6	*2.38*
7	*3.47*
Overall geomean (day 0-7)	***2.48***

The overall geometric mean measured concentration based on these numbers is 2.48 mg/L.

The originally reported geometric mean measured concentration of 3.02 mg/L was checked again.

The Excel spreadsheet as a failure did not consider all values for this calculation. The values for aged solutions on day 2 and 3 were not considered.

The originally reported result of 3.02 mg/L is not correct."

As the originally reported geometric means were confirmed to be incorrect, the RMS has removed these from the study summary.

The applicant estimated the exposure over time using a geometric mean. As there was both new and aged values for day 3 and day 5, the two values were geomeaned to give a single value for that day, before a geomean of each day's values was conducted to give the estimated exposure from day 0 to day 7. There is some uncertainty with this method in that it doesn't account for potential uneven exposure of the test organism if the test media was not replaced at 12 midday on renewal day. However, it is considered sufficient to establish an estimated exposure value for a long-term time period. Therefore, the recalculated geometric mean concentration for the highest tested concentration (this concentration was the only one recalculated as the endpoint was greater than the highest tested concentration) is considered suitable to define the E_rC_{50} endpoint to use in the aquatic risk assessment for *L.gibba:*

E_rC_{50}: > 2.48 mg a.s./L

B.9.2.8. Further testing on aquatic organisms

No futher studies were submitted.

B.9.3. EFFECTS ON ARTHROPODS

B.9.3.1. Effects on bees

Previous evaluation:	New data, submitted for purpose of review

Report:	KCA 8.3.1.1.1/01; Schmitzer, S.; 2014
Title:	Effects of BCS-CN88460 tech. (acute contact and oral) on honey bees (*Apis mellifera* L.) in the laboratory
Report No.:	89641035
Guideline(s):	OECD 213 and 214 (1998)
Guideline deviation(s):	none
GLP/GEP:	**yes**

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.
Reference item :	Perfekthion EC (active substance 400.9 g/L dimethoate, Batch no.: FRE-000926)
Test organism:	Female worker honeybees (*Apis mellifera*), obtained from a healthy and queen-right colony, bred by IBACON. Collected with glass or plastic tubes, from the outer honeycombs (away from the brood) without the use of smoke and without anaesthetics, collected on the morning of use.
Test duration:	48 hours
Test concentrations :	*Oral test:* Control: 50 % w/v sucrose solution (500 g sucrose/L tap water) and 50 % w/v sucrose solution (500 g sucrose/L tap water) containing solvent (5 % acetone). Treatment groups: 100.0 µg a.s./bee (nominal); 106.3 µg a.s./bee (measured). Refernce item: 0.30, 0.15, 0.08 and 0.05 µg dimethoate per bee (nominal); 0.32, 0.16, 0.08 and 0.05 µg dimethoate per bee (measured).

	Contact test: Control: tap water with 0.5 % Adhäsit and acetone control. Treatment groups: 100.0 µg a.s./bee. Reference item: 0.30, 0.20, 0.15 and 0.10 µg dimethoate per bee.
Application of the test item:	*Oral test:* The test item was diluted in acetone and then applied in 50 % w/v sucrose solution, which was used as carrier (food) in the oral test. The reference item was diluted in tap water and applied in 50 % w/v sucrose solution. For the control pure 50 % w/v sucrose solution was offered to the bees and for the solvent control 50 % w/v sucrose solution with 5 % acetone was offered to the bees. The treated food was offered in syringes, which were weighed before and after introduction into the cages (duration of uptake was 2 hours 55 minutes for the test item treatments). After a maximum of 2 hours 55 minutes, the uptake was complete and the syringes containing the treated food were removed, weighed and replaced by ones containing fresh, untreated food. Bees were starved for 15 minutes prior to application of the test item in all treatment groups. *Contact test:* The test item was applied as one 5 µL droplet of BCS-CN88460 tech. dissolved in acetone, placed on the dorsal bee thorax using a calibrated pipette. The reference item was applied as one 5 µL droplet of dimethoate dissolved in tap water containing 0.5 % Adhäsit. For the controls, one 5 µL droplet of tap water containing 0.5 % Adhäsit was used for the water control and pure acetone was used for the solvent control. Bees were anaesthetised with CO_2 for 20 seconds prior to application.
No. of individuals :	10
No. of replicates	5 per treatment group, control and reference item
Test units :	Stainless steel cages with 10 cm × 8.5 cm × 5.5 cm (length × height × width) with removal glass sheets, ventilation holes anf lined with filter paper.
Test conditions :	Temperature: 24 – 25 °C Relative humidity 28 – 72% Light: Darkness (except during observation) Ventilation: Yes
Feeding:	50 % w/v sucrose solution (500 g/L tap water) (provided as “household sugar”) ad libitum; was given directly after treatment. This was done with syringes that were inserted into the cages via an opening in the top of the test units and from which bees accessed the food directly. It was not necessary to supply fresh 50 % w/v sucrose solution during the test duration.
Observations:	Mortality and behavioural abnormalities were recorded after 4, 24 and 48 hours.
Statistical analysis:	Results obtained with the bees treated with the test item and the reference item were compared to those obtained with the control in both the contact and oral tests. The contact and oral LD_{50} values of the reference item were estimated using the binomial distribution. It was not necessary to correct the test item and the reference item mortality, since no control mortality occurred in either the contact or oral toxicity tests.

	The NOED was estimated using Fisher Exact Test (pairwise comparison, one-sided greater, $\alpha = 0.05$), which is a distribution-free test and does not require testing for normality or homogeneity prior to analysisThe software used to perform the statistical analysis was ToxRat Professional version 2.10.05.

Results

Validity criteria

Validity criteria	**Oral (according to OECD 213)**		**Contact (accoring to OECD 214)**	
	Required	**Obtained**	**Required**	**Obtained**
Control mortality	< 10 %	Sucrose control: 0 % Solvent control: 0 %	< 10 %	Sucrose control: 0 % Solvent control: 0 %
24 h LD_{50} of reference substance	0.10 - 0.35 µg a.s./bee	0.12 µg a.s./bee	0.10 - 0.30 µg a.s./bee	0.24 µg a.s./bee

All validity criteria were met for the test.

Biological findings:

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.

Table B.9.3.1-1: Mortality and behavioural abnormalities of the bees 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

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.

Table B.9.3.1-2: Mortality and behavioural abnormalities of the bees in the contact 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 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

Conclusion

The following endpoints were derived from this study:

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, $\alpha = 0.05$).

RMS comments

This study was conducted according to GLP and following OECD 213 and 214 guidelines. All validity criteria were met.

The following was noted by the RMS:

A volume of 5µL was used in the contact toxicity test for application to bees; this exceeds the 1µL specified in the test guidelines; however as the control and reference item groups performed adequately, whlist this is considered a deviation it is not considered to invalidate the test.

Relative humidity ranged from 28 – 72% during the test; according to the study guidelines it should be 50 – 70%, however as all the validity criteria were met this is not considered to have significantly impacted the test.

This study is valid and will be considered further in the risk assessment section.

The endpoints are confirmed as:

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**

Previous evaluation:	New data, submitted for purpose of review

Report:	KCA 8.3.1.1.1/02; Schmitzer, S.; Haupt, S.; 2013
Title:	Effects of BCS-CN88460 tech. (acute contact and oral) on honey bees (*Apis mellifera* L.) in the laboratory
Report No.:	83991035
Guideline(s):	OECD 213 and 214 (1998)
GLP/GEP:	yes

Material and methods:

Test item:	BCS-CN88460 technical: 98.0 % w/w; Customer Order No.: TOX 09897-01, batch no.: NLL 8674-28-2.
Reference item :	Perfekthion EC (active substance 411.7 g/L dimethoate, Batch no.: 0001017331)
Test organism:	Female worker honeybees (*Apis mellifera*), obtained from a healthy and queen-right colony, bred by IBACON. Collected with glass or plastic tubes, from the outer honeycombs (away from the brood) without the use of smoke and without anaesthetics, collected on the morning of use.
Test duration:	48 hours
Test concentrations :	*Oral test:* Control: 50 % w/v sucrose solution (500 g sucrose/L tap water) and 50 % w/v sucrose solution (500 g sucrose/L tap water) containing solvent (5 % acetone). Treatment groups: 100.0 µg a.s./bee (nominal); 109.5 µg a.s./bee (measured). Reference item: 0.30, 0.15, 0.08 and 0.05 µg dimethoate per bee (nominal); 0.32, 0.16, 0.08 and 0.06 µg dimethoate per bee (measured). *Contact test:* Control: tap water with 0.5 % Adhäsit and acetone control.

	Treatment groups: 100.0 µg a.s./bee. Reference item: 0.30, 0.20, 0.15 and 0.10 µg dimethoate per bee.
Application of the test item:	*Oral test:* The test item was diluted in acetone and then applied in 50 % w/v sucrose solution, which was used as carrier (food) in the oral test. The reference item was diluted in tap water and applied in 50 % w/v sucrose solution. For the control pure 50 % w/v sucrose solution was offered to the bees and for the solvent control 50 % w/v sucrose solution with 5 % acetone was offered to the bees. The treated food was offered in syringes, which were weighed before and after introduction into the cages (duration of uptake was 2 hours 5 minutes for the test item treatments). After a maximum of 2 hours 5 minutes, the uptake was complete and the syringes containing the treated food were removed, weighed and replaced by ones containing fresh, untreated food. Bees were starved for 15 minutes prior to application of the test item in all treatment groups. *Contact test:* The test item was applied as one 5 µL droplet of BCS-CN88460 tech. dissolved in acetone, placed on the dorsal bee thorax using a calibrated pipette. The reference item was applied as one 5 µL droplet of dimethoate dissolved in tap water containing 0.5 % Adhäsit. For the controls, one 5 µL droplet of tap water containing 0.5 % Adhäsit was used for the water control and pure acetone was used for the solvent control. Bees were anaesthetised with CO_2 for 20 seconds prior to application.
No. of individuals :	10
No. of replicates	5 per treatment group, control and reference item
Test units :	Stainless steel cages with 10 cm × 8.5 cm × 5.5 cm (length × height × width) with removal glass sheets, ventilation holes anf lined with filter paper.
Test conditions :	Temperature: 24 – 25 °C Relative humidity 50 – 72% Light: Darkness (except during observation) Ventilation: Yes
Feeding:	50 % w/v sucrose solution (500 g/L tap water) (provided as "household sugar") ad libitum; was given directly after treatment. This was done with syringes that were inserted into the cages via an opening in the top of the test units and from which bees accessed the food directly. It was not necessary to supply fresh 50 % w/v sucrose solution during the test duration.
Observations:	Mortality and behavioural abnormalities were recorded after 4, 24 and 48 hours.
Statistical analysis:	Results obtained with the bees treated with the test item and the reference item were compared to those obtained with the control in both the contact and oral tests. The contact and oral LD_{50} values of the reference item were estimated using Probit Analysis. It was not necessary to correct the test item and the reference item mortality, since no control mortality occurred in either the contact or oral toxicity tests. The NOED was estimated using Fisher Exact Test (pairwise comparison, one-sided greater, $\alpha = 0.05$), which is a distribution-free test and does not require testing for

	normality or homogeneity prior to analysisThe software used to perform the statistical analysis was ToxRat Professional version 2.10.05.

Results

Validity criteria

Validity criteria	**Oral (according to OECD 213)**		**Contact (accoring to OECD 214)**	
	Required	**Obtained**	**Required**	**Obtained**
Control mortality	< 10 %	Sucrose control: 0 % Solvent control: 0 %	< 10 %	Sucrose control: 0 % Solvent control: 0 %
24 h LD_{50} of reference substance	0.10 - 0.35 µg a.s./bee	0.15 µg a.s./bee	0.10 - 0.30 µg a.s./bee	0.16 µg a.s./bee

All validity criteria were met for the test.

Biological findings:

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 (see Table B.9.3.1-3).

Table B.9.3.1-3: Mortality and behavioural abnormalities of the bees 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

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 after this point (see Table B.9.3.1-4 below).

Table B.9.3.1-4: Mortality and behavioural abnormalities of the bees in the contact 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 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

Conclusion:

Endpoints from the test are presented below:

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, α = 0.05).

RMS comments

This study was conducted according to GLP and following OECD 213 and 214 guidelines. All validity criteria were met.

The following was noted by the RMS:

A volume of 5µL was used in the contact toxicity test for application to bees; this exceeds the 1µL specified in the test guidelines; however as the control and reference item groups performed adequately, whlist this is considered a deviation it is not considered to invalidate the test.

This study is valid and will be considered further in the risk assessment section.

The endpoints are confirmed as:

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**

Previous evaluation:	New data, submitted for purpose of review

Report:	KCA 8.3.1.1.1/03; Taenzler, V.; 2015
Title:	BCS-CN88460 tech.: Effects (acute oral) on bumble bees (*Bombus terrestris* l.) in the laboratory
Report No.:	97632105
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

Material and methods:

Test item:	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.
Reference item :	BAS 152 11 I (active substance 420.3 g/L dimethoate, Batch no.: FRE-001226)
Test organism:	Female worker bumble bees (*Bombus terrestris*), obtained from a healthy and queen-right colony, bred by a commercial bumble bee breeding company (Biobest Belgium N.V.). The colony was arranged in a plastic box, which was packed in a cardboard box. Underneath the plastic nest box containing the brood, a bottle with sugar water was located at the bottom of the hive. A wick transported the food out of the bottle to the brood compartment where it was sucked by the bumble bees. Collection was carried out with plastic cages from the upper, non-nest area of the plastic box under red light without the use of anaesthetics one day before application.
Acclimation	The bumble bees were acclimatised to the test conditions over night for 22 hours with *ad libitum* access to an untreated 50 % w/v sucrose solution.
Bumble bee weighing:	After collection from the hive the bumble bees were kept in test units (one individual per test unit). Care was taken that bumble bee size and variation in size was as similar as technically possible in all treatment groups, by visual inspection. Each bumble bee was weighed individually after anesthetisation with CO_2.
Test duration:	48 hours
Test concentrations :	Control: 50 % w/v sucrose solution (500 g sucrose/L tap water) and 50 % w/v sucrose solution (500 g sucrose/L tap water) containing solvent (4 % acetone and 1% Tween80). Treatment groups: 200 µg a.s./bee (nominal); 200.2 µg a.s./bee (measured). Reference item: 4 µg dimethoate per bee (nominal); 3.9 µg dimethoate per bee (measured).
Application of the test item:	The following food solutions were prepared: For the test item treatment, BCS-CN88460 tech. was diluted in 50 % w/v sucrose solution containing maximum 4 % acetone and 1 % Tween80. For the reference treatment, dimethoate was diluted in 50 % w/v sucrose solution. For the water control, 50 % w/v sucrose solution was used. For the solvent control, 50 % w/v sucrose solution containing 4 % acetone and 1 % Tween80 was used. The treated food was offered in syringes, which were weighed before and after introduction into the cages (duration of uptake was 6 hours for the test item treatment). After a maximum of 6 hours, the syringes containing the treated food were removed, weighed and replaced by ones containing fresh, untreated food. Bees were starved for 180 - 200 minutes prior to application of the test item in all treatment groups.
No. of individuals :	1 bumble bee per test unit

No. of replicates	Control: 69 Solvent Control: 72 Test item: 37 Reference item: 45
Test units :	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 bumble bees were kept in the above mentioned test units for the whole duration of the test. The test units were laid on a plate, the small opening was closed by a rubber plug holding a syringe for *ad libitum* feeding of a sugar solution. The large opening was closed by a lid.
Test conditions :	Temperature: 25.1 °C (mean) Relative humidity: 58.9 % (mean) Light: Darkness (except during observation) Ventilation: Yes
Feeding:	50 % w/v sucrose solution *ad libitum;* was given directly after treatment using syringes.
Observations:	Mortality and behavioural abnormalities were recorded after 4, 24 and 48 hours.
Statistical analysis:	Results obtained with the bees treated with the test item and the reference item were compared to those obtained with the controls. The NOED was estimated using Fisher Exact Test (pairwise comparison, one-sided greater, $\alpha = 0.05$), which is a distribution-free test and does not require testing for normality or homogeneity prior to analysis. The software used to perform the statistical analysis was ToxRat Professional version 2.10

Results

Validity criteria:
The test was not conducted to OECD 247 (2017) guidelines as these were not published at the time of the test; however the test results have been compared to the validity criteria according to OECD 247 (2017):

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 µg /bumble bee (in total 45 bumble bees out of 80)

All validity criteria of the test were met.

Biological findings:
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 as shown in Table B.9.3.1-5 below.

Table B.9.3.1-5: Mortality and behavioural abnormalities of the bumble bees in the oral toxicity 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)

Conclusion:

Endpoints from the study are presented below:

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, α = 0.05).

RMS comments

This study was conducted according to GLP and following the guidelines of OECD 213 (1998), Van der Steen (2001). Validity criteria were compared to those in OECD 247 (2017). All validity criteria were met.

The following was noted by the RMS:

According to Van Der Steen (2001) bumblebees must have consumed the test item within 2 hours, however according to the study report the test item was consumed within 6 hours. This may have overestimated the LD50 as the amount of food consumed within 2 hours may have been less than that consumed in 2 hours. As the study was conducted as a limit test and no adverse effects of the test item were noted, this deviation is considered acceptable.

This study is valid and will be considered further in the risk assessment.

The endpoints are confirmed as:

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**

Report:	KCA 8.3.1.1.2/03; Haupt, S.; 2015
Title:	Effects of BCS-CN88460 tech. (acute contact) on bumblebees (*Bombus terrestris* L.) in the laboratory
Report No.:	90221105
Guideline(s):	No specific guidelines available; study design based on OECD 214 (1998) Van der Steen (2001) and ICPPR non-apis group (2014)
GLP/GEP:	**yes**

Material and methods:

Test item:	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.
Reference item :	BAS 152 11 I (active ingredient 400.9 g/L dimethoate, Batch no.: FRE-000926)
Test organism:	Female worker bumble bees (*Bombus terrestris*), obtained from a healthy and queen-right colony, bred by a commercial bumble bee breeding company (Biobest Belgium N.V.). Collection was carried out with plastic cages from the upper, non-nest area of the plastic box under red light without the use of anaesthetics one day before application.
Acclimation	The bumble bees were acclimatised to the test conditions over night for 21 hours 35 minutes with *ad libitum* access to an untreated 50 % w/v sucrose solution.
Bumble bee weighing:	After collection from the hive the bumble bees were kept in test units (one individual per test unit). Care was taken that bumble bee size and variation in size was as similar as technically possible in all treatment groups, by visual inspection. Each bumble bee was weighed individually after anesthetisation with CO_2.
Test duration:	48 hours
Test concentrations :	*Control:* Water control: Tap water with 0.5 % Tween80; Solvent control: acetone. *Treatment groups:* 100 µg BCS-CN88460 tech. a.s./bumblebee. *Reference item:* 12 µg dimethoate per bumblebee (nominal).
Application of the test item:	One single 5 µL droplet of BCS-CN88460 tech. in an appropriate carrier (acetone) was placed on the dorsal bumblebee thorax using a pipette. The reference item was applied in 5 µL carrier (dimethoate made up in tap water containing 0.5 % Tween80*). As control one 5 µL droplet containing tap water with 0.5 % Tween80 was used. An additional control with the solvent (pure acetone) was used. Bumblebees were anaesthetised with CO_2 prior to application.
No. of individuals :	1 bumble bee per test unit
No. of replicates	50
Test units :	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. Contact application was conducted outside of the test units. The test units were laid on a plate, the small opening was closed by a rubber plug holding the feeding solution. The large opening was closed by a lid.
Test conditions :	Temperature: 25 - 26°C Relative humidity: 57 - 66 % Light: Darkness (except during observation) Ventilation: Yes

Feeding:	50 % w/v sucrose solution *ad libitum;* was given directly after treatment using syringes.
Observations:	Mortality and behavioural abnormalities were recorded after 4, 24 and 48 hours.
Statistical analysis:	Results obtained with the bees treated with the test item and the reference item were compared to those obtained with the controls. The NOED was estimated using Fisher Exact Test (pairwise comparison, one-sided greater, $\alpha = 0.05$), which is a distribution-free test and does not require testing for normality or homogeneity prior to analysis. The software used to perform the statistical analysis was ToxRat Professional version 2.10

Results

Validity criteria:

The test was not conducted to OECD 247 (2017) guidelines as these were not published at the time of the test; however the test results have been compared to the validity criteria according to OECD 247 (2017):

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 %

All validity criteria of the test were met.

