Draft Assessment Report

Evaluation of Active Substances

Plant Protection Products

Prepared according to **Regulation (EC) 1107/2009**
as it applies in Great Britain (GB PPP)

Cinmethylin (BAS 684 H)

Volume 3 – B.9 (AS)

Ecotoxicology

Great Britain

November 2020

Version History

When	What
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Table of contents

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

Report: CA 8.1.1.1/1
[redacted], 2016 a
BAS 684 H: Acute oral toxicity test (LD_{50}) with northern bobwhite (*Colinus virginianus*)
2016/7005980
Guidelines: EPA 850.2100
GLP: yes
(certified by United States Environmental Protection Agency)

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 684 H (Reg. No. 900202), CAS No.: 87818-31-3, batch identification: COD-002038, purity 93.0% (tolerance ± 1%)

B. STUDY DESIGN

Test species: Bobwhite quail (*Colinus virginianus*), before their first egg-laying season, visually indistinguishable from wild birds. Age of 44 weeks, 2 days at dosing. Source: [redacted]. Test animals were acclimatized to individual test chambers and study rooms for 14 days prior to testing.

Housing: Test cage dimensions were 53 x 25.2 x 20.5 to 25cm and were made of epoxy-coated wire mesh bottoms, fronts, backs and tops with solid galvanized metal partitions. Feed was provided by aluminum mini load pans and water was provided by automatic drip waterers with cups.

Test design: Birds were administered single doses of 0 and 2000 mg a.s./kg b.w. of the test substance BAS 684 H. The liquid test substance was delivered via gavage. Control animals received distilled water in volume comparable to that given birds of similar weight in the treatment group. 5 males and 5 females per dose group were used. The birds were observed for regurgitation, mortality, general condition, overt signs of toxicity and abnormal behavior at least for 1 hour after dosing and at three additional time points during day 0. An observation period of 14 days followed, during which mortality, general condition and overt signs of toxicity were recorded twice on day 1 and once daily, thereafter. Individual body weights were determined and group mean body weights calculated on the day of dosing and on days 7 and 14 after dosing. Mean food consumption (g/bird/day) was calculated from the weekly food consumption/cage for the first and second week after dosing. Spillage was prevented by the design of the feed cups used. A gross post-mortem examination was conducted for four birds (two males, two females) from the treatment group and the control group, respectively. The examination included the digestive tract, liver, kidney, lungs, gall bladder, breast muscles, heart and spleen.

Endpoints: Mortality, clinical signs of toxicity, feed consumption, body weight (b.w.), behavior and gross-pathological examinations. Calculation of LD_{50} and NOEL.

Test concentrations: 0 (Control) and 2000 mg a.s./kg body weight

Test conditions:	Birds were fasted during the overnight dark hours before administration of the test substance; temperature: 16°C (minimum) and 24°C (maximum); relative humidity: 65% and 83%; photoperiod: 10 hours light, 14 hours dark at 27.4 foot-candles average illumination.
Analytics:	No analytical determinations of the test substance in the carrier were necessary since the test substance was applied without carrier.
Statistics:	Body weight data and weekly food consumption were analyzed using a t-test (α = 0.05). No statistical calculation of the LD_{50} and NOEL was performed since no mortality was observed in the tested dose.

II. RESULTS AND DISCUSSION

Validity criteria

The validity criteria outlined in the study guideline were met as follows:

- Birds were randomly assigned to treatment and control pens.
- No more than 10% of the control birds died during the test.
- A minimum of ten birds were used for each dose level of the test substance and control.
- The test substance was orally administered, via either capsule or gavage.

Analytical measurements

No analytical determinations of the test substance in the carrier were necessary since the test substance was applied without carrier.

Biological results

Highest dose tested causing no mortality was 2000 mg a.s./kg b.w. The following acute oral LD_{50} value of the test substance in bobwhite quail was determined at the end of the observation period: LD_{50} > 2000 mg a.s./kg b.w. No regurgitation was observed during the first day after dosing.

There were no treatment related toxic symptoms seen.

There were no statistically significant differences among the treatment and control group in mean body weight and body weight change. No statistically differences were detected in food consumption per bird per day during either week 1 or week 2 of the study.

No unusual findings were noted at post-mortem examinations among the surviving birds examined at study termination. The relevant data and endpoints are summarized in the table below.

Table B.9.1.1.1-1: Acute toxicity of BAS 684 H to the northern bobwhite (*Colinus virginianus*)

	Dose rate [mg a.s./kg b.w.]	
	0 (control)	2000
Number of birds per dose group	10	10
Number of dead birds	0	0
Dead birds percentage [%]	0	0
Endpoints	Dose [mg a.s./kg b.w.]	
Highest dose causing no substance-related mortality	2000	
LD_{50} (14 d)	> 2000	
NOEL	> 2000	

III. CONCLUSION

The acute oral median lethal toxicity (LD_{50}) of BAS 684 H was > 2000 mg active substance / kg body weight. The "No Observed Effect Level" (NOEL) for mortality was 2000 mg active substance / kg body weight.

HSE evaluator comments:

The study was well reported and conducted with good adherence to OCSPP 850.2100: Avian Acute Oral Toxicity Test (2012).

Summary of validity criteria: OCSPP 850.2100 (2012) and OECD 223 (2010):

Criteria	Trigger value	Study value	Criteria met? Y/N	Guideline
1. Birds assignment to treatment and control pens	Random	Random	Y	OCSPP 850.2100
2. Control birds died or became moribund	≤ 10%	0%	Y	OCSPP 850.2100
3. Number of birds used for each dose level of the test substance and control	≥ 10	10 per group	Y	OCSPP 850.2100
4. Test substance method of administration	Orally, by capsule or gavage	Orally by gavage	Y	OCSPP 850.2100
5. In a definitive test only, number of treatments tested	≥ 5 + control	Limit test with 1 treatment	No, limit test	OCSPP 850.2100
6. Control mortality	≤ 10%	0%	Y	OECD 223

It was noted that in the guideline humidity is recommended to be between 45-70% and in the study it peaked outside of this range at 83%. However, the guideline specifically states that 'relative humidity is not as critical is some other variables' and since the validity criteria relating to mortality in the control group was met this is not considered to be a detrimental factor in terms of validity of the study.

It was noted that the study reported that no analytical measurements were carried out despite it being recommended in the guideline. This was considered acceptable since the active substance was applied alone and the purity of the substance was established.

The endpoint for consideration in the risk assessment is:

- **LD_{50} > 2000 mg active substance/kg b.w.**

Report: CA 8.1.1.1/2
[redacted], 1983 a
Acute oral LD_{50} - bobwhite quail sd95481
CI-505-001
Guidelines: none
GLP: no

Applicant comment:

The study was conducted under non-GLP conditions with no analytical verification of the test substance. Thus, the study is considered of limited acceptability and is only presented for reasons of completeness and as supportive information.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Technical SD 95481, Code 5-4-0-0 1/6/83 Exp: date 1/6/85

B. STUDY DESIGN

Test species: Bobwhite quail (*Colinus virginianus*). Seven months old, hatched and reared form egg by [redacted].

Test design: Birds were administered single doses of 0, 398, 631, 1000. 1590 and 2510 mg a.s./kg b.w. of the test substance. The liquid test substance was delivered via gavage. Control animals received corn oil in corresponding volume. 5 males and 5 females per dose group were used. An observation period of 14 days followed, during which mortality, general condition and overt signs of toxicity were recorded. Observations were conducted once daily. Body weights were recorded individually at initiation and by pen at 3 days, 7 days and at termination of the study. Mean food consumption (g/bird/day) was assessed weekly for the first and second week after dosing.

Endpoints: Mortality, clinical signs, feed consumption, body weight (b.w.), behavior and gross-pathological examinations. Calculation of LD_{50}.

Test concentrations: 0 (Control) 398, 631, 1000, 1590 and 2510 mg a.s./kg body weight

Test conditions: Birds were fasted during 16 hours before administration of the test substance; temperature: 65°F (18.3°C) (minimum) and 80°F (26.7°C (maximum); photoperiod: 14 hours light, 10 hours dark.

Analytics: No analytical determinations of the test substance concentration in the carrier were necessary since the test substance was applied without carrier.

Statistics: No statistical analysis for body weight data and weekly food consumption and no calculation of the LD_{50} was performed since no treatment-related mortality was observed in the tested doses.

II. RESULTS AND DISCUSSION

Analytical measurements:

No analytical determinations of the test substance in the carrier were necessary since the test substance was applied without carrier.

Biological results:

Highest dose tested causing no test substance related mortality was 2510 mg a.s./kg b.w. The following acute oral LD_{50} value of the test substance in bobwhite quail was determined at the end of the observation period: LD_{50} > 2510 mg a.s./kg b.w. On day 9 one female was found dead at the 398 mg a.s./kg b.w. with internal lesions associated with egg impaction. On day 10 one male was found dead at the 631 mg a.s./kg b.w. with injuries, apparently the result of striking the head on the pen top or side.

Highest dose tested causing no toxic signs was 398 mg a.s./kg b.w. At the 631 and 1000 mg a.s./kg b.w. dose a male and a female, respectively, showed lethargy and lower limb weakness on day 1. From day 1 to day 5 some birds showed reduced reaction to external stimuli, lower limb weakness, lethargy, wing droop, ruffled appearance and loss of coordination at the 1590 and 2510 mg a.s./kg b.w. dose.

Table B.9.1.1.1-2: Acute toxicity of BAS 684 H to the northern bobwhite quail (*Colinus virginianus*)

	Dose [mg a.s./kg b.w.]					
	0 (control)	398	631	1000	1590	2510
Number of birds per dose group	10	10	10	10	10	10
Number of dead birds	0	1	1	0	0	0
Dead birds percentage [%]	0	10	10	0	0	0
Endpoints	Dose [mg a.s./kg b.w.]					
Highest dose causing no substance-related mortality	2510					
LD_{50} (14 d)	> 2510					

HSE evaluator comments:

It should be noted that 'SD 95481' as described in the summary above is an alternative name for the active substance cinmethylin.

The reported results do not indicate any adverse findings to the valid, GLP-compliant acute test available. However, the study was not conducted in line with Good Laboratory Practice nor to any specific guideline.

The evaluator considers this study invalid and not suitable for incorporation in the risk assessment.

No endpoint was determined from this study.

B.9.1.1.2. Short-term dietary toxicity to birds

Report: CA 8.1.1.2/1
[redacted], 2018a
BAS 684 H: A dietary LC_{50} study with the northern bobwhite
2017/7008676
Guidelines: EPA 850.2200, OECD 205
GLP: yes
(certified by United States Environmental Protection Agency)

Applicant comment:

The short-term dietary study with northern bobwhite was conducted to meet data requirements outside the European Union. The study is provided for the sake of completeness.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 684 H (Reg. No. 900202), Batch No. COD-002038, purity: 93.0%.

B. STUDY DESIGN

Test species: Bobwhite quail (*Colinus virginianus*), chicks, hatched from eggs of animals indistinguishable from wild birds. Age: 12 days old at start of substance feeding; Source: [redacted].

Test design: The test substance was administered via the diet for 5 days at a concentration of 5620 mg active substance / kg diet to two groups with 5 birds each, of 12-day old northern bobwhite quails. The birds were not sexed since sex determination is very uncertain and difficult at that age. In a dietary exposure period of 5 days plus a

post exposure observation period of 3 days; assessment of mortality and signs of clinical toxicity was carried out two times daily; assessment of body weight was carried out on days 0, 5 and 8; Average feed consumption values were determined daily during the exposure period (Days 0-5) and during the post-exposure observation period (Days 5-8) for the treatment group and the control group. Feed consumption was determined by measuring the change in the weight of the feed presented to the birds over a given period of time and dividing it by bird days (number of birds and number of days). A gross necropsy was performed on three birds from the test group and the control group at test termination. Gross necropsy included a general examination of the exterior of the bird and an examination of the thoracic and abdominal cavities, including cardiovascular and respiratory systems, liver, spleen, gastro-intestinal tract, and urogenital system.

Endpoints: Mortality, clinical signs, feed consumption, body weight (b.w.), and gross-pathological examinations. Determination of LC_{50}, LDD_{50} and NOEL.

Test concentrations: 0 (Control) and 5620 mg a.s./kg body weight (nominal concentration based on active substance/kg diet).

Test conditions: Chicks were administered via treated feed for 5 consecutive days followed by a post-exposure period of 3 days on basal diet ad libitum without test substance; temperature: 26.4 ± 1.0 °C; relative humidity: 32 ± 10%, photoperiod: 16 hours light, 8 hours dark, light intensity 402 Lux, the light source was fluorescent lights that closely approximated the colour spectrum of noon-day sunlight.

Analytics: The test substance concentrations were analysed using HPLC with a flame ionization detector (FID). The method used for the analysis of BAS 684 H in avian diet was based upon a methodology developed by EAG Laboratories-Easton (see EAG Laboratories Project Number 147C-175, BASF DocIDs 2016/7001370 and 2017/7017248).

Statistics: There was no mortality observed at the limit test concentration for this study. Therefore, it was not possible to perform the calculation of an LC_{50} value. The LC_{50} value was determined to be greater than the limit dietary concentration tested. Body weight data were compared by 2-sample t-test using TOXSTAT®. Estimated test substance intakes, or daily dietary dose, for Northern Bobwhite were calculated for the treatment group during the exposure period using the following formula:

$$\text{Daily dose (mg/kg b.w./day)} = \frac{\text{Test Concentration (mg a.s./kg diet) x Mean Feed Consumption (g/bird/day)}}{\text{Mean body weight (g/bird)}}$$

II. RESULTS AND DISCUSSION

Analytical results:

The concentration control analyses in the feed yielded concentration was 93.0% of the nominal concentrations. The values indicated that the measured concentrations were in good agreement with the nominal concentrations and that the concentration of the test substance in the diet mix did not decrease under study conditions during the exposure period.

Biological results:

There were no mortalities in the control group or in the 5620 mg a.s./kg diet treatment group during the course of the test. The LDD_{50} calculated on the basis of daily doses was greater than 1283 mg a.s./kg b.w./day. No clinical signs of toxicity were observed in the control group and in the treatment group.

When compared to the control group, there were no apparent differences in feed consumption or body weight at 5620 mg a.s./kg diet test concentration for the exposure and post-exposure period. No substance-related

macroscopic abnormalities were detected in the gross-pathological post-mortem examination. The relevant endpoints are summarized in the table below.

Table B.9.1.1.2-1: Avian dietary toxicity of BAS 684 H to the bobwhite quail (*Colinus virginianus*)

Parameter	**Dose groups [mg a.s./kg diet]**	
	Control	**5620**
Mortality [dead/survivor]	0/20	0/10
Daily dose [mg a.s./kg b.w./d] [1)]	not applicable	>1283
Mean feed consumption during (exposure) days 1 to 5 [g feed/bird/day]	8	7
Mean body weight on days 0, 5 and 8 [g/bird]	25 / 37 / 46	25 / 36 / 45
Clinical signs	n.d.	n.d.
Endpoints [mg a.s./kg diet]		
LC_{50}	>5620	
NOEC	5620	
Endpoints [mg a.s./kg b.w./day]		
LDD_{50}	>1283	
NOEL	1283	

a.s. = active substance
b.w. = body weight
n.d. = no symptoms detected
[1)] Based on measured concentration of active substance in the diet

III. CONCLUSION

Under the conditions of this study the LC_{50} for chicks of the bobwhite quail (*Colinus virginianus*) was greater than 5620 mg a.s./kg diet. The LDD_{50} is greater than 1283 mg a.s./kg b.w./day. The NOEL for sublethal effects was 1283 mg a.s./kg b.w./day.

HSE evaluator comments:

The study was well reported and was conducted predominantly in line with both OECD 205 (1984) and EPA 850.2200 (2012) guidelines for testing avian dietary toxicity in terms of a limit test.

Environmental conditions in the study deviated from those recommended in both guidelines (see table below), however it was considered acceptable due to the control group behaving as required.

Guideline/Study	**Temperature (°C)**	**Relative Humidity (%)**	**Light Regime**
OECD 205 (1984)	30-32	50-75	12 to 16 hours of light per day
EPA 850.2200 (2012)	Temperature gradient in the pen of approximately 38 °C- 22 °C to allow young birds to seek a proper temperature	45-70	14 hours light and 10 hours dark
Achieved in study	26.4 ± 1.0	32 ± 10	16 hours light, 8 hours dark

The validity criteria in EPA 850.2200 (2012) were met as stated in the study report:

1. Birds were randomly assigned to treatment and control pens.
2. Mortality in the control group did not exceed 10%.
3. Concentrations of the test substance were satisfactorily maintained in the diet (levels were at least 80% of the nominal concentration) throughout the exposure period (*i.e.*, the first 5 days).
4. Birds were administered the test substance in their daily diet.
5. A minimum of 10 young birds were used for each dietary concentration of the test substance.
6. The test substance was administered in the diet.

The concentration control analyses in the feed yielded concentration was 93.0% of the nominal concentrations. The method of analysis used in the study was validated by the chemistry specialist (see CA B5).

The endpoint confirmed from this study was:

- **LDD_{50} > 1283 mg a.s./kg b.w./day**

Report:	CA 8.1.1.2/2 [redacted], 1983 b An eight-day dietary LC_{50} in bobwhite quail with sd95481 CI-505-002
Guidelines:	none
GLP:	no

Applicant comment:

The study was conducted under non-GLP conditions with no analytical verification of the test substance and test concentrations in the diet. Thus, the study is considered of limited acceptability and is only presented for reasons of completeness and as supportive information.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Technical SD 95481, Code 5-4-0-0 1/6/83 Exp. Date 1/6/85

B. STUDY DESIGN

Test species: Bobwhite quail (*Colinus virginianus*), age at initiation of study: 14 days; hatched from eggs of animals visually indistinguishable from wild birds; source: [redacted].

Test design: The test substance was administered via the diet for 5 days at experimental concentrations of 0 (control), 562, 1000, 1780, 3160 and 5620 mg a.s./kg diet (and a positive control with dieldrin conducted prior to the test) to bobwhite quails, with a post-exposure period of 3 days; 10 birds per test substance concentration and 10 for the control group were used; assessment for mortality and clinical signs was carried out daily; Body weights were recorded per pen on days 0, 5 and 8. The mean body weight per bird was calculated for each of these days; Mean feed consumption per bird/day for the substance feeding was calculated from the mean food consumption of each pen.

Endpoints: Mortality, clinical signs, feed consumption, body weight (b.w.) Estimation of LC_{50}.

Test concentrations: 0 (Control), 562, 1000, 1780, 3160 and 5620 mg a.s./kg diet.

Test conditions: Temperature not reported; photoperiod: 14 hours light: 10 hours dark,

Analytics: No analytical measurements of the concentration in the diet were conducted.

Statistics: Probit-analysis according to Finney.

II. RESULTS AND DISCUSSION

Analytical measurements:

No analytical measurements were conducted.

Biological results:

No mortality was observed in the control group. The highest concentration tested causing no substance-related mortality was 5620 mg a.s./kg diet. No toxic signs were observed up to and the highest test concentration (5620 mg a.s./kg diet). No clinical signs of toxicity, related to the test substance, were observed in any treatment group.

Results are presented in the table below.

Table B.9.1.1.2-2: Avian dietary toxicity of BAS 684 H to the bobwhite quail (*Colinus virginianus*)

Parameter	**Dose group [mg a.s./kg diet]**					
	Control	**562**	**1000**	**1780**	**3160**	**5620**
Mortality [%] (n=10)	0.0	0.0	0.0	0.0	0.0	0.0
Mean feed consumption during days 0 to 5 [g feed/bird/day]	9.6	9	9	12	9	10
Mean feed consumption during days 6 to 8 [g feed/bird/day]	10	12	9	12	11	9
Mean body weight on day 0, 5 and day 8 [g/bird]	28.2 / 41.4 / 49.4	30 / 42 / 51	26 / 38 / 46	29 / 43 / 52	29 / 40 / 49	29 / 42 / 51
Clinical signs	None	None	None	None	None	None
	Endpoints [mg a.s./kg diet]					
LC_{50}	>5620					

a.s. = active substance
b.w. = body weight

HSE evaluator comments:

The study was not conducted in line with Good Laboratory Practice nor to any specific guideline and no analytical measurements were conducted.

The evaluator considers this study invalid and not suitable for incorporation in the risk assessment.

No endpoint was determined from this study.

Report: CA 8.1.1.2/3
[redacted], 2018b
BAS 684 H: A dietary LC_{50} study with the mallard
2017/7008678
Guidelines: EPA 850.2200, OECD 205

GLP: yes
(certified by United States Environmental Protection Agency)

Applicant comment:

The short-term dietary study with mallard was conducted to meet data requirements outside the European Union. The study is provided for the sake of completeness.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 684 H (Reg. No. 900202), Batch No. COD-002038, purity: 93.0%.

B. STUDY DESIGN

Test species: Mallard (*Anas platyrhynchos*), ducklings, hatched from eggs of animals indistinguishable from wild birds. Age: 5 days old at start of substance feeding; Source: [redacted].

Test design: The test substance was administered via the diet for 5 days at a concentration of 5620 mg active substance / kg diet to two groups of 5-day old mallards. The birds were not sexed since sex determination is very uncertain and difficult at that age. During dietary exposure period of 5 days plus a post exposure observation period of 3 days; assessment of mortality and signs of clinical toxicity was carried out two times daily; assessment of body weight was carried out on days 0, 5 and 8; Average feed consumption values were determined daily during the exposure (days 0-5) and during the post-exposure period (days 5-8). Average feed consumption values were determined daily during the exposure period (days 0-5) and during the post-exposure period (days 5-8). Feed consumption was determined by measuring the change in the weight of the feed presented to the birds over a given period of time and dividing it by bird days (number of birds and number of days). A gross necropsy was performed on three birds from the test group and the control group at test termination. A gross necropsy included a general examination of the exterior of the bird and an examination of the thoracic and abdominal cavities, including cardiovascular and respiratory systems, liver, spleen, gastro-intestinal tract, and urogenital system.

Endpoints: Mortality, clinical signs, feed consumption, body weight (b.w.), and gross-pathological examinations of some birds. Determination of LC_{50}, LDD_{50} and NOEL.

Test concentrations: 0 (Control) and 5620 mg a.s./kg body weight (nominal concentration based on active substance/kg diet).

Test conditions: Ducklings were administered treated feed for 5 consecutive days followed by a post-exposure period of 3 days basal diet ad libitum without test substance; temperature: 21.9 ± 1.1 °C; relative humidity: 50 ± 12%, photoperiod: 16 hours light, 8 hours dark, light intensity 566 Lux, the light source was fluorescent lights that closely approximated the colour spectrum of noon-day sunlight.

Analytics: The test substance concentrations were analysed using HPLC with a flame ionization detector (FID). The method used for the analysis of BAS 684 H in avian diet was based upon a methodology developed by EAG Laboratories-Easton (see EAG Laboratories Project Number 147C-175, BASF DocIDs 2016/7001370 and 2017/7017248).

Statistics: The LC_{50} value was determined to be greater than the limit dietary concentration tested. Body weight data were compared by 2-sample t-test using TOXSTAT®. Estimated test substance intakes, or daily dietary dose, for mallards were calculated for the treatment group during the exposure period using the following formula:

$$\text{Daily dose (mg/kg b.w./day)} = \frac{\text{Test Concentration (mg a.s./kg diet) x Mean Feed Consumption (g/bird/day)}}{\text{Mean body weight (g/bird)}}$$

II. RESULTS AND DISCUSSION

Analytical results:

The concentration control analyses in the feed yielded concentration was 93.0% of the nominal concentrations. The values indicated that the measured concentrations were in good agreement with the nominal concentrations and that the concentration of the test substance in the diet mix did not decrease under study conditions during the exposure period. The endpoint can therefore be expressed in terms of the nominal test concentration.

Biological results:

There were no mortalities in the control or in the 5620 mg a.s./kg diet treatment group during the course of the test. The LDD_{50} calculated on the basis of daily doses was greater than 2714 mg a.s./kg b.w./day. No clinical signs of toxicity were observed in the control group and in the 5620 mg a.s./kg diet treatment groups.

When compared to the control group, there were no apparent differences in feed consumption and bodyweight at the 5620 mg a.s./kg diet test concentration for the exposure and post-exposure period. There were no remarkable findings in any of the birds necropsied.

The relevant endpoints are summarized in the table below.

Table B.9.1.1.2-3: Avian dietary toxicity of BAS 684 H to the mallard (*Anas platyrhynchos*)

Parameter	**Dose groups [mg a.s./kg diet]**	
	Control	**5620**
Mortality [dead/survivor]	0/20	0/10
Daily dose [mg a.s./kg b.w./d] 1)	not applicable	>2714
Mean feed consumption during (exposure) days 1 to 5 [g feed/bird/day]	69	75
Mean body weight on days 0, 5 and 8 [g/bird]	93 / 212 / 304	96 / 216 / 313
Clinical signs	n.d.	n.d.
Endpoints [mg a.s./kg diet]		
LC_{50}	>5620	
NOEC	5620	
Endpoints [mg a.s./kg b.w./day]		
LDD_{50}	>2714	
NOEL	2714	

a.s. = active substance
b.w. = body weight
n.d. = no symptoms detected
1) Based on measured concentration of active substance in the diet

III. CONCLUSION

Under the conditions of this study the LC_{50} for ducklings of the mallard (*Anas platyrhynchos*) was greater than 5620 mg active substance/kg diet. The LDD_{50} calculated on the basis of daily doses was greater than 2714 mg a.s./kg b.w./day. The NOEL for sublethal effects calculated on the basis of daily dose was 2714 mg/kg b.w./day.

HSE evaluator comments:

The study was well reported and was conducted predominantly in line with both OECD 205 (1984) and EPA 850.2200 (2012) guidelines for testing avian dietary toxicity in terms of a limit test.

Environmental conditions in the study deviated from those recommended in both guidelines (see table below), however it was considered acceptable due to the control group behaving as required.

Guideline/Study	Temperature (°C)	Relative Humidity (%)	Light Regime
OECD 205 (1984)	32-35	60-85	12 to 16 hours of light per day
EPA 850.2200 (2012)	Temperature gradient in the pen of approximately 38 °C- 22 °C to allow young birds to seek a proper temperature	45-70	14 hours light and 10 hours dark
Achieved in study	21.9 ± 1.1	50 ± 12	16 hours light, 8 hours dark

The validity criteria in EPA 850.2200 (2012) were met as stated in the study report:

1. Birds were randomly assigned to treatment and control pens.
2. Mortality in the control group did not exceed 10%.
3. Concentrations of the test substance were satisfactorily maintained in the diet (levels were at least 80% of the nominal concentration) throughout the exposure period (*i.e.*, the first 5 days).
4. Birds were administered the test substance in their daily diet.
5. A minimum of 10 young birds were used for each dietary concentration of the test substance.
6. The test substance was administered in the diet.

The method of analysis used in the study was validated by the chemistry specialist (see CA B5).

The endpoint for consideration in the risk assessment is:

- **LC_{50} > 5620 mg a.s./kg diet i.e. >2714 mg a.s./kg bw/d**

Report: CA 8.1.1.2/4
[redacted], 1983 c
Eight day dietary LC50 - mallard duck with sd95481
CI-505-003
Guidelines: none
GLP: no
Deviations:

Applicant comment:

The study was conducted under non-GLP conditions with no analytical verification of the test substance and test concentrations in the diet. Thus, the study is considered of limited acceptability and is only presented for reasons of completeness and as supportive information.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Technical SD 95481, Code 5-4-0-0 1/6/83 Exp. Date 1/6/85

B. STUDY DESIGN

Test species: Mallard duck (*Anas platyrhynchos*), hatchlings; age at initiation of study: 14 days; hatched from eggs of animals visually indistinguishable from wild birds; source: [redacted].

Test design: The test substance was administered via the diet for 5 days at experimental concentrations of 0 (control), 562, 1000, 1780, 3160 and 5620 mg a.s./kg diet (and lab standard (dieldrin) concentrations of 72, 100, 139, 193 and 269 mg a.s./kg diet) to mallard ducklings, with a post-exposure period of 3 days; 10 birds per test substance concentration and 10 for the control group were used; assessment for mortality and clinical signs was carried out daily; Birds were weighed per pen on days 0 and 8. The mean body weight per bird was calculated for each of these days; Mean feed consumption per bird/day for the substance feeding was calculated from the mean food consumption of each group.

Endpoints: Mortality, clinical signs, feed consumption, body weight (b.w.) Estimation of LC_{50}.

Test concentrations: 0 (Control), 562, 1000, 1780, 3160 and 5620 mg a.s./kg diet (and lab standard (dieldrin) concentrations of 72, 100, 139, 193 and 269 mg a.s./kg diet).

Test conditions: Temperature: 75°F (ca. 23.9°C) during the 8-day study; photoperiod: 14 hours light: 10 hours dark.

Analytics: No analytical measurements of the concentration in the diet were conducted.

Statistics: Probit-analysis.

II. RESULTS AND DISCUSSION

Analytical measurements:

No analytical measurements in the diet were conducted.

Biological results:

No mortality was observed in the control group. The highest concentration tested causing no substance-related mortality was 5620 mg a.s./kg diet. No toxic signs were observed up to and the highest test concentration (5620 mg a.s./kg diet). A slight reduction in body weight gain at the 5620 mg a.s./kg diet was observed. No clinical signs of toxicity, related to the test substance, were observed in any of the treatment groups.

Results are presented in the table below.