Biological findings:

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.

Table B.9.3.1-6: Mortality and behavioural abnormalities of the bumblebees in the contact toxicity 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:

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 %

All validity criteria of the test were met.

Conclusion:

The endpoints from this study are confirmed as follows:

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 $LD_{10/20}$ 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, α = 0.05).

RMS comments

This study was conducted according to GLP and following the guidelines of OECD 213 (1998), Van der Steen (2001). Validity criteria were compared to those in OECD 246 (2017). All validity criteria were met.

The following was noted by the RMS:

A volume of 5µL was used in the contact toxicity test for application to bees; this exceeds the 1µL specified in the test guidelines; however as the control and reference item groups performed adequately, whlist this is considered a deviation it is not considered to invalidate the test.

According to Van Der Steen (2001) the observation period should be 72 hours; however in this study it was 48 hours. As no mortality occurred at 48 hours and no behavioural abnormalities were apparent, the RMS considers thia acceptable as it is unlikely that 50% mortality would be achieved in another 24 hour period. Furthermore, according to OECD 246 (2017), the test duration should be 48 hours and only extended if the mortality increases by ≥10% between 24 and 48 hours. As this was not the case here, the RMS considers the 48 hour test duration to be sufficient.

According to Van Der Steen (2001) the test solution should be administered to the ventral part of the thorax on between the second and third pair of legs to avoid grooming attempts; however in this study application was made to the dorsal part of the thorax. As the reference item groups performed appropriately, indicating that bees were sufficiently exposed, this is not considered to invalidate the test. Furthermore, according to OECD 246 (2017), the test item should be applied to the dorsal thorax, as such the application method is considered appropriate by the RMS.

This study is valid and will be considered further in the risk assessment.

The endpoints are confirmed as:

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**

Previous evaluation:	New data, submitted for purpose of review

Report:	KCA 8.3.1.2/01; Gossmann, A.; 2015
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
Guideline(s):	OECD 213 (1998) and CEB No. 230 with current recommendations of the ring test group (2014)
GLP/GEP:	**yes**

Material and methods:

Test item:	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)
Reference item :	Perfekthion EC (active substance 400.9 g/L dimethoate, Batch no.: FRE-000926)
Test organism:	Female worker honeybees (*Apis mellifera*), obtained from a healthy and queen-right colony, bred by IBACON. Two days old at test initiation. Brood frames from one colony with capped cells which are expected to hatch on the same day were kept without nurse bees in an excluder box in the hive until hatch. This comb contained pollen which was used as a first feeding source for the freshly hatched bees. The following day, freshly emerged worker bees were taken from the excluder box and transferred to the ready-prepared test units (cages) for acclimatisation.
Acclimitisation period:	One day under test conditions.
Test duration:	10 days
Test concentrations :	Control: 50 % w/v sucrose solution (500 g sucrose/L tap water) Treatment groups: 208, 417, 833, 1667 and 3333 ppm (mg a.s./kg feeding solution); equivalent to 6.25, 12.5, 25.0, 50.0 and 100 µg a.s./bee per day (nominal); 6.6, 12.4, 28.6, 49.9 and 89.7 µg a.s./bee per day (mean measured: the concentration was converted to a dose by weighing the syringes at test initiation and test termination to determine the amount of ingested solution per bee and then adjusting this based on the measured a.s. content of the test solution). Reference item: 0.03 µg a.s./bee per day (nominal); 0.02 µg a.s./bee per day (mean measured).
Application of the test item:	The test item and reference item was incorporated in a 50 % (w/v) sucrose/water solution which was used as carrier in the test to achieve a feeding solution with the targeted final concentration. Every day the feeding syringes containing the above mentioned different control, test and reference item feeding solutions were replaced by fresh ones. The treated and untreated food was offered *ad libitum* to each cage in syringes. The syringes were weighed daily before introduction into the cages and after the feeding interval.
No. of individuals :	10
No. of replicates	3 per treatment group, control and reference item
Test units :	Stainless steel cages 10 cm × 8.5 cm × 5.5 cm (length × height × width) with removable glass sheets, ventilation holes and lined with filter paper.
Test conditions :	Temperature: 32 – 34 °C Relative humidity 50 – 90%

	Light: Darkness (except during observation) Ventilation: Yes
Feeding:	The bees were fed continuously ad libitum containing either: The test item (test item treatment group) Reference item (reference item group) Pure 50 % (w/v) sucrose solution (control group) The feeding solutions were offered to the bees via syringes *ad libitum*. The Syringes were replaced daily.
Observations:	Mortality and behavioural abnormalities were recorded daily until test termination. The food consumption per bee was calculated by the number of surviving bees per assessment and the amount of food taken up on the following assessment day.
Statistical analysis:	Results obtained with the bees treated with the test item and the reference item were compared to those obtained with the control in both the contact and oral tests. The NOEC/NOEDD was estimated using Fisher Exact Test (pairwise comparison, one-sided greater, $\alpha = 0.05$), which is a distribution-free test and does not require testing for normality or homogeneity prior to analysis. The software used to perform the statistical analysis was ToxRat Professional version 2.10.05.
Analytical verification:	Triplicate specimens (corresponding to one sample) of the feeding solution of each concentration (5 concentrations) and the control were taken from day 0 to day 9 of the experiment and analysed using HPLC-MS/MS

Results

Validity criteria:

The test was not conducted to OECD 245 (2017) guidelines as these were not published at the time of the test; however the test results have been compared to the validity criteria according to OECD 245 (2017):

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 %

All validity criteria were met in this study.

Analytical findings:

The mean measured concentration of BCS-CN88460 in the feeding solutions was in a range of 80 – 113 % of the nominal concentrations. Endpoints are based on mean measured concentrations.

Biological findings:

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, $\alpha = 0.05$).

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. The results are presented in Table B.9.3.1-7 below.

Table B.9.3.1-7: Mortality and behavioural abnormalities of the bees in the chronic oral toxicity test

Concentration [mg a.s./kg sugar solution]	Dose level [µg a.s./bee/day]	Mortality (% mean)									
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
3333	89.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1667	49.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
833	28.6	0.0	0.0	0.0	6.7	6.7	6.7	10.0	10.0	10.0	10.0
417	12.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
208	6.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Water control	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Reference item	0.02	0.0	16.7	53.3	76.7	100	100	100	100	100	100*

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

Endpoints from this study are presented below:

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

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

Conclusion:

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.

RMS comments

This study was conducted according to GLP and following OECD 213 guidelines and CEB No. 230 with current recommendations of the ring test group (2014); validity criteria were compared to OECD 245 (2017) guidelines and all validity criteria were met.

The following was noted by the RMS:

It is noted that 10% mortality occurred in the 28.6 µg a.s./bee/day treatment group. This is not considered to be related to the test item since no mortality occurred in any of the other test item groups and the mortalities occurred in a single replicate; as such this is likely due to natutal variability or a variation in the test conditions in this replicate.

The method is acceptably validated in accordance with SANCO/3029/99 rev.4 and is suitable for the determination of isoflucypram in samples of sugar solution at levels between 0.01 and 5000 mg/kg (see section B5.1.2.6.3 of the CA document for further details).

This study is considered valid and acceptable for use in the risk assessment. The endpoints are confirmed as:

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**

Previous evaluation:	New data, submitted for purpose of review

Report:	KCA 8.3.1.3/01; Oberrauch, S.; 2017
Title:	BCS-CN88460 - Honey bee (*Apis mellifera* L.) larval toxicity test (repeated exposure)
Report No.:	S16-00461
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)
GLP/GEP:	Yes

Test item:	Test item: BCS-CN88460: 94.2 % w/w (analytical), Origin Batch No.: 2013-006492, Customer Order No.: TOX 20011-02; Material: BCS-CN88460, technical; Specification No.: 102000028196
Reference item :	Dimethoate BAS 152 I; Batch no. 35015A161
Test organism:	*Apis mellifera carnica* The larvae originated from three different bee hives maintained by Eurofins Agroscience Services EcoChem GmbH / Eurofins Agroscience Services Ecotox GmbH, Eutinger Straße 24, 75223 Niefern-Öschelbronn, Germany. The hives used for honey bee larvae collection were adequately fed, healthy, as far as possible parasite-free and queen-right. No chemical substances (such as antibiotics, anti-Varroa treatments, pesticides, etc.) have been used in the hive within 4 weeks preceding the start of the test.
Age of test organisms:	Synchronised first instar larvae (L1)
Acquisition of larvae:	Four days prior to the grafting of larvae, queens of eight colonies were confined in their own colony in an excluder cage containing a comb with empty cells. Three days prior to the grafting, the queens were released from the cages. The combs containing eggs were left in the excluder cages during the incubation stage until hatching on day 1. On day 1, the combs were transferred to the laboratory using an insulated container in order to avoid temperature variation. In the laboratory three out of eight combs were selected for grafting, containing the highest number of synchronised larvae.
Preparation of larval diet and feeding of larvae:	The larval diet was prepared freshly in advance and divided into aliquots using a multi stepper pipette. The aliquots were subsequently stored deep-frozen (< 18 °C) until use. Diets were prepared as follows using autoclaved deionised water and fresh commercial royal jelly: • Diet A: 50 % weight of fresh royal jelly + 50 % weight of an aqueous solution containing 2 % weight of yeast extract, 12 % weight of glucose and 12 % weight of fructose • Diet B: 50 % weight of fresh royal jelly + 50 % weight of an aqueous solution containing 3 % weight of yeast extract, 15 % weight of glucose and 15 % weight of fructose.

<table>
<tr><td></td><td>
Diet C: 50 % weight of fresh royal jelly + 50 % weight of an aqueous solution containing 4 % weight of yeast extract, 18 % weight of glucose and 18 % weight of fructose.
Larvae were fed with the above diets as follows:

20µL of untreated diet A on day 1.
20µL of treated or untreated diet B on day 3.
30µL of treated or untreated diet C on day 4.
40µL of treated or untreated diet C on day 5.
50µL of treated or untreated diet A on day 6.

</td></tr>
<tr><td>Grafting and re-grafting of larvae:</td><td>On day 1 the required amount of Diet A was dropped into each grafting cell of the well-plate. Using a grafting tool, one larva was transferred from the comb to each cell on the surface of the diet. The test was initiated with an excess number of larvae. Therefore four reserve plates were prepared containing larvae of the same replicate hives. The grafting was conducted on warming plates. When a well-plate was completed, it was placed into a hermetically sealed Plexiglas desiccator. Before first application of the test item on day 3, it was assured that all larvae used were of similar size and alive; any larvae not meeting these criteria were replaced.</td></tr>
<tr><td>Test duration:</td><td>22 days</td></tr>
<tr><td>Test concentrations :</td><td>Test item: 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.

Reference item: 48.0 mg dimethoate/kg diet equivalent to a cumulative dose of 7.39 µg dimethoate/larva per development period.

Controls: Water and solvent controls.</td></tr>
<tr><td>Preparation of the test item:</td><td>The test item was prepared in acetone for the stock solutions and dilutions. Due to the low solubility of the test item in acetone, the test solution was ultrasonicated in order to achieve an adequate dilution.

Dimethoate was dissolved in autoclaved water.

All solutions were homogenised by shaking.

The application schedule is given below:
<table>
<tr><th>Tretament group</th><th>Day</th><th>Concentration (mg/diet)</th><th>Cumulative dose (µg/larva per development period)</th></tr>
<tr><td>Control</td><td rowspan="8">3 - 6</td><td>-</td><td>-</td></tr>
<tr><td>Solvent control</td><td>-</td><td>-</td></tr>
<tr><td rowspan="5">Test item</td><td>10.4</td><td>1.60</td></tr>
<tr><td>26.0</td><td>4.00</td></tr>
<tr><td>65.0</td><td>10.0</td></tr>
<tr><td>162</td><td>24.9</td></tr>
<tr><td>406</td><td>62.5</td></tr>
<tr><td>Reference item (dimethoate)</td><td>48.0</td><td>7.39</td></tr>
</table>
</td></tr>
<tr><td>No. of individuals per replicate:</td><td>16</td></tr>
<tr><td>No. of replicates:</td><td>3</td></tr>
<tr><td>Test units :</td><td>Crystal polystyrene grafting cells, diameter 9mm, sterilised in ethanol before use.

Each cell was placed in a sterile 48-well cell culture plate and stored in a hermetically-sealed desiccator containing potassium sulphate from day 1 until day8.</td></tr>
</table>

	On day 8 the plates were transferred to another desiccator containing a dish filled with saturated sodium chloride solution. The desiccators were placed in an incubator with forced air circulation. On day 15 each plate was covered by a lid and transferred from the desiccator into an incubator. The incubator contained a dish filled wit deionised water in order to keep adequate relative air humidity.
Test conditions :	During the entire test period the test organisms were kept under constant darkness except during grafting, feeding and assessments. Test conditions were as follows: Temperature (°C): Day 1 - 8: 33.7 – 35.1 (min – max) Day 8 - 15: 33.9 – 34.4 (min – max) Day 15 – 22: 34.1 – 34.5 (min – max) Relative humidity (%) Day 1 – 8: 70.6 – 100 (min – max) Day 8 - 15: 41.2 – 86.7 (min – max) Day 15 – 22: 37.1 – 59.5 (min – max)
Observations:	Assessment of mortality during the larval phase was conducted before feeding from day 4 until day 8. On day 8 the presence of uneaten food was qualitatively recorded. Assessment of mortality during the pupation phase was conducted on day 15 and day 22. On day 15 dead larvae, pupae and larvae that have not transformed into pupae were recorded as dead. On day 22 pupae that had not emerged were recorded as dead. Assessment of adult emergence was conducted on day 22. Bees were counted as successfully emerged if they showed signs of adult eclosion. This included the presence of differentiated wings and hair or the absence of the pupal skin. At each assessment time dead larvae and pupae were removed for sanitary reasons. Other observations and any other adverse effects were qualitatively recorded to aid in the interpretation of mortality in comparison to the solvent control group.
Statistical analysis:	ToxRat professional, Version 3.2.1 was used for all analyses. Multiple Fisher's exact test with Bonferroni-Holm adjustment (one-sided greater, α = 0.05) was used to evaluate whether there was a significant difference between the test item groups and the solvent control group for larval mortality on day 8, larval and pupal mortality on day 15, pupal mortality from day 8 through 22 and for adult emergence on day 22. The NOEC was determined according to OECD Series on Testing and Assessment Number 54 (2006) for larval mortality on day 8 and adult emergence on day 22. Probit analysis using linear maximum likelihood regression was used to calculate the LC_{10}, LC_{20} and LC_{50} for larval mortality on day 8. The LC_{10} could not be determined due to the lack of a clear concentration-response relationship. The EC_{10} and EC_{20} for adult emergence on day 22 with 95 % confidence limits were calculated by Probit analysis using linear max. likelihood regression and compensation for solvent control response (20.8 %). The NOED, ED_{10}, ED_{20} and ED_{50} were calculated from the NOEC, EC10, EC20 and EC50 based on the test item concentration, the cumulative feeding volume per larva anfd the density of the diet (1.1 g/cm^3)
Analytical verification:	Diet samples from the controls and the test item groups were analysed for residues of BCS-CN88460 based on the following method description: “Analytical method 01475 for the determination of residues of BCS-CN88460 and its metabolite BCS-CR60082 in/on plant by HPLC-MS/MS.

Results

Validity criteria:

Validity criteria according to OECD GD 239	Obtained in this study
Cumulative larval mortality from day 3 to 8 in control(s): ≤ 15 % across all replicates.	Control: 0 % Solvent control: 0 – **18.8** %
Adult emergence rate on day 22 in control(s): ≥ 70 % across all replicates	Control: 87.5 - 100 % Solvent control: **56.3** - 100 %
For reference item dimethoate larval mortality at day 8: ≥ 50 % across all replicates.	75 - 100 %

Validity criteria in **bold** were not met

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 0.0 – 18.8 %, 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 87.5 – 100 and 56.3 - 100 %, 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 (Table B.9.3.1-8).

Table B.9.3.1-8: 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**		**Adult Emergence on Day 22** [a]	**Decreased compared to the solvent control**
					(%)	**Corr-ected (%)**	**(%)**	
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	-10.5
	26.0		4.00		4.2	-4.5	77.1	2.7
	65.0		10.0		12.5	4.6	77.1	2.7
	162		24.9		4.2	-4.5	70.8	10.6
	406		62.5		10.4	2.3	62.5	21.1
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

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.

EC_{10} and EC_{20} values for emergence were 160 and 380 mg a.s./kg diet respectively and the corresponding ED_{10} and ED_{20} values were 24.6 and 58.5 µg a.s./larva per development period respectively.

RMS comments

This study was conducted according to GLP and following OECD 239 guidance and US EPA OCSPP 850.SUPP. Although the study was conducted according to the version of OECD 239 amended in July 2015, this guideline was superseded by another version in July 2016. As this study was conducted in 2017, the RMS has compared the validity criteria to those in the most recent OECD 239 guidelines.

The following was noted by the RMS:

It is not stated how emerging bees were fed; according to OECD 239 (2016) bees should be fed *ad libitum* with syrup/sucrose solution.

According to OECD 239, the validity criteria must be met across all replicates. Although validity criteria were met considering the mean values for the control and solvent control, the validity criteria for larval mortality and mean adult emergence were not met across all replicates in the solvent control, where 18.8% mortality was observed in replicate 1 on day 8, and emergence rate in replicate 3 was 56.3%. Consequently, this undermines the reliability of the endpoint due to a lower quality /and health of the test organism taken from 1/3 colonies meaning effects seen may be amplified over those expected should a robust healthy batch of organisms have been used.

This study will be considered further in the risk assessment in section CP B.9.6.

B.9.3.2. Effects on non-target arthropods other than bees

Laboratory Tests

Previous evaluation:	None; new active substance application.

Report:	KCP 10.3.2.1/01; Waibel, J.; 2017;
Title:	Toxicity to the parasitoid wasp Aphidius rhopalosiphi (Hymenoptera: Braconidae) using a laboratory test BCS-CN88460 EC 50 g/L
Report No.:	CW16/036
Document No.:	M-593743-01-1
Guideline(s):	EU Directive 91/414/EEC Regulation (EC) No. 1107/2009 MEAD-BRIGGS ET AL. (2000) CANDOLFI ET AL. (2001)
Guideline deviation(s):	none
GLP:	yes

Objective:

The objective of this laboratory study was to investigate the lethal toxicity of BCS-CN88460 EC 50 g/L on the parasitoid wasp *Aphidius rhopalosiphi* when exposed on a treated glass surface.

Material and methods:

Test item: BCS-CN88460 EC 50, Supplier batch No: 2016-001002, Spec. no: 102000031262, analysed content of active substance isoflucypram: 5.18% w/w (50.46 g/L).

Toxic reference item: dimethoate, applied at 0.04 g a.s./ha in 200 L deionised water/ha was included.

Test species: Adult wasps of *Aphidius rhopalosiphi* (less than 48 h old at study start) were used as test organisms. The test organisms were supplied as mummies by Katz Biotech AG, 15837 Baruth, Germany. The original source of the wasps had been Rothamsted Experimental Station, Great Britain; the rearing in the laboratory of Katz Biotech started 1994 (rearing conditions: 20 - 25 °C, 60 - 80% rel. humidity, daylength 16:8 h with a light intensity of >3000 Lux, host: *Rhopalosiphum padi* or *Sitobion avenae*).

Test design: Fifteen *Aphidius rhopalosiphi* per replicate (four replicates for each concentration of the test item, the control and the reference item) were exposed to each test concentration, control and reference item for 48

hours to assess mortality. The test item was applied on glass plates at rates of 7.5, 13.3, 23.7, 42.2 and 75.0 g a.s./ha in 200 L deionised water/ha using a calibrated laboratory track sprayer (mean measured application rate: 198 L/ha). The effects of the test item on the parasitoid wasp *Aphidius rhopalosiphi* were compared to those of a deionised water treated control.

The condition of the test animals was recorded 2, 24 and 48 hours after application as follows: Live (alive and apparently unaffected), affected (abnormal behaviours), moribund (unable to walk, but legs or antennae moving), dead (no longer moving). For each time period, the number of moribund and dead wasps was summed for each replicate and calculated as a percentage. A correction for control mortality was performed.

Endpoints measured: LR_{50}

Test concentrations: Control, 7.5, 13.3, 23.7, 42.2 and 75.0 g a.s./ha in 200 L deionised water/ha.

Application method: The sprayer was calibrated beforehand to deliver 200 L/ha ±10 % by spraying glass plates of known weight and area and weighing them immediately after in order to determine the actual amount of water applied. The mean measured application rate was 198 L/ha calculated based on the values from 5 weighed glass plates. Both the test and reference item were diluted under conditions corresponding to those in the field and applied immediately afterwards. Homogeneity of the test and references substances was guaranteed by thorough shaking immediately before application.

The suspensions for the test and reference items were prepared on the day of application and applied to the glass plates using a linear cabinet track sprayer. After the spray coating had dried the glass plates were transferred into the corresponding frames, with the treated areas inside the frames.

Test conditions: Until the start of the study the mummies were stored at the temperature of 23.5 – 27.0 °C, 60 – 74 % relative humidity (for four hours the humidity decreased to 40%). The mummies obtained from the breeder were distributed to several glass tubes. Each tube was inserted in a hatching cage so that the hatched wasps could move into the cage. The insects were fed via a tube filled with a feeding solution which consisted of 3 parts of water + 1 part honey. Two days prior to the start of the study the tubes with still unhatched mummies were inserted in empty glass cages to isolate them from the already hatched ones. This procedure ensured that the newly hatched wasps were not older than 48 h.

The experiment was performed in controlled environment rooms at a temperature range of 19.5 – 20.5 °C and a relative humidity range of 65 - 83%. Short deviations of the test conditions (less than 2 h; e.g. due to handling of the test system) are considered being without consequence to the study outcome and were not reported. The climatic conditions were continuously recorded with a data logger (ELPRO Messtechnik GmbH). The light / dark cycle was 16:8 h. The light intensity range was 930 - 1250 measured once at study start using a Luxmeter (RS Components GmbH).

The test units consisted of two treated glass plates (100 x 100 x 3 mm) with corresponding cover glasses (100 x 100 mm) and an untreated acryl frame (inner size 92 x 92 x 14 mm) with 3 ventilation holes on each side, which were covered with gauze.

Statistics: The mortality data were analysed for significance using the Fisher Exact test (one-sided with Bonferroni-Holm adjustment; $\alpha = 0.05$), which is a distribution-free test method and does not require testing for normality or homogeneity of variance prior analysis.

The LR50 value (lethal rate causing 50% mortality) was calculated using Spearman-Karber method.

The computer program SAS (Version 9.4) was used to perform the statistical analyses.

Findings:

Validity criteria

Validity criteria	Recommended	Obtained
Mortality in control	≤ 13%	1.7%
Corrected mortality reference substance	≥ 50%	100%

All validity criteria were met for the study.

Biological findings

Mortality

After 48 h of the study 1.7% of the wasps were found dead in the control group. In all test item rates a statistically significant mortality was found (Fisher`s Exact test (one-sided, $\alpha = 0.05$).

In the test item rates of 7.5 and 13.3 g a.s./ha, a corrected mortality of 10.2% and 32.2% occurred, respectively. In all higher test item rates of 23.7, 42.2 and 75.0 g a.s./ha, the corrected mortality was 100%. (see table B.9.3.2-1)

Table B.9.3.2-1: Mortality of *Aphidius rhopalosiphi* following exposure to Isoflucypram EC 50, a deionised water control or a toxic reference substance (dimethoate) during a standard laboratory test.

Test item		BCS-CN88460 EC 50 g/L		
Test organism		*Aphidius rhopalosiphi*		
Exposure on		Glass plates		
		Mortality after 48 h [%]		
Treatment	g a.s./ha	Uncorrected	Corrected (*)	P-Value (**)
Control	0.0	1.7		
Test item	7.5	11.7	10.2	0.031 sign.
Test item	13.3	33.3	32.2	<0.001 sign.
Test item	23.7	100.0	100.0	<0.001 sign.
Test item	42.2	100.0	100.0	<0.001 sign.
Test item	75.0	100.0	100.0	<0.001 sign.
Reference item	0.04	100.0	100.0	

LR_{50}: 14.13 g a.s./ha; 95 % Confidence Interval: 12.76 - 15.66 (calculated with Spearman-Karber)
* Corrected mortality according to SCHNEIDER-ORELLI (1947)
** Fisher`s Exact test (one-sided, $\alpha = 0.05$), p-values are adjusted according to Bonferroni-Holm
sign. significant

Conclusion:

The LR_{50} was calculated to be 14.13 g a.s./ha. The NOER for mortality was 7.5 g a.s./ha.

The figures obtained fulfil the validity criteria of the laboratory method using glass plates (MEAD-BRIGGS ET AL., 2000).

RMS comments

This study was conducted appropriately and was compliant with GLP. The validity criteria of a < 13 % mortality rate of the water control and ≥ 50 % mortality of *Aphidius* following exposure to the reference test substance were met.

The application rate of the reference substance was lower than that recommended in the test guidelines, which suggest 0.12 g a.s. in 200 L/ha. However, given that the mean mortality of *Aphidius* was 100 % following exposure to the reference item, which is within the recommended mortality rate (75-100 %) specified in the guidelines, this is not considered to be an issue by the RMS and confirms that the test system was sufficiently sensitive.

Four replicates were carried out for each of the test concentrations, control and reference substance, with 15 wasps in each replicate. The guidelines by Mead-Briggs et al. (2000) recommend 10 wasps per replicate. Although there is the potential for overcrowding at a higher stocking density, the control group performed well with no mortality and no adverse symptoms observed. As such it can be concluded that the increased stocking density of organisms in each replicate was not detrimental to organism health. The effect of increased stocking density on exposure was also considered and it is concluded that it was acceptable to assume that all 15 wasps were adequately exposed to the residues on the glass plates. This is supported by the high and regular mortality observed in the test item groups showing that adequate exposure took place to reflect the toxicity of the test item. Furthermore, the greater size of the group improves the statistical robustness of the results. Therefore this is considered to be an acceptable minor deviation by the RMS.

Full validation data in accordance with SANCO/3029/99 rev.4 have not been presented for this method. But due to the simplicity of the method (dilution followed by analysis), its similarity to other versions of the same method that have been validated in a range of matrices and the fact that it was used to confirm nominal concentrations in larval diet rather than determine unknown residues, it is considered by the RMS that its use in this study is acceptable (see section B5.1.2.6.4 of the CA document for further details).

This study is considered valid and acceptable for use in the risk assessment. The endpoint is confirmed as an **LR_{50} value of 14.13 g a.s./ha.**

Previous evaluation:	None; new active substance application.

Report:	KCP 10.3.2.1/02; Waibel, J.; 2017
Title:	Toxicity to the predatory mite Typhlodromus pyri (Acari: Phytoseiidae) using a laboratory test BCS-CN88460 EC 50 g/L
Report No.:	CW16/035
Document No.:	M-593747-01-1
Guideline(s):	EU Directive 91/414/EEC Regulation (EC) No. 1107/2009 BLÜMEL ET AL. (2000) CANDOLFI ET AL. (2001)
Guideline deviation(s):	none
GLP:	yes

Objective:

The objective of this laboratory study was to investigate the lethal toxicity of BCS-CN88460 EC 50 g/L to the predatory mite *Typhlodromus pyri* when exposed to a treated glass surface.

Material and Methods:

Test item: BCS-CN88460 EC 50, Supplier batch No: 2016-001002, Spec. no: 102000031262, analysed content of active substance isoflucypram: 5.18% w/w (50.46 g/L).

Toxic Reference item: dimethoate, applied at 5.0 g a.s./ha in 200 L deionised water/ha.

Test species: Protonymphs of *Typhlodromus pyri* (less than 24 hours old at study start) were used as test organisms. The test organisms were supplied as eggs by Katz Biotech AG, 15837 Baruth, Germany. The original source of the mites had been Staatliche Lehr- und Versuchsanstalt, Weinsberg, Germany; the rearing in the laboratory of Katz Biotech started 1992 (rearing conditions: 20 - 25 °C, 60 - 80% rel. humidity, daylength 16:8 h with a light intensity of > 3000 Lux, food: apple pollen).

Test design: Twenty *T.pyri* per replicate (five replicates for each concentration of the test item, the control and the reference item) were exposed to each test concentration, control and reference item for 7 days to assess mortality. The test item was applied on glass plates at rates of 7.5, 13.3, 23.7, 42.2 and 75.0 g a.s./ha in 200 L deionised water/ha using a calibrated laboratory track sprayer (mean measured application rate: 189 L/ha).

After the test unts were set up the protonymphs were placed onto the exposure units by test group (within one and a half hours after application). The mites were transferred with a fine brush under a stereomicroscope and immediately afterwards were examined to ensure they were undamaged and in good condition. Then pollen (birch – pine mixture) was supplied as food and the units were maintained under the climatic conditions of the test. The effects of the test item on *T.pyri* were compared to those of a deionised water treated control.

The mortality was recorded at 4 and 7 days after study start by counting the number of living and dead mites. The number of escaped mites was calculated as the difference from the total number exposed.

Endpoint measured: LR_{50}

Test concentrations: Control, 7.5, 13.3, 23.7, 42.2 and 75.0 g a.s./ha in 200 L deionised water/ha.

The climatic test conditions during the study were 24.0 - 25.5 °C temperature and 60 - 72% relative humidity. The light / dark cycle was 16:8 h with a light intensity range of 119 - 540 Lux.

The LR_{50} value (lethal rate causing 50% mortality) was calculated by Probit analysis.

The computer program SAS (Version 9.4) was used to perform the statistical analyses.

Application method: The sprayer was calibrated beforehand to deliver 200 L/ha ±10 % by spraying glass plates of known weight and area and weighing them immediately after in order to determine the actual amount of water applied. The mean measured application rate was 189 L/ha calculated based on the values from 5 weighed glass plates. Both the test and reference item were diluted under conditions corresponding to those in the field and applied immediately afterwards. Homogeneity of the test and references substances was guaranteed by thorough shaking immediately before application.

The suspensions for the test and reference items were prepared on the day of application and applied to the glass plates using a linear cabinet track sprayer. After the spray coating had dried the test arenas were moved back to the plastic trays.

Test conditions: One unit consisted of two glass cover slides (24 x 60 mm) which were put together so that their longitudinal sides touch and leave a narrow gap. A rectangular piece of floral foam (approx. 48 x 60 x 40 mm) was placed in a plastic tray with water which was sucked up by the floral foam. On top of the floral foam first a tissue paper and second the cover slides were placed. To avoid the escaping of mites a barrier of sticky material was placed in the middle of the cover slides to form the test arena (Ø approx. 40 mm).

Each exposure unit was transferred to a plastic tray filled with deionised water. The narrow gap between the two cover slides was filled with water by capillary forces to provide the mites with water.

The units were prepared one day before application to make sure that the floral foam was saturated with water.

The experiment was performed in a controlled environment room at a temperature range of 24.0 - 25.5 °C and a relative humidity range of 60 - 72%. Short deviations of the test conditions (less than 2 h; e.g. due to handling of the test system) are considered being without consequence to the study outcome and were not reported. The climatic conditions were continuously recorded with data logger (ELPRO Messtechnik GmbH).

The light / dark cycle was 16:8 h with a light intensity range of 119 - 540 Lux, measured once using a Luxmeter (testo AG).

Statistics: The mortality data were analysed for significance using the Fisher Exact test (one-sided with Bonferroni-Holm adjustment), which is a distribution-free test method and does not require testing for normality or homogeneity of variance prior analysis.

The LR50 value was calculated using Probit analysis.

The computer program SAS (Version 9.4) was used to perform the statistical analyses.

Findings:

Validity Critiera

	Recommended	Obtained
MortEsc.-rate in the control group on day 7	≤ 20%	13.0%
Average corr. mortality in the reference item	≥ 50%	94.3%

All validity criteria were met.

Biological findings

The mortality / escaping rate in the control exposure units up to day 7 after treatment was 13.0%.

In the higher test item rates of 23.7, 42.2 and 75.0 g a.s./ha, a statistically significantly different mortality compared to the control was found (Fisher`s Exact test, one-sided). At the lower test item rates of 7.5 and 13.3. g a.s/ha, a corrected mortality of 2.3% and -6.9% has been observed, respectively. At the test item rates of 23.7, 42.2 and 75.0 g a.s./ha, the corrected mortality was 27.6%, 80.5% and 96.6%, respectively.

A summary of the effects observed in this study is given in Table B.9.3.2-2.

Table B.9.3.2-2: Mortality of *T.pyri* following exposure to Isoflucypram EC 50, a deionised water control or a toxic reference substance (dimethoate) during a standard laboratory test.

Test item		BCS-CN88460 EC 50 g/L		
Test organism		*Typhlodromus pyri*		
Exposure on		Glass plates		
		Mortality after 7 days [%]		
Treatment	g a.s./ha	Uncorrected	Corrected (*)	P-Value (**)
Control	0.0	13.0		
Test item	7.5	15.0	2.3	0.839 not significant
Test item	13.3	7.0	-6.9	0.951 not significant
Test item	23.7	37.0	27.6	<0.001 significant
Test item	42.2	83.0	80.5	<0.001 significant
Test item	75.0	97.0	96.6	<0.001 significant
Reference item	5.0	95.0	94.3	

LR_{50}: 30.6 g a.s./ha; 95 % Confidence Interval: 27.0 - 34.3 (calculated with Probit analysis)
* Corrected mortality according to SCHNEIDER-ORELLI (1947)
** Fisher`s Exact test (one-sided), p-values are adjusted according to Bonferroni-Holm

Conclusion:

The LR_{50} was calculated to be 30.6 g a.s./ha. The NOER for mortality was 13.3 g a.s./ha.

The figures obtained fulfil the validity criteria of the laboratory method for exposure on glass plates (BLÜMEL ET AL., 2000).

RMS Comments

This study was conducted appropriately and was compliant with GLP. The validity criteria were met.

This study is considered valid and acceptable for use in the risk assessment. The endpoint is confirmed as an **LR_{50} value of 30.6 g a.s./ha.**

Extended laboratory tests

This is considered to represent a data point for products according to (EU) No. 284/2013. These study summaries can therefore be found at CP B.9.5.2.2.

B.9.4. EFFECTS ON NON-TARGET SOIL MESO- AND MACROFAUNA

B.9.4.1. Earthworm – sub-lethal effects

Previous evaluation:	New data, submitted for purpose of review

Report:	KCA 8.4.1/01; Frommholz, U.; 2016
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
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
GLP/GEP:	Yes

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.
Reference item :	Carbendazim 360 g a.s./L (test conducted Oct 15 – December 11 2014, non-GLP).
Test organism:	Adult *Eisenia fetida (Eisenia fetida andrei*; strain of Prof. Graff, Forschungsanstalt für Landwirtschaft, 38104 Braunschweig, Germany*)*; mean bodyweight at the start of the test ranged from 0.25 to 0.47 g/worm.
Age of test organisms:	Approximately 3 months old (synchronised culture of earthworms). In order to obtain a synchronised culture of earthworms 30 adult worms were kept in a closed rearing vessel with substrate and food for a period of 4 weeks. After this time, the adult worms were selected out of the substrate and the offspring hatched from the cocoons. Adult worms, at least 2 months old, but not older than 1 year, with a well-developed clitellum were used for the study. The age of the worms from the synchronised culture differed not more than 4 weeks.
Acclimation:	One day prior to the test start, the test organisms were acclimated to the artificial soil and test temperature.
Test duration:	8 weeks (4 weeks exposure, 4 week reproductive phase).
Test concentrations :	*Test item*: 5.6, 10, 18, 32, 56, 100, 178 and 326 mg a.s./kg dws *Reference item:* 0, 1.25, 2.50, 5.00 mg carbendazim/kg dws (conducted at a different time to the main test) *Controls:* Water and solvent (acetone) controls.
Preparation and application of the test item:	The test item was applied as a test item-quartz sand mixture. All test mixtures were prepared freshly on the day of application. Prior to the start of the test, a test item stock solution of the highest concentration of the test item was prepared.