Table B.9.1.1.2-4: Avian dietary toxicity of BAS 684 H to the mallard duck (*Anas platyrhynchos*)

Parameter	Dose group [mg a.s./kg diet]					
	Control	562	1000	1780	3160	5620
Mortality [%] (n=10)	0.0	0.0	0.0	0.0	0.0	0.0
Mean feed consumption during days 1 to 5 [g feed/bird/day]	79-82	81	82	85	81	74
Mean body weight on day 0 and day 8 [g/bird]	252-287 / 467-493	287 / 497	274 / 492	255 / 472	282 / 503	265 / 443
Clinical signs	None	None	None	None	None	None
	Endpoints [mg a.s./kg diet]					
LC_{50}	>5620					

HSE evaluator comments:

The study was not conducted in line with Good Laboratory Practice nor to any specific guideline. No analytical verification of the test substance was undertaken.

The evaluator considers this study invalid and not suitable for incorporation in the risk assessment.

No endpoint was determined from this study.

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

Report: CA 8.1.1.3/1
[redacted], 2016 a
BAS 684 H: A reproduction study with the northern bobwhite
2016/7009945
Guidelines: EPA 850.2300, OECD 206
GLP: yes
(certified by United States Environmental Protection Agency)

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 684 H, Reg. No.: 900202, Batch No.: COD-002038, purity: 94.9%

B. STUDY DESIGN

Test species: Northern bobwhite quails (*Colinus virginianus*), phenotypically indistinguishable from wild type; adults, age: 34 weeks of age at the initiation of the test (before beginning of first egg-laying period); weight range 185-241 grams; supplier: [redacted]. Adult birds were identified by individual leg bands.

Test design: Northern bobwhite quails approaching their first breeding season were kept in a group of 1 male and 1 female in a pen per replicate. 18 pens were allocated to the control and each treatment group. All adult birds and their offspring were given feed (basal diet containing at least 27% protein and 2% crude fat and no more than 5% crude fibre) and water *ad libitum* during acclimation and testing. The study period was divided into five phases:

1. Acclimation to laboratory conditions – 15 weeks;
2. Pre-photostimulation – 8 weeks;
3. Pre-egg laying (with photostimulation) – 3 weeks;
4. Egg laying period – 10 weeks;
5. Post-adult termination (final incubation, hatching and 14-day offspring rearing period) – 6 weeks.

Eggs were collected daily from all pens and stored in a cold room until incubation. At the end of a weekly interval, all eggs were removed from the cold room, counted and eggs selected by indiscriminate draw for egg shell thickness measurement.

Cracked or abnormal eggs were recorded and discarded. All eggs not discarded or used for egg shell thickness measurements were placed in an incubator. On day 21 of incubation, eggs were moved to a hatcher. Young birds were maintained for 14 days. Adult birds were sacrificed after the egg-laying period, young birds after 14 days.

Observations: During acclimation all birds were observed daily and birds exhibiting abnormal behaviour or debilitating physical injuries were not used for the test. During the study all birds were observed daily for signs of toxicity or abnormal behaviour. Also offspring were observed daily from hatching until 14 days of age.

Necropsy: Adult birds that died, were euthanised during the study or survived the study were subjected to a gross necropsy.

Endpoints: Adult birds: mortalities, clinical observations, gross necropsy, adult body weight, adult feed consumption.

Reproductive parameters: Eggs laid/hen/day, eggs uncracked of eggs laid, fertile eggs of eggs set, viable embryos of eggs set, live 3-week embryos of viable embryos, live 3-week embryos, hatchlings of 3 week-embryos, 14-day old survivors of hatchlings, hatchlings of eggs set, hatchling of fertile eggs, 14-day old survivors/pen/day, 14-day old survivors of eggs set, hatchling body weight, 14-day old survivors body weight and egg shell thickness.

Test concentrations: 0 (Control), 300, 600 and 1200 mg a.s./kg diet BAS 684 H (nominal).

Test conditions: Adult bobwhite study room: Temperature 20.5 ± 1.1 °C (SD); relative humidity: 40 ± 14% (SD); ventilation: 15 times the room air /h; photoperiod: 8 hours light (week 1 – 8), lengthened photoperiod to 17 hours light (week 9 to the end of study). 310 lux illumination during the pre-photostimulation, 264 lux at post-photostimulation and during egg-laying approx. 197 lux.

Egg collection and storage: Collected daily, stored in cold room: temperature: 14.2 ± 0.3 °C (SD), relative humidity: 78 ± 8% (SD). Eggs set for incubation: temperature: 37.4 ± 0.0 °C (SD), relative humidity 55 ± 0.0 °C (SD); the eggs were transferred to the hatcher on day 21: temperature: 37.3 ± 0.0 °C (SD), relative humidity approximately 58 ± 0% (SD)

Hatchlings: Brooding compartment temperature approximately 38°C from hatching until the birds were 14 days of age; average ambient room temperature 26.4 ± 2.0 °C (SD), relative humidity: 46 ± 11%; photoperiod: 16 hours light per day

Diet Sampling: Homogeneity of the test substance in the diet was evaluated by collecting six samples from each treated diet and one from the control. Samples were collected from the top, middle and bottom of the let and right sections of the mixing vessel. Control and treatment group diet samples were also collected by composition feed from the feed troughs on Day 7 of weeks 1, 12 and 20 to assess stability of the test substance in test conditions.

Analytics: The test substance concentrations were analysed using HPLC with a flame ionization detector (FID) (see EAG Laboratories Project Number 147C-175, BASF DocIDs 2016/7001370 and 2017/7017248).

Statistics: William´s test multiple comparison was used when data were normally distributed with equal variances among all groups. If data were not normally distributed or showed unequal variances among treatment groups, the Jonkheer-Terpstra step-down trend test was used to determine statistically significant differences between groups.

II. RESULTS AND DISCUSSION

Validity criteria according to OECD 206 (1984):

- The mortality in the controls did not exceed 10 per cent at the end of the test (actual = 0%).
- The average number of 14-day-old survivors per hen in the controls should be at least 12 for bobwhite quail (actual = 31).
- The average egg shell thickness for the control group was at least 0.19 mm bobwhite quail (actual = 0.231 mm).

Validity criteria according to OCSPP 850.2300 (2012):

- Birds were randomly assigned to treatment and control pens.
- The mortality in the controls did not exceed 10 per cent at the end of the test (actual = 0%).
- The average number of eggs laid per hen in the control group was less than 29 (actual = 42).
- The number of viable embryos in the control group was ≥ 80% of the eggs set (actual = 94% at day 11).
- The number of 18-d-old embryos of eggs set in the control group was ≥ than 97% (actual = 99%).
- The number of normal hatchlings in the control group was ≥ 85% (actual = 96% hatchlings of live 3-week embryos).
- The number of normal hatchlings in the control group was ≥ 71% of the eggs set (actual = 89%).
- The number of 14-day old survivors in the control group was ≥ 77% of the normal hatchlings (actual = 94%).
- The average eggshell thickness in the control group is ≥ 0.20 mm (actual = 0.231mm).
- There are ≤ 13% cracked eggs in the control group (actual = 12%).

Analytical measurements:

The result of the analytical verification of the test substance was 94.9 %. Concentrations of the test substance in the diet were adjusted to 100% active substance. Mean concentrations and standard deviations for the three test concentrations were 291 ± 6.13, 580 ± 6.02, and 1160 ± 27.6 mg a.s./kg diet for the nominally 300, 600, and 1200 mg a.s./kg diet, respectively. These values represented 94%, 92% and 88% of nominal concentrations. This demonstrates dosing concentrations were within 20% of nominal and hence sufficiently maintained in line with guidance.

Analysis of diet samples collected from feeders after being held at ambient temperature for 7 days during week 1 averaged 100%, 99% and 106% of the Day 0 values for the 300, 600 and 1200 ppm a.s. test concentrations respectively.

Analysis of diet samples collected from feeders after being held at ambient temperature for 7 days during week 12 averaged 103%, 100% and 102% of the Day 0 values for the 300, 600 and 1200 ppm a.s. test concentrations respectively.

Analysis of diet samples collected from feeders after being held at ambient temperature for 7 days during week 20 averaged 95%, 96% and 105% of the Day 0 values for the 300, 600 and 1200 ppm a.s. test concentrations respectively.

Biological results:

Parental generation

One single adult mortality occurred during the test in the 300 mg a.s./kg diet treatment group. However, this mortality was not considered to be treatment related. No other mortalities occurred during the course of the study. All birds were normal in appearance and behaviour with the exception of incidental clinical observations associated to injuries. All surviving adults were subjected to gross necropsy and all findings observed were considered unrelated to treatment.

There were no apparent treatment-related effects upon adult body weight at any of the concentrations tested. No statistically significant differences between the control group and the 300, 600 and 1200 mg a.s./kg diet treatment groups were observed. There were no apparent treatment-related effects upon feed consumption at any of the concentrations tested. There were slight, but statistically significant ($p < 0.05$) increase in mean feed consumption at the 600 and 1200 mg a.s./kg diet test concentration during week 13 of the test. In addition, at the 300 mg a.s./kg diet test concentration, there were slight increases in mean feed consumption during different weeks of the test. However, the differences were not considered to be related to treatment, since they were neither consistent over time, nor concentration responsive (see Table B9.1.1.3-1).

Reproductive results

There were no apparent treatment related effects upon egg shell thickness at any of the concentrations tested. When compared to the control group, there were no statistically significant differences in egg shell thickness in the 300, 600, or 1200 mg a.s./kg diet treatment groups.

There were no treatment-related effects upon reproductive performance at the any of the concentrations tested. When compared to the control group, there were no statistically significant differences in any of the reproductive parameters at the 300, 600 and 1200 mg a.s./kg diet test concentrations. At the 300 mg a.s./kg diet treatment group, there appeared to be a slight reduction in the number of viable embryos, that was also evidenced in slight reductions of live 3-week embryos, hatchlings and 14-day old survivors as percentage of the number of eggs set. However, those reductions were primarily influenced by two replicates, from which viability was only 4%. Thus, the lower viability was not considered treatment related, but considered as pen specific partial infertility.

There were no apparent treatment related effects upon offspring body weight at any of the concentrations tested. When compared to the control group, there were no statistically significant differences in the body weight of hatchlings or 14-day old survivors from the 300, 600 or 1200 mg a.s./kg treatment group. Results are summarized in the tables below.

Table B.9.1.1.3-1: Effects of BAS 684 H on the parental generation of the northern bobwhite quail (*Colinus virginianus*)

Parameter	**Treatment group [mg a.s./kg diet]**			
	Control	**300**	**600**	**1200**
No. of replicates (1 male and 1 female per replicate/pen)	18	17	18	18
No. of substance-related mortalities of adult birds	0	0	0	0
Adult body weight [g] at the end of study (male/female)	219/248	232/248	222/248	223/243
Gain of adult body weight [g] at the end of study (male/female)	5/34	13/37	4/35	5/30

Table B.9.1.1.3-2: Effects of BAS 684 H on the reproduction of the northern bobwhite quail (*Colinus virginianus*)

Parameter	**Treatment group [mg a.s./kg diet]**			
	Control	**300**	**600**	**1200**
Number of surviving replicates	18	17	18	18
Total eggs laid	759	777	821	877
Eggs laid/hen	42	46	46	49

Parameter	Treatment group [mg a.s./kg diet]			
	Control	300	600	1200
Eggs laid/hen/day	0.46	0.50	0.50	0.54
Eggs uncracked	741	762	807	858
Mean egg shell thickness (mm)	0.231 ± 0.016	0.233 ± 0.015	0.233 ± 0.014	0.227 ± 0.012
Eggs set	657	683	722	764
Viable Embryos	621	590	711	712
Mean body weight (g) of hatchlings per group	6.3 ± 0.5	6.4 ± 0.4	6.4 ± 0.4	6.2 ± 0.3
Live 3-week embryos	615	587	705	710
Mean bodyweight (g) of 14-day old survivors	26 ± 3	28 ± 3	27 ± 3	26 ± 3
Hatchlings	593	544	687	678
14-day old survivors	552	519	655	636
14-day old survivors/pen	31	31	36	35

Table B.9.1.1.3-3: Effects of BAS 684 H on the reproduction of the northern bobwhite quail (*Colinus virginianus*) expressed as percentages

Parameter	Treatment group [mg a.s./kg diet]			
	Control	300	600	1200
% viable embryos/eggs set	94	82	99	100
% live 3-week embryos/viable embryos	99	99	99	100
% hatchlings/live 3-week old embryos	96	94	97	96
% hatchlings/eggs set	89	76	95	90
% 14-day old survivors/eggs set	84	72	91	85
% 14-day survivors of hatchlings	94	96	95	94
% uncracked eggs of eggs laid [1)]	98	98	98	97

[1)] Percent values represent replicate means for each experimental group.

III. CONCLUSION

There were no treatment-related mortalities, overt signs of toxicity or treatment-related effects upon body weight at any of the concentrations tested. In addition, there were no treatment-related effects upon any of the reproductive parameters measured at the 300, 600 or 1200 mg a.s./kg diet test groups. The no-observed-effect concentration (NOEC) for northern bobwhite quails exposed to BAS 684 H in the diet during the study was 1200 mg a.s./kg diet (99.1 mg a.s./kg b.w./day), the highest concentration tested.

HSE Evaluator comments

The study was well reported and adhered predominantly to OECD 206 (1984) and OCSPP 850.2300 (2012).

It was noted that the birds at test initiation were 34 weeks old whereas in OECD 206 (1984) it is recommended that Bobwhite quails be 20-24 weeks old at this point with a ± 1-week age range within a given test. OCSPP 850.2300 (2012) states *'Adult test birds used are those approaching their first breeding season and are at least 16 weeks old. All test birds should be the same age within one month'*. Given that all validity criteria were met for both guidelines demonstrating the control behaved as required, the age of the birds was not considered to have a negative impact on the study.

Validity criteria outlined in OECD 206 (1984) and OCSPP 850.2300 (2012) were all met as outlined in the summary above.

All analytical measurements (initial concentrations and those taken after 7 days at ambient temperature during weeks 1, 12 and 20) were within 20% of nominal test substance concentrations. Therefore, it is accepted that the test item would be sufficiently maintained in the test environment during the study enabling dosing as stated. It is

therefore considered appropriate to express any endpoint in terms of nominal values. The method of analysis used in the study was validated by the chemistry specialist (see CA B5).

The study showed no statistical effects of the test item on any of the parameters measured or calculated. However, the HSE evaluator notes that as pointed out in the study summary, at the lowest concentration of 300 mg a.s./kg diet there was a slight reduction in the number of viable embryos, live 3-week embryos, hatchlings and 14-day old survivors as percentage of the number of eggs set (reduction in 12% compared to the control). These were not statistically significant effects nor were they part of a dose-response as they were not observed in the two higher concentrations. Therefore the HSE evaluator does not consider this a cause for concern.

The endpoint for consideration in the risk assessment is:

- **NOEC = 1200 mg a.s./kg diet (99.1 mg a.s./kg b.w./day)**

Report:	CA 8.1.1.3/2 [redacted], 2018c BAS 684 H: A reproduction study with the mallard 2017/7016288
Guidelines:	EPA 850.2300, OECD 206
GLP:	yes (certified by United States Environmental Protection Agency)

Remarks: The reproduction study with mallard was conducted to meet data requirements outside the European Union. The study is provided for the sake of completeness.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 684 H, Reg. No.: 900202, Batch No.: COD-002038, purity: 93.0%

B. STUDY DESIGN

Test species: Mallard (*Anas platyrhynchos*), phenotypically indistinguishable from wild type; adults 28 weeks of age at the initiation of the test (before beginning of first egg-laying period); supplier: [redacted].

Test design: Mallards approaching their first breeding season were kept in a group of 1 male and 1 female in a pen per replicate. 18 pens were allocated to the control and each treatment group. All adult birds and their offspring were given feed and water *ad libitum* during acclimation and testing. The study period was divided into five phases:

1. Acclimation to laboratory conditions – 14 weeks;
2. Pre-photostimulation – 11 weeks;
3. Pre-egg laying (with photostimulation) – 0 weeks;
4. Egg laying period – 10 weeks;
5. Post-adult termination (final incubation, hatching and 14-day offspring rearing period) – 6 weeks.

Eggs were collected daily from all pens and stored in a cold room until incubation. At the end of a weekly interval, all eggs were removed from the cold room, counted and eggs selected by indiscriminate draw for egg shell thickness measurement.

Cracked or abnormal eggs were recorded and discarded. All eggs not discarded or used for egg shell thickness measurements were placed in an incubator. On day 21 of incubation, eggs were moved to a hatcher. Young birds were maintained for 14 days. Adult birds were sacrificed after the egg-laying period, young birds after 14 days.

Endpoints:	Adult birds: mortalities, clinical observations, gross necropsy, adult body weight and body weight gain, feed consumption Reproductive parameters: Eggs laid/hen/day, uncracked eggs of eggs laid, viable embryos of eggs set, live 3-week embryos of eggs set, live 3-week embryos of viable embryos, hatchlings of 3 week-embryos, 14-day old survivors of hatchlings, hatchlings of eggs set, hatchling of eggs laid, 14-day old survivors of hatchlings, 14-day old survivors of eggs set, hatchlings/pen/day, offspring body weight, and egg shell thickness
Test concentrations:	0 (Control), 300, 600 and 1200 mg a.s./kg diet BAS 684 H (nominal).
Test conditions:	Adult mallard study room: Temperature 20.7 ± 1.1 °C (SD); relative humidity: 46 ± 12% (SD); ventilation: 15 times the room air/h; photoperiod: 8 hours light (week 1 – 10), lengthened photoperiod to 17 hours light (week 11 to the end of study). 292 lux illumination during the pre-photostimulation, 306 lux at post-photostimulation and 349 lux at during egg-laying approx. Egg collection and storage: Collected daily, washed, stored in cold room: temperature: 14.3 ± 0.3 °C (SD), relative humidity: 82 ± 9% (SD). Eggs set for incubation: temperature: 37.4 ± 0.0 °C (SD), relative humidity 55 ± 0.0 °C (SD); the eggs were transferred to the hatcher on day 21: temperature: 37.4 ± 0.0 °C (SD), relative humidity approximately 55 ± 0% (SD) Hatchlings: Brooding compartment temperature approximately 37.3 °C from hatching until the birds were 14 days of age; average ambient room temperature 24.3 ± 1.0 °C (SD), relative humidity: 54 ± 16%; photoperiod: 16 hours light per day
Analytics:	The test substance concentrations were analysed using HPLC with a flame ionization detector (FID). The method used for the analysis of BAS 684 H in avian diet was based upon a methodology developed by EAG Laboratories-Easton (see EAG Laboratories Project Number 147C-175, BASF DocIDs 2016/7001370 and 2017/7017248).
Statistics:	Analysis of variance (ANOVA) was performed to determine statistically significant differences between groups. Dunnett's multiple comparison procedure was used to compare the three treatment means with the control group mean and assess the statistical significance of the observed differences. Sample units were the individual pens within each experimental group, except adult body weights where the sample unit was the individual bird. Percentage data were examined using Dunnett's method following arcsine square root transformation for reproductive parameters.

II. RESULTS AND DISCUSSION

Validity criteria according to OECD 206 (1984):

- The mortality in the controls did not exceed 10 per cent at the end of the test (actual = 0%).
- The average number of 14-day-old survivors per hen in the controls was at least 14 (actual = 30).
- The average egg shell thickness for the control group should be at least 0.34 mm (actual = 0.382 mm).

Validity criteria according to EPA 850.2300 (2012):

- Birds were randomly assigned to treatment and control pens.
- The mortality in the controls did not exceed 10 per cent at the end of the test (actual = 0%).
- The average number of eggs laid per hen in the control group was ≥29 (actual = 51).
- The number of viable embryos in the control group was ≥80% of the eggs set (actual = 84%).
- The number of 21-d-old mallard embryos of eggs set in the control group ≥ 94% (actual = 98%).

- The number of normal hatchlings in the control group was ≥ 52% of the viable embryos for mallard (actual = 84%).
- The number of normal hatchlings in the control group was ≥ 44% of the eggs set for mallard (actual = 69%).
- The number of 14-day old survivors in the control group was ≥ 94% of the normal hatchlings for mallard (actual = 100%).
- The average eggshell thickness in the control group is ≥ 0.316 mm for mallards (actual = 0.382 mm).
- There were less than 13% cracked eggs in the control group (actual = 1%).

Analytical measurements:

Concentrations of the test substance in the diet were adjusted to 100% active substance. Analytical measurements represented 95.7%, 96.2% and 100% of the nominally 300, 600, and 1200 mg a.s./kg diet. Analytical measurements of diet samples from feeders kept for 7 days at ambient temperature of week 1, 12 and 20 revealed values in between 85.2% – 96.9% of the initial test concentrations. This demonstrates dosing concentrations were within 20% of nominal and hence sufficiently maintained in line with guidance.

Biological results:

Parental generation

With the exception of incidental observations, all birds were normal in appearance and behaviour without overt signs of toxicity. All surviving adults were subjected to gross necropsy and all findings observed were considered unrelated to treatment.

There were no apparent treatment-related effects upon feed consumption and adult body weight at any of the concentrations tested. No statistically significant differences between the control group and the 300, 600 and 1200 mg a.s./kg diet treatment groups were observed at any of the feed consumption and body weight intervals. A slight, but statistically significant reduction in mean food consumption at the 300 mg as/kg diet during week 7 was noted, which was however not considered treatment related, as it was neither concentration responsive nor consistent over time (see Table B9.1.1.3-4).

Reproductive results

There were no apparent treatment related effects upon egg shell thickness at any of the concentrations tested. When compared to the control group, there were no statistically significant differences in egg shell thickness in the 300, 600, or 1200 mg a.s./kg diet treatment groups.

There were no treatment-related effects upon reproductive performance at the any of the concentrations tested. When compared to the control group, there were no statistically significant differences in any of the reproductive parameters at the 300, 600 and 1200 mg a.s./kg diet test concentrations. At the 300 and 600 ppm mg a.s./kg diet test concentrations there was a very slight, but statistically significant reduction in the number of 14-day old survivors as a percentage of the number of hatchlings (99% $\pm$ 2 and 99% $\pm$ 1, respectively). This difference was influenced by the high survival rate in the control group, with all 535 offspring that hatched surviving, while seven offspring from each of the 300 and 600 ppm mg a.s./kg diet did not survive to 14 days of age. In addition to being a very slight difference, the reduction was not concentration responsive and was therefore not considered to be treatment-related.

There were no apparent treatment related effects upon offspring body weight at any of the concentrations tested. When compared to the control group, there were no statistically significant differences in the body weight of hatchlings or 14-day old survivors from the 300, 600 or 1200 mg a.s./kg treatment group. Results are summarized in the tables below.

Table B.9.1.1.3-4: Effects of BAS 684 H on the parental generation of the mallard (*Anas platyrhynchos*)

Parameter	Treatment group [mg a.s./kg diet]			
	Control	300	600	1200
No. of replicates (1 male and 1 female per replicate/pen)	18	18	18	18
No. of substance-related mortalities of adult birds	0	0	0	0
Adult body weight [g] at the end of study (male/female) [1)]	1130/1108	1161/1128	1150/1162	1176/1144
Gain of adult body weight [g] at the end of study (male/female)[1)]	-6/85	25/107	23/141	32/116

1) Values from appendix X, table 4.

Table B.9.1.1.3-5: Effects of BAS 684 H on the reproduction of the mallard (*Anas platyrhynchos*)

Parameter	Treatment group [mg a.s./kg diet]			
	Control	300	600	1200
Number of surviving replicates	18	18	18	18
Total eggs laid	911	871	1008	965
Eggs laid/hen	51	48	56	54
Eggs laid/hen/day	0.66	0.63	0.73	0.70
Eggs uncracked	906	867	1006	963
Mean egg shell thickness (mm)	0.382 ± 0.019	0.392 ± 0.025	0.395 ± 0.026	0.386 ± 0.020
Eggs set	789	761	909	863
Viable Embryos	648	678	852	737
Live 3-week embryos	636	664	843	728
Mean body weight (g) of hatchlings per group	37 ± 2.3	37 ± 2.7	37 ± 2.5	37 ± 2.9
Mean bodyweight (g) of 14-day old survivors	297 ± 18	296± 15	298 ± 17	289 ± 15
Hatchlings	535	566	706	622
14-day old survivors	535	559	699	620
14-day old survivors/pen	30	31	39	34

Table B.9.1.1.3-6: Effects of BAS 684 H on the reproduction of the mallard (*Anas platyrhynchos*) expressed as percentages

Parameter	Treatment group [mg a.s./kg diet]			
	Control	300	600	1200
% viable embryos/eggs set	84	89	94	86
% live 3-week embryos/viable embryos	98	98	99	99
% hatchlings/live 3-week old embryos	84	85	84	86
% hatchlings/eggs set	69	75	77	73
% 14-day old survivors/eggs set	69	74	77	73
% 14-day survivors of hatchlings	100	99*	99*	100
% uncracked eggs of eggs laid [1)]	99	100	100	100

1) Percent values represent replicate means for each experimental group.
* Significantly different from control at $p \leq 0.05$ (Dunnett's t-test).

III. CONCLUSION

There were no treatment-related mortalities, overt signs of toxicity or treatment-related effects upon body weight at any of the concentrations tested. In addition, there were no treatment-related effects upon any of the reproductive parameters measured at the 300, 600 or 1200 mg a.s./kg diet test groups. The no-observed-effect concentration (NOEC) for mallards exposed to BAS 684 H in the diet during the study was 1200 mg a.s./kg diet (174 mg a.s./kg b.w./day), the highest concentration tested.

HSE Evaluator Comments

The study was well reported and adhered predominantly to OECD 206 (1984) and OCSPP 850.2300 (2012).

Validity criteria outlined in OECD 206 (1984) and OCSPP 850.2300 (2012) were all met as outlined in the summary above.

According to the report there was a single mortality at the 1200 ppm treatment group, however that is not reflected in any of the results tables, only as one statement. This discrepancy should be noted and the HSE evaluator assumes that there were no mortalities based on the numerical results reported.

It was noted that the birds at test initiation were 28 weeks old whereas in OECD 206 (1984) it is recommended that Mallard ducks be 9-12 months old at this point with a ± 2 -week age range within a given test. The test birds being younger than recommended in OECD 206 is not considered to have affected the test conduction or results in anyway seeing as the control group behaved as required to meet validity criteria. In addition, OCSPP 850.2300 (2012) as a more general outline of bird age for a reproduction study which has been adhered to; *'Adult test birds used are those approaching their first breeding season and are at least 16 weeks old. All test birds should be the same age within one month'*.

The method of analysis used in the study was validated by the chemistry specialist (see CA B5). All analytical measurements (initial concentrations and those taken after 7 days at ambient temperature during weeks 1, 12 and 20) were within 20% of nominal test substance concentrations. Therefore, it is accepted that the test item would be sufficiently maintained in the test environment during the study enabling dosing as stated. It is therefore considered appropriate to express any endpoint in terms of nominal values.

The study showed a statistical difference between the % of 14-day survivors of hatchlings at concentrations of 300 and 600 mg a.s./kg diet and the control group (see table B9.1.1.3-6 above). However the HSE Evaluator does not consider this to impact the NOEC due to the % difference being 1%.

The endpoint for consideration in the risk assessment is:

- **NOEC = 1200 mg a.s./kg diet (174 mg a.s./kg b.w./day)**

B.9.1.2. Effects on terrestrial vertebrates other than birds

B.9.1.2.1. Acute oral toxicity to mammals

All relevant studies are provided in Volume 3 – B.6 of the Review Assessment Report.

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

All relevant studies are provided in Volume 3 – B.6 of the Review Assessment Report.

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

According to the data requirements, substances with a log P_{OW} greater than 3 have potential for bioaccumulation and should be assessed for the risk of bioaccumulation in prey of birds and mammals. The log Kow of the active substance BAS 684 H is 4.5 at 20°C and pH=7 (see Volume B2). The risk from bioaccumulation to fish-eating and worm-eating birds and mammals has been carried out, see Vol. 3, CP B9.

New fish BCF studies are available, summaries are provided in Section B.9.2.8. In addition as part of the refined higher tier risk assessment for earthworm-eating birds and mammals an earthworm bioconcentration study was submitted, the summary and evaluation of which is presented below.

Report:	CA 8.1.3/1 Simon, M., 2019 Bioaccumulation of BAS 684 H in terrestrial oligochaetes 2019/1059201
Guidelines:	OECD 317 (2010)
GLP:	Yes (certified by Ministerium fur Arbeit, Integration und Soziales des Landes Nordrhein-Westfalen)

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 684 H

Mixture of non-radiolabelled cinmethylin (BAS 684 H, Reg. No. 900 202; batch no. COD-002038, purity 93.5%) and radiolabelled 14C-Cyclohexane 4-BAS 684 H/cinmethylin (batch-No. 1146-1001, specific activity 42.1 MBq/g, chemical purity: 99.3%, radiochemical purity: 99.4%); mixing ratio 2:1.

B. STUDY DESIGN

Test species: *Eisenia fetida andrei*; adult worms with clitellum and weight of 337 mg ±5.85 mg/worm, age range of two months to 1 year; source: Regenwurmfarm Tacke, Klosterdiek 61,46325 Borken followed by in-house culture at Fraunhofer IME.

Test design: The test design followed principles of OECD Test Guideline 317 “Bioaccumulation in Terrestrial Oligochaetes”. Pre-acclimatized period for 7 days in artificial substrate followed by a 21-day uptake phase; adults of *Eisenia fetida andrei* were exposed to BAS 684 H for 21 days in dry artificial substrate under static exposure conditions. For each sampling point 5 replicates with test item were set up with one worm/replicate (4 replicates with one worm/replicate for control); at each sampling point artificial substrate of the 5 replicates was analysed (per replicate in 3 technical measurements; 4 replicates for control).

The test item was incorporated in to the soil via water to achieve a nominal concentration of 3.5 mg a.s./kg dry substrate. The chosen test concentration represents less than factor 25 below the chronic NOEC of 87.8 mg a.s./kg dry soil (Friedrich, 2016a), as well as less than 1% of the LC_{50} which was determined before to occur at 466 mg a.s./kg dry soil (Friedrich, 2016b).

Glass vessels (150 mL with a diameter of 5 cm and a height of 9 cm) were used as containers for substrate and worms. At the start of the test, conditioned worms were weighed individually and placed into single vessels. Worms were washed prior to weighing and excess water was removed by placing them briefly on slightly moistened paper towels.

The duration of the uptake phase was chosen to ensure a reach of the equilibrium phase (“steady-state”) between worm and substrate. The concentration of the test item was recorded by repeated analyses of sampled individuals from the test population (C_{worm}), and in parallel, the concentration of test item in substrate (C_{soil}). As no further increase (±20%) of the concentration Cworm was observed amongst over at least 3 samplings in sequence, the steady-state bioaccumulation factor BAFsteady state can be concluded by the quotient of Cworm means /Csoil overall mean.

Immediately after weighing, the worms were frozen in liquid nitrogen until further analysis. Samples were stored at ≤ -18°C. Substrate samples were analyzed

	immediately or were stored as well frozen ≤ -18°C). Worms were analyzed individually for the test item per sampling date by 14 C-content. Samples for analysis of test item concentration (substrate and worms) were taken on days 0,1,4,7,10,14,17 and 21. Worm behaviour (burrowing behaviour) and Endpoints: Steady state bioaccumulation factor ($BAF_{steady\ state}$)
Reference item:	A reference item was not used.
Control item:	The control substrate was prepared with unspiked water with the respective WHC content.
Test concentrations:	Control, 3.5 mg BAS 684 H/kg dry soil (nominal)
Test conditions:	Artificial substrate with 10% sphagnum peat (air-dried), 20% kaolinite (air-dried) and 70% industrial quartz (air-dried); pH at start of uptake phase: pH 5.67 (control) and pH 5.69 (treatment); water content: 44.7% (control) and 44.5 (spiked substrate) % of its maximum water holding capacity (WHC); temperature: 19 C – 21°C; photoperiod: 16 hours light, 8 hours dark; earthworms were fed with horse manure.
Statistics:	Descriptive statistics
Analytical methods:	Sample preparation for total radioactive analysis in substrate and tissue Substrate samples were analysed immediately (homogeneity) or were stored frozen (< -18 °C) until analysis. For earthworm tissue, each replicate was analysed separately. After purging their gut overnight and weighing, each worm was placed in liquid nitrogen, transferred into a sampling tube and stored frozen (< -18 °C) until analysis. Combustion of substrate and tissue samples For the determination of the homogenous distribution of test item in substrate, five substrate samples were analysed in triplicates after homogenization. The combustion was performed for two minutes using an oxidizer (Zinsser OX 500) and the radioactivity was determined by subsequent LSC analysis. For earthworm analysis, frozen worms were split into two parts, as the sample material was too much for one combustion. Both parts of the worms were transferred directly into combustion boats and were combusted for four minutes using an oxidizer (Zinsser OX 500). Worms were not dried prior the analysis. The water content was determined from ten worms and these values were applied for the calculation of the dry weight values For substrate and tissue samples analysis, the combustion efficiency was determined at the start of each run of 30 samples and was in a range of 90 -110 %. Combustion efficiency was determined by combusting known amounts of standard solution in duplicates and determination of the radioactivity by LSC analysis. LSC analysis of the obtained solutions were carried out with a Packard Tri Carb 2910 TR.

II. RESULTS AND DISCUSSION

Analytical results

The regulatory Chemistry specialist advised that the appropriate unit of measure to be used for radioactivity in this study is **mg eq/kg** representing the amount of radioactivity measured that is expressed as the parent cinmethylin. The Applicant has not used this unit and therefore this has been amended.

Residues in soil

Residues of 0.0001 and 0.0008 mg a.s./kg were detected on days 0 and 21 respectively. The homogenous distribution of the blend on the test medium was determined by analysing five biological replicates of freshly spiked substrate (day -4). An initial average content of 2.80 ±0.087 mg a.s./kg (corresponding to a RSD of 3.10 %). During the exposure (day 0-21) a mean concentration of 2.66 mg a.s./kg with an overall SD of ±0.09 mg/kg (corresponding to RSD = 3.39 %) was determined in substrate, which corresponds to 76.0 % of the nominal target concentration of 3.5 mg a.s./kg. Measured concentrations varied from 2.55 - 2.77 mg a.s./kg dry substrate, indicating constant exposure conditions for the whole exposure period (see Table B.9.1.3-1 for details). The units of measured used are mg eq/kg representing the amount of radioactivity measured that is expressed as the parent cinmethylin.

Table B.9.1.3-1: Content of test item in spiked substrate over time. Biological replicates of the treatments were analysed with three technical replicates each (residue data for single days from Table 13 of BASF DocID 2019/1059201

Day (d)	Total mean a.s. (mg eq/kg)	SD mean (mg eq/kg)	RSD (%)
0	2.7704	0.0925	3.34
1	2.6481	0.1114	4.21
4	2.5502	0.0350	1.37
7	2.7538	0.0847	3.08
10	2.7575	0.0296	1.07
14	2.5694	0.0907	3.53
17	2.6448	0.0893	3.38
21	2.5835	0.0878	3.40
Mean (d0-d21)	**2.66**	**0.09**	**3.39**

SD : Standard deviation ; RSD : Relative standard deviation

Residues in earthworms

Residues of 0.0003 and 0.0141 mg eq/kg were found in earthworms on day 0 and 21 respectively. Worms displayed normal burrowing behaviour and showed no indication of substrate avoidance at test initiation or during the exposition. Control animals, as well as those in exposure vessels gained weight during the test, aside from the animals exposed for one day which displayed a weight variance of 93.5 - 122.9 % from the initial starting weight. No mortalities occurred during testing.

The dry mass content of worms was determined at the start of the test by analysing 10 reference worms. A mean dry mass content of 13.2 ±1.10 % was determined, which was further used as a reference for the calculation of test item concentrations. This additional analysis was necessary as the worms had to be analysed for test item without drying, as BAS 684 H was suspected to be volatile and sensitive to heat.

The mean lipid content of worms was initially determined from 10 representative specimen for normalization purposes of the BAF and the subsequent calculation of the BSAF. A mean lipid content of 1.89 ±0.215 % was determined for the used test population.

In earthworms, the measured concentrations varied between 0.0006 and 4.11 mg eq/kg biomass during the uptake phase, reaching a plateau after 10 days (Table B.9.1.3-2). The concentrations in worm tissue during day 10 to 21 were determined to be within a range of ± 20 % of the mean from this period, indicating a steady state had been reached. The mean steady state concentration in worms was 2.97 ±0.37 mg eq/kg (Table B.9.1.3-2).

Table B.9.1.3-2: Content of test item in exposed worms of the test group over time. Sampling events were monitored by means of five biological replicates (residue data for single days from Table 15 of BASF DocID 2019/1059201)

Day (d)	Total mean a.s. (mg/kg)	SD mean (mg/kg)	RSD (%)
0	0.0006	0.0008	143
1	1.4712	0.1984	13.5
4	2.8140	0.4498	16.0
7	4.1054	0.6038	14.7
10	2.5401	0.5463	21.5
14	3.2660	0.6025	18.4
17	2.8009	0.8840	31.6
21	3.2909	0.7348	22.3
Mean (d0-d21)	**2.97**	**0.37**	**--**

[1)] The concentrations in worm tissue during day 10 to 21 were determined to be within a range of ±20 % of the mean from this period, indicating the reach of a steady state. For details on determination of steady state please refer to the table and explanation below.

Table B.9.1.3-3: Determination of the steady state concentration in worms

Day (d)	Mean* a.s. (mg eq/kg)	Mean# of 3 consecutive days (mg eq/kg)	Relative standard deviation of day-specific means (*) from 3-day specific mean (#) over last consecutive samplings (%)				
			d1-4	d4-10	d7-14	d10-17	d14-21
0	0.0006						
1	1.4712		52.6				
4	2.8140		101	89.2			
7	4.1054	2.797	147	130	124		
10	2.5401	3.153		80.6	76.9	88.5	
14	3.2660	3.304			98.9	114	105
17	2.8009	2.869				97.6	89.8
21	3.2909	3.119					106
		Min (%)	**52.6**	**80.6**	**76.9**	**88.5**	**89.8**
		Max (%)	**147**	**130**	**124**	**114**	**106**

The mean concentration of worms from the last 3 sampling events (labeled with "#") were compared via the relative standard deviation of single means (labeled with "*") to the overall mean (light grey fields constitute an example). A steady state is reached by a variation less ±} 20%. The Min-Max analysis of the RSDs was used to determine when deviations from means# were in a range of 80-120 %, indicating the reach of the steady state (dark grey shading).

BAF and BSAF calculation

The mean concentration of BAS 684 H in substrate (2.66 ±0.09 mg a.s./kg) and the mean concentration of test item in worms in steady state (2.97 ±0.37 mg a.s./kg) were used to calculate the bioaccumulation factor (BAF). For the test item BAS 684 H the BAFsteady state in earthworms of the species *Eisenia fetida andrei* was calculated to be 1.12 (Table B.9.1.3-3:).

Table B.9.1.3-4: Steady state bioaccumulation factor for BAS 684 H in *Eisenia fetida Andrei*

	Steady state level DAT 10 – DAT 21)	
	$C_{soil\ (steady\ state)}$	$C_{worm\ (steady\ state)}$
Average (mg a.s./kg)	2.66 ±0.09	2.97 ±0.09
SD (mg a.s./kg)	±0.09	±0.09
$BAF_{steady\ state}$ [1)]	**1.12**	

1) Calculated as $BAF_{steady\ state} = C_{worm} / C_{soil}$

The normalization of the BAF based on an organic carbon (Corg) content of 4.08 % in substrate (without addition of food) and a lipid content (Clip) of 1.89 % in worms resulted in the respective biota-soil accumulation

factor (BSAF). For the test item BAS 684 H the BSAFsubstrate in earthworms of the species *Eisenia fetida andrei* was calculated to be 2.41 (Table B.9.1.3-4:).

Table B.9.1.3-5: Biota-soil accumulation factor for BAS 684 H in *Eisenia fetida Andrei*

	C_{org}	C_{lip}
Content (%)	4.08	1.89
$BSAF_{substrate}$ 1)	**2.42**	

1) Calculated as $BSAF_{substrate} = BAF_{steady\ state} * C_{org} / C_{lip}$

III. CONCLUSION

Following exposure of *Eisenia fetida andrei* to BAS 684 H at an application rate of 3.5 mg a.s/kg dry soil in an artificial soil substrate for 21 days, mean residues of BAS 684 H in soil were found to be 2.66 mg a.s./kg dry substrate (range of 2.55 – 2.77 mg a.s./kg dry substrate). The mean residues in earthworms after 21 days were found to be 2.97 mg a.s./kg (range of 0.0006 to 4.11 mg a.s./kg biomass). The relevant BAF was calculated as $BAF_{steady\ state} = 1.12$.

Evaluator comments

The study was well reported and conducted mostly in line with guideline OECD 317 (2010). Validity criteria that should be fulfilled for both controls and treatments that is set out in the guideline have been considered below:

Validity criteria for OECD 317 (2010)

1. Adult mortality at the end of the study (% value of total number of introduced worms) was ≤ 10% (actual was zero mortalities).

2. Mean mass loss as measured at the end of the uptake and at the end of the elimination phase did not exceed 20% compared to the initial fresh weight (f.w.) at start of each phase.

It was noted that there was a deviation to the original study plan. Only 4 instead of 5 replicates were available for the test item analysis in the treatment group at day 7, as one worm escaped during the defecation procedure overnight. The corresponding substrate sample was excluded from the analyses and hence is not considered detrimental to the study.

It was noted that an elimination phase was not included in the study which is a deviation from the guideline which states '*an elimination phase is always required unless uptake of the test substance during the exposure phase has been insignificant'*. Since the bioaccumulation factor (BAF) is the endpoint required from the study for use in the risk assessment and this can be calculated without an elimination period (at steady state), the Evaluator considers this to be an acceptable deviation from the study guideline.

Chemistry input regarding analysis of radioactivity :

The detection technique is not specific to parent cinmethylin, it detects all radioactivity, therefore it is inappropriate to say the results are specific to the active substance mg/kg. The radioactive technique used tells you the total radioactive residues in the sample *expressed as parent*. (i.e. MBq/mg – MBq of radioactivity per mg of parent cinmethylin). Therefore the appropriate units for the results are mg eq/kg (i.e. mg parent equivalents/kg).

A sentence to this effect has been added to the study summary under the *Analytical results* heading and all units have been changed accordingly.

The endpoint resulting from this study is a bioaccumulation factor (BAF) of 1.12.

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

No active substance data was submitted for this data point.

B.9.1.5. Potential for endocrine disruption

The scientific criteria for determining endocrine disrupting properties in the context of pesticide regulation[1] have been finalized and published. Under this amendment to the EU regulation for pesticides a substance shall be considered as having endocrine disrupting properties that might cause adverse effects on non-target organisms if it meets the following criteria, unless there is evidence that the adverse effects observed are not relevant at the (sub) population level:

(1) it shows an adverse effect in non-target organisms, which is a change in the morphology, physiology, growth, development, reproduction or life span of an organism, system or (sub)population that results in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress or an increase in susceptibility to other influences;
(2) it has an endocrine mode of action, i.e. it alters the function(s) of the endocrine system;
(3) the adverse effect is a consequence of the endocrine mode of action.

On the basis of these criteria there is a need to further consider the potential for the active substance cinmethylin to have endocrine disrupting properties in relation to non-target organisms according to such criteria, which are supported by a modern guidance document: Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009[2].

The assessment for cinmethylin is detailed below. Firstly all available data has been considered (literature review and submitted studies) followed by an ecotoxicology assessment for each of the non-target organism groups in relevant s (B.9.1.5 and B.9.2.3).

Literature review

The applicant provided the following summary of the literature review in relation to the endocrine disruption assessment, shown in italics.

A professional search for Scientific Peer-Reviewed Open Literature was conducted by BASF SE Bioscience Information (G-FLP/OIB, Germany). The employed search profile included specific search terms for endocrine mediated effects regarding ecotoxicology. Two searches were performed, a main search approximately one year ahead of submission (July 2017) and an update search approximately three months ahead of submission (February 2018). The Literature Search Report as prepared by BASF SE Bioscience Information (2018/1099008) documents the data bases and search terms applied, and the dates of search. This document was submitted with the EU dossier.

The search result provided by BASF SE Bioscience Information (G-FLP/OIB, Germany) was exported to Excel and evaluated regarding Relevance and Reliability (BAS_684_H_PeerRevLit_Ecotox_Final_DOSSIER.xls).

The evaluation of Peer Reviewed Scientific Literature satisfies the following requirements and recommendations:

- *Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009. EFSA Journal 2011; 9 (2): 2092.*
- *Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009.*
- *Scientific Peer-Reviewed Open Literature providing specific guidance on the evaluation of ecotoxicology studies, thus ranging beyond the EFSA documents. The details on evaluation are summarized in a separate Word document (2016/1139952) that was submitted with the EU dossier.*

HSE ecotoxicology comments: The literature review is described in detail in section B.9.10 of CA dossier, volume 3. HSE considers the literature review acceptable for the endocrine disruption ecotoxicology assessment.

[1] COMMISSION REGULATION (EU) 2018/605 of 19 April 2018 amending Annex II to Regulation (EC) No 1107/2009 by setting out scientific criteria for the determination of endocrine disrupting properties
[2] EFSA Journal 2018;16(6):5311

Only a single publication was identified as reliable that was based on two study reports; [redacted], 1983b; along with supporting study Lee, 1984a. These studies have been evaluated in section B.9.2.8 of CA dossier, volume 3. These studies investigated bioaccumulation effects in fish under a flow through experimental design. Both these studies were considered unreliable by HSE. In addition, only analytical samples were reported i.e. there were no other results reported e.g. body weight or length. Therefore this publication is not relevant for the endocrine disruption assessment and further consideration is not required.

Summary of studies submitted relevant to endocrine disruption

Beyond the literature review several studies were submitted that can be considered in the endocrine disruption assessment.

In accordance with EFSA/ECHA guidance only reliable studies have been considered further by HSE for the endocrine disruption ecotoxicology assessment. The two studies not considered reliable have been detailed below, including one study that was terminated early and not fully reported.

Fish early life stage study ([redacted], 1990):

The fish early life stage study ([redacted], 1990) was not considered suitable for use in the standard risk assessment for cinmethylin. The full evaluation is provided in CA dossier, volume 3, section 9. Briefly, the analytical method was not sufficiently validated and the validity criteria of OECD 210 were not met. In addition, another modern early life stage study ([redacted] 2017a) testing effects of cinmethylin on the same species following the identical guideline was considered reliable for use in the risk assessment. Therefore, for the endocrine disruption assessment of aquatic organisms the invalid ELS study ([redacted], 1990) has not been considered further.

Additional Xenopus Eleutheroembryonic Thyroid screening Assay (XETA) assay:

An additional assay (XETA) was initiated but terminated early. This was due to issues with the analytical test item verification. Following discussions at EU level, instead of repeating the screening test it was agreed to conduct an AMA study for cinmethylin. A summary report of the terminated XETA was requested by HSE (BASF DocID 2020/2031984).

The analytical recoveries in the first two runs ranged between 102 and 146 % of nominals in fresh solutions and below Limit of Detection (LoD) in the aged solutions (24 hours). Subsequently the recoveries of fresh solution on the third run were variable (between 33 and 126 %) and again below LoD after 24 hours. Since exposure could not be adequately demonstrated and due to variable analytical data the study was terminated. In accordance with OECD 248 (2019) a substance is considered to indicate activity on the thyroid modality in the XETA if:

> • *'In unspiked mode an active concentration is defined as a concentration giving a statistically significant fluorescence increase of 12% or greater compared to the test medium control.*
> • *In T3-spiked mode an active concentration is defined as a concentration giving a statistically significant fluorescence increase or decrease of 12% or greater compared to the T3 control.'*

Whilst the study was not completed or considered reliable based on the available study results there was no indication of effects on thyroid i.e. < 12 % compared to relevant controls. In addition, no statistically significant differences compared to controls were observed.

A summary of all submitted ecotoxicology studies suitable for consideration of endocrine disruption are shown in the table below, with the exception of mammalian toxicology data.

Table B.9.1.5-1: Studies for ED assessment of cinmethylin in non-target organisms other than mammals. The potential for endocrine disruption has been considered in section B.9.1.5 for birds/mammals and section B.9.2.3 for aquatic organisms.

Study ID*	Study type	Species	Guideline	Reference**
196	Avian reproduction test	Northern bobwhite quail (*Colinus virginianus*)	OECD 206 U.S. EPA OCSPP 850.2300	[redacted], 2016a
197	Avian reproduction test	Mallard duck (*Anas platyrhynchos*)	OECD 206 U.S. EPA OCSPP 850.2300	[redacted] 2018c
198	Early life stage test (35 d)	Fathead minnow (*Pimephales promelas*)	OECD 210 U.S. EPA OCSPP 850.1400	[redacted] 1990
199	Early life stage test (35 d)	Fathead minnow (*Pimephales promelas*)	OECD 210 U.S. EPA OCSPP 850.1400	[redacted] 2017a
202	Fish Short Term Reproduction Assay (FSTRA)	Zebrafish (*Danio rerio*)	OECD 229	[redacted] 2020
203	Amphibian Metamorphosis Assay (AMA)	African clawed Frog (*Xenopus laevis*)	OECD 231	[redacted], 2020
204	*Xenopus* Eleutheroembryonic Thyroid screening Assay (XETA)	African clawed Frog (*Xenopus laevis*)	OECD 248	BASF report 2020/2031984

*Study ID, ** Study evaluations provided in relevant sections of volume 3, CA cinmethylin dossier with exception of XETA.
Shading indicates study not reliable or terminated early (see discussion in previous section).

Where appropriate the reliable studies have been considered in the relevant sections for the different non-target organism groups (section B.9.1.5 for birds/mammals/reptiles and section B.9.2.3 for aquatic organisms).

Birds:

A summary of the results has been provided below. The format is in accordance with EFSA/ECHA guidance i.e. appendix E.

Table B.9.1.5-2: Reporting the lines of evidence for adverse effects from avian reproduction studies

Study ID Matrix	Effect classification	Effect target	Species	Exposure	Route	Lowest Effect dose	Doses	Dose unit	Assessment of each line of evidence
196	Sensitive to, but not diagnostic of, EATS	Cracked eggs	Northern bobwhite quail	21 Wks	Oral	n.a.	300, 600, 1200	mg a.s./ kg diet	No indication
197	Sensitive to, but not diagnostic of, EATS	Cracked eggs	Mallard duck	21 Wks	Oral	n.a.			
196	Sensitive to, but not diagnostic of, EATS	Egg fertility (embryonic day 8)	Northern bobwhite quail	21 Wks	Oral	n.a.	300, 600, 1200	mg a.s./ kg diet	No indication
197	Sensitive to, but not diagnostic of, EATS	Egg fertility (embryonic day 8)	Mallard duck	21 Wks	Oral	n.a.			
196 **	Sensitive to, but not diagnostic of, EATS	Egg production	Northern bobwhite quail	21 Wks	Oral	n.a.			
197	Sensitive to, but not diagnostic of, EATS	Egg production	Mallard duck	21 Wks	Oral	n.a.			
196 **	Sensitive to, but not diagnostic of, EATS	Egg viability (% viable embryo of egg set)	Northern bobwhite quail	21 wks	Oral	n.a.			No indication
197	Sensitive to, but not diagnostic of, EATS	Egg viability (% viable embryo of egg set)	Mallard duck	21 Wks	Oral	n.a.			
196	Sensitive to, but not	Eggshell thickness	Northern	21	Oral	n.a.			

Study ID Matrix	Effect classification	Effect target	Species	Exposure	Route	Lowest Effect dose	Doses	Dose unit	Assessment of each line of evidence
	diagnostic of, EATS		bobwhite quail	Wks					
197	Sensitive to, but not diagnostic of, EATS	Eggshell thickness	Mallard duck	21 Wks	Oral	n.a.			
196**	Sensitive to, but not diagnostic of, EATS	Embryo viability (embryonic day 15) *	Northern bobwhite quail	21 Wks	Oral	n.a.			
197	Sensitive to, but not diagnostic of, EATS	Embryo viability (embryonic day 15) *	Mallard duck	21 Wks	Oral	n.a.	300, 600, 1200	mg a.s./ kg diet	No indication
196	Sensitive to, but not diagnostic of, EATS	Gross pathology (bird)	Northern bobwhite quail	21 Wks	Oral	n.a.			
197	Sensitive to, but not diagnostic of, EATS	Gross pathology (bird)	Mallard duck	21 Wks	Oral	n.a.			
196**	Sensitive to, but not diagnostic of, EATS	Hatchability	Northern bobwhite quail	21 Wks	Oral	n.a.	300, 600, 1200	mg a.s./ kg diet	No indication
197	Sensitive to, but not diagnostic of, EATS	Hatchability	Mallard duck	21 Wks	Oral	n.a.			
196	Systemic toxicity	Body weight	Northern bobwhite quail	21 Wks	Oral	n.a.			
197	Systemic toxicity	Body weight	Mallard duck	21 Wks	Oral	n.a.			
196**	Systemic toxicity	Mortality	Northern bobwhite quail	21 Wks	Oral	n.a.	300, 600, 1200	mg a.s./ kg diet	No indication
197***	Systemic toxicity	Mortality	Mallard duck	21 Wks	Oral	n.a.			

* covers fertile eggs and viable embryos at day 11 and 18, n.a. = not applicable.
** Slight reduction compared to control for number of viable embryos, live 3-week embryos and 14-day old survivors as percentage of number of eggs set (maximum of 12 %). However, these changes were not statistically significant or part of a dose response- not observed at two higher concentrations hence not considered treatment related by HSE (see dossier section B.9.1.1. for full details).
*** Statistical difference between the percentage of 14-day survivors of hatchlings at concentrations of 300 and 600 mg a.s./kg diet and the control group. However, HSE does not consider this treatment related due to difference being a maximum of 1 % between treatment and control.

The applicant consideration for birds is provided in italics below:

'*In these two avian reproduction regulatory studies with cinmethylin, conducted according to standard guidelines, all parameters listed in section A of Table 17 of the ECHA/EFSA ED GD were investigated. Gross pathology examinations of the parent birds were included in both studies. Measurements of biochemical parameters like hormone levels and histopathological examinations of endocrine organs are not part of the guidelines for standard avian reproduction studies and thus, were not carried out.*
No adverse effects were reported for any of the measured endpoints in both reproduction studies, and the NOAEL was confirmed at 1200 mg a.s./kg bw, the highest dose tested. The avian reproduction test is classified as a CF (Conceptual Framework) level 4 study whose endpoints are sensitive to, but not diagnostic of EATS (estrogen/ androgen/ thyroid/ steroidogenic) modalities. Therefore, it is not possible to draw a final conclusion on EATS-mediated adverse effects for birds as no single parameter is considered diagnostic for a specific endocrine mediated activity (Table 17, EFSA 2018). However, based on the lack of adverse effects on the investigated parameter, it is considered highly unlikely that cinmethylin fulfils ED criteria in birds as the 'adversity' part of the definition is not fulfilled.

In line with the technical recommendations in the ECHA/EFSA ED GD (2018) no further avian experimental data should be requested and therefore no further studies with birds are proposed.'

'In the available avian reproduction studies with cinmethylin no adverse effects were reported for any of the investigated parameters. Gross-pathological investigations in the avian studies with cinmethylin did not provide evidence of changes in endocrine organs.
In the light of the content of the ED guidance document on birds, no further avian experimental data should currently be requested to address the ED issue for cinmethylin. Therefore, no additional studies are proposed by the applicant.
According to Commission Regulation (EU) 2018/605 and the technical recommendations in the ECHA/EFSA GD (2018) for birds, the studies required to address ED-relevant issues for birds are fulfilled for BAS 684 H. The information available shows that cinmethylin does not fulfill the ED criteria in birds and no additional studies should be required.'

HSE ecotoxicology conclusion for birds

When considering reproductive toxicity the NOAEL values were the highest test concentration of 1200 mg a.s./kg diet for both avian studies, equivalent to 99.1 mg a.s./kg b.w./day ([redacted] 2016a and [redacted] 2018c). No treatment related effects were observed based on the parameters measured that are considered sensitive but not diagnostic of EATS (shown in table B.9.1.5-2).

In accordance with EFSA/ECHA guidance the gross pathology findings should be reported. This was the case for both avian studies and no treatment related effects were observed.

Currently there are no further tests available for assessing endocrine activity in birds hence HSE agrees with the applicant that further testing is not required at this stage. Furthermore, as detailed in the mammal's (see section below) population level effects are not deemed likely providing supporting read-across information for the bird assessment.

Overall, HSE concludes that disruption in birds from cinmethylin resulting in population level effects is unlikely on the basis of the current dataset and guidance document scope.

The wild mammal endocrine disruption assessment is shown below.

Ecotoxicology consideration of wild mammals:

As an initial step the toxicology conclusions have been considered to inform the assessment of ED for mammals (as non-target organisms). Current indications are that if a substance is considered to meet the criteria for human health then they will also be met for mammals as non-target organisms so long as:

'The adverse effects on reproduction, growth/development, and other relevant adverse effects are likely to impact on (sub) populations' – as detailed in the implementing regulation (EU) 2018/605.

An exhaustive and detailed discussion of endocrine disruption for cinmethylin has been provided in the volume 3, CA section 6 dossier part II (B.6.8.3).

Overall conclusion for EAS modalities (toxicology):

The following conclusion was reached: *'Based on scenario 1a of the ECHA/EFSA guidance for the identification of endocrine disruptors in the context of Regulations (EU) 528/2012 and (EC) No 1107/2009, there is no indication of adversity for the EAS modalities. In addition, EAS adversity has been sufficiently investigated. There is also robust evidence from the ToxCast ER bioactivity model of a lack of endocrine activity for the E modality. The first condition of the ED criteria is not met; therefore, it is possible to conclude that cinmethylin does not meet the ED criteria for the EAS modalities and that these modalities have been sufficiently investigated.*'

'*HSE (toxicology) concludes that for the EAS modalities cinmethylin is not an ED and its ED potential has been sufficiently investigated.*'

HSE (ecotoxicology) considers that based on the toxicology conclusion the ED criteria are not met for mammals as non-target organisms when considering EAS modalities and that these modalities have been sufficiently investigated.