	The application solution of the highest test concentration was further diluted with acetone to obtain the desired test concentrations. 1 mL of the test item solution was mixed into quartz sand as homogeneously as possible for each replicate of the test concentrations. After evaporation of the acetone, the dry test item-quartz sand mixtures were applied to the artificial soil. Uniform amounts of the application mixtures were used for all replicates of the test concentrations. They were thoroughly mixed into the artificial soil of each replicate using a laboratory mixer in the order lowest test concentration to highest test concentration. Control and solvent control were treated in the same way but with quartz sand and acetone-quartz sand mixture only. Treatment and control were moistened with 129 mL deionised water.
No. of individuals per replicate:	10
No. of replicates:	8 per control and solvent control and 4 for the test item.
Test units :	Non-re-usable plastic boxes (length x width x height *ca*. 16.5 cm x 12 cm x 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. The test vessels were covered with transparent lids to prevent earthworms from escaping and to reduce evaporation during the test period. The lids were perforated to allow air exchange.
Test conditions :	pH: 5.97 – 6.14 (min – max across all test vessels) WHC: 51.81 – 59.60 (min – max across all test vessels) Light intensity: 564 – 579 lux Temperature: 19.4 – 25.3°C (the temparture could not be recorded for two of the test days). Duration: light : dark = 16 : 8
Feeding:	One day after the application (day 1), an amount of approximately 5 g finely ground, air dried animal manure was evenly distributed over the surface of the artificial soil in each test vessel. The food was moistened with deionised water (approximately 6 mL per 5 g food). The adult earthworms were fed once per week during the test period with approximately 5 g food/vessel. If food consumption was low (determined by visual inspection), the additional amount of food was reduced accordingly. The offspring were fed only once at the start of the second 4 weeks exposure period by mixing the food into the soil. The surface of the artificial soil was moistened with deionised water once per week in order to compensate evaporation.
Artificial soil:	The artificial soil was prepared with the following composition (percentage distribution on dry weight basis): • Sphagnum peat (shredded): 10 % • Kaolinite clay (content of Kaolinite = 30.2 %) 20 % • Industrial quartz sand (Particle size: 0.20 mm – 0.05 mm = 91.35 %): 70 % Calcium carbonate for the adjustment to pH 6.0 ± 0.5 (CaCO3) was added. The artificial soil was prepared by mixing the dry components intensely in a laboratory mixer. Then, the soil was pre-moistened with 5.2 L deionised water per 26 kg dry weight artificial soil.
Test procedure:	Directly after application the individually weighed adult earthworms were exposed to the treated artificial soil. Before weighing, the earthworms were quickly washed with water and surplus water was absorbed on filter paper.

	The sequence of inserting the test organisms into the test vessels followed a computer-generated random design. Only healthy animals were used. After a period of 4 weeks the adult earthworms were removed from the test vessels, the survivors were counted and their fresh weight was measured. From these data mortality and biomass effects were determined. The cocoons and juvenile earthworms remained in the vessels for additional four weeks. After this additional test period the reproduction was determined by counting the number of offspring hatched from the cocoons per test vessel.
Observations:	*Mortality:* After 4 weeks of exposure, the content of each test vessel was emptied and the living adult earthworms were counted and checked for any abnormal behaviour or other adverse effects (e.g. lack of movement, rigidity etc.). Those earthworms, which did not move after gentle mechanical stimulus, were considered to be dead. Also missing earthworms (compared to the number of initially placed test organisms) were considered to have died. Mortality of the adult earthworms in comparison to the initial placed test organisms was determined and expressed in % mortality. *Growth:* At the beginning (prior to exposure) and at the end of the 4 weeks of exposure, the adult test organisms of each vessel were weighed (at the start each individually, at the end together of each test vessel). Changes in body weight values of the surviving test organisms of the treatment groups during the test period were compared to the values of the solvent control group. *Reproduction:* At the end of the test after 8 weeks, the number of surviving juveniles per test vessel was determined. The test vessels were placed in a water bath at 50 - 60 °C. By this treatment, alive juvenile earthworms rose to the soil surface. The emerging earthworms were removed and counted. Afterwards the content of each test vessel was checked additionally by carefully stirring up the artificial soil with the help of tweezers. The reproduction of the surviving test organisms per test vessel at the end of the study was compared to the solvent control. Water holding capacity (WHC) of the soil was determined prior to test initiation, at day 0 and day 56. pH of the soil was determined prior to the test and at test termination. Temperature was measured daily and light intensity measured on day 0, 28 and 56.
Statistical analysis:	The calculation of mean, SD and percentage mortality of the control and treatment groups was conducted in Excel. The software used to perform the statistical analysis was ToxRat Professional 2.10 released February 20, 2010, (Ratte, 2010). Reproduction data were tested for normal distribution and homogeneity of variance using Shapiro-Wilk's-Test and Cochran's-Test ($\alpha = 0.05$) respectively. These conditions were met, therefore William's t-test, onesided- smaller, $\alpha = 0.05$ was used to determine the NOEC.

Results

Validity criteria:

Validity criteria according to OECD 222 (2004)	Obtained in this study
Adult mortality in the control ≤ 10 %	Water control: 0 % Solvent control : 5 %
Rate of reproduction of juveniles (earthworms per control vessel) ≥ 30	Water control: ≥ 62 Solvent control: ≥ 59
Coefficient of variation of reproduction in the control ≤ 30 %	Water control : 11.3 % Solvent control : 17.8%

All validity criteria were met in this study.

Toxic reference test:

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. The number of juveniles per test vessel of the two highest test concentrations of 2.50 and 5.00 mg a.s./kg dry weight artificial 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.

Biological results:

After 28 days of exposure, no mortality in the control group and 5% in the solvent control group was observed. A significant decarese in mortalirt in comparison to the control was observed at a concentration of 10 mg a.s./kg dws but not at any other test concentration.

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; however a reduction in juvenile number of 13.5, 10.2 and 12.4% was observed at 326, 178 and 100 mg a.s./kg dws respectively was observed.

A summary of the results is presented in Table B.9.4.1-1 below.

Table B.9.4.1-1: Mortality, body weight change and reproductive output of earthworms exposed to BCS-CN88460

Test object / **Test item**	***Eisenia fetida*** **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										
Replicate 1	90	83	43	78	76	79	73	68	100	44
Replicate 2	78	90	95	67	68	79	70	89	57	66
Replicate 3	88	66	78	117	89	69	96	60	55	58
Replicate 4	81	88	77	48	60	105	80	66	78	100
Replicate 5	83	78	-	-	-	-	-	-	-	-
Replicate 6	72	77	-	-	-	-	-	-	-	-
Replicate 7	62	59	-	-	-	-	-	-	-	-
Replicate 8	82	105	-	-	-	-	-	-	-	-
Mean	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				56[1]		

[1] Based on biological relevance
* 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

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 56 mg a.s./kg dws.

RMS comments

This study was conducted according to GLP and following OECD 222 guidelines.

The following was noted by the RMS :

The temperature during the test was outside the stated range in the test guidelines (20±2°C) and on two days the temperature could not be determined. However as the controls performed well, meeting all validity criteria, this is not considered to invalidate the test.

A statistically significant increase in mortality was observed at a test concentration of 10 mg a.s./kg dws. Whilst statistically signficant, the RMS does not consider this to be related to the test item. Looking at the individual test replicates, 90% mortality was observed in replicate 4 but not in any other replicate, indicating an issue with this

particular replicate rather than a clear effect of the test item.

Whilst not statistically significant, a reduction in juvenile number of 13.5, 10.2 and 12.4% was observed at 326, 178 and 100 mg a.s./kg dws respectively relative to the solvent control was observed, which could be potentially biologically relevant. Looking at the individual replicates, replicate 1 of 178 mg a.s./kg dws and replicate 4 of 326 mg a.s./kg dws contained 100 juveniles, whilst the other replicates contained between 55 – 78 and 44 – 66 juveniles at 178 and 326 mg a.s./kg dws respectively. The coeffcients of variation in these replicates are also relative large at 29.1 and 35.5% for test concentrations 178 and 326 mg a.s./kg dws respectively, due to the replicate containing 100 juveniles in each case, which may explain why no clear dose-response was observed at these test concentrations. The removal of these replicates results in a mean number of juveniles of 63.3 and 56 juveniles at 178 and 326 mg a.s./kg dws respectively. This results in a reduction in juvenile number of 30.7%, 21.7% and 12.4% at 326, 178 and 100 mg a.s./kg dws respectively relative to the solvent control and a clearer dose-response. As no EC_{10} or EC_{20} values were calculated from this study due to the lack of clear dose-response, the RMS considers it appropriate to conservatively set the NOEC based on reproductive output at 56 mg a.s./kg dws.

This study is considered valid and the endpoint confirmed for use in the risk assessment is as follows :

NOEC (reproductive output) = 56 mg a.s./kg dws

Previous evaluation:	New data, submitted for purpose of review

Report:	KCA 8.4.1/02; Frommholz, U.; 2017
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
Guideline(s):	EU Directive 91/414/EEC; Regulation (EC) No. 1107/2009; US EPA OCSPP Not Applicable
GLP/GEP:	**yes**

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).
Reference item :	Carbendazim 360 g a.s./L (test conducted August 25 – November 19 2015 and July 4 to December 20 2016, non-GLP).
Test organism:	Adult *Eisenia fetida (Eisenia fetida andrei*; strain of Prof. Graff, Forschungsanstalt für Landwirtschaft, 38104 Braunschweig, Germany*)*; mean body weight 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.
Age of test organisms:	The earthworms were adult with a well-developed clitellum and approximately 3 months old (1st run) and 5-6 months old (2nd run). In order to obtain a synchronised culture of earthworms 30 adult worms were kept in a closed rearing vessel with substrate and food for a period of 4 weeks. After this time, the adult worms were selected out of the substrate and the offspring hatched from the cocoons. Adult worms, at least 2 months old, but not older than 1 year, with a well-developed clitellum were used for the study. The age of the worms from the synchronised culture differed not more than 4 weeks.
Acclimation:	One day prior to the test start, the test organisms were acclimated to the artificial soil and test temperature.
Test duration:	8 weeks (4 weeks exposure, 4 week reproductive phase).
Test concentrations :	*Test item*: 1st run: 150 mg pure metabolite/kg dws; 2nd run: 10, 18, 32, 56 and 100 mg pure metabolite/kg dws *Reference item:* 0, 1.25, 2.50, 5.00 mg carbendazim/kg dws (conducted at a different time to the main test)

	Controls: Water and solvent (acetone) controls.
Preparation and application of the test item:	All test mixtures were prepared freshly on the day of application, by dissolving in acetone and mixing with quartz sand. The application solution of the highest test concentration was further diluted with acetone to obtain the desired test concentrations in the 2nd test run. 1 mL of the test item solution was mixed into quartz sand as homogeneously as possible for each replicate of the test concentrations. After evaporation of the acetone, the dry test item-quartz sand mixtures were applied to the artificial soil. Uniform amounts of the application mixtures were used for all replicates of the test concentrations. Control and solvent control were treated in the same way but with quartz sand and acetone-quartz sand mixture only. Treatment and control were moistened with deionised water.
No. of individuals per replicate:	10
No. of replicates:	*1st run:* 8 per control, solvent control and test item *2nd run*: 8 per control and solvent control and 4 for the test item
Test units :	Non-re-usable plastic boxes (length x width x height *ca*. 16.5 cm x 12 cm x 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. The test vessels were covered with transparent lids to prevent earthworms from escaping and to reduce evaporation during the test period. The lids were perforated to allow air exchange.
Test conditions :	pH: 1st run: 6.03 – 6.14; 2nd run: 6.04 – 6.64 (min – max across all test vessels) WHC: 1st run: 54.33 – 57.25%; 2nd run: 54.92 – 58.73% (min – max across all test vessels) Light intensity: 1st run: 522 – 607 lux; 2nd run: 584 – 671 lux Temperature: 19.4 – 25.3°C (the temperature could not be recorded for two of the test days). Duration: light : dark = 16 : 8
Feeding:	One day after the application (day 1), an amount of approximately 5 g finely ground, air dried animal manure was evenly distributed over the surface of the artificial soil in each test vessel. The food was moistened with deionised water (approximately 6 mL per 5 g food). The adult earthworms were fed once per week during the test period with approximately 5 g food/vessel. If food consumption was low (determined by visual inspection), the additional amount of food was reduced accordingly. The offspring were fed only once at the start of the second 4 weeks exposure period by mixing the food into the soil. The surface of the artificial soil was moistened with deionised water once per week in order to compensate evaporation.
Artificial soil:	The artificial soil was prepared with the following composition (percentage distribution on dry weight basis): • Sphagnum peat (shredded): 10 % • Kaolinite clay (content of Kaolinite = 30.2 %) 20 % • Industrial quartz sand (Particle size: 0.20 mm – 0.05 mm = 91.35 %): 70 % Calcium carbonate for the adjustment to pH 6.0 ± 0.5 (CaCO3) was added. The artificial soil was prepared by mixing the dry components intensely in a laboratory mixer. Then, the soil was pre-moistened with deionised water.

Test procedure:	Directly after application the individually weighed adult earthworms were exposed to the treated artificial soil. Before weighing, the earthworms were quickly washed with water and surplus water was absorbed on filter paper. The sequence of inserting the test organisms into the test vessels followed a computer-generated random design. Only healthy animals were used. After a period of 4 weeks the adult earthworms were removed from the test vessels, the survivors were counted and their fresh weight was measured. From these data mortality and biomass effects were determined. The cocoons and juvenile earthworms remained in the vessels for additional four weeks. After this additional test period the reproduction was determined by counting the number of offspring hatched from the cocoons per test vessel.
Observations:	*Mortality:* After 4 weeks of exposure, the content of each test vessel was emptied and the living adult earthworms were counted and checked for any abnormal behaviour or other adverse effects (e.g. lack of movement, rigidity etc.). Those earthworms, which did not move after gentle mechanical stimulus, were considered to be dead. Also missing earthworms (compared to the number of initially placed test organisms) were considered to have died. Mortality of the adult earthworms in comparison to the initial placed test organisms was determined and expressed in % mortality. *Growth:* At the beginning (prior to exposure) and at the end of the 4 weeks of exposure, the adult test organisms of each vessel were weighed (at the start each individually, at the end together of each test vessel). Changes in body weight values of the surviving test organisms of the treatment groups during the test period were compared to the values of the solvent control group. *Reproduction:* At the end of the test after 8 weeks, the number of surviving juveniles per test vessel was determined. The test vessels were placed in a water bath at 50 - 60 °C. By this treatment, alive juvenile earthworms rose to the soil surface. The emerging earthworms were removed and counted. Afterwards the content of each test vessel was checked additionally by carefully stirring up the artificial soil with the help of tweezers. The reproduction of the surviving test organisms per test vessel at the end of the study was compared to the solvent control. Water holding capacity (WHC) of the soil was determined prior to test initiation, at day 0 and day 56. pH of the soil was determined prior to the test and at test termination. Temperature was measured daily and light intensity measured on day 0, 28 and 56.
Statistical analysis:	Data of reproduction for the 1st run were statistically evaluated by using Student-t test for Homogeneous Variances, one-sided smaller, $\alpha = 0.05$. For the 2nd run data of reproduction were tested for normal distribution and homogeneity of variance using Kolmogorov - Smirnov -Test and Cochran's –Test, $\alpha = 0.05$ respectively. Data of reproduction were normally distributed and the homogeneity ofvariances was given. Therefore Williams multiple sequential-t test, one-sided smaller, $\alpha = 0.05$, was used to determine NOEC values.

Results

Validity criteria:

All validity criteria were met in this study.

Validity criteria according to OECD 222	**Recommended**	**Obtained**	
		1st run	**2nd run**
Adult mortality in the control	≤ 10 %	Control: 0 % Solvent control: 5 %	Control : 0 % Solvent control : 1.25 %
Rate of reproduction of juveniles (earthworms per control vessel)	≥ 30	Control : 62 to 90 Solvent control : 59 - 105	Control : 223 to 362 Solvent control : 205 to 300
Coefficient of variance of reproduction in the control	≤ 30 %	Control : 11.3 % Solvent control : 17.8 %	Control : 18.1 % Solvent control : 15.4%

Toxic reference test:

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.

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 (Table B.9.4.1-2); values in this table are rounded values).

Table B.9.4.1-2: Mortality, body weight chamge and reproductive output of earthworms exposed to BCS-CN88460-carboxylic acid

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	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	1113.1	104.7	114.7	106.3	-	-	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**	**100**
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).

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.

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). However a

reduction of 15.2% at 150 mg pure metabolite/kg dws in reproductive output in comparison to the solvent control was observed. 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, $\alpha = 0.05$) in comparison to the solvent control group..

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.

RMS comments

This study was conducted according to GLP and following OECD 222 guidelines.

The following was noted by the RMS :

The temperature during the test was outside the stated range in the test guidelines (20±2°C) and on two days the temeperture could not be determined. However as the controls performed well, meeting all validity criteria, this is not considered to invalidate the test.

A NOEC based on reproductive output of 150 mg pure metabolite/kg dws was proposed from the 1st run of the study; however whilst not statistically significant, a reduction of 15.2% in reproductive output was observed at this test concentration, which could be considered biologically relevant. As such, the RMS considers the NOEC based on reproductive output to be 100 mg pure metabolite/kg dws from the 2nd run of the study.

This study is considered valid and the endpoint confirmed for use in the risk assessment is:

NOEC growth/reproduction = 100 mg pure metabolite/kg dws

B.9.4.2. Effects on non-target soil meso- and macrofauna (other than earthworms)

Previous evaluation:	New data, submitted for purpose of review

Report:	KCA 8.4.2.1/01; Frommholz, U.; 2015
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
Guideline(s):	EU Directive 91/414/EEC Regulation (EC) No. 1107/2009 US EPA OCSPP not applicable
GLP/GEP:	Yes

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.
Reference item :	Boric acid (test conducted March 2015, non-GLP).
Test organism:	*Folsomia candida* (Collembolan, Isotomidae) bred at Bayer CropScience since January 2012. The strain was originally obtained from Ibacon, Institute for Analytic and Consulting, GmbH, 64380 Rossdorf, Germany.
Age of test organisms:	9 – 12 days old from a synchronised culture. Twelve days before starting the study, egg clusters from the breeding containers were transferred to fresh breeding substrate to obtain collembolans of a uniform age (9-12 days old at test start). After 3 days the egg clusters were removed and the remaining collembolans hatched from the eggs were fed with granulated dry yeast.
Breeding of the test organism:	The collembolans were bred on a mixture of Plaster of Paris and activated charcoal and demineralised water (10:1.25:12.5 w/w). Plastic vessels (9.5 cm diameter) were filled up to a height of approximately 1 cm with this mixture. The vessels, closed with plastic

	lids, were moistened and aerated regularly twice a week. Breeding vessels were kept at room temperature in permanent darkness and fed once per week with dried bakers yeast.
Test duration:	28 days
Test concentrations :	*Test item*: 99, 176, 313, 556, and 990 mg a.s./kg dws *Reference item:* 27, 37, 52, 72, 100, 139, 193 and 269 mg Boric acid/kg dws (conducted at a different time to the main test) *Controls:* Quartz sand without test item.
Preparation and application of the test item:	The test item was applied as a test-item-quartz-sand-mixture. All test mixtures were prepared freshly on the day of application. For each application rate 500 g instead of 495 g of artificial soil dry weight was treated. The test-item-quartz-sand-mixture was thoroughly mixed into 500 g artificial dry weight artificial soil of each application rate using a laboratory mixer. Treatment and control were moistened with 50 mL deionized water. Afterwards the treated artificial soil of control and treatment was portioned out. Each test vessel of the 8 control and the 4 treatment replicates plus the one for measurement purpose was filled up with 30±1 g wet weight artificial soil avoiding compression of the artificial soil.
No. of individuals per replicate:	10
No. of replicates:	8 per control and 4 for the test item.
Test units :	Test containers were reusable glass vessels (volume 140 mL, diameter 5 cm). Each test vessel contained 30±1 g wet weight artificial soil. The test vessels were covered with glass lids to prevent the collembolans from escaping but allowing aeration during the test period.
Feeding and water:	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 each test vessel was weighed for the determination of water loss. After 14 days the loss of water was determined by reweighing the test vessels. The vessels were rewetted with the approximately 2-fold amount of the missing water.
Artificial soil:	The artificial soil was prepared according to the guideline with the following constituents (percentage distribution on dry weight basis): - 75 % fine quartz sand (sort F 36, particle size 0.2 – 0.05 mm = 91.35 %) - 5 % Sphagnum peat, air dried and finely ground - 20 % Kaolin clay (content of Kaolinite: 30.2 %) - Calcium carbonate (CaCO3) for the adjustment to pH to 6.0 ± 0.5 The artificial soil was prepared by mixing the dry components in a laboratory mixer. Two days before test start the dry artificial soil was pre-moistened with 64 mL deionized water per 500 g dry weight artificial soil. During the application of the test item the water content was adjusted to a final water content of approximately 50 % of the maximum water holding capacity.
Test procedure:	Directly after application the 9-12 days old collembolans from the synchronised cohort were collected with an exhauster into small glass tubes. They were counted to ensure that 10 non-damaged individuals were introduced. Then the 10 collembolans were placed on the soil surface of the treated soil and the untreated control respectively. The sequence of inserting the test organisms into the test vessels followed a computer-generated random design. The test vessels were set up randomised in a climatic test room. After 7, 14 and 21 days the test vessels were re-randomised.