Overall HSE conclusion for T modality (toxicology):

The following conclusion was reached: '*Based on scenario 1b of the ECHA/EFSA guidance for the identification of endocrine disruptors in the context of Regulations (EU) 528/2012 and (EC) No 1107/2009, there is an overall indication of adversity. Some information from ToxCast on thyroid activity (direct thyroid MoAs) has been presented and direct thyroid MoAs have been excluded; however, more detailed information is required to substantiate the postulated indirect MoA.*'

'*HSE (toxicology) concludes in relation to the T modality a conclusion cannot be reached as further information is required.*'

HSE (ecotoxicology) has considered the available thyroid data, which is summarised in volume 3, CA section 6 dossier part II, B.6.8.3 table 6.8-25 (adversity). The adversity review of the T parameters determined in the existing toxicological database indicated that the thyroid is a target organ in the rat, but not in mice and dogs. The effects on the thyroid consisted of increased thyroid weights, increased incidences of follicular cell hypertrophy/hyperplasia and/or altered colloid (flaky appearance). A summary of the results has been provided in the table below.

Table B.9.1.5-3: Mammalian data investigating thyroid, summary of data where effects were observed, for full table see volume 3, section 6 dossier part II, B.6.8.3 and table 6.8-25.

ID	Effect	Effect target	Species	Duration of exposure	Duration unit	Route	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
30	EATS-mediated	Thyroid weight	Rat	13	Weeks	Oral	814	mg/kg bw/day	Increase	♀ high dose: abs. +8%, rel. +25%*. both outside HCD ==> partly sec. to lower body weight, corresponding liver changes at same or lower doses	Increased thyroid weight in rats.	Overall positive evidence for Thyroid effects in rats.	T T
90a	EATS-mediated	Thyroid weight	Rat	10 (♂ and ♀ premating) +3 (♀ gestation) +3 (♀ lactation)	weeks	Oral	394 to 481	mg/kg bw/day	Increase	High dose F0 & F1: ♂(w.HCD)/♀(slightly outside HCD) abs. (15%-22%) and rel. (+17%-24%), dose-dependently increased, correlating histopathological finding,			T
10	EATS-mediated	Thyroid histopathology	Rat	4	Weeks	Oral	477	mg/kg bw/day	Increase	Follicular hypertrophy/hyperplasia Hypertropy mean grading: ♂ : 0-0-1.3-1.6; ♀ : 0-0-1.0-1.8 ==>likely a consequence of liver enzyme induction	Increased follicular hypertrophy/hyperplasia in rat, accompanied by liver effects.		T
30	EATS-mediated	Thyroid histopathology	Rat	13	Weeks	Oral	211/ 240	mg/kg bw/day	Increase	Hypertrophy/Hyperplasia: Incidence in ♂: 0, 0, 4, 9; ♀: 0, 0, 1, 1 Mean severity grading in ♂: 0, 0, 1.5, 2; ♀: 0, 0, 1, 2; ==>likely a consequence of liver enzyme induction			T T
70b	EATS-mediated	Thyroid histopathology	Rat	104	Weeks	Oral	242	mg/kg bw/day	Increase	Hyperplasia, follicular cells: Incidence in ♂: 2, 2, 4, 10 no increase in tumors			T

90a	EATS-mediated	Thyroid histopathology	Rat	10 (♂ and ♀ premating) +3 (♀ gestation) +3 (♀ lactation)	weeks	Oral	394 - 481	mg/kg bw/day	Change	Follicular hypertrophy/hyperplasia Incidences: F0: ♂ :0, 2, 3, 10; ♀ : 0, 0, 0, 16; F1: ♂ : 0, 1, 1, 15; ♀ : 0, 0, 0, 8; ==> treatment-related in high dose.			T
10	EATS-mediated	Colloid area (thyroid histopathology)	Rat	4	Weeks	Oral	1522/ 1331	mg/kg bw/day	Increase	Altered colloid: ↑minimal to slight flaky appearance of colloid at top dose Incidence in ♂: 1,2,2,3 ♀:0,0,0,2; Mean severity grading ♂ : 1-1-1-1.3; ♀ :0-0-0-1.0	Increased incidence of altered colloid (flaky appearance) in rats.		T
30	EATS-mediated	Colloid area (thyroid histopathology)	Rat	13	Weeks	Oral	792/ 814	mg/kg bw/day	Increase	Altered colloid: Incidence ♂: 1, 1, 0, 8; ♀: 0, 1, 0, 2; Mean severity grading ♂: 1, 1, 0, 1.4; ♀: 0, 1, 0, 1;			T
70a	EATS-mediated	Colloid area (thyroid histopathology)	Rat	52	Weeks	Oral	51	mg/kg bw/day	Change	Altered colloid in mid and high dose ♂ : 1,2,4,8;			T
70a	EATS-mediated	Colloid area (thyroid histopathology)	Rat	52	Weeks	Oral	351	mg/kg bw/day	Change	Altered colloid in high dose ♀:0,1,1,6;			T
70b	EATS-mediated	Colloid area (thyroid histopathology)	Rat	104	Weeks	Oral	242	mg/kg bw/day	Increase	Altered colloid in high dose: Incidence in ♂: 7, 4, 12, 24** Mean severity grading in ♂: 1.6, 1.8, 1.3, 1.8;			T
70b	EATS-mediated	Colloid area (thyroid histopathology)	Rat	104	Weeks	Oral	317	mg/kg bw/day	Increase	Altered colloid: Incidence in ♀: 5, 6, 7, 33* Mean severity grading in ♀: 1.0, 1.3, 1.3, 1.3;			T

10	Target organ toxicity	Liver weight	Rat	4	Weeks	Oral	477	mg/kg bw/day	Increase	abs. & rel. (% change to Ctrl) in ♂ and ♀: >10%, dose dep., with hypertrophy at higher dose	Liver weight increased in rat		T
30	Target organ toxicity	Liver weight	Rat	13	Weeks	Oral	211/ 240	mg/kg bw/day	Increase	abs. & rel. (% change to Ctrl) in ♂ and ♀: ≥12% / ≥11% , with hypertrophy at higher dose			T
70a	Target organ toxicity	Liver weight	Rat	52	Weeks	Oral	265	mg/kg bw/day	Increase	abs. & rel. (% change to Ctrl) in ♂ 16% (w. HCD)/ 14%, with histopath correlate, treatment-related;			T
70a	Target organ toxicity	Liver weight	Rat	52	Weeks	Oral	351	mg/kg bw/day	Increase	rel. (% change to Ctrl) in ♀: +17% considered secondary to body weight reduction (-13%). However, hypertrophy indicates liver as being induced			T
70b	Target organ toxicity	Liver weight	Rat	104	Weeks	Oral	45	mg/kg bw/day	Increase	rel. (% change to Ctrl) in ♂ at mid dose +5% secondary to body weight reduction, at high dose +13%, treatment related, at high dose with histopath Correlate, no neoplastic findings			T
70b	Target organ toxicity	Liver weight	Rat	104	Weeks	Oral	59	mg/kg bw/day	Increase	rel. In mid dose (+11%), and abs. & rel. (% change to Ctrl) in high dose ♂ 6%/ 20%, with histopath correlate, treatment-related;			T
90a	Target organ toxicity	Liver weight	Rat	10 (♂ and ♀ premating) +3 (♀ gestation) +3 (♀ lactation)	weeks	Oral	81 to 97	mg/kg bw/day	Increase	abs. &rel. (% change to Ctrl) in Adult F0 ♂ and ♀ from mid dose onwards:≥ 8 %/≥ 4%), being adaptive first, adverse at higher dose levels			T

90a	Target organ toxicity	Liver weight	Rat	10 (♂ and ♀ premating) +3 (♀ gestation) +3 (♀ lactation)	weeks	Oral	384 to 481	mg/kg bw/day	Increase	abs. &rel. (% change to Ctrl) in Adult F1 ♂ and ♀ at high dose:≥ 19 %/≥ 19%), treatment related			T
100a	Target organ toxicity	Liver weight	Rat	1.5 (GD6-15)	weeks	Oral	1000	mg/kg bw/day	Increase	abs. &rel. (% change to Ctrl) in ♀ from 1000 mg/kg bw onwards:≥ 12 %/≥ 16%), treatment related			T
120	Target organ toxicity	Liver weight	Rat	7	weeks	Oral	87/ 99	mg/kg bw/day	Increase	abs. &rel. (% change to Ctrl) in ♂ & ♀ at top dose:≥ 9 %/≥ 8.5%), treatment related,			T
10	Target organ toxicity	Liver histopathology	Rat	4	Weeks	Oral	1522/ 1331	mg/kg bw/day	Increase		Increased hepatocellular hypertrophy, cytoplasmatic alterations, fatty change and pigment storage.		T
30	Target organ toxicity	Liver histopathology	Rat	13	Weeks	Oral	211/ 240	mg/kg bw/day	Increase				T
70a	Target organ toxicity	Liver histopathology	Rat	52	Weeks	Oral	265/ 351	mg/kg bw/day	Change				T
70b	Target organ toxicity	Liver histopathology	Rat	104	Weeks	Oral	242	mg/kg bw/day	Change	Cytoplasmatic alterations, periportal pigment storage in ♂			T
70b	Target organ toxicity	Liver histopathology	Rat	104	Weeks	Oral	59	mg/kg bw/day	Change	Hypertrophy in all high dose ♂ & ♀.			T
													T
120	*In vivo* mechanistic	Phase I enzyme induction (*in vivo*)	Rat	7	weeks	Oral	26.4 / 29.2	mg/kg bw/day	Induction	slight CYP induction	The ability for cinmethylin to induce hepatic CYP activities in rats cannot be excluded.	Evidence for liver enzyme induction.	T

STBNDO*: Sensitive to, but not diagnostic of, EATS; *: p≤0.05; **: p≤0.01; T = Thyroid, N = Not assignable to a specific modality, HCD = Historical Control Data

HSE ecotoxicology conclusion for wild mammals:

It is noted that from a toxicological perspective, that the potential effects of cinmethylin on thyroid activity is unresolved and further clarification has been requested. HSE considers that until this issue is fully addressed, no conclusion can be drawn on the ecotoxicological element of this assessment. Applicant has conducted further testing, mode of action analysis for toxicology that will be submitted and evaluated by HSE. Hence the conclusion is pending.

Reptiles:

No publications or studies assessing effects on reptiles were submitted for cinmethylin. Currently investigation of ED properties in these taxa is hampered by a lack of test methods investigating endocrine specific endpoints. Indeed, the joint EFSA/ECHA guidance document sets as a recommendation for future research work to gain a better understanding of the endocrinology of reptiles and whether extrapolations from other vertebrate groups can be scientifically justified. As such HSE judges that no conclusion can be drawn with regards to the ED properties of cinmethylin in relation to reptiles.

B.9.2. EFFECT ON AQUATIC ORGANISMS

B.9.2.1. Acute toxicity to fish

B.9.2.1.1 Active substance: Cinmethylin:

Report:	CA 8.2.1/1 [redacted], 1983 a Acute toxicity of technical sd95481 to rainbow trout *Salmo gairdneri* CI-511-003
Guidelines:	None quoted in study report.
GLP:	No

I. MATERIAL AND METHODS

A. MATERIALS

Test item:	Cinmethylin BAS 684 H (SD 95481, Reg. no.: 900 202), batch no.: 5-4-0-0; purity: 92 %.

B. STUDY DESIGN

Test species:	Rainbow trout (*Salmo gairdneri, syn. Oncorhynchus mykiss*); Lot #4882; mean body length: 3.9 cm; mean body weight: 0.85 g; supplied by [redacted].
Test design:	Static system (96 h); 5 test item concentrations, a dilution water control and a solvent control (acetone), 10 fish per aquarium; assessment of mortality and adverse effects after 24, 48, 72 and 96 hours after start of exposure.
Endpoints:	LC_{50} and NOEC based on mortality and adverse effects.
Test concentrations:	Control (dilution water), solvent control (acetone) 1.0, 1.8, 3.2, 5.6 and 10 mg a.s./L corresponding to mean measured concentrations of 0 (control), 0 (solvent control), 0.43, 0.89, 2.7, 0.90, 4.8 and 9.2 mg a.s./L.
Test conditions:	5 gallon glass aquaria, test volume: 15 L; test water: soft reconstituted well water; hardness: 40 - 45 mg $CaCO_3$/L (test water); total alkalinity: 30 – 35 mg/L; temperature: 12 °C; pH 7.1 – 7.6; oxygen content: 8.5 – 9.2 mg/L; photoperiod 16 h light: 8 h dark; no feeding.

Analytics: Analytical verification of test item concentrations was conducted using a HPLC-method with UV detection.

Statistics: Descriptive statistics; Probit, binomial and moving average analysis for calculation of LC_{50}.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of cinmethylin concentrations was conducted in each test item concentration at the beginning of the test and at the end of the test. The mean measured concentrations in the test item treatments were 0.43, 0.89, 2.7, 4.8 and 9.2 mg a.s./L. The analysed contents of cinmethylin ranged from 52 to 98 % of nominal concentrations at test initiation, from 41 to 87 % of nominal concentrations at test termination. Noting the report did not state whether precipitation of the test item was observed. Whilst the study author based the concentrations on mean measured concentrations, the HSE evaluator has calculated the geometric mean measured concentrations given the rate of decline in the majority of test concentrations (noting the highest test concentration was maintained within ± 20 % of nominal). When comparing the geometric mean measured values to those used by the study author they are identical. Therefore, the HSE evaluator agrees with the values used to derived LC_{50} values. It was not stated in the study report whether precipitation of the test item was assessed.

The results are shown in the table below:

Table B.9.2.1.1-1: Measured concentrations during study

Nominal Concentration (mg a.s./L)	Mean measured concentration (mg a.s./L)				Geometric mean measured concentration (mg a.s./L)
	0 hour	% of nominal	96 hours	% of nominal	
Control	< 0.02	na	< 0.08	na	na
1.0	0.519	52	0.344	34	0.4
1.8	1.03	57	0.744	41	0.9
3.2	3.11	97	2.29	72	2.7
5.6	5.46	98	4.15	74	4.8
10	9.72	97	8.71	87	9.2

na = not applicable.

Validity criteria:

This study was conducted before the most recent OECD guideline. Nonetheless in order to determine whether the study is valid the criteria in OECD 203 (1992) have been considered below:

- The mortality in the control(s) should not exceed 10 per cent (or one fish if less than ten are used) at the end of the test. Obtained: 0 %.
- The dissolved oxygen (DO) concentration must have been at least 60 per cent of the air saturation value throughout the test. Obtained: Whilst the DO was reported based on mg/L when considering the test conditions (fresh water at 12 ℃) the values generated (minimum 8.5 mg/L) are equivalent to > 60 % air saturation.
- There must be evidence that the concentration of the substance being tested has been satisfactorily maintained, and preferably it should be at least 80 per cent of the nominal concentration throughout the test. During study: Analytical data was reported and test concentrations were based on geometric mean measured concentrations.

During the study the above criteria were met.

Biological results: After 96 hours of exposure, no mortality was observed in the controls and at test item concentrations of up to and including 4.8 mg a.s./L, whereas 100 % mortality was observed at 9.2 mg a.s./L. The results are summarised in Table B.9.2.1.1-2.

Table B.9.2.1.1-2: Acute toxicity (96 h) of BAS 684 H to rainbow trout (*S. gairdneri*)

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**1.0**	**1.8**	**3.2**	**5.6**	**10**
Concentration [mg a.s./L] (geometric mean measured)	**--**	**--**	**0.4**	**0.89**	**2.7**	**4.8**	**9.2**
Mortality [%] (96 h)	0	0	0	0	0	0	100
Behavioural observations	N	N	N	N	N	N	LOE
	Endpoints [mg a.s./L] (geometric mean measured*)						
LC_{50} (96 h)	6.60 (95 % confidence limits: 4.8 – 9.2)						
NOEC (96 h)	4.8						

N = described as 'Normal' by study author, LOE = Loss of equilibrium applies to all fish in the highest test concentration (10 mg a.s./L nominal).
* Study author reported mean measured which is equivalent to geometric mean measured concentrations calculated by HSE evaluator, see table B.9.2.1-1.

III. CONCLUSION

In a 96-hour static acute toxicity study with rainbow trout the LC_{50} of cinmethylin was 6.60 mg a.s./L based on geometric mean measured concentrations. The NOEC was determined to be 4.8 mg a.s./L.

HSE evaluator comments:

It was noted that the above study was not conducted to GLP. Furthermore, it was not possible to validate the analytical method in accordance with SANCO/3029/99 (see volume 3, CA, section B5 for full details). The following deficiencies were noted:

i) It is not possible to accept the linearity of the method as the example chromatograms for the highest concentration of the linear range show overloading of the sample, with the detector being saturated giving a broad flat top peak.
ii) To assess accuracy and precision, at least 5 recoveries should be provided for at least 2 fortification levels.
iii) The LOQ is not supported by 5 recovery determinations.
iv) Procedural recoveries have not been completed.

Therefore, this study has not been considered further in the risk assessment section.

Report: CA 8.2.1/2
[redacted], 2017 a
BAS 684 H (Cinmethylin) - Acute toxicity study in rainbow trout (*Oncorhynchus mykiss*)
2017/1134335
Guidelines: EC 440/2008 C.1 Acute Toxicity for Fish, OECD 203, EPA 72-1, EPA 850.1075
GLP: Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Cinmethylin (Reg. no.: 900 202), batch no. COD-002038, purity: 93 ± 1.0 %.

B. STUDY DESIGN

Test species: Rainbow trout (*Oncorhynchus mykiss*), age: approx. 3 months; mean body length of fish: 4.6 cm; mean wet weight of fish: 0.73 g; originally obtained from [redacted].

Test design:	Static system (96 h); 5 test item concentrations plus a control, 2 replicates per treatment; 10 fish per aquarium (loading 0.365 g fish/L); assessment of mortality and sub-lethal effects 1, 6, 24, 48, 72 and 96 hours after start of exposure.
Endpoints:	LC_{50}, NOEC, mortality and sub-lethal effects.
Test concentrations:	Control, 1.25, 2.5, 5, 10 and 20 mg a.s./L corresponding to mean measured concentrations of 0 (control), 0.601, 1.28, 2.65, 5.65 and 12.85 mg a.s./L, respectively.
Test conditions:	24 L stainless steel aquaria; test volume 20 L; dilution water: non-chlorinated charcoal filtered drinking water mixed with deionized water; hardness: 1.2 mmol/L; temperature: 11.7 – 11.9 °C; pH 7.9 – 8.4; oxygen content: 7.0 – 10.5 mg/L; conductivity: 221 µS/cm; photoperiod 16 h light: 8 h dark; no aeration; no feeding.
Analytics:	Analytical verification of test item concentrations was conducted using a HPLC-method with MS detection.
Statistics:	Descriptive statistics; Probit analysis for calculation of LC_{50} and Fishers Exact Binomial Test with Bonferroni correction for determination of NOEC values (α = 0.05, one-sided greater).

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of cinmethylin concentrations was conducted in each test item concentration and replicate at the beginning of the test, after 48 h and at the end of the test. The concentrations were not maintained within ± 20 % of nominals. No undissolved test substance was observed in the stock solution or test concentrations during the study.

The results are shown in the table below:

Table B.9.2.1.1-3: Measured concentrations during study

Nominal Concentration (mg a.s./L)	**Mean measured concentration (mg a.s./L)**						**GM (mg a.s./L)***	**MM (mg a.s./L)****
	0 hour	**% of nominal**	**48 hours**	**% of nominal**	**96 hours**	**% of nominal**		
Control	--	na	--	na	--	na	na	na
1.16	0.82	70.7	0.52	44.8	0.46	39.7	**0.58**	**0.601**
2.33	1.57	67.4	1.19	51.1	1.09	46.8	**1.27**	**1.28**
4.65	2.95	63.4	2.62	56.3	2.37	51.0	**2.64**	**2.65**
9.3	6.03	64.8	5.70	61.3	5.21	56.0	**5.64**	**5.65**
18.6	13.63	73.3	12.07	64.9	--	--	**12.8** (up to day 2[#])	**12.85**

na = not applicable. – not reported. Measured values were corrected by study author based on actual content i.e. 93 % purity, * Geometric mean measured concentration calculated by HSE evaluator, ** mean measured concentration during study calculated by study author, [#] = only monitored until day 2 as mortality reached 100 % after 24 hours for this test concentration.

The HSE evaluator has calculated the geometric mean test concentrations and notes they are comparable to the mean measured concentrations calculated by the study author. Therefore, the HSE evaluator considers the endpoints reported (based on mean measured concentration) acceptable.

Validity criteria:

The criteria in OECD 203 (1992) have been considered below:

- The mortality in the control(s) should not exceed 10 per cent (or one fish if less than ten are used) at the end of the test. Obtained: 0 %

- The dissolved oxygen (DO) concentration must have been at least 60 per cent of the air saturation value throughout the test. Obtained: Whilst the DO was reported based on mg/L when considering the test conditions (fresh water at approximately 12 ℃) the values generated (minimum 7.9 mg/L) are equivalent to > 60 % air saturation.
- There must be evidence that the concentration of the substance being tested has been satisfactorily maintained, and preferably it should be at least 80 per cent of the nominal concentration throughout the test. During study: Analytical data was reported and test concentrations were based on geometric mean measured concentrations.

During the study the above criteria were met.

Biological results: After 96 hours of exposure, no mortality was observed in the control and at test item concentrations of up to and including 5.65 mg a.s./L, whereas, at the highest tested concentration, all fish were dead after 96 hours of exposure. Sub-lethal effects (i.e. swimming at the bottom) were found at 5.65 mg a.s./L after 96 hours. The results are summarised in Table B.9.2.1.1-4.

Table B.9.2.1.1-4: Acute toxicity (96 h) of BAS 684 H to rainbow trout (*Oncorhynchus mykiss*)

Concentration [mg a.s./L] (nominal)	**Control**	**1.25**	**2.5**	**5**	**10**	**20**
Concentration [mg a.s./L] (mean measured)	**--**	**0.601**	**1.28**	**2.65**	**5.65**	**12.85**
Mortality [%] (96 h)	0	0	0	0	0	100 [1)]
Symptoms (after 96 h) *	none	none	None	none	D(8)	n.d.
Endpoints [mg a.s./L] (mean measured)						
LC_{50} (96 h)	8.49 (95 % confidence limits: n.c.)					
NOEC (96 h)	5.65					

n.d. = not determined due to 100 % mortality; n.c. = not calculated due to mathematical reasons
* Symptoms after 96 h: D = swimming at the bottom
[1)] Statistical significant difference compared to control (Fishers Exact Binomial Test with Bonferroni correction, $\alpha = 0.05$, one-sided greater)

III. CONCLUSION

In a 96-hour static acute toxicity study with rainbow trout the LC_{50} of cinmethylin was 8.49 mg a.s./L based on mean measured concentrations. The NOEC (96 h) was determined to be 5.65 mg a.s./L.

HSE evaluator comments:

As this was an additional vertebrate study the following justification was provided by the applicant (required under Regulation 1107): '*The acute fish study on rainbow trout was conducted to meet data requirements outside the European Union. On request of the RMS CTGB, the study is provided for the sake of completeness.'* The HSE evaluator considers the justification provided appropriate.

It was noted the temperature was below guideline recommendations (13 – 17 °C) at 11.7 – 11.9 °C. However, this is considered a minor deviation by the HSE evaluator particularly given the validity criteria were met and not sufficient reason to invalidate the study.

The above study was conducted to GLP and considered valid. Furthermore, the analytical method was validated in accordance with SANCO/3029/99 with an LOQ of 2.5 ng/mL (see volume 3, CA, section B5 for full details). As stated above the HSE evaluator considers the endpoint should have been expressed as geometric mean measured however these values are comparable as shown in table B.9.2.1.1-3 with mean measured concentrations. Therefore the following endpoint will be considered in the risk assessment:

- Technical cinmethylin 96-hour LC_{50} = **8.49 mg a.s./L** (based on mean measured concentration)

Report: CA 8.2.1/3
[redacted], 2017 a
BAS 684 H - Carp, acute toxicity test
2016/1063240
Guidelines: OECD 203 (1992)
GLP: Yes

Report: CA 8.2.1/4
[redacted], 2018 b
Amendment No. 1 to the final report - BAS 684 H - Carp, acute toxicity test
2018/1068368
Guidelines: OECD 203 (1992)
GLP: Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Cinmethylin (Reg. no.: 900 202), batch no. COD-002038, purity: 93 ± 1.0 %.

B. STUDY DESIGN

Test species: Common carp (*Cyprinus carpio*), age: approx. 3 months; body length of control fish 4.74 cm (4.5 – 5.0 cm); body weight of control fish: 2.79 g (2.11 g – 3.12 g); supplied by [redacted].

Test design: Static system (96 h); 5 test item concentrations plus a dilution water control, 1 replicate per treatment; 10 fish per aquarium (loading 0.80 g fish/L/day); assessment of mortality and sub-lethal effects within 3 hours after start of exposure and 6, 24, 48, 72 and 96 hours after start of exposure.

Endpoints: LC_{50}, NOEC, mortality and sub-lethal effects.

Test concentrations: Control (dilution water), 2.0, 3.0, 4.4, 5.6 and 10 mg a.s./L corresponding to geometric mean measured concentrations of 1.51, 2.22, 3.74, 4.81 and 8.64 mg a.s./L.

Test conditions: 35 L glass aquaria; test water: conditioned tap water; hardness: 63.6 mg $CaCO_3/dm^3$ (dilution water); temperature: 20.5 – 23.3 °C (control); pH 6.62 – 7.34; oxygen content: 81 – 100 % of air saturation value (control); conductivity: 229 µS/cm (dilution water); photoperiod 16 h light: 8 h dark; gentle aeration; no feeding.

Analytics: Analytical verification of test item concentrations was conducted using a LC-method with DAD detection.

Statistics: Descriptive statistics; Spearman-Karber method for determination of LC value, Step-down Cochran-Armitage Test for LOEC and NOEC determination ($\alpha = 0.05$, one-sided greater).

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of cinmethylin concentrations was conducted in each test item concentration at the beginning of the test and at the end of the test. The geometric mean measured concentrations in the test item treatments were 1.51, 2.22, 3.74, 4.81 and 8.64 mg a.s./L. In samples collected at exposure initiation the determined concentration of the test item was in the range of 93.2 – 98.9 % of nominal concentration. In samples collected at exposure termination the determined concentration of the test item was in the range of 57.8 – 78.2 % of nominal concentration. Therefore, the following biological results are based on geometric mean measured concentrations. Test solutions were visibly clear during the study.

The results are shown in the table below:

Table B.9.2.1.1-5: Measured concentrations during study

Nominal (mg a.s./L)	0 hours		96 hours		Geometric mean (mg a.s./L)*
	Mean measured (mg a.s./L)	% of nominal*	Mean measured (mg a.s./L)	% of nominal*	
0.0	< LoD	--	< LoD	--	0.0
2.0	1.977	98.9	1.156	57.8	1.51
3.0	2.795	93.2	1.759	58.6	2.22
4.4	4.324	98.3	3.240	73.6	3.74
5.6	5.479	97.8	4.219	75.3	4.81
10	9.539	95.4	7.824	78.2	8.64

-- = not applicable LoQ = 0.002 mg a.s./L, LoD = 0.001 mg a.s./L
* Calculated by study author following OECD 23, annex II equation.

Validity criteria:

The criteria in OECD 203 (1992) have been considered below:

- The mortality in the control(s) should not exceed 10 per cent (or one fish if less than ten are used) at the end of the test. Obtained: 0 %
- The dissolved oxygen (DO) concentration must have been at least 60 per cent of the air saturation value throughout the test. Obtained: 81 – 100 % air saturation value
- There must be evidence that the concentration of the substance being tested has been satisfactorily maintained, and preferably it should be at least 80 per cent of the nominal concentration throughout the test. During study: Analytical data was reported and test concentrations were based on geometric mean measured concentrations

During the study the above criteria were met.

Biological results: After 96 hours of exposure, no mortality was observed in the control and at test item concentrations of up to and including 3.74 mg a.s./L, whereas 10 and 100 % mortality was observed at 4.81 and 8.64 mg a.s./L, respectively. Sub-lethal effects (i.e. unbalanced swimming) was found in the test item concentrations of 4.81 mg a.s./L. A statistically significant difference compared to the control was observed at the highest concentration of 8.64 mg a.s./L (Step-down Cochran-Armitage Test, $\alpha = 0.05$, one-sided greater). The results are summarised in Table B.9.2.1.1-6.

Table B.9.2.1.1-6: Acute toxicity (96 h) of BAS 684 H to common carp (*Cyprinus carpio*)

Concentration [mg/L] (nominal)	**Control**	2.0	3.0	4.4	5.6	10
Concentration [mg a.s./L] (geometric mean)	**--**	1.51	2.22	3.74	4.81	8.64
Mortality [%] (96 h)	0	0	0	0	10	100 [1)]
Symptoms (after 96 h) *	none	none	none	none	3 U	n.d.
	Endpoints [mg a.s./L] (geometric mean)					
LC_{50} (96 h)	5.75 (95 % confidence limits: 5.31 – 6.22)					
NOEC (96 h)	4.4					

n.d. = not determined due to 100% mortality; n.c. = not calculated due to mathematical reasons
* Symptoms after 96 h: U = unbalanced swimming,
[1)] Statistically significant difference compared to control (Step-down Cochran-Armitage Test, $\alpha = 0.05$, one-sided greater)

III. CONCLUSION

In a static acute toxicity study with common carp the LC_{50} (96 h) of cinmethylin was 5.75 mg a.s./L based on geometric mean measured concentrations. The NOEC (96 h) was determined to be 4.4 mg a.s./L.

HSE evaluator comments:

As this was an additional vertebrate study the following justification was provided by the applicant (required under Regulation 1107): '*The acute fish study on carp was conducted to meet data requirements outside the European Union. On request of the RMS CTGB, the study is provided for the sake of completeness.'* The HSE evaluator considers the justification provided appropriate.

It was noted the length of fish was above the recommendation in OECD 203 (2 – 4 cm) with values ranging between 4.5 and 5 cm. However, the difference is relatively low and as the validity criteria were met the HSE evaluator considers this deviation acceptable.

The above study was conducted to GLP and considered valid. Furthermore, the analytical method was validated in accordance with SANCO/3029/99 with an LOQ of 0.002 mg/L (see volume 3, CA, section B5 for full details). The following endpoint will be considered in the risk assessment:

- 96-hour LC_{50} = **5.75 mg a.s./L** (based on geometric mean measured concentration)

Report:	CA 8.2.1/5 [redacted], 2017 b BAS 684 H (Cinmethylin) - Acute toxicity study in the fathead minnow (*Pimephales promelas*) 2017/1111618
Guidelines:	EC 440/2008 C.1 Acute Toxicity for Fish, OECD 203, EPA 72-1, EPA 850.1075
GLP:	Yes

Report:	CA 8.2.1/6 [redacted], 2018 a Amendment 1: BAS 684 H (Cinmethylin) - Acute toxicity study in the fathead minnow (*Pimephales promelas*) 2018/1044871
Guidelines:	EC 440/2008 C.1 Acute Toxicity for Fish, OECD 203, EPA 72-1, EPA 850.1075
GLP:	Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test item:	Cinmethylin (Reg. no.: 900202), batch no. COD-002038, purity: 99 ± 1.0 %.

B. STUDY DESIGN

Test species:	Fathead minnow (*Pimephales promelas*), age: approx. 3 months; mean body length of fish: 2.5 cm (2.0 – 3.0 cm); mean wet weight of fish: 0.14 g (0.09 – 0.19 g); in-house breeding.
Test design:	Static (96 h); 5 test item concentrations plus a dilution water control, 2 replicates per treatment; 10 fish per aquarium (loading 0.14 g fish/L); assessment of mortality and sub-lethal effects within 1 hour after start of exposure and 6, 24, 48, 72 and 96 hours after start of exposure.
Endpoints:	LC_{50}, NOEC, mortality and sub-lethal effects.
Test concentrations:	Control (dilution water), 1.25, 2.5, 5, 10 and 20 mg a.s./L (nominal) corresponding to 0 (control), 0.504, 1.08, 2.365, 4.884 and 11.739 mg a.s./L (mean measured concentrations corrected for purity).

Test conditions:	10 L stainless steel aquaria, test volume: 10 L; dilution water: non-chlorinated charcoal-filtered drinking water mixed with deionized water; hardness: approx. 1.13 mmol/L; temperature: 22.1 – 22.6 ℃; pH 7.7 - 8.1; oxygen content: > 60 % of air saturation value (6.7 – 8.7 mg/L); conductivity: 285 µS/cm; photoperiod 16 h light: 8 h dark; light intensity: approx. 128 – 400 lux; no aeration; no feeding.
Analytics:	Analytical verification of test item concentrations was conducted using a LC-method with MS detection.
Statistics:	Descriptive statistics; Probit analysis for calculation of LC_{50} and Fishers Exact Binomial Test with Bonferroni Correction for determination of NOEC ($\alpha = 0.05$, one-sided greater).

II. RESULTS AND DISCUSSION

Analytical measurements: It is noted an amendment ([redacted], 2018 a) was submitted that added an additional table of measured values not corrected for purity.

Analytical verification of cinmethylin concentrations was conducted in each test item concentration at the beginning of the test, after 48 h and at the end of the test. The geometric mean measured concentrations of the test item were 0 (control), 0.50, 1.08, 2.37, 4.88 and 11.74 mg a.s./L. The analysed contents of cinmethylin ranged from 51.2 to 62.1 % of nominal concentrations at test initiation, from 41.6 to 69.6 % after 48 h and from 37.5 to 57.7 % of nominal concentrations at test termination. The following biological results are based on geometric mean measured concentrations. Test solutions were visibly clear during the study.

The results are shown in the table below:

Table B.9.2.1.1-7: Measured concentrations during study

Nominal Concentration (mg a.s./L)	Mean measured concentration (mg a.s./L)						GM (mg a.s./L)*	MM (mg a.s./L)**
	0 hour	% of nominal	48 hours	% of nominal	96 hours	% of nominal		
Control	--	na	--	na	--	na	na	na
1.16	0.59	51.2	0.48	41.6	0.44	37.5	**0.50**	**0.50**
2.33	1.24	53.4	1.01	43.5	0.97	41.7	**1.07**	**1.08**
4.65	2.70	58.0	2.32	49.9	2.08	44.7	**2.35**	**2.37**
9.3	5.69	61.2	4.45	47.9	4.51	48.5	**4.85**	**4.88**
18.6	11.55	62.1	12.94	69.6	10.73	57.7	**11.70**	**11.74**

na = not applicable. – not reported. Measured values were corrected by study author based on actual content i.e. 93 % purity, * Geometric mean measured concentration calculated by HSE evaluator, ** mean measured concentration during study calculated by study author

The HSE evaluator has calculated the geometric mean test concentrations and notes they are comparable to the mean measured concentrations calculated by the study author. Therefore, the HSE evaluator considers the endpoints reported (based on mean measured concentration) acceptable.

Validity criteria:

The criteria in OECD 203 (1992) have been considered below:

- The mortality in the control(s) should not exceed 10 per cent (or one fish if less than ten are used) at the end of the test. Obtained: 0 %
- The dissolved oxygen (DO) concentration must have been at least 60 per cent of the air saturation value throughout the test. Obtained: > 60 % air saturation value
- There must be evidence that the concentration of the substance being tested has been satisfactorily maintained, and preferably it should be at least 80 per cent of the nominal concentration throughout the test. During study: Analytical data was reported and test concentrations were based on geometric mean measured concentrations

During the study the above criteria were met.

Biological results: After 96 hours of exposure, no mortality was observed in the dilution water control and at test item concentrations of up to and including 2.37 mg a.s./L (geometric mean measured concentration), whereas 10 and 100 % mortality was observed at 4.88 and 11.74 mg a.s./L respectively (geometric mean measured). Statistically significant effects were determined at the highest test item concentration of 11.74 mg a.s./L (Fishers Exact Binomial Test with Bonferroni Correction, $\alpha = 0.05$, one-sided greater). At the highest tested concentrations, all fish were dead after 24 hours of exposure. Sub-lethal effects (i.e. swimming at the bottom and tottering) were found at 4.88 mg a.s./L (geometric mean measured concentration) after 24 hours. The results are summarized in Table B.9.2.1.1-8.

Table B.9.2.1.1-8: Acute toxicity (96 h) of BAS 684 H to fathead minnow (*Pimephales promelas*)

Concentration [mg a.s./L] (nominal)	**Control**	**1.25**	**2.5**	**5**	**10**	**20**
Concentration [mg a.s./L] (geometric mean measured)	**--**	**0.50**	**1.08**	**2.37**	**4.88**	**11.74**
Mortality [%] (96 h)	0	0	0	0	10	100 #
Symptoms (individuals after 96 h) *	none	none	none	none	T(3), D(4)	n.d.
Endpoints [mg a.s./L] (geometric mean measured)						
LC_{50} (96 h)	5.84 (95 % confidence limits: n.c.)					
NOEC (96 h)	4.88					

n.d. = not determined due to 100 % mortality; n.c. = not calculated due to mathematical reasons
* Symptoms after 96 h: D = swimming at the bottom; T = tottering
#' Significantly different compared to control after Fishers Exact Binomial Test with Bonferroni Correction ($\alpha = 0.05$, one-sided greater).

III. CONCLUSION

In a static acute toxicity study with fathead minnow the LC_{50} (96 h) of cinmethylin was 5.84 mg a.s./L based on mean measured concentrations. The NOEC (96 h) was determined to be 4.884 mg a.s./L.

HSE evaluator comments:

As this was an additional vertebrate study the following justification was provided by the applicant (required under Regulation 1107): '*The acute fish study on fathead minnow was conducted to meet data requirements outside the European Union. On request of the RMS CTGB, the study is provided for the sake of completeness.'* The HSE evaluator considers the justification provided appropriate.

The above study was conducted to GLP and considered valid. Furthermore, the analytical method was validated in accordance with SANCO/3029/99 with an LOQ of 2.5 ng/mL (see volume 3, CA, section B5 for full details). As stated above the HSE evaluator considers the endpoint should have been expressed as geometric mean measured however these values are comparable as shown in table B.9.2.1.1-7 with mean measured concentrations. The following endpoint will be considered in the risk assessment:

- 96-hour LC_{50} = **5.84 mg a.s./L** (based on mean measured concentration)

Report: CA 8.2.1/7
[redacted], 1983 a
Acute toxicity of technical SD 95481 to sheepshead minnows (*Cyprinodon variegatus*)
CI-511-001
Guidelines: none
GLP: no

I. MATERIAL AND METHODS

A. MATERIALS

Test item:	Technical SD 95481 (Cinmethylin, Reg. no. 900 202), Code 5-4-0-0, purity: 92 %.

B. STUDY DESIGN

Test species:	Sheepshead minnows (*Cyprinodon variegatus*), age: 8-11 days; BMRL culture.
Test design:	Static system (96 h); 5 test item concentrations plus an untreated control and a solvent control, 1 replicate per treatment; 10 fish per aquarium (loading 0.009 g fish/L); assessment of mortality and sub-lethal effects 24, 48, 72 and 96 hours after start of exposure.
Endpoints:	LC_{50}, mortality and sub-lethal effects.
Test concentrations:	Control, solvent control (0.25 % acetone), 0.62, 1.25, 2.5, 5.0, and 10 mg a.s./L.
Test conditions:	3.8 L glass jars; test volume 3 L; dilution water: filtered seawater with adjustments of salinity; Salinity: 24 ‰; temperature: 22 – 23 °C; pH at test start 7.9, pH at test termination: 7.8; dissolved oxygen content ≥ 58 – 100 %; no aeration; no feeding.
Analytics:	Analytical verification of test item concentrations was performed at the highest tested concentration and the concentration where 100 % mortality occurs (not reported).
Statistics:	Descriptive statistics; moving average angle analysis, Probit analysis and binomial probability. The 96-h LC_{50} value were determined via the moving average angle method.

II. RESULTS AND DISCUSSION

Analytical measurements: not reported and not stated whether precipitation of the test item was assessed.

Validity criteria:

This study was conducted before the most recent OECD guideline. Nonetheless in order to determine whether the study is valid the criteria in OECD 203 (1992) have been considered below:

- The mortality in the control(s) should not exceed 10 per cent (or one fish if less than ten are used) at the end of the test. Obtained: 0 %
- The dissolved oxygen (DO) concentration must have been at least 60 per cent of the air saturation value throughout the test. Obtained: ≥ 58 – 100 % air saturation. Whilst this is below the requirement the difference is negligible and unlikely to invalidate the study.
- There must be evidence that the concentration of the substance being tested has been satisfactorily maintained, and preferably it should be at least 80 per cent of the nominal concentration throughout the test. Obtained: Analytical data were not reported.

During the study the above criteria were <u>not</u> met.

Biological results: After 96 hours of exposure, mortality ranged from 10 % in the 0.62 mg a.s./L (nominal) test concentration to 100 % in test concentrations ≥ 5.0 mg a.s./L (nominal). There was no mortality in either control. No sub-lethal effects were observed in living fish after 96 hours. The results are summarised in Table B.9.2.1.1-9.

Table B.9.2.1.1-9: Acute toxicity (96 h) of technical SD 95481 (BAS 684 H) to sheepshead minnow (*Cyprinodon variegatus*)

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent Control**	**0.62**	**1.25**	**2.5**	**5**	**10**
Mortality [%] (96 h)	0	0	10	40	60	100	100
Symptoms (after 96 h)	none	none	none	none	none	n.d.	n.d.
	Endpoints [mg a.s./L] (nominal)						
LC_{50} (96 h)	1.6 (95 % confidence limits: 1.1 – 2.3)						

n.d. = not determined due to 100 % mortality

III. CONCLUSION

In a 96-hour static acute toxicity study with sheepshead minnow (*Cyprinodon variegatus*) the LC_{50} of technical cinmethylin was 1.6 mg a.s./L based on nominal concentrations.

HSE evaluator comments:

The above study was not conducted to GLP. In addition, the study report was brief (length/weight of fish and other details were missing), and it was not possible to determine whether test concentrations were adequately maintained as analytical data were not reported, hence the relevant validity criteria were not met. Furthermore, the analytical method was not sufficiently validated in accordance with SANCO/3029/99 (see volume 3, CA, section B5 for full details). The following deficiencies were noted:

i) It is not possible to accept the linearity of the method as the example chromatograms for the highest concentration of the linear range show overloading of the sample, with the detector being saturated giving a broad flat top peak.
ii) To assess accuracy and precision, at least 5 recoveries should be provided for at least 2 fortification levels.
iii) The LOQ is not supported by 5 recovery determinations.
iv) Procedural recoveries have not been completed.

Based on the above deficiencies this study is not considered suitable for use in the risk assessment. Therefore, further consideration is not required.

Report: CA 8.2.1/8
[redacted], 1983 b
Acute toxicity of technical SD95481 to bluegill sunfish *Lepomis macrochirus*
CI-511-002
Guidelines: none
GLP: no

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Technical Cinmethylin (SD 95481, Reg. no.: 900 202), purity: 92 %

B. STUDY DESIGN

Test species: Bluegill sunfish (*Lepomis macrochirus*); Lot #3882; mean body length: 2.5 cm; mean body weight: 0.4 g; supplied by [redacted].

Test design: Static system (96 h); 5 test item concentrations, a dilution water control and a solvent control (acetone), 10 fish per aquarium; assessment of mortality and adverse effects after 24, 48, and 96 hours after start of exposure.

Endpoints:	LC_{50} and NOEC based on mortality.
Test concentrations:	Control (dilution water), solvent control (acetone) 1.0, 1.8, 3.2, 5.6 and 10 mg a.s./L, corresponding to mean measured concentrations of 0 (control), 0 (solvent control), 0.9, 2.0, 3.0, 4.7 and 8.8 mg a.s./L.
Test conditions:	5 gallon glass aquaria, test volume: 15 L; test water: soft reconstituted well water; hardness: 40 - 45 mg $CaCO_3$/L (test water); temperature: 23 °C; pH 6.9 – 7.7; oxygen content: 5.8 – 9.4 mg/L; photoperiod 16 h light: 8 h dark; no feeding.
Analytics:	Analytical verification of test item concentrations was conducted using a HPLC-method with UV detection.
Statistics:	Descriptive statistics; Probit, binomial and moving average analysis for calculation of LC_{50}.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of technical cinmethylin was conducted in each test item concentration at the beginning of the test and at the end of the test. The mean measured concentrations in the test item treatments were 0.9, 2.0, 3.0, 4.7 and 8.8 mg a.s./L. The analysed contents of cinmethylin (ranged from 87.3 to 121.7 % of nominal concentrations at test initiation, from 70.6 to 99.4 % of nominal concentrations at test termination. Thus, the following biological results are based on mean measured concentrations. It was not stated in the study report whether precipitation of the test item was assessed.

The results are shown in the table below. It should be noted that behavioural observations were not recorded during the study.

Table B.9.2.1.1-10: Measured concentrations during study

Nominal (mg a.s./L)	0 hours		96 hours		Geometric mean (mg a.s./L)*	Mean measured during study (mg a.s./L)**
	Mean measured (mg a.s./L)	% of nominal*	Mean measured (mg a.s./L)	% of nominal*		
0.0	< 0.05	--	< 0.11	--	0.0	0.0
1.0	1.01	101.0	0.794	79.4	0.90	0.90
1.8	2.19	121.7	1.79	99.4	1.98	2.0
3.2	3.38	105.6	2.26	70.6	2.76	3.0
5.6	4.89	87.3	4.56	81.4	4.72	4.7
10	9.04	90.4	8.56	85.6	8.80	8.8

* Geometric mean measured concentrations calculated by HSE evaluator.
** Mean measured concentrations reported by study author. It is noted these are comparable to the geometric mean concentrations calculated by HSE evaluator. Therefore, the use of the mean measured values calculated by study author to derive endpoints is considered acceptable.

Validity criteria:

This study was conducted before the most recent OECD guideline. Nonetheless in order to determine whether the study is valid the criteria in OECD 203 (1992) have been considered below:

- The mortality in the control(s) should not exceed 10 per cent (or one fish if less than ten are used) at the end of the test. Obtained: 0 %
- The dissolved oxygen (DO) concentration must have been at least 60 per cent of the air saturation value throughout the test. Obtained: DO was reported in terms of mg/L. It was stated in the study report that under test conditions 100 % was equivalent to 8.8 mg/L. The minimum value reported during the study was 5.8 mg/L equivalent to approximately 66 %.
- There must be evidence that the concentration of the substance being tested has been satisfactorily maintained, and preferably it should be at least 80 per cent of the nominal concentration throughout the

test. During study: Analytical data was reported, and test concentrations were based on geometric mean measured concentrations

During the study the above criteria were met.

Biological results: After 96 hours of exposure, no mortality was observed in the controls and at test item concentrations of up to and including 4.7 mg a.s./L, whereas 100 % mortality was observed at 8.8 mg a.s./L. Adverse effects (i.e. surfacing, loss of equilibrium and dark discoloration) were found in the at test item concentrations of 4.7 mg a.s./L. The results are summarized in Table B.9.2.1.1-11. It should be noted that behavioural observations were not made during the study.

Table B.9.2.1.1-11: Acute toxicity (96 h) of cinmethylin to bluegill sunfish (*L. macrochirus*)

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**1.0**	**1.8**	**3.2**	**5.6**	**10**
Concentration [mg a.s./L] (mean measured)	**--**	**--**	**0.9**	**2.0**	**3.0**	**4.7**	**8.8**
Mortality [%] (96 h)	0	0	0	0	0	0	100
	Endpoints [mg a.s./L] (geometric mean measured)						
LC_{50} (96 h)	6.40 (95 % confidence limits: 4.7 – 8.8)						
NOEC (96 h)	3.0						

III. CONCLUSION

In a 96-hour static acute toxicity study with bluegill sunfish the LC_{50} of cinmethylin was 6.40 mg a.s./L based on geometric mean measured concentrations. The NOEC was 3.0 mg a.s./L (geometric mean measured concentration).

HSE evaluator comments:

The following justification was provided by the applicant for conducting this study: '*The acute fish study on bluegill sunfish was conducted to meet data requirements outside the European Union. On request of the RMS CTGB, the study is provided for the sake of completeness.'* The HSE evaluator considers the justification provided appropriate, noting the study was completed before the implementation of regulation 1107/2009.

It was noted that the above study was not conducted in accordance with GLP. Furthermore, the analytical method was not sufficiently validated in accordance with SANCO/3029/99 (see volume 3, CA, section B5 for full details). The following deficiencies were noted:

i) It is not possible to accept the linearity of the method as the example chromatograms for the highest concentration of the linear range show overloading of the sample, with the detector being saturated giving a broad flat top peak.
ii) To assess accuracy and precision, at least 5 recoveries should be provided for at least 2 fortification levels.
iii) The LOQ is not supported by 5 recovery determinations.
iv) Procedural recoveries have not been completed.

Therefore, this study has not been considered further in the risk assessment section.

Report: CA 8.2.1/9
[redacted], 1983 a
Dynamic acute toxicity of sd95481 to bluegill sunfish *Lepomis macrochirus*
CI-512-001
Guidelines: none
GLP: No

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 684 H (SD 95481, Reg. no.: 900 202), purity: 92 %

B. STUDY DESIGN

Test species: Bluegill sunfish (*Lepomis macrochirus*); Lot #183; mean body length: 2.0 cm; mean body weight: 0.19 g; supplied by [redacted].

Test design: Flow through system (7 d); 5 test item concentrations and a dilution water control and a solvent control, 20 fish per aquarium; assessment of mortality and adverse effects after 0 and every 24 hours after start of exposure.

Endpoints: LC_{50} and NOEC based on mortality and adverse effects.

Test concentrations: Control (dilution water), 0.96, 1.8, 2.2, 4.6 and 10 mg a.s./L corresponding to mean measured concentrations of 0 (control), 0.63, 1.8, 2.1, 4.3 and 13 mg a.s./L.

Test conditions: 40 L glass aquaria, test volume: 30 L; test water: soft reconstituted well water; flowrate: 210 ml/minute; hardness: 255 ppm (test water); temperature: 21°C; pH 7.9 – 8.1; oxygen content: 7.0 – 9.6 mg/L; photoperiod 16 h light: 8 h dark; aerated well water.

Analytics: Analytical verification of test item concentrations was conducted using a HPLC-method with UV detection.

Statistics: Descriptive statistics; Probit, binomial and moving average analysis for calculation of LC_{50}.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of cinmethylin concentrations was conducted in each test item concentration at the beginning of the test and at the end of the test. The mean measured concentrations in the test item treatments were 0.63, 1.8, 2.1, 4.3 and 13 mg a.s./L. The analysed contents of cinmethylin ranged from 62 to 118 % of nominal concentrations at test initiation and from 68.8 to 135 % of nominal concentrations at test termination. Thus, the following biological results are based on mean measured concentrations. The HSE evaluator considers the use of mean measured concentrations acceptable as this was a flow through study and in accordance with OECD 23 (2000). It was not stated in the study report whether precipitation of the test item was assessed.

The results are shown in the table below:

Table B.9.2.1.1-12: Measured concentrations during study

Nominal (mg a.s./L)	0 hours		Day 7		Mean measured during study (mg a.s./L)
	Mean measured (mg a.s./L)	% of nominal*	Mean measured (mg a.s./L)	% of nominal*	
0.0	< 0.01	--	< 0.01	--	0.0
0.96	0.595	62.0	0.660	68.8	0.63
1.8	1.67	92.8	1.90	105.6	1.8
2.2	2.06	93.6	2.14	97.3	2.1
4.6	4.32	93.9	4.36	94.8	4.3
10	11.8	118.0	13.5	135.0	13

Validity criteria:

This study was conducted before the most recent OECD guideline. Nonetheless in order to determine whether the study is valid the criteria in OECD 203 (1992) have been considered below:

- The mortality in the control(s) should not exceed 10 per cent (or one fish if less than ten are used) at the end of the test. Obtained: 0 %
- The dissolved oxygen (DO) concentration must have been at least 60 per cent of the air saturation value throughout the test. Obtained: DO was reported in terms of mg/L. It was stated in the study report that under test conditions 100 % was equivalent to 8.8 mg/L. The minimum value reported during the study was 7.0 mg/L equivalent to approximately 80 %.
- There must be evidence that the concentration of the substance being tested has been satisfactorily maintained, and preferably it should be at least 80 per cent of the nominal concentration throughout the test. During study: Analytical data was reported, and test concentrations were based on mean measured concentrations

During the study the above criteria were met.

Biological results: The biological results are based on mean measured concentrations of the test item. After 7 days of exposure, no mortality was observed in the controls. Mortalities of 10 % and 100 % were observed at test item concentrations of 4.3 and 13 mg a.s./L (mean measured) respectively. The results are summarised in Table B.9.2.1.1-13.

Table B.9.2.1.1-13: Acute toxicity (7-day) of cinmethylin to bluegill sunfish (*L. macrochirus*)

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**0.96**	**1.8**	**2.2**	**4.6**	**10**
Concentration [mg a.s./L] (mean measured)	**--**	**--**	**0.63**	**1.8**	**2.1**	**4.3**	**13**
Mortality [%] (day 7)	0	0	0	0	0	10	100
Behavioural observations	N	N	N	N	10Q	9Q	N/A
Endpoints [mg a.s./L] (mean measured)							
LC_{50} (7 day)	5.8 (95 % confidence limits: 4.3 – 13)						
NOEC (7 d)	0.1*						

* NOEC value based on expert judgement- details were not reported but it was concluded by the study author that 0.1 mg a.s./L was a safe concentration based on mortality, weight and length data, N = Normal, D = dormancy/inactivity (quiescent), N/A = not applicable as 100 % mortality had occurred after 48 hours. Note the number before behavioural observation indicates how many fish were affected.

III. CONCLUSION

In a 7 day-flow through acute toxicity study with bluegill sunfish the LC_{50} of cinmethylin was 5.8 mg a.s./L based on mean measured concentrations. The NOEC was determined by the study author to be 0.1 mg a.s./L (mean measured concentration) when considering weight, length and mortality data. It should be noted based on the study report it was unclear how this value was derived and what length/weight data was used to reach this conclusion.

HSE evaluator comments:

The following justification was provided by the applicant for conducting this study: '*The acute fish study on blue gill sunfish was conducted to meet data requirements outside the European Union. On request of the RMS CTGB, the study is provided for the sake of completeness.'* The HSE evaluator considers the justification provided appropriate, noting the study was completed before the implementation of regulation 1107/2009.

It was noted that the above study was not conducted to GLP and the duration of the study was beyond the standard of four days (seven days in total). Furthermore, the analytical method was not sufficiently validated in accordance with SANCO/3029/99 (see volume 3, CA, section B5 for full details). The following deficiencies were noted:

i) It is not possible to accept the linearity of the method as the example chromatograms for the highest concentration of the linear range show overloading of the sample, with the detector being saturated giving a broad flat top peak.
ii) To assess accuracy and precision, at least 5 recoveries should be provided for at least 2 fortification levels.
iii) The LOQ is not supported by 5 recovery determinations.
iv) Procedural recoveries have not been completed.

Therefore, this study has not been considered further in the risk assessment section.

B.9.2.1.2 Metabolite Cineole alcohol (M684H003):

Report:	CA 8.2.1/10 [redacted], 1988 a Cineole alcohol: Acute toxicity to rainbow trout *Salmo gairdneri* and *Daphnia magna* CI-570-001
Guidelines:	EEC 79/831 A V C, EEC 79/831 A V C 2
GLP:	Yes

It should be noted this study reports data for both an acute fish and invertebrate study. The following summary details the fish study. The invertebrate study has been summarised in section B.9.2.4.2, acute toxicity to aquatic invertebrates.

I. MATERIAL AND METHODS

A. MATERIALS

Test item:	Cineole alcohol (M684H003, Metabolite of cinmethylin, CAS: 87172-89-2), Code no. SD3853, purity: 98 ± 2.0 %.

B. STUDY DESIGN

Test species:	Rainbow trout (*Oncorhynchus mykiss, former Salmo gairdneri*), batch no. RT 78, finglerings; mean body length of fish: 4.2 cm (3.7 – 4.9 cm); mean weight of fish: 0.58 g (0.39 – 0.95 g); originally obtained from [redacted].
Test design:	Semi static system (96 h); daily renewal of test substance; 6 test item concentrations plus a control, 1 replicate per treatment; 10 fish per aquarium; assessment of mortality and sub-lethal effects 24, 48, 72 and 96 hours after start of exposure.
Endpoints:	LC_{50}, mortality and sub-lethal effects.
Test concentrations:	Control, 53, 95, 171, 309, 556 and 1000 mg metabolite/L.
Test conditions:	Glass aquaria; test volume 10 L; dilution water: filter and dechlorinated ground water; hardness: 236 - 248 mg/L; temperature: 14.3 – 14.7 °C; pH 7.6 – 7.9; oxygen content: 8.4 – 10.2 mg/L; photoperiod 16 h light: 8 h dark; gentle aeration; no feeding.
Analytics:	Analytical verification of test item concentrations was conducted using a GC-method with MS detection.
Statistics:	Descriptive statistics.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of cineole alcohol concentrations was conducted in each test item concentration and replicate at the beginning and end of each water renewal (every 24 h) of the test. Recoveries ranged from 56 to 131 % of nominals during study. It was not stated in the study report whether precipitation of the test item was assessed.

The results are shown in the table below:

Table B.9.2.1.2-1: Measured concentrations during study

N (mg/L)	0 hours	24 hours		48 hours		72 hours		96 hours
	'Fresh' %*	'Old' %*	'Fresh' %*	'Old' %*	'Fresh' %*	'Old' %*	'Fresh' %*	'Old' %*
0	na	na	na	na	na	na	na	na
53	104	102	91	90	104	107	92	108
95	102	99	89	100	94	92	88	99
171	99	85	85	103	88	103	88	100
309	92	98	95	56	**	**	87	131
556	93	95	85	99	94	93	91	102
1000	98	102	87	95	85	107	84	108

* Percentage of nominal concentration, N = Nominal concentration, na = not applicable, 'Fresh' = freshly prepared test solutions, 'Aged' = Aged samples at end of static period (semi-static study design)
** Concerns raised in study report about analytical results, one sample appeared to be an anomaly with particularly low recovery (58 % of nominal) which was attributed to poor mixing prior to analysis. Given the stability of other test concentrations and there was higher recovery in the aged sample (91 %) the HSE evaluator agrees with the study authors omission of these results. Similarly, the aged sample at 48 hours at 309 mg metabolite/L demonstrated low recovery which appears to be an anomaly.