	After 28 days, the soil of each replicate was transferred into a plastic vessel (volume: 200 mL; surface: 75 cm^2). Each portion was stirred up with 80 mL of deionized water and the collembolans drifted to the surface. The water was coloured with 10 mL black ink in order to increase the contrast between the water and the white collembolans. From each vessel a digital image was taken and checked by visual inspection. The adult collembolans were visually counted and marked on the digital image, followed by confirmation of the automatically generated juvenile count, which was manually corrected if necessary. The extraction efficiency of this method was determined to be 97 % in a separate extraction run in vessels containing a known number of adult and juvenile collembolans kept in untreated artificial soil.
Observations:	Mortality and reproduction was recorded at test termination. Missing adults (compared to the number of initially placed test organisms) were considered to be dead Water holding capacity (WHC) of the soil was determined at day 0 and day 28. pH of the soil was determined at test initiation and at test termination. Temperature was measured continuously and light intensity measured on day 0, 14 and 28.
Statistical analysis:	The calculation of mean, SD and percentage mortality of the control and treatment groups was conducted in Excel. Data of reproduction were tested for outliers using outlier test after Dixon & Hartley. One outlier in the control group was found and excluded from further calculations and statistics. Afterwards data of reproduction were tested for normal distribution and homogeneity of variance using Kolmogorov - Smirnov -Test and Cochran's -Test $\alpha = 0.05$) respectively. Data of reproduction were normally distributed and homogeneity of variances was given. Therefore William's t-test (one-sided-smaller, $\alpha = 0.05$) was used to determine the NOEC. The software used to perform the statistical analysis was ToxRat Professional 2.10 released February 20, 2010, (Ratte, 2010).

Results

Validity criteria:

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 %

All validity criteria were met in this study.

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.

Experimental conditions:

The following table (Table B.9.4.2-1) show the experimental conditions during the test :

Table B.9.4.2-1: Experimental conditions during the test.

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

Light intensity was 593 – 647 lux, temperature was 20±2°C and collembolans were kept under a 16:8 hour light:dark cycle.

Biological results:

The following table (Table B.9.4.2-2) shows a summary of the mortality and reproduction results during the test :

Table B.9.4.2-2: Mortality and reproductive output of *Folsomia candida* following exposure to BCS-CN88460

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	

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.

RMS comments

This study was conducted according to GLP and following OECD 232 (2016) test guidelines. The following was noted by the RMS :

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. This deviation is considered to be acceptable by the RMS.

Collembolans were bred under complete darkness, rather than the 12 :12 hour light :dark cycle recommended in the test guidelines. As sufficient synchronised collembolans were bred for the test using this method, this is not considered to be an issue by the RMS.

Replicate 7 of the untreated control was excluded from analysis as it was deemed at outlier. Looking at the raw data, the RMS would agree with this conclusion and removal of this replicate due to the high mortality observed.

The reference test with boric acid resulted in an EC_{50} of 77 mg boric acid/kg dws ; this is lower than the 100 mg boric acid/dws recommended in the test guidelines. However, as this indicates that the test organisms are more sensitive, this is not considered to be an issue by the RMS.

No $EC_{10/20}$ values could be calculated from this test as a clear dose-response was not observed. As the NOEC is protective of 10% effects on reproductive output, this is considered to be acceptable by the RMS.

This study is considered valid and the endpoint confirmed for use in the risk assessment is :

$NOEC_{reproduction}$: 99 mg a.s./kg dws

Previous evaluation:	New data, submitted for purpose of review

Report:	KCA 8.4.2.1/02; Friedrich, S.; 2017
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
Guideline(s):	EU Directive 91/414/EEC Regulation (EC) No 1107/2009 (2009) US EPA OCSPP Not Applicable
GLP/GEP:	Yes

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.
Reference item :	Boric acid
Test organism:	*Folsomia candida* (Collembolan, Isotomidae). The strain was originally obtained from "Biologische Bundesanstalt (BBA)", Berlin-Dahlem, Germany in May 2000 and then bred in the test laboratory under ambient laboratory conditions.
Age of test organisms:	9 – 12 days old from a synchronised culture. Twelve days before starting the study, egg clusters from the breeding containers were transferred to fresh breeding substrate to obtain collembolans of a uniform age (9-12 days old at test start). After 3 days the egg clusters were removed and the remaining collembolans hatched from the eggs were fed with granulated dry yeast. After a further 9 days the test organisms were collected and used for the test.
Breeding of the test organism:	The collembolans were bred on a mixture of Plaster of Paris and activated charcoal and demineralised water (8:1:9). Plastic vessels (9.5 cm diameter) were closed with plastic lids and kept at 20±2 °C with a light:dark cycle of 16:8 hours and fed twice per week with granulated dried yeast. Vessels were aerated twice per week and moistened automatically with an absorbent wick.
Test duration:	28 days
Test concentrations :	*Test item*: 18, 32, 56, 100, 178, 316, 562, 1000 mg pure metabolite/kg dws *Reference item:* 44, 67, 100, 150 and 225 mg Boric acid/kg dws (conducted at a different time to the main test) *Controls:* Untreated (quartz sand only)

Preparation and application of the test item:	On the day of the test, the test item was mixed with a small quantity of finely ground quartz sand (10 g treated sand per treatment group), such that the required test concentration was achieved once mixed with the artificial soil. The control substrate contained the corresponding amount of untreated quartz sand only. After thorough mixing, 30 g (wet weight) of the test substrate was placed into each vessel, avoiding compression.
No. of individuals per replicate:	10
No. of replicates:	8 per control and 4 for the test item.
Test units :	Glass container (approximately 150 mL) covered with a lid; surface area of soil: 18.9 cm^2. Test vessels contained 30 g of soil (wet weight).
Feeding and water:	Collembolans were fed with 2mg of granulated dry yeast at test start and after 14 days. At test start each test vessel was weighed for the determination of water loss. The water content was checked weekly by reweighing the additional test vessels. Water loss was compensated if exceeding 2 % of the initial water content.
Artificial soil:	The artificial soil was prepared according to the guideline with the following constituents (percentage distribution on dry weight basis): 5 % sphagnum peat; origin: Torfwerk Moorkultur Ramsloh, 26683 Saterland, Germany, classified according to DIN 11540 (as close to pH 5.5-6.0 as possible, no visible plant remains, finely ground, dried to measured moisture content) - 20 % kaolin clay (kaolinite content > 30 %); type: Kaolin W, origin: ERBSLÖH Lohrheim GmbH, 65558 Lohrheim, Germany - 0.3 % calcium carbonate; origin: MERCK KGaA, 64271 Darmstadt, Germany - 74.7 % industrial quartz sand; type: Millisil W3, origin: Quarzwerke GmbH, 50207 Frechen, Germany (fine sand is dominant with more than 50 % of the particles between 50 and 200 μ m) - deionised water The artificial soil was prepared by mixing the dry components in a laboratory mixer and moistened to 57.8 – 58.0 % of water holding capacity (WHC) two days before the start of the test.
Test conditions:	Water content: test start: 24.9 – 25.0 (equivalent to 57.8 – 58.0 % of WHC); test end: 24.4 – 24.8 (equivalent to 56.6 – 57.5 % of WHC). pH: test start: 6.04 – 6.10; test end: 5.82 – 5.92. Temperature: 19.1 – 21.2 °C. Light intensity: 570 lux, 16:8 hour light:dark cycle.
Test procedure:	Ten test organisms were introduced to each vessel, using an aspirator. After addition of the test organisms, the test vessels were positioned randomly in a controlled-environment test room, and these positions were re-randomized weekly. The test containers were tightly covered with a lid and briefly opened twice a week for aeration. Four weeks after introducing the test organisms the parental and juvenile collembolans in the test and control vessels were counted. The test substrate of each replicate was poured into an individual container (with a volume of about 200 mL) and the test organisms were floated off the substrate by the addition of water. To improve the contrast between the white collembolans and surrounding water surface, the water was stained dark with ink. After gentle stirring the numbers of parental and juvenile collembolans floating on the surface were determined. Missing parental collembolans were assumed to have died during the test period. Surviving adults and juveniles were counted using a digital image processing system (LemnaTec Scanalyzer), an automated counting technique based on a video camera connected to a digital image storage and

	analysis system. The extraction efficiency of the extraction method was determined to be 97 % in a separate extraction run using vessels containing a known number of juvenile kept in untreated test substrate.
Observations:	Mortality and reproduction was recorded at test termination. Missing adults (compared to the number of initially placed test organisms) were considered to be dead Water holding capacity (WHC) of the soil, pH and light intensity was determined at day 0 and day 28. Temperature was measured continuously.
Statistical analysis:	The statistical analysis was performed with the software ToxRat Professional 3.2.1 (Ratte 2015). The Multiple sequentially-rejective Fisher Test after Bonferroni-Holm and the Multiple sequentially-rejective U-test after Bonferroni-Holm were used to compare the control with the independent test item group. The LCx and ECx values were calculated by Logit analysis using linear maximum likelihood regression and Probit analysis using linear maximum likelihood regression, respectively. Confidence limits (95 %) of the LCx and ECx values were computed by normal approximation.

Results

Validity criteria:

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 %

All validity criteria were met in this study.

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.

Biological results

Mortality rates of 0 % - 100 % were recorded in the test item treatment groups. 2.5 % parental mortality was observed in the control. Statistically significant effects on mortality compared to the control were observed at concentrations of 32, 56, 100, 178, 316, 562 and 1000 mg pure metabolite/kg dws. No effects on behaviour of the collembolans were observed during the test. The NOEC for mortality was determined to be 18 mg pure metabolite/kg dws. The LC_{10} and LC_{20} values for mortality were calculated to be 24 and 31 mg pure metabolite/kg dws, respectively (based on Logit analysis).

The mean number of juvenile collembolans counted four weeks after introduction of the parental collembolans into the test vessels was 738 in the control and 755, 417, 183, 181, 114, 123, 72 and 46 at concentrations of 18, 32, 56, 100, 178, 316, 562 and 1000 mg pure metabolite/kg soil d.w., respectively (Table B.9.4.2-3). Statistically significant effects on the number of juveniles compared to the control group were recorded at concentrations of 32, 56, 100, 178, 316, 562 and 1000 mg pure metabolite/kg dws. The NOEC for reproduction was determined to be 18 mg pure metabolite/kg dws. The EC_{10} and EC_{20} values for reproduction were calculated to be 13 and 20 mg pure metabolite/kg dws, respectively (based on Probit analysis).

A summary of the mortality and reproductive output results form the test is provided in Table B.9.4.2-3 below :

Table B.9.4.2-3: Effects of BCS-CN88460-carboxylic acid (BCS-CY26497) on mortality and reproductive output of *Folsomia candida*

Test item **Test object** **Exposure**	**BCS-CN88460-carboxylic acid (M12)** ***Folsomia candida*** **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
						Mortality	**Reproduction**
LC_{10}[1)]/EC_{10}[2)] [mg pure metabolite/kg dry weight artificial soil] 95% confidence limits						24 (14 – 40)	13 (4.9 – 37)
LC_{20}[1)]/EC_{20}[2)] [mg pure metabolite/kg dry weight artificial soil] 95% confidence limits						31 (20 – 47)	20 (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)

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 NOEC for mortality was determined to be 18 mg pure metabolite/kg dws. 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, NOEC for reproduction was determined to be 18 mg pure metabolite/kg dws. An EC_{10} value of 13 mg pure metabolite/kg dws was calculated.

RMS comments

This study was conducted according to GLP and following OECD 232 (2016) test guidelines. The following was noted by the RMS :

Collembolans were bred under a 16:8 hour light:dark cycle, rather than the 12:12 hour light:dark cycle recommended in the test guidelines. As sufficient synchronised collembolans were bred for the test using this method, this is not considered to be an issue by the RMS.

The EC_{10} value based on reproductive output (13 mg pure metabolite/kg dws) calculated from this study is lower than the NOEC (18 mg pure metabolite/kg dws) where the number of juveniles actually increased relative to the control. As such, the RMS considers this value inaccurate and the NOEC will be used in the risk assessment.

This study is considered valid and the endpoint considered suitable for use in the risk assessment is as follows:

$NOEC_{reproduction}$: 18 mg pure metabolite/kg dws

Previous evaluation:	New data, submitted for purpose of review

Report:	KCA 8.4.2.1/03; Larnaudie-Lopez, M.; 2015
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
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
GLP/GEP:	yes

Material and methods

Test item:	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).
Reference item :	Dimethoate (non-GLP, conducted January 05, 2015).
Test organism:	*Hypoaspis aculeifer* bred at Bayer CropScience AG since 2002. The strain was originally obtained from ECT Oekotoxikologie GmbH, 65439 Flörsheim a. M.
Age of test organisms:	30 days old (adult females) from a synchronised culture. In order to obtain adult, female *Hypoaspis aculeifer* of a uniform age on 2015-01-05, 2 x 300 adult, female *Hypoaspis aculeifer* were transferred to fresh breeding vessels. On 2015-01-08, after three days of egg laying, these females were removed. The *Hypoaspis aculeifer* hatched from the eggs were fed with *Panagrellus redivivus.*
Breeding of the test organism:	The mites were bred on a mixture of Plaster of Paris and activated charcoal and demineralised water (10:1.25:12.5 w/w). Plastic vessels (9.5 cm diameter) were filled up to a height of approximately 1 cm with this mixture and are closed with lids. Mites were fed with *Panagrellus redivivus* (nematodes) which were bred on watered oak flakes. The breeding culture was kept under the following conditions: Temperature: room temperature Light cycle: permanent dark
Test duration:	14 days (plus 2 days for extraction of mites)
Test concentrations :	*Test item*: 99, 176, 313, 556 and 990 mg a.s./kg dws *Reference item:* 1.0, 1.8, 3.2, 5.6 and 10.0 mg dimethoate/kg dws (conducted at a different time to the main test) *Controls:* Untreated (quartz sand only)
Preparation and application of the test item:	The test item was applied as a test-item-quartz-sand-mixture. All test mixtures were prepared freshly on the day of application. For each application rate 505 g of artificial soil dry weight was treated. Prior to the start of the test stock mixtures were prepared. The 5 g test-item-quartz-sand-mixture was thoroughly mixed into the pre-moistened 500 g artificial dry weight artificial soil of each application rate using a laboratory mixer. Treatment and control were moistened with 50 mL deionised water. Afterwards the treated artificial soil of control and treatment was portioned out. Each test vessel of the 8 control and the 4 treatment replicates plus the one for measurement purpose was filled up with 20±1 g dry weight artificial soil avoiding compression of the artificial soil.

No. of individuals per replicate:	10
No. of replicates:	8 per control and 4 for the test item. There was one additional vessel for each applicationrate for measurement of pH value and moisture of the artificial soil at the end of the test not loaded with *Hypoaspis aculeifer*.
Test units :	Test containers were reusable glass vessels (Weck Mini-Sturzglas, 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 test vessels were covered with glass lids to prevent *Hypoaspis aculeifer* from escaping but allowing aeration during the test period.
Feeding and water:	Directly after the addition of the *Hypoaspis aculeifer*, they were fed with nematodes (*Panagrellus redivivus*). Nematodes were bred on watered oak flakes in the laboratory. During the continuation of the test the soil mites were fed 2, 6 and 9 days after test start with the nematodes. Between 58 and 128 mg food per test vessel was added. Each test vessel was weighed for the determination of water loss. Seven days after test start water loss was determined and replenished.
Artificial soil:	The artificial soil was prepared according to the guideline with the following constituents (percentage distribution on dry weight basis): - 75 % fine quartz sand (sort F 36, particle size 0.2 – 0.05 mm = 91.35 %) - 5 % Sphagnum peat, air dried and finely ground - 20 % Kaolin clay (content of Kaolinite: = 30.2 %) - Calcium carbonate (CaCO3) for the adjustment to pH to 6.0 ± 0.5 The artificial soil was prepared by mixing the dry components in a laboratory mixer. Two days before test start the dry artificial soil was pre-moistened with 64 mL deionised water per 500 g dry weight artificial soil. During the application of the test item the water content was adjusted to a final water content of approximately 50 % of the maximum water holding capacity by mixing 50 mL deionised water into 500 g dry weight artificial soil for the control group and 50 mL test item solution for the treatment groups.
Test conditions:	Water content: test start:17.89 – 18.51 (equivalent to 46.16 ‑ 48.11 % of WHC); test end: 17.40 ‑ 18.48 (equivalent to 44.61 – 48.02 % of WHC). pH: test start: 5.74 ‑ 6.07; test end: 5.53 ‑ 6.64. Temperature: 20 ± 2 °C. Light intensity: 555 – 642 lux, 16:8 hour light:dark cycle.
Test procedure:	Directly after application of the test item, the adult, fertilized, females (30 days after start of egg laying for three days) were exposed to the control and treatment vessels. This was achieved by putting 10 females individually onto the surface of the artificial soil using a fine brush. The transfer of the test animals was finished within two hours after the application of the test item. After a period of 14 days, the surviving adults and the living juveniles per test vessel were extracted, applying a temperature gradient. The content of each test vessel was carefully transferred to sieve vessels (mesh size approximately 0.8 mm). The vessels were positioned in MacFadyen- Extractor. The temperature was increased from approximately 25 to 40 °C within two days. Extracted mites were collected in a fixing solution (20 % ethylene glycol, 80 % deionised water; 2 g detergent/L). The extracted mites in the fixing solution were stored in a refrigerator until the start of the counting of surviving adults and juveniles. All *Hypoaspis aculeifer* (adult females and juveniles) were counted under a binocular.
Observations:	Mortality and reproduction was recorded at test termination. Missing adults (compared to the number of initially placed test organisms) were considered to be dead.