Validity criteria:

This study was conducted before the most recent OECD guideline. Nonetheless in order to determine whether the study is valid the criteria in OECD 203 (1992) have been considered below:

- The mortality in the control(s) should not exceed 10 per cent (or one fish if less than ten are used) at the end of the test. Obtained: 0 %
- The dissolved oxygen (DO) concentration must have been at least 60 per cent of the air saturation value throughout the test. Obtained: DO was reported in terms of mg/L however based on the test conditions the minimum value of 8.4 mg/L is considered to be > 60 % air saturation by the HSE evaluator.
- There must be evidence that the concentration of the substance being tested has been satisfactorily maintained, and preferably it should be at least 80 per cent of the nominal concentration throughout the test. During study: Analytical data was reported, at the nominal concentration of 309 mg metabolite/L there were low recoveries at 48 and 72 hours. Based on the study report this appears to be due to poor mixing of samples and these results seem to be anomalies. Therefore, the HSE evaluator agrees with the study authors proposal of using nominal test concentrations.

During the study the above criteria were met.

Biological results: After 96 hours of exposure, no mortality was observed in the control and at test item concentrations of up to and including 556 mg metabolite/L (nominal), whereas, at the highest tested concentration (1000 mg metabolite/L (nominal)), 4 out of 10 fish were dead after 96 hours of exposure. Sub-lethal effects were found at 556 mg/L (i.e. abnormal swimming) and 1000 mg/L (i.e. immobilization) after 96 hours. The results are summarised in Table B.9.2.1.2-2.

Table B.9.2.1.2-2: Acute toxicity (96 h) of cineole alcohol (M684H003, metabolite of cinmethylin) to rainbow trout (*Salmo gairdneri*)

Concentration [mg metabolite/L] (nominal)	**Control**	**53**	**95**	**171**	**310**	**556**	**1000**
Mortality [%] (96 h)	0	0	0	0	0	0	40
Symptoms (number after 96 h) *	none	none	none	none	none	A (2)	I (6)
Endpoints [mg metabolite/L] (nominal)							
LC_{50} (96 h)	> 1000 (95 % confidence limits: n.c.)						

n.c. = not calculated due to mathematical reasons
* Symptoms after 96 h: A = abnormal swimming, I = immobilization

III. CONCLUSION

In a 96-hour static acute toxicity study with rainbow trout the LC_{50} of cineole alcohol (M684H003, metabolite of cinmethylin) was > 1000 mg metabolite/L based on nominal concentrations.

HSE evaluator comments:

The following justification was provided by the applicant for conducting this study: '*Fish is not the most sensitive species to BAS 684 H, however, the acute study with the metabolite M684H003 on rainbow trout was conducted in order to fulfil data requirements at the time of the study performance.'* The HSE evaluator considers the justification provided appropriate, noting the study was completed before the implementation of regulation 1107/2009.

It was noted that some of the analytical data had been omitted by the study author based on poor mixing prior to analysis. In addition, some results did not appear to be consistent e.g. at 309 mg metabolite/L in the 'fresh' sample at 72 hours there was 87 % of nominal but the aged solution at 96 hours had higher recovery i.e. 131 % of nominal. This generates some uncertainty regarding the analytical results particularly at the nominal concentration of 309 mg metabolite/L. Furthermore, the analytical method was not sufficiently validated in accordance with SANCO/3029/99 (see volume 3, CA, section B5 for full details). The following deficiencies were noted:

i) Missing data on the specificity (no chromatograms were provided)
ii) Linearity not fully addresses as no calibration curve, equation or standards were provided

Whilst the analytical method was not sufficiently validated given this is the only GLP fish (vertebrate) study submitted that tested the relevant metabolite M684H003 the HSE evaluator has considered in the risk assessment as supporting information.

- Cineole alcohol (M684H003) 96-hour LC_{50} = **>1000 mg metabolite/L** (based on nominal) noting some concerns raised regarding analytical data and method of analysis, hence this study should be used as **supporting information only**.

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

B.9.2.2 Active substance: Cinmethylin:

Report: CA 8.2.2.1/1
[redacted], 1990 a
WL95481 (Argold): An early life stage test with the fathead minnow (*Pimephales promelas*) RAFINESQUE
CI-512-002
Guidelines: EPA 540/9-82-024, EPA 72-4
GLP: Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Cinmethylin 'Argold' (WL 95481, Reg.no.:900 202), WRC Tox. Sample No.: 513F, purity: 91.9 - 93.1 % (analyzed).

B. STUDY DESIGN

Test species: Fathead minnow (*Pimephales promelas*), embryos, source: in-house rearing, stock (batch PP88/2) originally obtained from [redacted].

Test design: Flow through system (35 d); 6 test item concentrations plus a dilution water control, 4 replicates per treatment with thirty *P. promelas* embryos were exposed in cylindrical glass vessels (egg cups); surviving larvae were released to their respective test vessels continuously through a nylon mesh. The test solution flowed continuously from a stainless-steel reservoir into a proportional dilutor which continuously prepared the required serial dilutions. The test media was passed into 10 L glass reservoirs (one per treatment level). Test media from the glass reservoir was continuously supplied to the test chambers via peristaltic pumps. On day 35 fish were sacrificed with a solution of 3-aminobenzoate and methanesulfonic acid salt (benzocaine) and the body length and weight of surviving individuals as well as their fork length were determined. Daily assessment of mortality, numbers of normal and abnormal after hatch.

Endpoints: NOEC and EC_{10} values based on embryo survival, hatching, overall survival and fry growth.

Test concentrations: Control (dilution water), 0.1, 0.2, 0.5, 1.0, 2.0 and 5.0 mg a.s./L (nominal), corresponding to mean measured concentrations of 0.002 (control), 0.094, 0.20, 0.52, 0.97, 2.1 and 5.3 mg a.s./L.

Test conditions: Test vessels: glass aquaria (170 x 120 x 130 mm), water volume: 1 L; egg cups: 85 mm glass tube, diameter 45 cm, 1 mm nylon mesh base. Dilution water: aerated, filtered and dechlorinated main water; temperature 24 - 25°C; pH 7.1 – 7.5; oxygen content 6.4 - 8.2 mg/L; water hardness: 194 - 236 mg $CaCO_3$/L; conductivity: 410 - 530 µS/cm; acidity: 13 - 24 mg/L; Light intensity: 22 - 28 lux; photoperiod: 16 hours light : 8 hours dark; flow rates: 13.9 mL/min/test vessel (± 10 %). Feeding: unoiled Pruteen (day 2 – 8, ICI PLC, Billingham) < 24 h old *Artemia salina* (day 4 – 16) and < 48 h old *Artemia salina* (day 17 – 34), aeration via dilution water.

Analytics: Analytical verification of test item concentrations was conducted using an GC-method with MS detection.

Statistics: Descriptive statistics; Wet weight, dry weight and fork length data were analyzed using two-way analysis of variance followed by Williams' test to compare treatment means with controls ($p \geq 0.05$). Overall survival data were analyzed using two-way analysis of covariance. EC_{10} value was recalculated in addition using the analysis software ToxRat.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentration was conducted in all test item concentrations, on one of four replicate test vessels at each day, at beginning of the test and at days 3, 7, 14, 21, 26, 27, 28 and 35. The determined concentrations were in the range 88 to 132 % of the nominal values throughout the exposure period except for Day 26 where a malfunction of the diluter resulted in a range of 60 –

70 % of the nominal values. Since concentration was in the necessary range the day after, Day 26 result were excluded from the analysis by study author and endpoints were based on nominal concentrations. It was not stated in the study report whether precipitation of the test item was assessed.

The results are shown in the table below.

Table B.9.2.2-1: Measured concentrations during study

Nominal concentration (mg a.s./L)	Measured concentration mg a.s./L (% of nominal)								
	Day 0	**Day 3**	**Day 7**	**Day 14**	**Day 21**	**Day 26**	**Day 27**	**Day 28**	**Day 35**
0	0.001	0.002	0.002	0.002	0.002	0.001	0.001	0.002	0.001
0.1	0.12 (128)	0.10 (107)	0.09 (96)	0.09 (96)	0.09 (96)	0.06 (64)	0.10 (107)	0.10 (107)	0.094 (0.015)
0.2	0.24 (122)	0.19 (96)	0.18 (91)	0.19 (96)	0.20 (102)	0.13 (66)	0.21 (107)	0.21 (107)	0.22 (112)
0.5	0.61 (117)	0.50 (96)	0.51 (97)	0.55 (105)	0.47 (90)	0.32 (61)	0.57 (109)	0.56 (107)	0.60 (115)
1.0	1.2 (124)	0.94 (97)	0.90 (93)	0.94 (97)	0.88 (91)	0.62 (64)	1.1 (113)	1.1 (113)	1.2 (124)
2.0	2.4 (117)	1.9 (92)	1.9 (92)	2.0 (97)	1.8 (88)	1.4 (68)	2.4 (117)	2.5 (122)	2.6 (126)
5.0	6.2 (117)	5.2 (98)	5.6 (106)	4.9 (92)	4.4 (83)	3.1 (58)	6.1 (115)	6.4 (121)	6.6 (124)

Validity criteria:

This study was conducted before the most recent OECD guideline. Nonetheless in order to determine whether the study is valid the criteria in OECD 210 (2013) have been considered below:

- The dissolved oxygen concentration should be >60 % of the air saturation value throughout the test. Obtained: Range between 74 and 99 % (reported in appendix).

- The water temperature should not differ by more than + 1.5 ºC between test chambers or between successive days at any time during the test and should be within the temperature ranges specified for the test species; 25 ± 1.5 ºC. Obtained: 24 – 25 ºC.
- The analytical measure of the test concentrations is compulsory. In this study analytical measurements were taken, noting the malfunction reported on day 26 resulting in low recoveries. However, as described in the HSE evaluator comments the analytical method was not sufficiently validated.
- Overall survival of fertilised eggs and post-hatch success in the controls and, where relevant, in the solvent controls should be greater than or equal to 70 and 75 % respectively. Obtained: Overall survival in the control vessels was 75 % (53 – 83 %) at the end of the study. For the individual test vessels, control survival was only unacceptable in block 3 (replicate 3) with a survival rate of 53 %, which had been excluded from further calculations by the study author. When considering the raw data based on number of fertilised eggs at beginning and live embryos and fry at the end of day 5, survival was a minimum of 77.5 %.

The HSE evaluator considers the above validity criteria have not been fully met.

Biological results: First hatch was recorded in the test vessels 2 to 3 days after the start of the experiment and the last hatch was recorded 4 to 5 days after the start of the experiment. The times for first and last hatch did not appear to be affected by the test item treatments. The study report stated that no effects on hatch or survival were detected at any of the concentrations tested. Actual hatching success values were not clearly stated in the study report and it was unclear whether these could be calculated based on the raw data in the appendix.

It was not stated in the study report whether precipitation of the test item was assessed.

Effects on wet weights were assessed using pooled replicates. Significant treatment effects compared to the control appeared in the two highest concentrations tested (2 and 5 mg a.s./L, nominal, Williams` test, $p \leq 0.01$).

Effects on dry weight were assessed using replicates 3 and 4. Analysis of variance was performed in the same manner as for the wet weights with reciprocal transformation of the data. Significant treatment effects compared to the control appeared in the two highest concentrations tested (2 and 5* mg a.s./L, nominal, Williams` test, $p \leq 0.05$, * $p \leq 0.01$).

Effects of treatment on the fork length of fry were assessed using pooled replicates. Because the variances appeared to be constant across the treatments the data were not transformed before two-way analysis of variance. Significant treatment effects compared to the control appeared in the two highest concentrations tested (2 and 5 mg a.s./L, nominal, Williams` test $p \leq 0.01$).

No significant treatment related fry abnormalities were recorded. The results are summarised in Table B.9.2.2-2.

Table B.9.2.2-2: Chronic toxicity of 'cinmethylin' to fathead minnow (*Pimephales promelas*) in a fish early life stage test (35 d)

Concentration (nominal) [mg a.s./L]	**Control**	**0.1**	**0.2**	**0.5**	**1.0**	**2.0**	**5.0**
Concentration (time weighted average) [mg a.s./L]	**<0.002**	**0.094**	**0.20**	**0.52**	**0.97**	**2.1**	**5.3**
Number of dead embryos until day 5 [n]	1	0	3	3	1	2	1
Number of dead larvae until day 5 [n]	26	13	9	8	9	1	8
Mean survival from day 0 to test termination (35 d) [%]	75	86	81	85	67	96	79
Mean wet weight (35 d) [mg]	83	75	77	72	71	52**	18**
Mean dry weight (35 d) [mg]	19	16	16	15	16	11*	3.7**
Mean fork length (35 d) [mm]	21	20	20	20	20	18**	13**

Parameter	**Endpoints [mg a.s./L] (nominal)**
Fork length EC_{10} (35 d) based on recalculation [1)]	1.677 (95 % confidence limits: 1.206 – 2.148)
Fork length EC_{20} (35 d) based on recalculation [1)]	2.918 (95 % confidence limits: 2.468 – 3.369)
Dry weight EC_{10} (35 d) based on recalculation [1)#]	1.273 (95 % confidence limits: 0.835 – 1.711)
Dry weight EC_{20} (35 d) based on recalculation [1)#]	1.582 (95 % confidence limits: 1.151 – 2.012)
Wet weight EC_{10} (35 d) based on recalculation [1)]	**0.976** (95 % confidence limits: 0.540 – 1.411)
Wet weight EC_{20} (35 d) based on recalculation [1)]	1.437 (95 % confidence limits: 0.973 – 1.901)
$NOEC_{overall}$ (35 d) based on wet weight, dry weight and fork length	1.0

* Statistically significant differences compared to the control (Williams` test, $p \leq 0.05$).
** Statistically significant differences compared to the control (William´s test, $p \leq 0.01$).
1) EC_{10} value has been recalculated with raw data from the present study (Doc ID CL-512-002). This report was provided by the applicant following a request for information by the HSE evaluator.
It should be noted that only two of the four replicates were measured in terms of dry weight. The other two replicates were then estimated by the applicant based on wet weight data (see HSE evaluator comments for further details).
-- not applicable, + = decrease compared to control, - = increase compared to control

III. CONCLUSION

In an early life stage study with fathead minnow (*Pimephales promelas*) the overall NOEC (35 d) for cinmethylin was determined to be 1.0 mg a.s./L based on nominal concentrations by the study author. The 35 d-EC_{10} value was calculated to be 0.976 mg a.s./L.

HSE evaluator comments:

The HSE evaluator requested further information regarding the results for dry weight observed during the study. Whilst the results are not statistically significant there is a decrease compared to control in all treatment groups (minimum of 16 %). The following response was received, noting an extrapolation approach was used to calculate $EC_{10/20}$ values: '*In contrast to fresh weight and fork length, dry weight was only measured in replicates (i.e. blocks) 3 and 4 of each treatment. Hence, the dry weight data presented in the original report does not adequately reflect the effects of BAS 684 H on P. promelas. This is particularly important as the mean wet*

weights observed in the control replicate 1 and 2 were meaningfully lower than those measured in replicate 3 and 4 (up to 40 %). However, the strong and significant linear dependency ($p < 0.001$; $R2 = 0.98$) between mean fresh weight and mean dry weight data in replicate 3 and 4 allows for interpolation of the missing dry weights of replicate 1 and 2.' The HSE evaluator considers this approach generates some uncertainty for the dry weight results.

Based on the analytical data test concentrations were maintained within ± 20 % of nominal concentrations except for all treatment groups on day 26 (range of 58 – 68 %). This was due to equipment failure during the study. The endpoints have been based on nominal concentrations but this issue on day 26 adds uncertainty regarding exposure and the derived endpoint. Furthermore, the analytical method was not sufficiently validated in accordance with SANCO/3029/99 (see volume 3, CA, section B5 for full details). The following deficiencies were noted:

i) It is not possible to accept the linearity of the method as the example chromatograms for the highest concentration of the linear range show overloading of the sample, with the detector being saturated giving a broad flat top peak.
ii) To assess accuracy and precision, at least 5 recoveries should be provided for at least 2 fortification levels.
iii) The LOQ is not supported by 5 recovery determinations.
iv) Procedural recoveries have not been completed.

The validity criteria in terms of overall survival were not met for all replicates. Specifically, one replicate, had survival rates lower than limits stated in OECD 2010 (2013). Whilst this replicate was excluded from analysis this does add some uncertainty regarding the study results.

Given the uncertainty regarding dry weight data, exposure and validity criteria the HSE evaluator considers this study is not suitable for use in the risk assessment. In addition, a modern GLP early life stage study ([redacted], 2017a) for the same species has been submitted that generated more conservative values i.e. NOEC of 0.25 mg a.s./L (based on mean measured concentration).

Report: CA 8.2.2.1/2
[redacted], 2017 a
BAS 684 H (Cinmethylin) - Early-Life-Stage toxicity test on the fathead minnow (*Pimephales promelas*) in a flow through system
2017/1176649
Guidelines: OECD 210, EPA 850.1400
GLP: Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Cinmethylin (Reg.no.: 900 202), batch no. COD 002038, purity: 93.0 % (tolerance: ± 1.0 %)

B. STUDY DESIGN

Test species: Fathead minnows (*Pimephales promelas*), < 4 h old embryos; source: in-house egg production; parental fathead minnows obtained from: "[redacted].

Test design: Flow through system (35 d); 5 test item concentrations plus a dilution water control, 4 replicates per treatment with 25 fertilized eggs in each. Eggs and larvae were exposed in cylindrical glass vessels; The test solution flowed continuously from the mixing tank into a flow splitter into 4 equal parts for the 4 replicate test aquaria. On day 35 fish were sacrificed and the body length and weight of surviving individuals were determined. Daily assessment of hatch, swim-up, survival, signs of toxicity and abnormal behaviour.

Endpoints:	NOEC and ECx values based on hatching success, juvenile survival, time to hatch, toxic signs and growth.
Test concentrations:	Control (dilution water), 0.25, 0.56, 1.24, 2.73 and 6.00 mg a.s./L (nominal), corresponding to mean measured concentrations of <LoQ (Limit of quantification), 0.25, 0.59, 1.19, 3.01 and 6.61 mg a.s./L.
Test conditions:	Test vessels: stainless steel aquaria (29 x 21 x 22 cm), water volume: 9 L; egg cups: cylindrical glass vessels, diameter 19 cm (water volume: 1.7 L); dilution water: non-chlorinated, charcoal filtered drinking water (UV-sanitized; diluted with deionized water); temperature 24.6 - 26 °C; pH 7.7 – 8.1; oxygen content 5.7 mg/L - 8.1 mg/L; water hardness: 1.10 – 1.18 mmol/L; conductivity: 266 - 278 µS/cm; total organic carbon: 1.1 mg/L; acid capacity: 2.42 mmol/L – 2.46 mmol/L; light intensity: 107 - 199 lux; photoperiod: 16 hours light: 8 hours dark; flow rates: 1.125 L/hour/test vessel. Feeding: milled live Artemia nauplii and commercial fish diet (Tetramin, supplied by Tetra-Werke; Germany); slight aeration from day 18 on.
Analytics:	Analytical verification of test item concentrations was conducted using an HPLC-method with MS detection.
Statistics:	Descriptive statistics; one-sided Jonkheere-Terpstra for embryo, larvae and juvenile fish survival; one-sided William´s test for weight and length data, one-sided Wilcoxon-test and Dunnett`s test for variability between replicates.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentration was conducted in all test item concentrations at test initiation. Additionally, from alternating replicate per test group on days 6, 13, 20, 27 and 35. Additional samples were collected in those cases when deviations > 20 % from the nominal values were obtained to confirm that the deviations were transient and that the concentration had returned to nominal on the subsequent days. Mean measured concentrations of cinmethylin ranged from 96 to 110 % of nominal over the exposure period.

All stock solutions were visibly clear with the exception of the one for day 6 where a slightly turbid solution with oily film was observed. However, the analysis confirmed appropriate doses i.e. within ± 20 % of nominal values. A flow through design was utilised during the study.

The results are shown in the table below.

Table B.9.2.2-3: Measured concentrations during study

N (mg a.s./L)	Day 0 MC (%#)						Day 6	Day 13 MC (%#)			Day 14	Day 20	Day 27	Day 35	MM**
	R1	R2	R3	R4	RS1	RS2	MC (%#)	R1	RS1	RS2	MC (%#)	MC (%#)	MC (%#)	MC (%#)	
0	< LOQ	< LOQ	< LOQ	< LOQ	--	--	< LOQ	< LOQ	--	--	--	< LOQ	< LOQ	< LOQ	0
0.27	0.251 (93.0)	0.279 (103.3)	0.251 (93.0)	0.275 (101.9)	--	--	0.304 (112.6)	0.29 (107.4)	--	--	--	0.255 (94.4)	0.254 (94.1)	0.27 (100.0)	0.25
0.60	0.597 (99.5)	0.685 (114.2)	0.650 (108.3)	0.693 (115.5)	--	--	0.639 (106.5)	0.718 (119.7)	0.694 (115.7)	0.733 (122.2)	0.638 (106.3)	0.537 (89.5)	0.603 (100.5)	0.65 (108.3)	0.59
1.33	1.287 (96.8)	1.317 (99.0)	1.338 (100.6)	1.343 (101.0)	--	--	1.16 (87.2)	1.377 (103.5)	--	--	--	1.188 (89.3)	1.285 (96.6)	1.33 (100.0)	1.19
2.93	3.061 (104.5)	3.369 (115.0)	3.541 (120.9)	3.236 (110.4)	3.024 (103.2)	3.058 (104.4)	3.13 (106.8)	3.575 (122.0)	3.956 (135.0)	4.13 (141.0)	2.991 (102.1)	2.995 (102.2)	2.951 (100.7)	3.494 (119.2)	3.01
6.45	6.757 (104.8)	6.893 (106.9)	7.247 (112.4)	8.073 (125.2)	6.351 (98.5)	6.659 (103.2)	6.752 (104.7)	7.593 (117.7)	--	--	--	--	--	--	6.61

N = Nominal concentration (mg a.s./L), MC = measured concentration (mg a.s./L), MM = Mean measured concentration (mg a.s./L) calculated using mean values at day 0, na = not applicable, * Percentage of nominal concentration, ** = calculated by study author and corrected for percentage purity i.e. 93 %, LOQ = Limit of quantification: 2.5 ng a.s./L, R = replicate, RS = Reserve samples- these were analysed when flow through system was adjusted after measured concentration deviated by > ± 20 %, -- = not tested, # = percentage of nominal shown in brackets.

It was noted that when mean measured values were calculated by the study author some samples were excluded e.g. when reserve samples were taken. In order to assess the difference, the HSE evaluator has calculated the mean measured values when all samples are included (both reserve and original) and obtained the following values; 0, 0.25, 0.61, 1.19, 2.97 and 6.62 mg a.s./L when corrected for purity. These values are either identical or comparable therefore the HSE evaluator agrees with the use of the mean measured values calculated by the study author.

Validity criteria:

To determine whether the study is valid the criteria in OECD 210 (2013) have been considered below:

- The dissolved oxygen concentration should be >60 % of the air saturation value throughout the test. Obtained: 100 % saturation at the test temperature is equivalent to 8.38 mg/L, minimum recorded in this study was 68 %.

- The water temperature should not differ by more than + 1.5 ºC between test chambers or between successive days at any time during the test and should be within the temperature ranges specified for the test species; 25 ± 1.5 ºC. Obtained: 25 – 26 ºC.
- The analytical measure of the test concentrations is compulsory. In this study analytical measurements were taken.
- Overall survival of fertilised eggs and post-hatch success in the controls and, where relevant, in the solvent controls should be greater than or equal to 70 and 75 % respectively. Obtained: minimum of 80 % hatching success and 95 % juvenile survival, noting overall survival (day 35) related to eggs was a minimum of 80 %.

During the study the above criteria were met.

Biological results: Hatching started simultaneously in all test groups on day 2 and was complete by day 6. Hatching success ranged from 80 – 84 % for the replicates of the control group. Hatching success was statistically significantly decreased in comparison to the control group in the highest treatment group 5 (6.61 mg a.s./L mean measured and 6.00 mg a.s./L nominal). From the end of hatch to the end of exposure (day 6 – 35) survival was 95 – 100 % for the replicates of the control group. The survival from the end of hatch to the end of exposure (day 6 – 35) was statistically significantly decreased in the treatment groups ≥ 1.19 mg a.s./L (mean measured concentration) and ≥ 1.24 mg a.s./L (nominal concentration) in comparison to the control group.

The study author stated that juvenile mortality in test groups 3 and 4 (1.24 and 2.73 mg a.s./L based on nominal concentration) did not follow a concentration response pattern and may not be related to the test substance. Mortality occurred predominantly in the time after hatch in the highest treatment group 5 (6.61 mg a.s./L mean measured and 6.00 mg a.s./L nominal) and reached 100 % on day 17. The overall survival from insertion on day 0 to termination on day 35 was statistically significantly decreased in comparison to the control group in treatment group 5 (6.61 mg a.s./L mean measured and 6.00 mg a.s./L nominal). There was no statistically significant test substance-related effect on survival in test concentrations ≤ 0.59 mg a.s./L mean measured concentration corresponding to ≤ 0.56 mg a.s./L nominal. Since the juvenile survival data in test groups 3 and 4 did not follow a concentration response pattern and no effects on hatching success or overall survival were detected in these test groups, the survival effects are considered incidental and overall survival was used to determine the survival NOEC by the study author.

A reduced growth was observed in the treatment group 4 (3.01 mg a.s./L mean measured and 2.73 mg a.s./L nominal) starting on day 28 to the end of exposure. There were no other observed morphological abnormalities or signs of toxicity.

In comparison to the control group the mean wet weights of the surviving fish at the end of the exposure period were statistically significantly decreased in the test groups ≥3 (1.19 mg a.s./L mean measured and 1.24 mg a.s./L nominal). The total body lengths of the surviving fish at the end of the exposure period were statistically significantly decreased in comparison to the control group in the test groups ≥2 (0.59 mg a.s./L mean measured and 0.56 mg a.s./L nominal). Overall weight was affected to a greater degree than length as a percent of the control value. Consequently, the EC_{10} value for growth as weight is considered the most relevant effect metric for this endpoint. The results are summarized in Table B.9.2.2-2.

Table B.9.2.2-4: Chronic toxicity of cinmethylin to fathead minnow (*Pimephales promelas*) in a fish early life stage test (35 d)

Concentration corrected for purity (nominal) [mg a.s./L]	**Control**	**0.25**	**0.56**	**1.24**	**2.73**	**6.00**
Concentration corrected for purity (mean measured) [mg a.s./L]	**<LoQ**	**0.25**	**0.59**	**1.19**	**3.01**	**6.61**
Average % Hatch (range)	83 (80 – 84)	86 (84 – 88)	83 (76 – 88)	94 (84 – 100)	87 (80 – 100)	**77* 3)** **(76 – 80)**

Survival of young fish (day 6 - 35) [%]	99 (95 – 100)	98 (95 – 100)	97 (91 – 100)	**86** [2)] **(80 – 91)**	**91** [2)] **(85 – 100)**	**0** [2)] **(0 – 0)**
Survival from day 0 to test termination (35 d) [%] (range)	82 (80 – 84)	84 (83 – 84)	80 (76 – 84)	81 (76 – 84)	79 (68 – 88)	**0** [2)] **(0 – 0)**
Symptoms	none	none	none	none	3G	--
Mean weight (35 d) [mg] (SD)	173 (± 41.9)	177 (± 40.7)	168 (± 40.2)	**145** [1)] (± 36.2)	**80** [1)] (± 23.5)	--
Mean length (35 d) [cm] (SD)	2.7 (± 0.22)	2.7 (± 0.20)	**2.6*** [1)] (± 0.20)	**2.5** [1)] (± 0.25)	**2.0** [1)] (± 0.20)	--

Parameters	**Endpoints [mg a.s./L]**	
	Nominal	**Mean measured**
$EC_{20\ body\ weight}$ (35 d)	1.35 (95 % CI 1.22 – 1.50)	1.34 (95 % CI 1.21 – 1.50)
$EC_{20\ body\ length}$ (35 d)	2.38 (95 % CI 2.29 – 2.46)	2.57 (95 % CI 2.47 – 2.68)
$EC_{10\ body\ weight}$ (35 d)	0.971 (95 % CI 0.84 – 1.13)	0.92 (95 % CI 0.78 – 1.08)
$EC_{10\ body\ length}$ (35 d)	1.45 (95 % CI 1.36 – 1.55)	1.47 (95 % CI 1.37 – 1.56)
$NOEC_{mortality}$ (35 d) [4)]	2.73	3.01
Overall NOEC (35d) based on all parameters	0.56	0.59

Values printed in **bold** show statistically significant differences compared to the control; LoQ: limit of quantification (LoQ < 2.5 ng a.s./ml); Symptoms: G: reduced growth; --: all fish dead, CI = confidence limits, SD = Standard Deviation- calculated by HSE evaluator for all replicates, study report calculated values for replicates

[1)] Statistically significant differences compared to the control (one-sided William´s test, * $p \leq 0.05$; ** $p \leq 0.01$). [2)] Statistically significant differences compared to the control (one-sided Jonkheere-Terpstra test, * $p \leq 0.05$; ** $p \leq 0.01$). [3)] Statistically significant differences compared to the control (one-sided Wilcoxon test, * $p \leq 0.05$; ** $p \leq 0.01$). [4)] NOEC value was based on the higher endpoint mortality by study author since juvenile survival does not follow a dose-response relationship.

III. CONCLUSION

In an early life stage study with fathead minnow (*Pimephales promelas*) the mortality NOEC (35 d) for cinmethylin was determined by the study author to be 3.01 mg a.s./L based on mean measured concentrations.

HSE evaluator comments:

The following justification was provided by the applicant for conducting this study: *'The following new chronic early life stage study on fathead minnow was conducted to meet data requirements outside the European Union. On request of the RMS CTGB, the study is provided for the sake of completeness.'* The HSE evaluator considers the justification provided appropriate.