	Water holding capacity (WHC) of the soil and pH was determined at day 0 and day 14. Light intensity was measured at test initiation and after each feeding of the test organisms. Temperature was measured continuously.
Statistical analysis:	The statistical analysis was performed with the software ToxRat Professional 2.10 (Ratte 2010). For the determination of normal distribution and homogeneity of variance Kolmogorov-Smirnov Test and Cochran's Test (α = 0.05), respectively were used. Data of reproduction were normally distributed and homogeneity of variances was given. Therefore William's-t test (one-sided-smaller, α = 0.05) was used to determine NOEC and LOEC values.

Results

Validity criteria:

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 %

All validity criteria were met.

Toxic reference test:

In a separate study (Maria Ivonne Larnaudie Lopez, LAR/HR-O-16/14, January 05, 2015) performed with the reference item dimethoate at test concentrations 1.0, 1.8, 3.2, 5.6 and 10.0 mg dimethoate/kg dry weight artificial soil, 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 (3.0 – 7.0 mg dimethoate/kg dws).

Experimental conditions:

pH and water content/WHC of the soil during the test is shown in Table B.9.4.2-4 below:

Table B.9.4.2-4: pH, water content and water holding capacity of the test soil

[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. A summary of the results is presented in Table B.9.4.2-5 below :

Table B.9.4.2-5: The effect of BCS-CN88460 on mortality and reproductive output of on mortality and reproductive output of *Hyposapis aculeifer* following exposure in artificial soil.

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	-
33	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				

Calculations were done with un-rounded values.
(*)=William's-t.-test one sided smaller; α=0.05; "-": non-significant; "+": significant

Conclusion

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

RMS comments

This study was conducted according to GLP and following OECD 226 (2008) guidance.

According to the study guidelines (OECD 226) extraction efficiency must be validated once or twice a year in controls with known numbers of adults and juveniles. Efficiency should be above 90% on average combined for all developmental stage. However these details are not given in the study report. It is noted that in the study conducted for the product testing *H.aculeifer* an extraction efficiency of 91% was reported in the study report; as the studies were conducted by the same laboratory, the RMS considers this suitable for confirming that the extraction efficiency was acceptable for this study.

All validity criteria were met and the study is considered acceptable for use in the risk assessment. The endpoint is confirmed as follows :

$NOEC_{reproduction}$ = 990 mg a.s./kg dws

Previous evaluation:	New data, submitted for purpose of review

Report:	KCA 8.4.2.1/04; Larnaudie-Lopez, M. I.; 2015
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
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
GLP/GEP:	Yes

Material and methods

Test item:	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).
Reference item :	Dimethoate (non-GLP, conducted January 05, 2015).
Test organism:	*Hypoaspis aculeifer* bred at Bayer CropScience AG since 2002. The strain was originally obtained from ECT Oekotoxikologie GmbH, 65439 Flörsheim a. M.
Age of test organisms:	30 days old (adult females) from a synchronised culture. In order to obtain adult, female *Hypoaspis aculeifer* of a uniform age on 2015-01-05, 2 x 300 adult, female *Hypoaspis aculeifer* were transferred to fresh breeding vessels. On 2015-01-08, after three days of egg laying, these females were removed. The *Hypoaspis aculeifer* hatched from the eggs were fed with *Panagrellus redivivus.*
Breeding of the test organism:	The mites were bred on a mixture of Plaster of Paris and activated charcoal and demineralised water (10:1.25:12.5 w/w). Plastic vessels (9.5 cm diameter) were filled up to a height of approximately 1 cm with this mixture and are closed with lids. Mites were fed with *Panagrellus redivivus* (nematodes) which were bred on watered oak flakes. The breeding culture was kept under the following conditions: Temperature: room temperature Light cycle: permanent dark
Test duration:	14 days (plus 2 days for extraction of mites)
Test concentrations :	*Test item*: 990 mg pure metabolite/kg dws *Reference item:* 1.0, 1.8, 3.2, 5.6 and 10.0 mg dimethoate/kg dws (conducted at a different time to the main test) *Controls:* Untreated (quartz sand only)
Preparation and application of the test item:	The test item was applied as a test-item-quartz-sand-mixture. The test mixture was prepared freshly on the day of application. To reach a concentration of 990 mg pure metabolite/kg dry weight artificial soil 0.5062 g test item was mixed with 4.4626 g quartz sand. This mixture was used for the treatment. The control was treated in the same way but with 5.0016 g quartz sand only. The 5 g test-item-quartz-sand-mixture was thoroughly mixed into the pre-moistened 500 g artificial dry weight artificial soil of each application rate using a laboratory mixer. Treatment and control were moistened with 50 mL deionised water. Afterwards the treated artificial soil of control and treatment was portioned out. Each test vessel of the 8 control and the 4 treatment replicates plus the one for measurement purpose was filled up with 20±1 g dry weight artificial soil avoiding compression of the artificial soil.
No. of individuals per replicate:	10
No. of replicates:	8 per control and 4 for the test item. There was one additional vessel for each applicationrate for measurement of pH value and moisture of the artificial soil at the end of the test not loaded with *Hypoaspis aculeifer*.
Test units :	Test containers were reusable glass vessels (Weck Mini-Sturzglas, 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 test vessels were covered with glass lids to prevent *Hypoaspis aculeifer* from escaping but allowing aeration during the test period.

Feeding and water:	Directly after the addition of the *Hypoaspis aculeifer*, they were fed with nematodes (*Panagrellus redivivus*). Nematodes were bred on watered oak flakes in the laboratory. During the continuation of the test the soil mites were fed 2, 6 and 9 days after test start with the nematodes. Between 58 and 128 mg food per test vessel was added. Each test vessel was weighed for the determination of water loss. Seven days after test start water loss was determined and replenished.
Artificial soil:	The artificial soil was prepared according to the guideline with the following constituents (percentage distribution on dry weight basis): - 75 % fine quartz sand (sort F 36, particle size 0.2 – 0.05 mm = 91.35 %) - 5 % Sphagnum peat, air dried and finely ground - 20 % Kaolin clay (content of Kaolinite: = 30.2 %) - Calcium carbonate (CaCO3) for the adjustment to pH to 6.0 ± 0.5 The artificial soil was prepared by mixing the dry components in a laboratory mixer. Two days before test start the dry artificial soil was pre-moistened with 64 mL deionised water per 500 g dry weight artificial soil. During the application of the test item the water content was adjusted to a final water content of approximately 50 % of the maximum water holding capacity by mixing 50 mL deionised water into 500 g dry weight artificial soil for the control group and 50 mL test item solution for the treatment groups.
Test conditions:	Water content: test start:17.89 – 18.63 (equivalent to 46.16 ‑ 48.48 % of WHC); test end: 17.49 ‑ 17.65 (equivalent to 44.88 – 45.40 % of WHC). pH: test start: 5.93 ‑ 6.07; test end: 5.58 ‑ 5.62. Temperature: 20 ± 2 °C. Light intensity: 555 – 642 lux, 16:8 hour light:dark cycle.
Test procedure:	Directly after application of the test item, the adult, fertilized, females (30 days after start of egg laying for three days) were exposed to the control and treatment vessels. This was achieved by putting 10 females individually onto the surface of the artificial soil using a fine brush. The transfer of the test animals was finished within two hours after the application of the test item. After a period of 14 days, the surviving adults and the living juveniles per test vessel were extracted, applying a temperature gradient. The content of each test vessel was carefully transferred to sieve vessels (mesh size approximately 0.8 mm). The vessels were positioned in MacFadyen- Extractor. The temperature was increased from approximately 25 to 40 °C within two days. Extracted mites were collected in a fixing solution (20 % ethylene glycol, 80 % deionised water; 2 g detergent/L). The extracted mites in the fixing solution were stored in a refrigerator until the start of the counting of surviving adults and juveniles. All *Hypoaspis aculeifer* (adult females and juveniles) were counted under a binocular.
Observations:	Mortality and reproduction was recorded at test termination. Missing adults (compared to the number of initially placed test organisms) were considered to be dead. Water holding capacity (WHC) of the soil and pH was determined at day 0 and day 14. Light intensity was measured at test initiation and after each feeding of the test organisms. Temperature was measured continuously.
Statistical analysis:	The statistical analysis was performed with the software ToxRat Professional 2.10 (Ratte 2010). For the determination of normal distribution and homogeneity of variance Kolmogorov-Smirnov Test and Cochran's Test ($\alpha = 0.05$), respectively were used. Data of reproduction were normally distributed and homogeneity of variances was given. Therefore William's-t test (one-sided-smaller, $\alpha = 0.05$) was used to determine NOEC and LOEC values.

Results

Validity criteria:

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 %

All validity criteria were met in this study.

Toxic reference test:

In a separate study (Maria Ivonne Larnaudie Lopez, LAR/HR-O-16/14, January 05, 2015) performed with the reference item dimethoate at test concentrations 1.0, 1.8, 3.2, 5.6 and 10.0 mg dimethoate/kg dry weight artificial soil, 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 (3.0 – 7.0 mg dimethoate/kg dws).

Biological findings:

For reproduction, no significant difference between control and treatment group detected.

A summary of the results is presented in Table B.9.4.2-6 below :

Table B.9.4.2-6: The effect of BCS-CN88460 on mortality and reproductive output of on mortality and reproductive output of *Hyposapis aculeifer* following exposure in artificial soil.

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]				≥ 990
$LOEC_{reproduction}$ [mg pure metabolite/kg dry weight artificial soil]				> 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.

Experimental conditions:

pH and water content/WHC of the soil during the test is show in Table B.9.4.2-7 below:

Table B.9.4.2-7: pH, water content and water holding capacity of the test soil

[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

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. 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.

RMS comments

This study was conducted according to GLP and following OECD 226 (2008) guidance.

According to the study guidelines (OECD 226) extraction efficiency must be validated once or twice a year in controls with known numbers of adults and juveniles. Efficiency should be above 90% on average combined for all developmental stage. However these details are not given in the study report. It is noted that in the study conducted for the product testing *H.aculeifer* an extraction efficiency of 91% was reported in the study report; as the studies were conducted by the same laboratory, the RMS considers this suitable for confirming that the extraction efficiency was acceptable for this study.

All validity criteria were met and the study is considered acceptable for use in the risk assessment. The endpoint is confirmed as follows :

$NOEC_{reproduction}$ = 990 mg pure metabolite/kg dws

B.9.5. EFFECTS ON SOIL NITROGEN TRANSFORMATION

Previous evaluation:	New data, submitted for purpose of review

Report:	KCA 8.5/01; Schulz, L.; 2015
Title:	BCS-CN88460 a.s.: Effects on the activity of soil microflora (Nitrogen transformation test)
Report No.:	15 10 48 032 N
Guideline(s):	OECD 216; 2000, OECD
GLP/GEP:	Yes

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 10421-02, Specification No.: 102000028196, CAS No.: 1255734-28-1, Article No.: 81782172, Certificate No.: MZ 00994, analysed purity: 94.2 % w/w.
Reference item :	Dinoterb (conducted from 06.01.2015 to 03.02.2015)
Origin of soil used in test:	Origin: Wassergut Canitz Country: Germany Federal state: Saxony Municipality: Canitz Field name: Schlag 34/3 Land owner: Wassergut Canitz GPS-position 12.694435960 degrees East, 51.403774567 degrees North No fertilisers applied since 2003 and no plant protection products since 1990. The soil was removed to a depth of 20 cm as a mixed sample. Afterwards, the soil was dried at room temperature, passed through a 2 mm mesh sieve and then stored at a temperature of approx. 4°C in containers under aerobic conditions in the dark. The soil was adapated to test conditions prior to application of the test item.
Soil characteristics:	pH: 6.1 Organic carbon: 1.32 % Humus content: 2.27% Carbon content of microbial biomass: 40.87 mg C/100 g soil d.w = 3.10% of organic carbon content

	N_{min}: 4.11 mg/100g soil d.w. Total N = 0.20% Soil type: Loamy sand (according to DIN 11277); 10.6% clay; 36.6% silt; 52.9% sand Water holding capacity: 39.54 g/100g soil d.w. Water content: 12.95 g/100g soil d.w. Cation exchange capacity: 11.2 $cmol^+$/kg soil
Test duration:	28 days
Test concentrations :	*Test item*: 0.075 kg a.s./ha, corresponding to 0.080 kg test item/ha (0.11 mg a.s./kg dws) and 0.375 kg a.s./ha, corresponding 0.398 kg test item/ha (0.53 mg a.s./kg dws) *Reference item:* 6.80 mg, 16.00 mg and 27.00 mg Dinoterb/kg dws (conducted at a different time to the test) *Controls:* Untreated (quratz sand only)
Application and incubation of the test soil:	200 g soil dry weight (= one sub-sample) per test vessel was weighed. The soil was mixed with 0.5 % (i.e. 1.0 g/200 g soil d.w.) lucerne meal by means of a hand-stirrer (the C/N ratio of the lucerne meal was 13.2/1). One additional soil sample (without lucerne meal) was used for determination of the initial NO3-N-content. The initial NO3-N-content was 6.16 mg /100 g soil d.w. Since the water solubility was not available, the test item was thoroughly mixed with quartz sand. Subsequently the obtained mixture was added and mixed with the soil by means of a hand stirrer. For an optimum distribution of the test item in the soil, the test item quartz sand mixture was applied at a ratio of about 10 g per kilogram soil dry weight. Water was added to the soil to achieve a water content of approximately 45 % of WHC.
No. of replicates:	3
Test units :	The incubation of the prepared soil was carried out in wide mouth glass flasks (500 mL) with screw caps to permit air exchange.
Test conditions:	Water content: test start:18.01 – 18.41 g/100 g soil d.w.; test end: 17.61 ‑ 18.04 g/100 g soil d.w pH: test start: 5.8 – 5.9 ‑ 6.07; test end: 5.6 ‑ 5.7. Temperature: 19.2 – 21.5 °C.
Sampling and analysis:	Soil samples (10 g soil d.w. per replicate) were taken at intervals of 3 hours, 7, 14 and 28 days after application and the NH4-N-, NO3-N- and NO2-N-contents were determined. Soil was extracted by adding 50 mL 1 M KCl solution to the equivalent of 10 g soil d.w. and mixing on a rotator at 150 rpm for 60 minutes. The mixtures were centrifuged and stored deep-frozen prior to analysis at -20 ± 5 °C. For the quantitative determination of the mineralized part of nitrogen the Autoanalyzer was used. The Autoanalyzer is a continuous flow analysis system. Ammonium reacts with salicylate and dichloroisocyanuric acid to form an indophenoleblue compound. The intensity of the formed compound is colorimetrically measured at a wavelength of 625 nm. Nitrate is reduced to nitrite by hydrazinesulphate. The nitrite reacts with sulphanilamide in an acidic solution to form a diazocompound. The diazotized product is then coupled with naphthylamine. The intensity of the formed azodye, which is proportional to the sum of the nitrate and nitrite originally present in the sample, is colorimetrically measured at a wavelength of 525 nm.

	Thee autoanalyzer was calibrated before each measurement series by establishing a calibration curve.
Observations:	A sample of each replicate of each treatment was taken at intervals of 3 hours, 7, 14 and 28 days and the mineral nitrogen content of the soil was determined. 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. The pH-values of the soil used in the test were measured at test start (after application) and at the final sampling on day 28.
Statistical analysis:	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).

Results

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 %

Reference item

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.

Biological effects

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).

Table B.9.5-1: Effects on nitrogen transformation in soil after treatment with BCS-CN88460

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 ≤ 0.05)

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 a.s/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).

RMS comments

This study was conducted according to GLP and following OECD 216 (2000) guidance.

All validity criteria were met.

This study is considered acceptable for use in the risk assessment and the endpoint is confirmed as:

<25% effects on nitrogen transformation at up to 0.53 mg a.s./kg dws

Previous evaluation:	New data, submitted for purpose of review

Report:	KCA 8.5/02; Schulz, L.; 2015
Title:	BCS-CN88460-carboxylic acid (BCS-CY26497): Effects on the activity of soil microflora (nitrogen transformation test)
Report No.:	15 10 48 033 N
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

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.
Reference item :	Dinoterb (conducted from 06.01.2015 to 03.02.2015)
Origin of soil used in test:	Origin: Wassergut Canitz Country: Germany Federal state: Saxony Municipality: Canitz Field name: Schlag 34/3 Land owner: Wassergut Canitz GPS-position 12.694435960 degrees East, 51.403774567 degrees North No fertilisers applied since 2003 and no plant protection products since 1990. The soil was removed to a depth of 20 cm as a mixed sample. Afterwards, the soil was dried at room temperature, passed through a 2 mm mesh sieve and then stored at a temperature of approx. 4°C in containers under aerobic conditions in the dark. The soil was adapated to test conditions prior to application of the test item.
Soil characteristics:	pH: 6.1 Organic carbon: 1.32 % Humus content: 2.27% Carbon content of microbial biomass: 40.87 mg C/100 g soil d.w = 3.10% of organic carbon content N_{min}: 4.11 mg/100g soil d.w. Total N = 0.20% Soil type: Loamy sand (according to DIN 11277); 10.6% clay; 36.6% silt; 52.9% sand

	Water holding capacity: 39.54 g/100g soil d.w. Water content: 12.95 g/100g soil d.w. Cation exchange capacity: 11.2 $cmol^+$/kg soil
Test duration:	28 days
Test concentrations :	*Test item*: 0.081 kg pure metabolite/ha, corresponding to 0.082 kg pure metabolite/ha (0.11 mg a.s./kg dws) and 0.403 kg pure metabolite/ha, corresponding 0.408 kg pure metabolite/ha (0.54 mg a.s./kg dws) *Reference item:* 6.80 mg, 16.00 mg and 27.00 mg Dinoterb/kg dws (conducted at a different time to the test) *Controls:* Untreated (quratz sand only)
Application and incubation of the test soil:	200 g soil dry weight (= one sub-sample) per test vessel was weighed. The soil was mixed with 0.5 % (i.e. 1.0 g/200 g soil d.w.) lucerne meal by means of a hand-stirrer (the C/N ratio of the lucerne meal was 13.2/1). One additional soil sample (without lucerne meal) was used for determination of the initial NO3-N-content. The initial NO3-N-content was 6.16 mg /100 g soil d.w. Since the water solubility was not available, the test item was thoroughly mixed with quartz sand. Subsequently the obtained mixture was added and mixed with the soil by means of a hand stirrer. For an optimum distribution of the test item in the soil, the test item quartz sand mixture was applied at a ratio of about 10 g per kilogram soil dry weight. Water was added to the soil to achieve a water content of approximately 45 % of WHC.
No. of replicates:	3
Test units :	The incubation of the prepared soil was carried out in wide mouth glass flasks (500 mL) with screw caps to permit air exchange.
Test conditions:	Water content: test start:17.36 – 18.32 g/100 g soil d.w.; test end: 16.98 ‑ 18.03 g/100 g soil d.w (42.94 to 46.34 % of $WHC_{max)}$ pH: test start: 5.9; test end:5.7. Temperature: 19.2 – 21.5 °C.
Sampling and analysis:	Soil samples (10 g soil d.w. per replicate) were taken at intervals of 3 hours, 7, 14 and 28 days after application and the NH4-N-, NO3-N- and NO2-N-contents were determined. Soil was extracted by adding 50 mL 1 M KCl solution to the equivalent of 10 g soil d.w. and mixing on a rotator at 150 rpm for 60 minutes. The mixtures were centrifuged and stored deep-frozen prior to analysis at -20 ± 5 °C. For the quantitative determination of the mineralized part of nitrogen the Autoanalyzer was used. The Autoanalyzer is a continuous flow analysis system. Ammonium reacts with salicylate and dichloroisocyanuric acid to form an indophenoleblue compound. The intensity of the formed compound is colorimetrically measured at a wavelength of 625 nm. Nitrate is reduced to nitrite by hydrazinesulphate. The nitrite reacts with sulphanilamide in an acidic solution to form a diazocompound. The diazotized product is then coupled with naphthylamine. The intensity of the formed azodye, which is proportional to the sum of the nitrate and nitrite originally present in the sample, is colorimetrically measured at a wavelength of 525 nm. Thee autoanalyzer was calibrated before each measurement series by establishing a calibration curve.
Observations:	A sample of each replicate of each treatment was taken at intervals of 3 hours, 7, 14 and 28 days and the mineral nitrogen content of the soil was determined.

	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. The pH-values of the soil used in the test were measured at test start (after application) and at the final sampling on day 28.
Statistical analysis:	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).

Results

Validity criteria:

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 %

Reference item

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.

Biological effects

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).

Table B.9.5-2: Effects on nitrogen transformation in soil after treatment with BCS-CN88460- carboxylic acid

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	±	0.36	+ 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, $p \leq 0.05$)

Conclusion:

BCS-CN88460-carboxylic acid (M12) caused no adverse effects at concentrations up to 0.54 mg test item/kg soil dry weight (difference to control < 25 %, OECD 216) on soil nitrogen transformation (expressed as NO_3-N-production) at the end of the 28-day incubation period.

RMS comments

This study was conducted according to GLP and following OECD 216 (2000) guidance.

All validity criteria were met.

This study is considered acceptable for use in the risk assessment and the endpoint is confirmed as:

<25% effects on nitrogen transformation at up to 0.54 mg pure metabolite/kg dws

B.9.6. EFFECTS ON TERRESTRIAL NON-TARGET HIGHER PLANTS

The study summaries for this data point can be found at CP B.9.11.

B.9.7. EFFECTS ON OTHER TERRESTRIAL ORGANISMS (FLORA AND FAUNA)

No data submitted.

B.9.8. EFFECTS ON BIOLOGICAL METHODS FOR SEWAGE TREATMENT

Report:	KCA 8.8/01; Neuhahn, A.; 2018;
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

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.

Test design: 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 fed daily with a standard amount synthetic sewage (composition matches that recommended in OECD 209 (2010).

Measurements: 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 (0.8 – 1.7 L/min) 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 (inoculated samples 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.

Test conditions: The test temperature during exposure was 20 ± 2°C (18.1 – 20.4°C). The pH was 8.3 – 8.4 during the study. The incubation time was 3 hours with permanent aeration.

Statistical analysis: 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.

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 (Table B.9.8-1) and 16.6 % respiration inhibition at a test item concentration of 1000 mg/L for heterotrophic respiration (Table B.9.8-2).

Table B.9.8-1: Results of the test item isoflucypram without ATU (total respiration)

Treatment [mg/L]	Respiration rate [mg/L × h]	CV (%)	Mean Temp. [°C]	pH-value	Inhibition [%]
Control 1	34.592	7.7	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	2.9	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	1.2	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	5.5	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	5.3	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	3.3	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 3,5-Dichlorophenol	25.992		19.3	8.2	16.563
5 mg/L 3,5-Dichlorophenol	28.375		19.1	8.1	8.913
10 mg/L 3,5-Dichlorophenol	20.469		19.3	8.0	34.292
20 mg/L 3,5-Dichlorophenol	11.088		19.2	8.1	64.408
40 mg/L 3,5-Dichlorophenol	7.148		18.6	8.2	77.054

* Statistically- significant difference from the control (Dunnett's Multiple t-test procedure).
CV= coefficient of variation

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.

Table B.9.8-2: Results of the test item isoflucypram with ATU (heterotrophic respiration)

Treatment [mg/L]	Respiration rate [mg/L × h]	CV (%)	Mean Temp. [°C]	pH-value	Inhibition [%]
Control 1	33.790	10.3	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	3.1	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	3.2	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	26.171	1.0	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	5.8	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	2.3	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 3,5-Dichlorophenol	29.917		19.6	8.5	2.737
5 mg/L 3,5-Dichlorophenol	27.525		19.4	8.3	10.514
10 mg/L 3,5-Dichlorophenol	23.967		19.5	8.4	22.080
20 mg/L 3,5-Dichlorophenol	14.563		19.3	8.4	52.655
40 mg/L 3,5-Dichlorophenol	9.369		18.9	8.5	69.541

* Statistically- significant difference from the control (Dunnett's Multiple t-test procedure).
CV= coefficient of variation

Table B.9.8-3: 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	-338.071
1000 mg/L test item	27.450	25.662	1.788	-353.807
2.5 mg/L 3,5-Dichlorophenol.	25.992	29.917	-3.925	1096.193

5 mg/L 3,5-Dichlorophenol	28.375	27.525	0.850	-115.736
10 mg/L 3,5-Dichlorophenol	20.469	23.967	-3.498	987.817
20 mg/L 3,5-Dichlorophenol	11.088	14.563	-3.475	981.980
40 mg/L 3,5-Dichlorophenol	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	n.d. **
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)
** n.d. as there was statistically significant difference at the lowest tested dose (10 mg/L)

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.

RMS Comments

This study was conducted to OECD 209 (2010) and was considered valid. There were some deviations to the guidelines which are discussed below:

The pH of the test solutions ranged between 8.3 – 8.4 during the study, this is in deviation to the recommendation in OECD 209 that the pH should be 7.5 ± 0.5. As the pH was relatively consistent across replicates and test concentrations/controls, it is not considered to have affected the endpoints of this study.

The aeration rate in the study was 0.8 – 1.7 L/min. While OECD 209 suggests 0.5 – 1.0 L/min, it is noted that the minimum aeration was achieved. No adverse effects are considered likely from the aeration reaching higher than the top of the range in the guidelines.

OECD 209 (2010) recommends 5 replicates, whereas only 3 replicates have been used in the current study, reducing the statistical power of the test. The RMS calculated the Coefficient of variation for the controls and test

concentrations and as all of these were low (maximum was 10.3%), the replicates are considered representative and the means accurate.

The RMS confirms the endpoint of this study as **EC_{50} >1000 mg/L. No NOEC could be determined.**

Risk Assessment

As the PECsw value of 9.63 µg a.s/L is less than the EC_{50} of 1 000 000 µg/L, the risk to activated sludge is considered acceptable.

B.9.9. MONITORING DATA

No data submitted.

B.9.10. BIOLOGICAL ACTIVITY OF METABOLITES POTENTIALLY OCCURRING IN GROUNDWATER

No data submitted.

B.9.11. LITERATURE REVIEW

A literature review was carried out for isoflucypram and its metabolites according to the requirements of the Regulation (EU) No 844/2012, which itself refers to Article 8(5) of Regulation (EC) No 1107/2009. The review itself is in line with the EFSA Guidance document as published in EFSA Journal 2011; 9(2):2092.

The key objective of the literature review was to establish whether any scientific peer-reviewed open literature published within the last ten years before the date of submission of the dossier would be relevant for the risk assessment of isoflucypram and its metabolites in the context of side-effects on health, the environment and non-target species. However this section will only assess the review with relevance to ecotoxicology.

Databases used in the literature review

The list of databases searched totalled 13 and covered an appropriate time period (01-01-2008 – 18-10-2017) and appropriate range of databases. The list of databases searched is provided in Table B.9.11-1 below.

Table B.9.11-1: Databases searched in the literature review of isoflucypram

Database Name	Date of last Database Update
Agricola	2017-10-12
Biosis	2017-10-11
CABA	2017-10-18
Chemical Abstracts	2017-10-17
Derwent Drug File (DRUGU)	2017-10-13
EMBASE	2017-10-17
Esbiobase	2017-10-18
IPA	2017-10-09
Medline	2017-10-17
PQSciTech	2017-09-27
Scisearch	2017-10-16
Toxcenter	2017-10-16
FSTA	2017-10-13

Search parameters

Search parameters/queries used during the literature review are presented in Table B.9.11-2 below.

The information used for screening the selected databases to identify all relevant publications consists of IUPAC name, CAS name/number, common names, codes and abbreviations, molecular structure, molecular formula, molar mass and other names/codes, as far as available.

Name	Search terms
Active substance	
a.s.	(ISOFLUCYPRAM OR 1255734-28-1 OR CHLORO(3T)ISOPROPYLBENZYL(2T)CYCLOPROPYL(2T)DIFLUOROMETHYL(2T)FLUORO(2T)METHYL(5T)(PYRAZOLE(2T)CARBOXAMIDE) OR CYCLOPROPYL(2T)DIFLUOROMETHYL(2T)FLUORO(3T)CHLORO(3T)ISOPROPYLBENZYL(2T)METHYL(5T)(PYRAZOLE(2T)CARBOXAMIDE)) NOT P/DT AND PY>=2008 AND ED>=20080101
Metabolites	
M01	(CHLORO(3T)HYDROXYPROPAN(3T)BENZYL(3T)CYCLOPROPYL(2T)DIFLUOROMETHYL(2T)FLUORO(2T)METHYL(5T)(PYRAZOLE(2T)CARBOXAMIDE)) NOT P/DT AND PY>=2008 AND ED>=20080101
M02	(CHLORO(3T)HYDROXYPROPAN(3T)BENZYL(3T)CYCLOPROPYL(2T)DIFLUOROMETHYL(2T)FLUORO(2T)METHYL(5T)(PYRAZOLE(2T)CARBOXAMIDE)) NOT P/DT AND PY>=2008 AND ED>=20080101
M06	(CHLORO(3T)HYDROXYPROPAN(3T)BENZYL(3T)CYCLOPROPYL(2T)DIFLUOROMETHYL(2T)FLUORO(5T)(PYRAZOLE(2T)CARBOXAMIDE)) NOT P/DT AND PY>=2008 AND ED>=20080101
M07	(CHLORO(3T)DIHYDROXYPROPAN(3T)BENZYL(3T)CYCLOPROPYL(2T)DIFLUOROMETHYL(2T)FLUORO(5T)(PYRAZOLE(2T)CARBOXAMIDE)) NOT P/DT AND PY>=2008 AND ED>=20080101

M10	(CHLORO(3T)CYCLOPROPYL(3T)DIFLUOROMETHYL(2T)FLUORO(2T)METHYL(3T)(PYRAZOL (4T)CARBONYL(2T)AMINO(2T)METHYL(2T)PHENYL)(3T)HYDROXYPROPANOIC ACID) NOT P/DT AND PY>=2008 AND ED>=20080101
M11	(CHLORO(3T)CYCLOPROPYL(3T)DIFLUOROMETHYL(2T)FLUORO(3T)(PYRAZOL (4T)CARBONYL(2T)AMINO(2T)METHYL(2T)PHENYL)(3T)PROPANOIC ACID) NOT P/DT AND PY>=2008 AND ED>=20080101
M12	(CHLORO(3T)CYCLOPROPYL(3T)DIFLUOROMETHYL(2T)FLUORO(2T)METHYL(3T)(PYRAZOL (4T)CARBONYL(2T)AMINO(2T)METHYL(2T)PHENYL)(3T)PROPANOIC ACID) NOT P/DT AND PY>=2008 AND ED>=20080101
M13	(CHLORO(3T)ISOPROPYLBENZYL(2T)CYCLOPROPYL(2T)DIFLUOROMETHYL(2T)FLUORO (5T)(PYRAZOLE(2T)CARBOXAMIDE)) NOT P/DT AND PY>=2008 AND ED>=20080101
M19	(CHLORO(3T)CYCLOPROPYL(3T)DIFLUOROMETHYL(2T)FLUORO(2T)METHYL(3T)(PYRAZOL(4T)CARBONYL(2T)AMINO(2T)METHYL(2T)PHENYL)(3T)PROPYL (2T)GLUCOPYRANOSIDURONIC ACID) NOT P/DT AND PY>=2008 AND ED>=20080101
M20	(CHLORO(3T)CYCLOPROPYL(3T)DIFLUOROMETHYL(2T)FLUORO(2T)METHYL(3T)(PYRAZOL(4T)CARBONYL(2T)AMINO(2T)METHYL(2T)PHENYL)(3T)(PROPAN OR PROPYL)(4T)GLUCOPYRANOSIDURONIC ACID) NOT P/DT AND PY>=2008 AND ED>=20080101
M23	(CHLORO(3T)CYCLOPROPYL(3T)DIFLUOROMETHYL(2T)FLUORO(2T)METHYL(3T)(PYRAZOL (4T)CARBONYL(2T)AMINO(2T)METHYL(2T)PHENYL)(3T)(PROP(2T)EN(2T)YL OR PROPEN(2T)YL OR PROP(2T)ENYL OR PROPENYL)(3T) GLUCOPYRANOSIDURONIC ACID) NOT P/DT AND PY>=2008 AND ED>=20080101
M25	(CHLORO(3T)CYCLOPROPYL(3T)DIFLUOROMETHYL(2T)FLUORO(2T)METHYL(3T)(PYRAZOL (4T)CARBONYL(2T)AMINO(2T)METHYL(2T)PHENYL)(3T)(PROPAN OR PROPYL)(2T)HYDROGEN SULFATE) NOT P/DT AND PY>=2008 AND ED>=20080101
M26	(CHLORO(3T)CYCLOPROPYL(3T)DIFLUOROMETHYL(2T)FLUORO(3T)(PYRAZOL (4T)CARBONYL(2T)AMINO(2T)METHYL(2T)PHENYL)(3T)(PROPAN OR PROPYL)(4T)GLUCOPYRANOSIDURONIC ACID) NOT P/DT AND PY>=2008 AND ED>=20080101
M31	(CHLORO(3T)ISOPROPYLBENZYL(2T)CYCLOPROPYL(2T)DIFLUOROMETHYL(2T)FLUORO(4T)GLUCOPYRANURONOSYL(5T)(PYRAZOLE(2T)CARBOXAMIDE)) NOT P/DT AND PY>=2008 AND ED>=20080101
M35	(CHLORO(3T)(DIHYDOXYPROPANYL OR DIHYDROXYPROPAN(2T)YL)(2T)BENZYL(2T)CYCLOPROPYL(2T)DIFLUOROMETHYL(2T)FLUORO(4T)GLUCOPYRANURONOSYL(5T)(PYRAZOLE(2T)CARBOXAMIDE)) NOT P/DT AND PY>=2008 AND ED>=20080101
M36	(CHLORO(3T)(DIHYDOXYPROPANYL OR DIHYDROXYPROPAN(2T)YL)(2T)BENZYL(2T)CYCLOPROPYL(2T)DIFLUOROMETHYL(2T)FLUORO(4T)GLUCOPYRANURONOSYL(5T)(PYRAZOLE(2T)CARBOXAMIDE)) NOT P/DT AND PY>=2008 AND ED>=20080101
M37	(CHLORO(3T)(DIHYDOXYPROPANYL OR DIHYDROXYPROPAN(2T)YL)(2T)BENZYL(2T)CYCLOPROPYL(2T)DIFLUOROMETHYL(2T)FLUORO(4T)GLUCOPYRANURONOSYL(5T)(PYRAZOLE(2T)CARBOXAMIDE)) NOT P/DT AND PY>=2008 AND ED>=20080101

M38	(CHLORO(3T)CYCLOPROPYL(3T)DIFLUOROMETHYL(2T)FLUORO(3T)(PYRAZOL (4T)CARBONYL(2T)AMINO(2T)METHYL(2T)PHENYL)(3T)PROPYL(3T)CARBOXYACETYL(2T)HEXOPYRANOSIDE) NOT P/DT AND PY>=2008 AND ED>=20080101
M41	(GLUTAMYL(5T)CHLORO(3T)ISOPROPYLBENZYL(2T)CYCLOPROPYL(2T)CARBAMOYL(2T)DIFLUOROMETHYL (2T)METHYL(3T)PYRAZOL(5T)(CYSTEINYL(2T)ALANINE)) NOT P/DT AND PY>=2008 AND ED>=20080101
M44	(CARBOXYACETYL(5T)CHLORO(3T)ISOPROPYLBENZYL(2T)CYCLOPROPYL(2T)CARBAMOYL(2T)DIFLUOROMETHYL(2T)METHYL(3T)PYRAZOL(5T)CYSTEINE) NOT P/DT AND PY>=2008 AND ED>=20080101
M45	(CHLORO(3T)ISOPROPYLBENZYL(2T)CYCLOPROPYL(2T)CARBAMOYL(2T)DIFLUOROMETHYL(2T)METHYL(3T)PYRAZOL(5T)SULFANYL(3T)HEXOPYRANOSYLOXY(2T)PROPANOIC ACID) NOT P/DT AND PY>=2008 AND ED>=20080101
M47	(CYCLOPROPYL(2T)DIFLUOROMETHYL(2T)FLUORO(2T)METHYL(5T)(PYRAZOLE(2T)CARBOXAMIDE)) NOT P/DT AND PY>=2008 AND ED>=20080101
M49	(1255735-09-1 OR DIFLUOROMETHYL(2T)FLUORO(2T)METHYL(5T)(PYRAZOLE(2T)CARBOXYLIC ACID)) NOT P/DT AND PY>=2008 AND ED>=20080101
M50	(CYCLOPROPYL(2T)DIFLUOROMETHYL(2T)FLUORO(3T)(PYRAZOLE(2T)CARBOXAMIDE)) NOT P/DT AND PY>=2008 AND ED>=20080101
M58	(CYCLOPROPYLCARBAMOYL(2T)DIFLUOROMETHYL(2T)FLUORO(3T)(PYRAZOL(5T)ALANINE)) NOT P/DT AND PY>=2008 AND ED>=20080101
M66	(CYCLOPROPYLCARBAMOYL(2T)DIFLUOROMETHYL(3T)(PYRAZOL(5T)ALANINE)) NOT P/DT AND PY>=2008 AND ED>=20080101
M66	(CHLORO(3T)CYCLOPROPYL(3T)DIFLUOROMETHYL(2T)FLUORO(2T)METHYL(3T)(PYRAZOL (4T)CARBONYL(2T)AMINO(2T)METHYL(2T)PHENYL)(3T)(PROP(2T)EN(2T)YL OR PROPEN(2T)YL OR PROP(2T)ENYL OR PROPENYL)(3T) GLUCOPYRANOSIDURONIC ACID) NOT P/DT AND PY>=2008 AND ED>=20080101

Search results

A total of 0 publications were found for Isoflucypram and 0 for the metabolites. As RMS considers the search dates, search terms and databases appropriate, it is satisfied that there is no further assessment to do in this area.