It is noted that the test item concentration was not maintained within ± 20 % of the mean measured test item concentration during the study as required by the validity criteria under OECD 210 (2013). However, the biological results have been presented in terms of the arithmetic mean concentration of the test substance. This is in line with the 'Guidance document on aquatic toxicity testing of difficult substances and mixtures' (2000) for flow through study designs. Therefore, the results are considered suitable for use in risk assessment.

The study author reported the NOEC based on survival as 3.01 mg a.s./L (mean measured). However, given there were 13 % effects at this concentration along with significant effects on juvenile mortality (noting lack of dose response) the HSE evaluator has considered the NOEC further. Based on survival an appropriate NOEC is 0.59 mg a.s./L.

When considering both the statistical analysis and symptoms the overall NOEC (all parameters measured) is 0.25 mg a.s./L (mean measured). It should be noted this value can be considered conservative as the only statistically significant effect at 0.59 mg a.s./L was based on length where the reported mean values; 2.7 (± 0.22) cm in control and 2.6 (± 0.22) cm in treatment group. Hence it could be argued as this difference is relatively low between control and treatment it may not be treatment related and the HSE evaluator considers an overall NOEC of 0.59 mg a.s./L appropriate.

$EC_{10/20}$ values were not calculated based on survival during the study or for juveniles. However, given the most sensitive parameters are weight and length based on the study the HSE evaluator considers this acceptable.

The analytical method was validated in accordance with SANCO/3029/99 with an LOQ of 2.5 ng a.s./mL (for full details see volume 3, CA, section B5).

The above study was conducted to GLP and the following endpoints (most sensitive) will be considered in the risk assessment section:

- Overall NOEC (considering survival, body length and weight) = **0.59** mg a.s./L (based on mean measured concentration)
- EC_{10} (body weight most conservative of parameters measured) = 0.92 mg a.s./L (based on mean measured concentration)
- EC_{20} (body length most conservative of parameters measured) = 2.57 mg a.s./L (based on mean measured concentration)

B.9.2.3. Potential for endocrine disruption

Consideration of EAS modalities (aquatic organisms):

Two studies have been submitted, the FSTRA and ELS, noting that the latter measures parameters that are 'sensitive too but not diagnostic of EATS'. The studies that are valid have been summarised in the table below. The full study evaluations are shown in sections B.9.2.2. and B.9.2.3.

Table B.9.2.3-1: Brief overview of aquatic studies relevant to assessment of EAS modalities:

Details	**ELS ([redacted], 2017a)**	**FSTRA ([redacted], 2020)**
Study ID[a]	199	202
Species tested:	Fathead minnow (*Pimephales promelas*)	Zebra fish (*Danio rerio*)
Exposure method and duration:	Flow through 35 days	Flow through 21 days
Test concentrations:	Test concentrations not maintained within ± 20 % of nominal concentrations hence measured concentrations used; 0.25, 0.59, 1.19, 3.01, 6.61 mg a.s./L	Test concentrations not maintained within ± 20 % of nominal concentrations hence measured concentrations used; 0.11, 0.37, 1.17 mg a.s./L
Guideline followed:	OECD 210 (2013)	OECD 229 (2012)
Parameters measured:	Survival, behavioural/abnormalities, body weight/length	Histology samples taken but not analysed*, egg production, body weight (males/females), vitellogenin (males/females), behavioural/abnormalities

***** Histological analysis is an optional requirement under OECD 229. Samples were taken but not analysed due to lack of effects observed based on vitellogenin levels in treatment groups compared to control.
[a] Study ID used in table B.9.2.3-4.

The study results have been discussed below for each study.

ELS study ([redacted] 2017a):

A summary of the results from the ELS study are shown in the table below.

Table B.9.2.3-2: Chronic toxicity of cinmethylin to fathead minnow (*Pimephales promelas*) in a fish early life stage test (35 d)

Concentration corrected for purity (mean measured) [mg a.s./L]	**Control (<LoQ)**	**0.25**	**0.59**	**1.19**	**3.01**	**6.61**
Average % Hatch (range)	83 (80 – 84)	86 (84 – 88)	83 (76 – 88)	94 (84 – 100)	87 (80 – 100)	**77* 3) (76 – 80)**
Survival of young juvenile fish (day 6 - 35) [%]	99 (95 – 100)	98 (95 – 100)	97 (91 – 100)	**86** 2) (80 – 91)**	**91** 2) (85 – 100)**	**0** 2) (0 – 0)**
Survival from day 0 to test termination (35 d) [%] (range)	82 (80 – 84)	84 (83 – 84)	80 (76 – 84)	81 (76 – 84)	79 (68 – 88)	**0** 2) (0 – 0)**
Symptoms	None	none	none	none	3G	--
Mean weight (35 d) [mg] (SD)	173 (± 41.9)	177 (± 40.7)	168 (± 40.2)	**145** 1) (± 36.2)**	**80** 1) (± 23.5)**	--
Mean length (35 d) [cm] (SD)	2.7 (± 0.22)	2.7 (± 0.20)	**2.6* 1)** (± 0.20)	**2.5** 1)** (± 0.25)	**2.0** 1)** (± 0.20)	--

Parameters	**Endpoints [mg a.s./L]**
	Mean measured
$EC_{20\ body\ weight}$ (35 d)	1.34 (95 % CI 1.21 – 1.50)
$EC_{20\ body\ length}$ (35 d)	2.57 (95 % CI 2.47 – 2.68)
$EC_{10\ body\ weight}$ (35 d)	0.92 (95 % CI 0.78 – 1.08)
$EC_{10\ body\ length}$ (35 d)	1.47 (95 % CI 1.37 – 1.56)
$NOEC_{mortality}$ (35 d) 4)	3.01
Overall NOEC (35d) based on all parameters	0.59

Values printed in **bold** show statistically significant differences compared to the control; LoQ: limit of quantification (LoQ < 2.5 ng a.s./ml); Symptoms: G: reduced growth; --: all fish dead, CI = confidence limits, SD = Standard Deviation- calculated by HSE for all replicates, study report calculated values for replicates
1) Statistically significant differences compared to the control (one-sided William´s test, * $p \leq 0.05$; ** $p \leq 0.01$). 2) Statistically significant differences compared to the control (one-sided Jonkheere-Terpstra test, * $p \leq 0.05$; ** $p \leq 0.01$). 3) Statistically significant differences compared to the control (one-sided Wilcoxon test, * $p \leq 0.05$; ** $p \leq 0.01$). 4) NOEC value was based on the higher endpoint mortality by study author since juvenile survival does not follow a dose-response relationship.

Consideration of ELS results ([redacted], 2017a):

In the study hatching started in all test groups on day 2 and was complete by day 6. Statistically significant effects on hatching and survival from study initiation to termination (days 0 to 35) were observed at the highest test concentration (6.61 mg a.s./L) with 0 % survival. In terms of survival for 'young juvenile fish' (day 6 to 35) statistically significant effects were observed at the three highest test concentrations (from 1.19 mg a.s./L upwards) reaching 100 % mortality at the highest test concentration of 6.61 mg a.s./L. However, the changes in survival were variable at 1.19 and 3.01 mg a.s./L and only at the highest test concentration was there a clear decrease.

Reduced growth was observed at a concentration of 3.01 mg a.s./L. Statistically significant effects on weight and length were noted from concentrations of 1.19 and 0.59 mg a.s./L respectively. HSE considered an overall NOEC of 0.59 mg a.s./L appropriate despite the statistically significant effects on length recorded at 0.59 mg a.s./L. This is because the change was relatively low; means of 2.6 and 2.7 cm in the treatment and control respectively. This decision is further supported by the EC_{10} based on body length of 1.47 mg a.s./L. It was noted that at the higher concentration of 3.01 mg a.s./L there was a relatively large decrease in length.

FSTRA study ([redacted] 2020):

The results from the FSTRA 21-day flow through GLP study are shown in the tables below for the parameters assessed.

Table B.9.2.3-3: Biological results during the in-life phase of the study

Parameters measured		Mean measured concentration cinmethylin [mg a.s./L]			
		Control	**0.11**	**0.37**	**1.17**
Total egg no. per day and female [n] [A]	**Mean**	**49**	**59**	**62**	**45**
	SD	29	17	4	3
	RSD	60.1	29.3	6.3	7.6
	% CV	59.2	28.8	6.5	6.7
Wet weight males at study termination [mg] [A]	**Mean**	**0.451**	**0.445**	**0.415**	**0.450**
	SD	0.030	0.039	0.073	0.015
	RSD	6.6	8.7	17.6	3.4
Wet weight females at study termination [mg] [A]	**Mean**	**0.483**	**0.488**	**0.433**	**0.506**
	SD	0.030	0.009	0.007	0.028
	RSD	6.2	1.8	1.6	5.6
VTG / total protein [ng/µg]; males at study termination [A]	**Mean**	**0.04**	**0.04**	**0.04**	**0.04**
	SD	0.02	0.01	0.01	0.01
	RSD	46.4	24.0	40.0	42.3
VTG / total protein [ng/µg]; females at study termination [A]	**Mean**	**820.1**	**1122.8**	**423.5**	**758.2**
	SD	240.5	472.7	45.9	410.3
	RSD	29.3	42.1	10.8	54.1
	Range	650 – 990.2	788.5 – 1457.1	391.0 – 455.9	468.1 – 1048.4

SD = Standard deviation
RSD = Relative standard deviation
CV = Coefficient of Variation
VTG = Vitellogenin
[A]: No statistically significant difference between controls and treatments, Bonferroni-Welch t-test, one-sided smaller, $\alpha = 0.05$

Egg production and wet weight results:

When considering the egg production results shown in table B.9.2.3-3 the CV in the control and lowest treatment rate are relatively high (59.2 and 28.8 % respectively). However, the CV for the control is in-line with the range stated in OECD 229 of 20 to 60 %. Noting at the higher end of the range the ability of the assay to detect a significant decrease in egg production < 70 % is limited. For egg production there was an increase compared to control at treatment rates of 0.11 and 0.37 mg a.s./L (mean measured concentrations). However, these changes were not statistically significant and there were no clear treatment related effects on egg production. Similarly no statistically significant effects on wet weight for males and females compared to control were observed during the study.

Consideration of vitellogenin results:

As stated in OECD 229 and EFSA/ECHA guidance 2018 vitellogenin levels can be impacted by general toxicity, non-endocrine toxic modes of action and confounding factors such as diet or infection. In terms of vitellogenin (VTG) levels in this study for males' values were comparable to control across all treatment groups. For females (table B.9.2.3-3) there were no statistically significant effects. However, it was noted there were relatively high differences compared to control at mean measured concentrations of 0.11 and 0.37 mg a.s./L with an increase and decrease respectively. The ranges of levels are also shown in table B.9.2.3-3. At the mean measured concentration of 0.11 mg a.s./L one fish was within the range of the control. It is only at 0.37 mg a.s./L where the range of vitellogenin levels in females does not overlap with the control. In addition, the mean level for females at the highest test concentration is broadly comparable to control. Furthermore, in the OECD validation report for 21 day fish screening assay (2007) the following is stated: '*there is typically high variability of VTG values (high SD's) but true responses are sensitive and dramatic, thus high enough to easily reach statistical significance*'. In this study there were no clear treatment related effects on VTG levels and the results were not statistically significant compared to control (requirement under OECD 229) for both males and females. Therefore, HSE considers there are no clear treatment related effects of cinmethylin on endocrine activity at the highest concentration tested based on the submitted FSTRA study.

Histological analysis:

Histological analysis is an optional requirement under OECD 229. Samples were taken but not analysed due to lack of effects observed based on vitellogenin levels in treatment groups compared to control.

EAS summary of parameters:

A summary of the results from both the FSTRA and ELS studies has been provided in the table below. The format is in accordance with EFSA/ECHA guidance i.e. appendix E. The results summarised are for the EAS modalities.

Table B.9.2.3-4: Reporting the lines of evidence for adverse effects from fish studies (EAS modalities), note the effect classification for parameters is in-line with EFSA/ECHA guidance 2018

Effect classification	Effect target	Study ID Matrix	Study type	Species	Duration of exposure (days)	Exposure route	Lowest Effect dose (mg a.s./L)	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
In vivo mechanistic	Vitellogenin (VTG) in females	202	FSTRA	Zebrafish	21	Uptake from water	> 1.17	Not stat. sig	Not stat. sig* No indication		No indication	E, A, S
	Vitellogenin (VTG) in males	202	FSTRA	Zebrafish	21	Uptake from water	> 1.17	No effect	No indication			E, A, S
Sensitive to, but not diagnostic of, EATS	Reproduction (fecundity, fertility)	202	FSTRA	Zebrafish	21	Uptake from water	> 1.17	No effect	No indication		Effects on body weight, length and hatching success observed.	N
	Embryo time-to-hatch	199	ELS	Fathead minnow	35	Uptake from water	> 6.61	No effect	No indication			N
	Hatching success	199	ELS	Fathead minnow	35	Uptake from water	6.61	Decrease	Decrease observed at highest test concentration			N
	Morphological abnormalities / behaviour	199	ELS	Fathead minnow	35	Uptake from water	> 3.01	No effect	No indication**			N
		199	ELS	Fathead minnow	35	Uptake from water	> 3.01	No effect				N
		202	FSTRA	Zebrafish	21	Uptake from water	> 1.17	No effect				N
	Body weight (fish)	199	ELS	Fathead minnow	35	Uptake from water	1.19; EC_{10} = 0.92	Decrease	Reduced body weight	Decrease (weight and length) observed in		N
		202	FSTRA	Zebrafish	21	Uptake from	> 1.17	No effect	No effect			N

Effect classification	Effect target	Study ID Matrix	Study type	Species	Duration of exposure (days)	Exposure route	Lowest Effect dose (mg a.s./L)	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
						water			(based on wet weight)	ELS study		
	Length (fish)	199	ELS	Fathead minnow	35	Uptake from water	1.19***; EC_{10} = 1.47	Decrease	Reduced length			N N
Systemic toxicity	Survival (fish)	199	ELS	Fathead minnow (embryos, larvae, juvenile)	35	Uptake from water	6.61	Decrease	Reduced survival of all exposed life stages at highest concentration		Evidence of systemic toxicity at the highest tested concentration in ELS study. No evidence in FSTRA	-
		202	FSTRA	Zebrafish (mature spawning fish)	21	Uptake from water	> 1.17	No effect	No indication			-

* HSE considers there were no clear treatment related effects on vitellogenin levels, supported by statistical analysis

** Reduced growth observed at 3010 µg a.s./L. However this is not a morphological abnormality or behavioural effects and is captured by the body weight and length parameters.

*** Statistically significant effects observed at 0.59 mg a.s./L. However, due to the relatively low difference between control and test concentration, this difference was not considered treatment related by HSE. The averages were 2.7 and 2.6 cm for the control and 0.59 mg a.s./L respectively.

E = Estrogen, A = Androgen, S = Steroidogenesis, N = Not assignable to a specific modality, - = not applicable, stat sig = Statistically Significant

HSE ecotoxicology consideration of aquatic organisms (EAS modalities):

The applicant consideration for aquatic organisms (EAS modalities) is provided in italics below:

'The analyses of EAS-mediated parameters are sufficiently addressed and do not indicate endocrine activity of BAS 684 H and thus allows to conclude that ED criteria are not met for the estrogen, androgen and steroidogenesis modalities.'

A FSTRA study ([redacted], 2020) was provided that investigated EAS activity of cinmethylin. Whilst this study was considered valid, HSE has concerns regarding MTC (nominal 1.24 mg a.s./L/1.17 mg a.s./L mean measured) as detailed below.

The results from the ELS study ([redacted] 2017a) were used to derive the Maximum Tolerated Concentration (MTC) in the FSTRA. The MTC is defined as the highest test concentration which results in less than 10 % mortality. The selected MTC and FSTRA has been considered further below.

FSTRA study ([redacted] 2020) highest concentration tested (MTC):

HSE has considered the MTC used in the FSTRA study testing zebrafish. In [redacted], 2020 the study author stated the following:

'*The highest test concentration was chosen as a maximum tolerated concentration (MTC) based on the results of an early life stage study with fathead minnow. The chosen MTC (nominal 1.24 mg a.s./L) is a factor higher of 2.2 higher than the growth LOEC identified in the ELS study. At 1.24 mg a.s./L the study reported clear effects on fish growth (as length and weight) and some effect on post hatch survival which was considered incidental. For a FSTRA study the MTC should give clear systemic toxicity [1], yet with < 10 % mortality [2] and without compromising physiological functions [3]. The concentration of 1.24 mg a.s./L was considered the highest possible concentration that could meet these opposing considerations.*'

The relevant sections of the guidance/guidelines referenced by study author are shown in the table below:

B.9.2.3-5: References quoted by study author in FSTRA.

[1] OECD Conceptual framework	*The top dose or concentration should be sufficiently high to give clear systemic (i.e. non endocrine-specific) toxicity in order to ensure that a wide range of exposures (high to low) is tested. However, endocrine effects observed solely in the presence of clear systemic toxicity should be interpreted with caution and may be disregarded when sufficiently justified to be caused by secondary effects which are unlikely to be due to endocrine activity. The reason for this advice is a concern that some endocrine active substance (EAS) sensitive assays are being run at doses / concentrations of EASs that are too low to trigger direct impacts on the endocrine system. This GD is not the place to address this issue directly, but it should be considered when EAS-sensitive test guidelines (TGs) are revised in the future.*
[2] OECD 229 FSTRA method	*For the purposes of this test, the highest test concentration should be set by the maximum tolerated concentration (MTC) determined from a range finder or from other toxicity data, or 10 mg/L, or the maximum solubility in water, whichever is lowest. The MTC is defined as the highest test concentration of the chemical which results in less than 10 % mortality. Using this approach assumes that there are existing empirical acute toxicity data or other toxicity data from which the MTC can be estimated. Estimating the MTC can be inexact and typically requires some professional judgment.*
[3] EFSA/ECHA guidance document 2018	*In principle, the top dose/concentration selected for the conduction of the (eco)toxicological studies should provide information on substance toxicity at an exposure of the tested agent that should be tolerated without inducing significant chronic physiological dysfunctions, be compatible with animal survival and permits data interpretation in the context of the use of the study. The concepts of maximum tolerated dose (MTD) and maximum tolerated concentration (MTC) are then useful for top dose/concentration selection and should be considered as a starting point for the evaluation of changes which could be due to excessive systemic toxicity.* *The aim of the MTD is to produce a minimum toxic effect over the course of the study. Elements to consider are alterations in physiological function, including: no more than 10 % decrease in body weight gain relative to control, target organ toxicity and alterations in clinical pathological parameters.*

	Although these parameters can only be considered indicative and expert judgement is necessary to define the MTD on a case-by-case basis. Elements which indicate that the MTD has been exceeded are reported in the OECD Guidance on the Recognition, Assessment and Use of Clinical Signs as Humane Endpoints for Experimental Animals Used in Safety Evaluation (OECD, 2000). *Equally, in ecotoxicology, the MTC is defined as the highest test concentration of the chemical which results in less than 10 % mortality (Hutchinson et al., 2009; Wheeler et al., 2013; Ankley and Jensen, 2014). For tests on aquatic organisms, the maximum solubility in water, or the limit concentration as defined in the relevant OECD guidelines should be considered.*

HSE notes that the ELS study has been used by the applicant to set the MTC. However, it is noted that the ELS study tested fathead minnow and the FSTRA zebrafish. Therefore, there is some uncertainty regarding 'read across' between species toxicity for determining MTC. It is unclear whether the use of growth LOEC and multiplication factor of 2.2 is suitably protective of other species.

Overall conclusion regarding MTC used in FSTRA:

The justification for MTC, provided by FSTRA study author, is not fully in-line with OECD 150, EFSA/ECHA guidance or OECD 229. This is further demonstrated by no systemic toxicity observed in the FSTRA at the highest test concentration (see FSTRA results section below), noting clear systemic toxicity is a requirement stated in conceptual framework (OECD 150) for OECD 229 study. HSE notes there is supporting information to justify the chosen MTC (nominal 1.24 mg a.s./L) based on mortality results in ELS i.e. > 10 % mortality for young fish at 1.19 mg a.s./L. However, the ELS study tested a different species i.e. fathead minnow not zebrafish resulting in further uncertainty.

The conclusion regarding MTC is **pending**. The applicant plans to provide further information supporting the chosen MTC, including toxicity data for other fish species. This information will be evaluated in due course by HSE.

Consideration of submitted EAS data:

The key points regarding the ELS and FSTRA studies are discussed below.

ELS testing fathead minnow ([redacted], 2017a)

When considering the ELS testing fathead minnow ([redacted], 2017a) systemic toxicity was demonstrated in the highest test concentration with 100 % mortality at 6.61 mg a.s./L. However, there were effects on body length and weight recorded with EC_{10} values of 1.47 and 0.92 mg a.s./L respectively. These parameters are considered sensitive too but not diagnostic of EATS modalities.

FSTRA testing zebrafish ([redacted] 2020)

Vitellogenin levels (males and females) was the only parameter measured in available studies that is indicative of EAS modalities. Based on EFSA/ECHA guidance, for the FSTRA (OECD 229) other parameters indicative of these modalities are; specific gonad histopathology (optional) and Secondary Sex Characteristics (SSC). Regarding SSC, HSE notes that the test species was zebrafish and a method for SSC has not been validated. Furthermore, the following is stated in OECD 229: '*Secondary sex characteristics in male fish of certain species are externally visible, quantifiable and responsive to circulating levels of endogenous androgens; this is the case for the fathead minnow and the medaka - but not for zebrafish which does not possess quantifiable secondary sex characteristics.'* Therefore, the omission of SSC is considered acceptable by HSE when testing zebrafish. Histology samples are available but the applicant has not analysed due to lack of effects on vitollegin, noting the conclusion of toxicology for EAS modalities that cinmethylin is not an endocrine disruptor.

Overall EAS conclusion:

Currently a final conclusion has not been reached for aquatic organisms based on EAS modalities as further information regarding MTC used in FSTRA will be provided by the applicant.

HSE ecotoxicology consideration of aquatic organisms (T modality):

AMA study:

An Amphibian Metamorphosis Assay (AMA) [redacted], 2020a was submitted and considered valid. There were 4 replicates per treatment group and the GLP study was conducted following OECD 231, flow through 21 day study.

Analytical verification:

Concentrations of the test substance were not maintained within 20 % of the nominal values and mean measured values were calculated, which is considered by HSE to be appropriate.

It was noted that the analytical recovery in replicate A of the 0.019 mg/L treatment level at the day 0 interval was out of specification (>200 % of target). Based on the acceptable recovery in the adjacent aquarium (0.019 mg/L replicate B), the replicate A aquarium was replaced as a conservative measure on test day 2 by the Study Director. HSE considers this acceptable as it occurred at test initiation and therefore the study organisms were exposed to the concentration with acceptable recovery (within 20 % of nominal) for the majority of the study. In addition, growth stages of this replicate did not deviate from other replicates in this test concentration throughout the study and all measured output variables were within the ranges of other replicates in this test concentration.

MTC concentration:

The MTC concentration was based on a range finding study testing tadpoles that achieved a 7 day LC_{50} value of 5.7 mg a.s./L. The MTC was then derived using one third of the LC_{50} i.e. 1.9 mg a.s./L. HSE agrees with the approach taken as it is in-line with OECD 231.

Results

The results from this 21 day flow through GLP AMA are provided below.

Spinal deformities:

On day 7 spinal deformities (i.e., scoliosis, bent tail) were observed in 50 % of control animals and in 55, 55, and 10 % of tadpoles exposed to the 0.023, 0.21, and 1.9 mg/L treatment levels, respectively. On day 21 spinal deformities were observed in 27 % of control animals and in 26, 15, and 8 % of tadpoles exposed to the 0.023, 0.21, and 1.9 mg/L treatment levels, respectively. For the entire exposure spinal deformities were observed for 33 % of control animals and for 33, 25, and 9 % of tadpoles exposed to the 0.023, 0.21, and 1.9 mg/L treatment levels, respectively. The study author stated that the spinal deformities did not impact any endpoint collected for this assay or growth/survival of tadpoles and was not attributed to cinmethylin exposure.

Developmental Stage

The day 21 developmental stage distribution profile was analysed by applying the multi-quantal Jonckheere-Terpstra's Step-Down Test to the 20^{th} through the 80^{th} percentiles for all treatment levels compared to the control. The overall multi-quantal procedure determined a significant reduction in day 21 percentile developmental stage at 1.9 mg/L and corroborated the standard comparison test result for day 7 developmental stage. The 10^{th} and 90^{th} percentile of day 21 developmental stage distribution was 3.1 stages (maximum allowable spread $\leq$ 4 stages).

Hind Limb Length Normalized by Snout-Vent Length

No significant reduction in day 7 or day 21, Nieuwkoop and Faber (NF) stage >60 tadpoles was shown for hind limb length normalized by SVL among tadpoles exposed to any of the treatment levels tested compared to the control. A significant difference in day 21 hind limb length normalized by SVL for NF stage $\leq$ 60 tadpoles exposed to the 1.9 mg a.s./L treatment level compared to the control as indicated below.

Table B.9.2.3-6: Bioassay for *Xenopus laevis* tadpoles after flow-through exposure to cinmethylin

Parameters		Targeted concentration [mg a.s./L]			
		Control	**0.019**	**0.19**	**1.9**
		Mean measured concentration [mg/L]			
		Control	**0.023**	**0.21**	**1.9**
No of replicates		4	4	4	4
Survival [%]	Day 21	100	100	98	100
Median developmental stage Range shown in brackets	Day 7	54 (53-54)	54 (53-54)	54 (53-54)	54 (53-54)
	Day 21	60 (57-61)	59 (57-62)	59 (57-61)	59 (57-61)

Table B.9.2.3-7: Endpoints for *Xenopus laevis* tadpoles after flow-through exposure to cinmethylin

Endpoints

Parameters			Targeted concentration [mg a.s./L]			
			Control	**0.019**	**0.19**	**1.9**
			Mean measured concentration [mg a.s./L]			
			Control	**0.023**	**0.21**	**1.9**
Mean hind limb length [mm] a)	Day 7		0.127	0.123	0.127	0.124
	Day 21	NF ≤ 60	0.693	0.697	0.647	0.537*
		NF ≥ 61	1.048	1.050	1.040	0.880
Mean snout-vent length [mm]	Day 7		16.86	16.94	17.38	16.27
	Day 21	NF ≤ 60	24.01	23.63	23.17	22.55
		NF ≥ 61	19.44	18.53	17.57	17.88
Mean whole body wet weight [g]	Day 7		0.3803	0.3812	0.4051	0.3308
	Day 21	NF ≤ 60	1.0612	1.0225	0.9519	0.8381
		NF ≥ 61	0.7907	0.7791	0.6661	0.6416

a) Normalized by Snout-Vent Length (SVL)

***** statistically significant difference between controls and treatments determined, using a two-factor ANOVA with nested variance structure

NF = Nieuwkoop and Faber

Based on statistical analysis there was a significant effect on hind limb length at day 21 for NF ≤ 60 tadpoles, noting lengths were normalized by SVL. It is unclear that normalisation by SVL is required in OECD 231. Therefore the raw data for replicates (hind limb length and whole body wet weight) are shown in the table below.

Table B.9.2.3-8: Hind Limb Length and whole body wet weight following 21-day flow-through exposure to cinmethylin of tadpoles NF stage ≤ 60

Mean Measured Concentration (mg a.s./L)	Replicate ID	Hind Limb Length (mm)			Whole Body Wet Weight (g)		
		Mean	Median	SD	Mean	Median	SD
Control	A	15.01	15.39	3.41	0.9951	0.9877	0.1620
	B	18.44	18.24	2.52	1.1532	1.0996	0.3033
	C	15.72	15.53	2.31	1.2121	1.1918	0.2190
	D	16.64	16.51	1.94	0.8843	0.8691	0.1911
	Mean	**16.45**	**16.42**	**1.483**	**1.0612**	**1.0370**	**0.1493**
0.023	A	16.72	16.61	3.06	1.0596	1.1508	0.2169
	B	15.94	15.73	3.00	0.9251	0.9751	0.1223
	C	14.36	14.23	3.05	0.8354	0.7954	0.1519
	D	18.01	18.09	2.09	1.2699	1.3055	0.2702
	Mean	**16.26**	**16.16**	**1.526**	**1.0225**	**1.0567**	**0.1889**
0.21	A	13.87	14.24	2.17	0.8340	0.7973	0.1558
	B	15.45	14.35	3.55	1.1538	1.1849	0.1449
	C	13.00	13.20	3.40	0.5453	0.5119	0.1036
	D	17.24	17.01	1.68	1.2744	1.2210	0.2235
	Mean	**14.89**	**14.70**	**1.865**	**0.9519**	**0.9288**	**0.3286**
1.9	A	9.41	9.23	2.41	0.4984	0.5146	0.0718
	B	13.43	13.04	2.84	1.0203	1.0333	0.1533
	C	11.68	12.63	2.99	0.6418	0.6575	0.1347
	D	14.00	14.35	2.17	1.1920	1.1712	0.1889
	Mean	**12.129**	**12.313**	**2.067**	**0.8381**	**0.8442**	**0.3227**

SD = Standard Deviation
CV = Coefficient of Variation

Thyroid gland histology

The histopathology analysis was conducted in-line with OECD 231 guideline (and separate guideline for histopathology OECD 2007). Samples should be graded according to 4 levels; with 0 being no remarkable effects. In this study remarkable effects were graded in-line with OECD guidance i.e. Grade 1 ('mild' with 30 – 50 % of tissue affected), Grade 2 ('moderate' with 60 – 80 % of tissue affected) and Grade 3 ('severe' with > 80 % of tissue affected).