When literature searches produce results, these are then assessed for relevance and reliability. In this instance, there were no results, therefore the rapid and detailed assessment criteria were not deployed. However, the criteria that the applicant proposed to use in case of search results is discussed below, for clarity.

Evaluation of studies for inclusion/exclusion based on relevance

Initial rapid assessment – The following rapid assessment criteria were used:

- Efficacy
- Analytical method development
- New ways of synthesis
- Studies on a molecular level, which cannot be related to environmental RA
- Non-EU monitoring studies

- Abstract refers to a conference contribution and does not contain data, full text not available
- Not relevant due to missing information: Studies with target organisms

These criteria are considered acceptable, with regard to ecotoxicology.

Criteria for relevance – The following detailed assessment criteria were used:

Not relevant, because:

- Target substance not a test item
- **Conversion into units useful for RA not possible**
- Study design / test system not sufficiently described
- **Study design / test system not adequate**
- **Study design / test system not relevant to EU data requirements**
- Test system not relevant to representative uses/GAPs
- **Test method does not cover the right targets**
- Test material deviates from composition of BCS active ingredient / product
- **Findings not related to a certain test system**
- **no endpoint can be derived**
- observations are not attributable (i.e. ecotox) to a specific substance
- effects are caused by a non-relevant route of exposure
- **observations cannot be transferred into an endpoint**

The criteria highlighted in bold text are not considered transparent. They do not provide clear and consistent features to establish the relevance of a study, and a wide range of interpretation is possible based on individual judgement.

Additionally the relevance criteria include both consideration of relevance and reliability.

However, as the criteria were not deployed, the RMS has not asked for more information from the applicant.

Conclusion

The literature searches generated no publications for either the active substance or it's metabolites. The RMS is satisfied that searches were appropriate and thorough. Therefore, the RMS accepts that there is no literature data on isoflucypram or it's metabolites that needs to be considered in this dossier.

B.9.12. REFERENCES RELIED ON

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KCA 8.1.1.1/01	[redacted]	2015	Toxicity of BCS-CN88460 technical	Y	Y	New data for a new active substance	Bayer	No

			during an acute oral LD50 with the northern bobwhite quail (Colinus virginianus) [redacted] Bayer Report No.: EBLNN006 Edition Number: M-535551-01-1 Date: 2015-09-14 GLP/GEP: Yes, unpublished					
KCA 8.1.1.1 / 02	[redacted]	2016	Toxicity of BCS-CN88460 technical during an acute oral LD50 with the canary (Serinus canaria) [redacted] Bayer Report No.: [redacted] Edition Number: M-547051-01-1 Date: 2016-01-27 GLP/GEP: Yes, unpublished	Y	Y	New data for a new active substance	Bayer	No
KCA 8.1.1.3 / 01	[redacted]	2017	Toxicity of BCS-CN88460 on reproduction in the mallard duck (Anas platyrhynchos) [redacted]	Yes	Yes	New data for a new active substance	Bayer	No

			[redacted] Bayer Report No.: [redacted] Edition Number: M-597500-01-1 Date: 2017-07-20 GLP/GEP: Yes, unpublished					
KCA 8.2.1 / 01	[redacted]	2018	Amendment no. 1: BCS-CN88460 (tech.) - Acute toxicity to fish (Pimephales promelas) under static conditions [redacted] Bayer Report No.: EBLNN356 Edition Number: M-542897-02-1 Date: 2015-12-03 **... amended: 2018-01-18** GLP/GEP: Yes, unpublished	Yes	Yes	New data for a new active substance	Bayer	No
KCA 8.2.1 / 02	[redacted]	2015	BCS-CN88460 (tech.) - Acute toxicity to fish (Oncorhynchus mykiss) under static conditions Bayer Report No.: EBLNN024 Edition Number: M-543443-01-1 Date: 2015-11-09 GLP/GEP: Yes, unpublished	Yes	Yes	New data for a new active substance	Bayer	No

KCA 8.2.1 / 03	[redacted]	2015	Acute toxicity of BCS-CN88460 technical to the sheepshead minnow (Cyprinodon variegatus) under static conditions [redacted] Bayer Report No.: EBLNN023 Edition Number: M-537137-01-1 Date: 2015-10-23 GLP/GEP: Yes, unpublished	Yes	Yes	New data for a new active substance	Bayer	No
KCA 8.2.1 / 04	[redacted]	2017	BCS-CN88460-carboxylic-acid (BCS-CY26497) - Acute toxicity to rainbow trout (Oncorhynchus mykiss) under static conditions - Final report [redacted] Bayer Report No.: EBLNN193 Edition Number: M-587655-01-1 Date: 2017-04-26 GLP/GEP: Yes, unpublished	Yes	Yes	New data for a new active substance	Bayer	No
KCA 8.2.2.1 / 01	[redacted]	2017	Early-life stage toxicity of BCS-CN88460	Yes	Yes	New data for a new active substance	Bayer	No

			(tech.) to fish (Pimephales promelas) [redacted] Bayer Report No.: EBLNN029 Edition Number: M-580247-01-1 Date: 2017-02-03 GLP/GEP: Yes, unpublished					
KCA 8.2.2.1 / 02	[redacted]	2016	Early life stage toxicity of BCS-CN88460 technical to the sheepshead minnow (Cyprinodon variegatus) under flow-through conditions [redacted] Bayer Report No.: [redacted] Edition Number: M-575119-01-1 Date: 2016-12-07 GLP/GEP: Yes, unpublished	Yes	Yes	New data for a new active substance	Bayer	No
KCA 8.2.2.3 / 01	[redacted]	2017	[pyrazole-4-14C] BCS-CN88460 - Aqueous exposure bioconcentration fish test and biotransformation in fish (Lepomis macrochirus)	Yes	Yes	New data for a new active substance	Bayer	No

			[redacted] Bayer Report No.: EBLNN359 Edition Number: M-610008-01-1 Date: 2017-12-14 GLP/GEP: Yes, unpublished **... also filed: KCA 6.2.5 / 01**					
KCA 8.2.4.1 / 01	Kuhl, K.	2016	Acute toxicity of BCS-CN88460 (tech.) to the waterflea Daphnia magna in a static laboratory test system - Final Report - Bayer Report No.: EBLNN033 Edition Number: M-574184-01-1 Date: 2016-12-06 GLP/GEP: Yes, unpublished	No	Yes	New data for a new active substance	Bayer	No
KCA 8.2.4.1 / 02	Riebschlaeger, T	2016	Acute toxicity of BCS-CN88460-carboxylic-acid (BCS-CY26497) to the waterflea Daphnia magna in a static laboratory test system Bayer Report No.: EBLNN198 Edition Number: M-573296-01-1 Date: 2016-11-25	No	Yes	New data for a new active substance	Bayer	No

			GLP/GEP: Yes, unpublished					
KCA 8.2.4.2 / 01	Brougher, D. S.; Siddiqui, A. I.; Gallagher, S. P.	2016	BCS-CN88460: A 96-hour static-renewal acute toxicity test with the saltwater mysid (Americamysis bahia) Wildlife International, Ltd., Easton, MD, USA Bayer Report No.: 149A-257B Edition Number: M-547041-01-1 Date: 2016-01-11 GLP/GEP: Yes, unpublished	No	Yes	New data for a new active substance	Bayer	No
KCA 8.2.5.1 / 01	Bruns, E.	2017	Effects of BCS-CN88460 (tech.) on development and reproductive output of the waterflea Daphnia magna in a static renewal laboratory test system Bayer AG, Crop Science Division, Monheim, Germany Bayer Report No.: EBLNN031 Edition Number: M-593961-01-1 Date: 2017-06-29 GLP/GEP: Yes, unpublished	No	Yes	New data for a new active substance	Bayer	No
KCA 8.2.5.2 / 01	Milligan, A. L.; Siddiqui,	2016	BCS-CN88460: A flow-through	No	Yes	New data for a new active substance	Bayer	No

	A. I.; Gallagher, S. P.; Krueger, H. O.		life-cycle toxicity test with the saltwater mysid (Americamysis bahia) Wildlife International, Ltd., Easton, MD, USA Bayer Report No.: 149A-256 Edition Number: M-567966-01-1 Date: 2016-10-05 GLP/GEP: Yes, unpublished					
KCA 8.2.5.2 / 03	Brougher, D. S.; Siddiqui, A. I.; Gallagher, S. P.	2016	BCS-CN88460: A 96-hour shell deposition test with the eastern oyster (Crassostrea virginica) Wildlife International, Ltd., Easton, MD, USA Bayer Report No.: 149A-258 Edition Number: M-547035-01-1 Date: 2016-01-12 GLP/GEP: Yes, unpublished	No	Yes	New data for a new active substance	Bayer	No
KCA 8.2.5.4 / 01	Bradley, M. J.	2017	Life-cycle toxicity test exposing midges (Chironomus dilutus) to BCS-CN88460 technical applied to sediment under static-renewal conditions following EPA test methods	No	Yes	New data for a new active substance	Bayer	No

			Smithers Viscient, Wareham, MA, USA Bayer Report No.: 13798.6405 Edition Number: M-596883-01-1 Date: 2017-08-03 GLP/GEP: Yes, unpublished					
KCA 8.2.6.1 / 01	Kuhl, K.	2017	Pseudokirchneriella subcapitata growth inhibition test with BCS-CN88460 (tech.) Bayer AG, Crop Science Division, Monheim, Germany Bayer Report No.: EBLNN050 Edition Number: M-586715-01-1 Date: 2017-03-31 GLP/GEP: Yes, unpublished	No	Yes	New data for a new active substance	Bayer	No
KCA 8.2.6.1 / 02	Kuhl, K.	2017	Pseudokirchneriella subcapitata growth inhibition test with BCS-CN88460-carboxylic-acid (BCS-CY26497) Bayer AG, Crop Science Division, Monheim, Germany Bayer Report No.: EBLNN290	No	Yes	New data for a new active substance	Bayer	No

			Edition Number: M-587659-01-1 Date: 2017-04-24 GLP/GEP: Yes, unpublished					
KCA 8.2.6.2 / 01	Arnie, J. R.; Siddiqui, A. I.; Porch, J. R.; Martin, K. H.	2017	BCS-CN88460: A 96-hour toxicity test with the cyanobacteria (Anabaena flos-aquae) EAG, Inc., Easton, Maryland, USA Bayer Report No.: 149P-111 Edition Number: M-605074-01-1 Date: 2017-10-16 GLP/GEP: Yes, unpublished	No	Yes	New data for a new active substance	Bayer	No
KCA 8.2.6.2 / 02	Arnie, J. R.; Siddiqui, A. I.; Porch, J. R.; Martin, K. H.	2017	BCS-CN88460: A 96-hour toxicity test with the marine diatom (Skeletonema costatum) EAG, Inc., Easton, Maryland, USA Bayer Report No.: 149P-113 Edition Number: M-604811-01-1 Date: 2017-10-16 GLP/GEP: Yes, unpublished	No	Yes	New data for a new active substance	Bayer	No
KCA 8.2.6.2 / 03	Arnie, J. R.; Siddiqui, A. I.; Porch, J.	2017	BCS-CN88460: A 96-hour toxicity test with the	No	Yes	New data for a new active substance	Bayer	No

	R.; Martin, K. H.		freshwater diatom (Navicula pelliculosa) EAG, Inc., Easton, Maryland, USA Bayer Report No.: 149P-112A Edition Number: M-604809-01-1 Date: 2017-10-16 GLP/GEP: Yes, unpublished					
KCA 8.2.7 / 01	Kuhl, K.	2017	Lemna gibba G3 - Growth inhibition test with BCS-CN88460 under semi-static conditions Bayer AG, Crop Science Division, Monheim, Germany Bayer Report No.: EBLNN016 Edition Number: M-593965-01-1 Date: 2017-06-22 GLP/GEP: Yes, unpublished	No	Yes	New data for a new active substance	Bayer	No
KCA 8.3.1.1.1 / 01	Schmitzer, S.	2014	Effects of BCS-CN88460 tech. (acute contact and oral) on honey bees (Apis mellifera L.) in the laboratory IBACON GmbH, Rossdorf, Germany Bayer Report No.: 89641035	No	Yes	New data for a new active substance	Bayer	No

			Edition Number: M-503824-01-1 Date: 2014-11-24 GLP/GEP: Yes, unpublished **... also filed: KCA 8.3.1.1.2 / 01**					
KCA 8.3.1.1.1 / 03	Taenzler, V.	2015	BCS-CN88460 tech.: Effects (acute oral) on bumble bees (Bombus terrestris l.) in the laboratory IBACON GmbH, Rossdorf, Germany Bayer Report No.: 97632105 Edition Number: M-542774-01-1 Date: 2015-12-14 GLP/GEP: Yes, unpublished	No	Yes	New data for a new active substance	Bayer	No
KCA 8.3.1.1.2 / 01	Schmitzer, S.	2014	Effects of BCS-CN88460 tech. (acute contact and oral) on honey bees (Apis mellifera L.) in the laboratory IBACON GmbH, Rossdorf, Germany Bayer Report No.: 89641035 Edition Number: M-503824-01-1 Date: 2014-11-24 GLP/GEP: Yes, unpublished **... also filed: KCA 8.3.1.1.1 / 01**	No	Yes	New data for a new active substance	Bayer	No

KCA 8.3.1.1.2 / 02	Schmitzer, S.; Haupt, S.	2013	Effects of BCS-CN88460 tech. (acute contact and oral) on honey bees (Apis mellifera L.) in the laboratory IBACON GmbH, Rossdorf, Germany Bayer Report No.: 83991035 Edition Number: M-472468-01-1 Date: 2013-12-11 GLP/GEP: Yes, unpublished **... also filed: KCA 8.3.1.1.1 / 02**	No	Yes	New data for a new active substance	Bayer	No
KCA 8.3.1.1.2 / 03	Haupt, S.	2015	Effects of BCS-CN88460 tech. (acute contact) on bumblebees (Bombus terrestris L.) in the laboratory IBACON GmbH, Rossdorf, Germany Bayer Report No.: 90221105 Edition Number: M-509048-01-1 Date: 2015-01-06 GLP/GEP: Yes, unpublished	No	Yes	New data for a new active substance	Bayer	No
KCA 8.3.1.2 / 01	Gossmann, A.	2015	Chronic oral toxicity test of BCS-CN88460 SC 200 (200 G/L) on the honey bee (Apis mellifera L.) in the laboratory IBACON GmbH,	No	Yes	New data for a new active substance	Bayer	No

			Rossdorf, Germany Bayer Report No.: 93851136 Edition Number: M-540173-01-1 Date: 2015-11-17 GLP/GEP: Yes, unpublished					
KCA 8.4.1 / 01	Frommholz, U.	2016	BCS-CN88460 a.s.: Effects on survival, growth and reproduction on the earthworm Eisenia fetida tested in artificial soil Bayer Report No.: E 312 4704-1 Edition Number: M-548749-01-1 Date: 2016-02-04 GLP/GEP: Yes, unpublished	No	Yes	New data for a new active substance	Bayer	No
KCA 8.4.1 / 02	Frommholz, U.	2017	BCS-CN88460-carboxylic acid (BCS-CY26497): Effects on survival, growth and reproduction of the earthworm Eisenia fetida tested in artificial soil Bayer AG, Crop Science Division, Monheim, Germany Bayer Report No.: E 312 4705-2 Edition Number: M-579263-01-1 Date: 2017-01-26	No	Yes	New data for a new active substance	Bayer	No

			GLP/GEP: Yes, unpublished					
KCA 8.4.2.1 / 01	Frommholz, U.	2015	BCS-CN88460 a.s.: Influence on the reproduction of the collembolan species Folsomia candida tested in artificial soil Bayer Report No.: E 314 4697-4 Edition Number: M-522863-01-1 Date: 2015-05-20 GLP/GEP: Yes, unpublished	No	Yes	New data for a new active substance	Bayer	No
KCA 8.4.2.1 / 02	Friedrich, S.	2017	BCS-CN88460-carboxylic acid (BCS-CY26497): Effects on mortality and reproduction of the collembolan species Folsomia candida tested in artificial soil BioChem agrar GmbH, Gerichshain, Germany Bayer Report No.: 16 10 48 262 S Edition Number: M-587760-01-1 Date: 2017-05-02 GLP/GEP: Yes, unpublished	No	Yes	New data for a new active substance	Bayer	No
KCA 8.4.2.1 / 03	Larnaudie-Lopez, M.	2015	BCS-CN88460 a.s.: Influence on mortality and reproduction of the soil mite	No	Yes	New data for a new active substance	Bayer	No

			species Hypoaspis aculeifer tested in artificial soil Bayer Report No.: E 428 4700-5 Edition Number: M-528194-01-1 Date: 2015-07-15 GLP/GEP: Yes, unpublished					
KCA 8.4.2.1 / 04	Larnaudie-Lopez, M. I.	2015	BCS-CN88460-carboxylic acid (BCS-CY26497): Influence on mortality and reproduction of the soil mite species Hypoaspis aculeifer tested in artificial soil Bayer Report No.: E 428 4699-2 Edition Number: M-524464-01-1 Date: 2015-05-21 GLP/GEP: Yes, unpublished	No	Yes	New data for a new active substance	Bayer	No
KCA 8.5 / 01	Schulz, L.	2015	BCS-CN88460 a.s.: Effects on the activity of soil microflora (Nitrogen transformation test) BioChem agrar GmbH, Gerichshain, Germany Bayer Report No.: 15 10 48 032 N Edition Number: M-532055-01-1 Date: 2015-08-19	No	Yes	New data for a new active substance	Bayer	No

			GLP/GEP: Yes, unpublished					
KCA 8.5 / 02	Schulz, L.	2015	BCS-CN88460-carboxylic acid (BCS-CY26497): Effects on the activity of soil microflora (nitrogen transformation test) BioChem agrar GmbH, Gerichshain, Germany Bayer Report No.: 15 10 48 033 N Edition Number: M-538059-01-1 Date: 2015-10-20 GLP/GEP: Yes, unpublished	No	Yes	New data for a new active substance	Bayer	No
KCA 8.8 / 01	Neuhahn, A.	2018	Activated sludge, respiration inhibition test with isoflucypram technical Currenta GmbH & Co. OHG, Leverkusen, Germany Bayer Report No.: 2018/0009/01 Edition Number: M-617426-01-1 Date: 2018-03-16 GLP/GEP: No, unpublished	No	Yes	Data requirement of Regulation EC 1107/2009	Bayer	No