Histopathologic findings in the thyroid glands are shown in the table below.

Table B.9.2.3-9: Thyroid Gland Histology for *Xenopus laevis* tadpoles after 21-day flow-through exposure to cinmethylin

Cinmethylin Treatment Group (mg a.s./L)		**0.0 (control)**					**0.023**					**0.21**					**1.9**				
Replicate		A	B	C	D	**T**[a]	A	B	C	D	**T**	A	B	C	D	**T**	A	B	C	D	**T**
Number examined		5	5	5	5	**20**	5	5	5	5	**20**	5	5	5	5	**20**	5	5	5	5	**20**
Follicular cell hyperplasia	Mild	3	2	2	1	**8**	3	3	0	3	**9**	1	2	0	4	**7**	0	3	2	3	**8**
Follicular cell hypertrophy	Mild	4	4	3	2	**13**	5	4	1	4	**14**	1	4	4	3	**12**	0	4	0	4	**8**
	Moderate	0	1	0	1	**2**	0	0	0	1	**1**	0	1	0	2	**3**	0	1	0	1	**2**
Thyroid hypoplasia/atrophy	Mild	0	0	0	0	**0**	0	0	0	0	**0**	0	0	0	0	**0**	2	0	0	0	**2**
Follicular lumen area (previously colloid area)		0	0	0	0	**0**	0	0	0	0	**0**	0	0	0	0	**0**	0	0	0	0	**0**
Colloid quality		0	0	0	0	**0**	0	0	0	0	**0**	0	0	0	0	**0**	0	0	0	0	**0**

[a] T = Total

A description of the histology effects observed is provided below based on OECD, 2007 guidelines.

Observation	**Description**
Thyroid gland hypertrophy/atrophy	*Increases (hypertrophy) or decreases (atrophy) in the overall size of the thyroid gland are consequent of changes in follicular cell size and number. The severity of either hypertrophic or atrophic observations is to be graded on an overall, general appearance of the thyroid gland. Because the diagnosis of hypertrophy or atrophy is dependent on a comparison to controls, it is necessary to establish the normal variability of thyroid gland sizes in control tadpoles prior to making determinations on thyroid gland size in dose groups.*
Follicular cell hypertrophy:	*Hypertrophic follicular cells, defined as tall columnar cells, are to be graded based on the percentage of the cells exhibiting this feature. It is recognized that follicular cell hypertrophy may present as a generalized lesion and interpreted thus. Because normal amphibian thyroid glands show heterogeneity in follicular cell shape, ranging from squamous to tall columnar, severity is determined by the change in percentage of cells exhibiting tall columnar structure.*
Follicular cell hyperplasia:	*Follicular cell hyperplasia is diagnosed when there is follicular cell crowding, stratification (multiple layers), or papillary infolding of single or multiple layers of follicular cells. The severity grading scheme for follicular cell hyperplasia is based on the percentage of follicles that exhibit hyperplasia, and/or the percentage of tissue that is affected.*

T summary of parameters:

A summary of the results from AMA has been provided in the table below. The format is in accordance with EFSA/ECHA guidance i.e. appendix E. The results summarised are for the T modality.

Table B.9.2.3-10: Lines of evidence for T modality (aquatic organisms)

Effect	Effect target	Study ID	Species	Study type	Duration of exposure	Exposure route	Lowest Effect dose	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
EATS-mediated	Developmental stage	203	African Clawed Frog	AMA	21 Days	Uptake from water	1.9 mg a.s./L	Decrease Stat sig	Decrease compared to control at day 21	Indication of delay in development	Evidence of delay in development stage and decrease in hind limb length. Remarkable effects also observed in histology treatment samples and not control.	T
	Hind limb length	203	African Clawed Frog	AMA	21 Days	Uptake from water	1.9 mg a.s./L	Decrease	Decrease compared to control at day 21 NF ≤ 60	Indication of decrease in hind limb length, NF ≤ 60		T

Effect	Effect target	Study ID	Species	Study type	Duration of exposure	Exposure route	Lowest Effect dose	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
	Thyroid histopathology (amphibian)	203	African Clawed Frog	AMA	21 Days	Uptake from water	1.9 mg a.s./L	Increase	Prevalence of thyroid hypoplasia/ atrophy (mild) in 2 tadpoles	Evidence of mild hypoplasia/ Atrophy. Other effects were observed in control and treatment groups.		T
Sensitive to, but not diagnostic of, EATS	Behaviour (amphibian)	203	African Clawed Frog	AMA	21 Days	Uptake from water	> 1.9 mg a.s./L	No effect	No indication		No evidence for treatment related effects. Spinal deformities observed in control and treatment groups at day 7 and 21.	N
	Body weight (amphibian)	203	African Clawed Frog	AMA	21 Days	Uptake from water	> 1.9 mg a.s./L	No effect (not stat. sig)	No indication			N
	Malformations (spinal deformities)	203	African Clawed Frog	AMA	21 Days	Uptake from water	-	Spinal Def	Spinal Def in control and treatments.	No indication		N
	Snout-vent length/growth	203	African Clawed Frog	AMA	21 Days	Uptake from water	> 1.9 mg a.s./L	No effect	No indication			N
Systemic toxicity	Mortality (amphibian)	203	African Clawed Frog	AMA	21 Days	Uptake from water	> 1.9 mg a.s./L	No effect	No indication		No evidence for systemic toxicity	-

AMA = Amphibian Metamorphosis Assay, T = Thyroid, N = Not assignable to a specific modality, - = not applicable, stat sig = statistically significant, NF = Nieuwkoop and Faber developmental stage. Spinal Def = Spinal deformities

Applicant comments for AMA study:

The applicant's discussion of the AMA study is shown below in italics.

'In this AMA study, a wide range of parameters was investigated. After 21 days the mean hind-limb length in tadpoles NF stage ≤ 60 was significantly reduced, which in itself does not indicate thyroid activity. Mean body weight at day 21 was reduced at the highest test concentration by 15 % for NF stage ≤ 60 tadpoles and by 18 % for NF ≥ 60 tadpoles, however the differences were not statistically significant. This reduction could indicate general toxicity. Thyroid gland histopathology for Xenopus laevis tadpoles after 21 day identified thyroid hypoplasia/atrophy in 2 tadpoles from one replicate of the highest treatment group.

A decreased follicular cell hypertrophy and thyroid gland hypoplasia/atrophy as observed in the highest treatment level (1.9 mg/L) are suggestive of reduced thyroid stimulating hormone (TSH) activity. Reductions in TSH can occur following administration of exogenous thyroid hormone, for example. However, administration of a thyroid hormone agonist (e.g., thyroxine) that causes follicular cell hypertrophy and thyroid gland hypoplasia/atrophy would also likely result in accelerated metamorphic development. The opposite response occurred in the 1.9 mg/L treatment level, in which development was delayed relative to controls. The hypoplasia/atrophy findings in two tadpoles of the highest treatment appear to be incidental as the findings were observed only in one replicate (replicate A) in which the tadpoles were significantly lighter than in the other replicates of the highest treatment, see Table B.9.2.3-7. In fact, the mean weight of the replicate was reduced by 54% compared to the mean of the control replicates.

Therefore, one cannot exclude confounding effects of general toxicity and it is presently unclear how to frame the results. Further data, e.g. repetition of the highest treatment of the experiment, may help interpret the results.

The examination of the thyroid modality and in particular the findings of thyroid gland hypoplasia/atrophy in two of 20 tadpoles from a single replicate of the highest treatment group could be interpreted as thyroid activity. However, the decreased development of the tadpoles indicates general toxicity, and the results of the terminated XETA study, as well as results obtained in mammalian toxicology studies are not in line with this finding and possibly even contradicting it. Therefore, based on the Xenopus laevis histopathology findings, a thyroid activity for BAS 684 H can at present not be concluded. Further data, such as a repeat experiment to confirm the presence or absence of thyroid activity, are deemed necessary for a robust conclusion.'

HSE ecotoxicology conclusion for aquatic organisms (T modality):

As stated both in OECD 231 and EFSA/ECHA guidance the following decision tree should be used to interpret the AMA screening test.

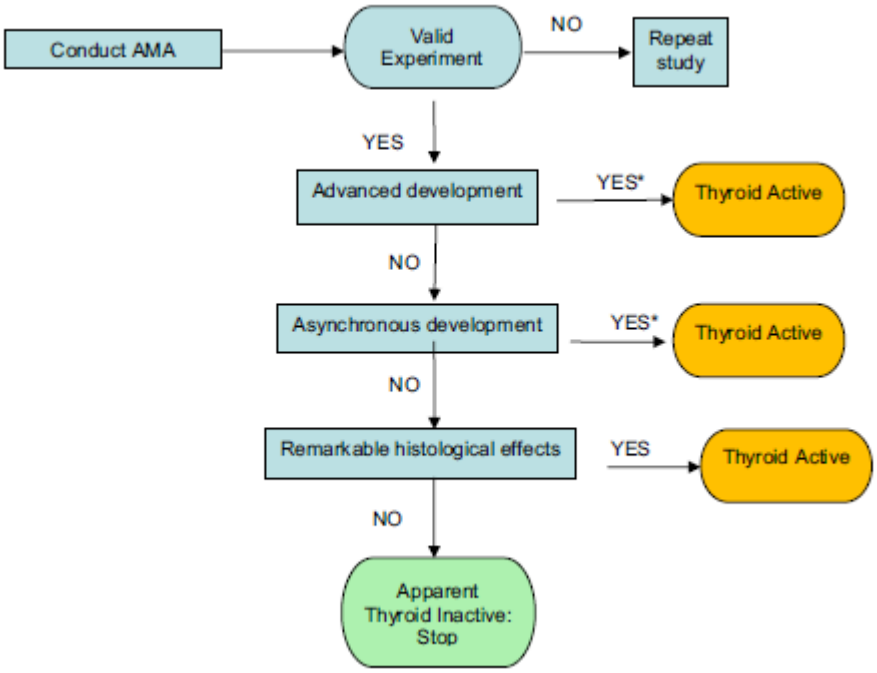


Figure B.9.2.3-1: Decision tree for evaluating thyroidal effects in AMA.

Using the above decision tree there is evidence of thyroid activity for cinmethylin based on histological analysis.

In addition based on the following section of OECD 150 conceptual framework the delayed development observed also requires consideration: '*According to OECD TG 231, there is disagreement about the implications of the different endpoints in this larval development screen. Some experts accept that changes in one of the thyroid-relevant apical endpoints (advanced development; asynchronous development; delayed development in absence of non-specific systemic toxicity) may on their own provide information on thyroid activity, while others will only reach this conclusion if one of the apical endpoints is accompanied by significant thyroid histopathology, such as moderate or severe follicular hypertrophy and/or hyperplasia (OECD, 2007). Note that the AMA is subject to indirect thyroid effects such as those that result from cytochrome P450 induction (e.g. phenobarbital, the model compound for the latter effect, tests positive in the AMA). Therefore, interpretation of the AMA may be complicated.*'

HSE has considered the results further below.

Whilst there were statistically significant effects at day 21 in terms of delayed development at highest tested concentration it was not clear when considering the development stage NF ranges at study termination (table B.9.2.3-6). Nonetheless, there is some evidence of delayed development at the highest treatment rate (1.9 mg a.s./L).

In terms of hind limb length, there was a statistically significant reduction compared to control in highest test concentration when considering tadpoles at NF ≤ 60. When considering the ranges (table B.9.2.3-8), the highest reduction is in replicate A, noting all replicates were outside the range of the control.

It was noted that spinal deformities occurred at comparable levels in control and two treatment groups (exception being highest test concentration). These did reach relatively high levels at day 7 in control (50 %) compared to 10 % at highest treatment rate. The occurrence dropped by day 21 to 27 %. There was no evidence of treatment related effects based on spinal deformities but there were relatively high levels in control during study.

The histological analysis demonstrated evidence of effects at highest test concentration that did not occur in control. Specifically mild (30 – 50 % of tissue) thyroid hypoplasia/atrophy (decrease in overall size of thyroid gland). As the applicant highlights this occurred in a single replicate at highest treatment

rate (1.9 mg a.s./L) for two tadpoles. It was noted there was also a relatively high reduction in body weight (same replicate) and statistically significant decrease in hind limb length at NF ≤ 60 (apical endpoints) at 1.9 mg a.s./L. No systemic toxicity was observed during the study.

HSE also notes that other histological effects were observed in the AMA but were present in both control and treatment groups. In the control data 8 of 20 tadpoles had mild (30 – 50 % of tissue) follicular cell hyperplasia, 13 of 20 mild (30 – 50 % of tissue) and 2 of 20 moderate (60 – 80 % of tissue) follicular cell hypertrophy. This raises some uncertainty about the ability of the study to detect treatment related effects for these other observations (e.g. follicular cell hypertrophy) when there were relatively high levels in control organisms, noting previously described spinal deformities also observed in controls. The applicant plans to provide historical control data to support the study.

In addition thyroid activity was highlighted in the mammalian toxicology data set (from ToxCast) that requires further consideration. It was noted that opposite effects to the AMA were observed i.e. increased thyroid weights rather than atrophy. Other effects observed in toxicology data included increased incidences of follicular cell hypertrophy/hypertrophy and/or altered colloid (see table B.9.1.5-3 for full details).

The study author conclusion based on histological analysis in the AMA was: '*Although only two of 20 tadpoles exhibited hypoplasia/atrophy, it cannot be excluded that this may be exposure-related. Further data (e.g., additional histopathology on preserved tadpoles from this experiment and/or a repeat experiment) may be needed to clarify if this effect is indeed treatment related or incidental.*'

HSE has discussed the AMA study with applicant. There are indications of thyroid endocrine activity following exposure to cinmethylin that require further consideration. The thyroid hypoplasia/atrophy observed may be caused by poor health (not treatment related), general toxicity or endocrine activity.

The applicant is providing further information that will be evaluated by HSE. This includes further histopathological analysis of tadpoles from study, mode of action analysis in-line with EFSA/ECHA endocrine disruption guidance 2018 and historical control data analysis (histopathology). Currently no further vertebrate testing is planned.

Overall HSE ecotoxicology conclusion for non-target organisms (endocrine disruption):

Overall, HSE concludes that based on current guidance that cinmethylin does not meet the criteria of being an endocrine disruptor (ED) for birds. For non-target wild mammals and aquatic organisms the **conclusion is pending following submission of further information by applicant**.

Endocrine disruption studies tested fish and amphibians.

Report:	CA 8.2.3/2 [redacted], 2020 Zebrafish (*Danio rerio*) - Short term reproduction assay, Flow through conditions 2019/2054638 (report no. CCO–007/4-46/A)
Guidelines:	OECD 229
GLP:	yes (certified by Ministerium fuer Arbeit, Gesundheit und Soziales des Landes Nordrhein-Westfalen Duesseldorf)
Deviations from current test guideline	No

II. MATERIAL AND METHODS

Test item:	BAS 684 H (Cinmethylin); purity 90.7 % (batch no. COD-002038)
Test species:	Zebrafish (*Danio rerio*) (reproductive adult fish were obtained from the [redacted]
Exposure design:	Continuous flow through exposure for 21-days in 25 L glass aquaria as test vessels
Endpoints:	Mortality, fecundity, wet weight by sex, plasma VTG by sex
Test concentrations Control;	0.12, 0.39 and 1.24 mg a.s./L (nominal). The highest test concentration (1.24 mg a.s./L) was chosen as a maximum tolerated concentration (MTC) based on the results of an early life stage (ELS) study. The chosen MTC is a factor of 2.2 higher than the ELS ([redacted], 2017a) growth LOEC, but was not expected to induce more than 10 % mortality.
Replication	2 replicates per test concentration, each with 5 female and 5 male fish
Test conditions:	Light: 12-h light / 12-h dark (ca. 1000 lux). Water temperature 26.0 °C ± 2.0 °C. Dissolved oxygen > 60 % air saturation. Mean pH value between 7.45 - 7.73 in test vessels. Daily feeding and observations of mortality, spawning, and behaviour.

Test procedure

Table B.9.2.3-11: Study timeline

Time of exposure	Phase	Course	Endpoints
Pre-exposure phase	Fish, Short term reproduction assay (FSTRA)	Start with 5 males & 5 females per vessel, daily introduction of spawning trays, feeding *ad libitum*, Determination of wet weight at test start	Mortality / fecundity
exposure		Transfer to exposure vessels	
Day 1-21		Daily introduction of spawning trays, feeding *ad libitum*, sex determination of dead fish	Mortality / fecundity
Day 21		End of in life phase, test termination, sex determination	Individual wet weight of all remaining fish / biomarker evaluation (VTG)

Test performance

At test start, five male and five female fish taken from the main batch were allocated to each of 10 aquaria as spawning groups where they were held for 43 days to record spawning success during the pre-treatment phase. Regular spawning was observed. The spawning groups for the main test were composed of randomised distribution of males and females (50 of each sex).

The weight of the fish at test start was determined from a subsample of five males and five females taken from a spawning group not chosen for the exposure period. Females were in the range of 0.65g ± 20 % and 0.4 g ± 20 % for males.

Animals were fed *ad libitum* with TetraMin® (Tetra Werke, Melle, Germany) and brine shrimp nauplii (*Artemia salina*).

Observed effect criteria

Observations of behaviour, general toxicity signs including hyperventilation, uncoordinated swimming, loss of equilibrium and atypical quiescence or non-feeding were recorded daily. Additionally, external abnormalities (such as haemorrhage, discolouration) were recorded. Daily egg counts were undertaken. After 21 days the fish were anesthetised with chloro-butanol and a blood sample was taken by cardia puncture. The fish were euthanized humanely by a dorsal cut according to the German Animal Welfare Act (2006). Afterwards the blotted wet weight was determined. Blood plasma was separated from the blood sample and measured for vitellogenin for both males and females. Fish heads were removed, weighted and frozen in liquid nitrogen to allow for a second determination of vitellogenin if necessary.

After termination all fish were placed in an appropriate fixative to enable an optional histopathological evaluation of the fish tissue.

Histotechnical processing and histopathology

The histological processing was performed at the test site BASF SE Experimental Toxicology and Ecology, 67056 Ludwigshafen, Germany. Paraplast blocks were prepared by the lab and are available should a histopathology evaluation be required at a later date since this is an optional requirement of OECD 229 (2012).

Chemical analysis/ analytical method

Analytical verifications of the test item concentrations were performed under GLP using LC-MS/MS (BASF analytical method L0361/01) on day 0, 8, 14 and 21 (test end).

Data evaluation and statistical analysis

All biological data were evaluated separately by sex to determine significant differences to control responses. All statistics were calculated using ToxRat® Professional 3.3. The Bonferroni-Welch t-test was used as the residuals of the ANOVA were normally distributed and treatment variances were not homogeneous. Prior to use of parametric procedures, results from tests of normality and homogeneity of variance were considered. Failure to confirm assumptions of normality and homogeneity of variance resulted in the use of a suitable non-parametric test for the data involved.

RESULTS

Analytical measurements

Mean concentrations of cinmethylin during the course of the study ranged from 73.0 % - 102.9 % of the nominal concentrations. At study initiation (day 0), the concentrations in samples from the highest treatment level were not within the desired range of 80 – 120 % of nominal values. Thus, the biological effects are based on mean measured concentrations (0.11, 0.37 and 1.17 mg a.s./L). HSE notes the use of mean measured concentrations is in accordance with OECD 23, for flow through test systems which was used in this study.

Table B.9.2.3-12: Mean concentrations of cinmethylin in [µg a.s./L] and [% of nominal] during the in-life phase of the study

Nominal concentration Cinmethylin [µg a.s./L]	Day	Measured concentration of cinmethylin		Mean measured concentration per test group [µg a.s./L]	
		[µg a.s./L] / test group	[%] / vessel		
		Mean	Mean	Mean	SD
Control	0	< LoQ	-	< LoQ	-
	8	< LoQ	-		
	14	< LoQ	-		
	21	< LoQ	-		
120	0	107.1	89.3	111.2	1.8
	8	110.0	91.7		
	14	118.6	98.8		
	21	109.1	90.9		
390	0	344.0	88.2	369.8	4.7
	8	383.2	98.3		
	14	365.7	93.8		
	21	382.5	98.1		
1240	0	905.8	73.0	1168.5	3.1
	8	1267.2	102.2		
	14	1276.5	102.9		
	21	1224.5	98.8		

[%] = Percentage of nominal concentration. LoQ = Limit of Quantification, - = not applicable or determined.

Biological results

No mortality or abnormal behaviour was observed during the in-life phase of the study. The parameters measured and results are shown in the table below.

Table B.9.2.3-13: Biological results during the in-life phase of the study

Parameters measured		Nominal concentration cinmethylin [mg a.s./L]			
		Control	**0.12**	**0.39**	**1.24**
		Mean measured concentration cinmethylin [mg a.s./L]			
		Control	**0.11**	**0.37**	**1.17**
Total egg no. per day and female [n] [A]	**Mean**	**49**	**59**	**62**	**45**
	SD	29	17	4	3
	RSD	60.1	29.3	6.3	7.6
	% CV	59.2	28.8	6.5	6.7
Wet weight males at study termination [mg] [A]	**Mean**	**0.451**	**0.445**	**0.415**	**0.450**
	SD	0.030	0.039	0.073	0.015
	RSD	6.6	8.7	17.6	3.4
Wet weight females at study termination [mg] [A]	**Mean**	**0.483**	**0.488**	**0.433**	**0.506**
	SD	0.030	0.009	0.007	0.028
	RSD	6.2	1.8	1.6	5.6
VTG / total protein [ng/µg]; males at study termination [A]	**Mean**	**0.04**	**0.04**	**0.04**	**0.04**
	SD	0.02	0.01	0.01	0.01
	RSD	46.4	24.0	40.0	42.3
VTG / total protein [ng/µg]; females at study termination [A]	**Mean**	**820.1**	**1122.8**	**423.5**	**758.2**
	SD	240.5	472.7	45.9	410.3
	RSD	29.3	42.1	10.8	54.1
	Range	650 – 990.2	788.5 – 1457.1	391.0 – 455.9	468.1 – 1048.4

SD = Standard deviation
RSD = Relative standard deviation
CV = Coefficient of Variation
VTG = Vitellogenin
[A]: No statistically significant difference between controls and treatments, Bonferroni-Welch t-test, one-sided smaller, $\alpha = 0.05$

Conclusion

Statistical evaluation revealed no significant effect of cinmethylin on reproduction in terms of total egg numbers, on wet weight of males and females and on VTG content in males and females.

HSE evaluator comments

The study was well reported and conducted predominantly in line with recommended guideline OECD 229 (2012).

It was noted that in this study the fish were euthanized humanely by a dorsal cut according to the German Animal Welfare Act (2006). However OECD 229 (2012) states that the fish should be euthanized with appropriate amounts of Tricaine, 100-500 mg/L buffered with 300 mg/L $NaHCO_3$ to reduce mucous membrane irritation. This deviation is considered acceptable by HSE.

Consideration of the relevant validity criteria is shown below.

Table B.9.2.3-14: Validity criteria in OECD 229 (2012)

Validity criteria according to OECD TG 229	**Obtained in this study:**	**Compliance**
The mortality should not exceed 10% in each control group.	0%	Yes
The dissolved oxygen should be at least 60 % of the air saturation value throughout the exposure period.	>90%	Yes
The water temperature did not differ by more than ± 1.5 °C between test vessels at any time during the test and should be within 26 ± 2 °C	25.7 °C - 26.6°C	Yes
Evidence that concentrations of test item in solution were maintained within ± 20% of the mean measured values	Slight deviation in highest treatment only; 73.0 % of nominal at study initiation	Acceptable deviation since overall mean measured values are used for discussing affects seen in the study, noting flow through study design
Evidence that fish were actively spawning in all replicates prior to initiation and in control replicates during the test	Pre-exposure: 50 eggs/female/day Test: 49 eggs/female/day	Yes

Statistical analysis

OECD 229 (2012) provides a decision flowchart for statistical analysis of data produced from this study. The study report indicates that tests were run to check whether the data showed normal distribution and homogeneity of variance and the resulting recommended test for each output variable was the Multiple Sequentially-rejective Welch-t-test After Bonferroni-Holm. Although this test is not specifically referenced in the schematic in OECD 229 (2012), HSE considers this to be acceptable.

Analytical verification

Effects are described in with reference to the mean measured test concentrations of the test substance which is the most accurate way of interpreting results from this study. Although the guideline states that all measured concentrations should be within 20 % of the mean measured values, HSE has the view that the above described slight deviation is acceptable since the calculated mean measured values take account of this deviation. Furthermore, the use of mean measured values is in-line with OECD 23 when considering flow through systems.

The Chemistry specialist evaluated the method of analysis used in this study (full evaluation of method at KCA 4.1.2/39) and concluded the following: *The method was considered fully validated in accordance with SANCO/3029/99 rev. 4 in tap water and M4-medium, under study CA 4.1.2/40, and has is shown be acceptably validated in support of the current study with an LOQ of 1.0 µg a.s./L.*

This study and results have been considered further in the endocrine disruption hazard assessment for cinmethylin (start of section B.9.2.3.).

Report:	CA 8.2.3/1 [redacted]. 2020a BAS 684 H - Amphibian Metamorphosis Assay with African Clawed Frog (*Xenopus laevis*)
Guidelines:	OECD 231 (2009)
GLP:	yes
Document No	BASF DocID 2020/2032686 (report no. 986.6337)
Deviations from guideline	Compared to OECD 231 (2009): Minor deviations - reduced feeding regime - inter-replicate differential pH of 0.6 instead of 0.5 (only once on day 16 in the control replicate D) These deviations did not have a negative impact on the results or interpretation of this study. Major deviations None

MATERIAL AND METHODS

Test item:	BAS 684 H (Cinmethylin) Purity: 90.7 % Batch No.: COD-002038 CAS No.: 87818-31-3
Test species:	African clawed frog *(Xenopus laevis)* stage 51 tadpoles Source: [redacted] Feeding: *Xenopus* Express tadpole food (Brooksville, Florida) Continuous flow through exposure for 21-days
Exposure design:	
Endpoints (observation time points)	Daily: mortality Day 7 and 21: developmental stage, Snout-Vent Length (SVL), hind limb length Day 21: thyroid gland histology and whole body wet weight
Test concentrations	Dilution water control (Iodide concentration 6.5 at test start and 7.0 µg/L at test end; within OECD 231 recommended range of 0.5 to 10 µg/L) Test item nominal: 0.019, 0.19 and 1.9 mg a.s./L Test item measured: 0.023, 0.21 and 1.9 mg a.s./L The highest test concentration (1.9 mg a.s./L) was chosen as a maximum tolerated concentration (MTC) based on 1/3 of a 7d-LC_{50} for *Xenopus laevis* (non GLP range finding toxicity test)
Exposure system	Consisted of an intermittent-flow proportional diluter, a temperature-controlled water bath and a set of 16 exposure aquaria. The exposure system was designed to provide three concentrations of the test substance and a dilution water control to four replicate test aquaria (flow through).

Test units

The test chambers (made of glass, silicone sealant) were 2.5 gallon exposure aquaria measuring 30 × 14.5 × 20 cm with a 12.5 cm high side drain that maintained a constant exposure solution volume of approximately 5.5 L. Flow-splitting cells were employed to equally distribute the solutions to the four replicate vessels for each concentration or control group at a rate of 250 mL of test solution per vessel per cycle. Flow splitting accuracy of the diluter cells was within ± 20 % of the targeted value.

Prior to exposure initiation, an FMI pump, in conjunction with a 200-L HDPE carboy, was calibrated to deliver approximately 32 mL/min (211 mL/cycle) of the 10 mg/L diluter stock solution to the diluter system's mixing chamber at each cycle. The stock solution flow rate, in combination with the calibrated dilution water cell (1000 mL/cycle), filled the mixing chamber at each cycle to a total volume of 1.110 L. After consultation with the Study Sponsor following pre-exposure analytical data, it was determined that the measured concentrations were slightly below expectations. Therefore, the decision was made on pre-exposure day 6 to intentionally increase the flow of stock solution to the mixing chamber by 25 %. Therefore, the FMI pump was recalibrated from 32 mL/min (209 mL/cycle) to 40 mL/min (264 mL/cycle). This was implemented to increase recoveries of the test substance in an effort to approximate target concentrations (i.e., to achieve 80 to 120% of the target concentration).

The mixing chamber was positioned over a magnetic stir plate and the solution was continuously stirred with a Teflon-coated stir bar throughout the test. The solution in the mixing chamber was equivalent to that of the highest targeted test concentration (1.9 mg a.s./L) and was proportionally diluted by a factor of 10 to produce the remaining targeted test concentrations (0.19 and 0.019 mg a.s./L).

A set of control vessels was also established in the same water bath which contained the same dilution water and was maintained under the same conditions as the treatment level vessels and contained no BAS 684 H. The exposure system was operating properly for 6 days prior to exposure initiation to allow equilibration of the test substance in the diluter apparatus and exposure aquaria.

The diluter delivered the control and test solutions to the exposure aquaria at a rate sufficient to provide approximately 10 aquarium volumes per 24-hour period.

Replication

4 replicates, each with 20 tadpoles

Test conditions:

Photoperiod: 12 h light/ 12 h dark
Light intensity: 650 - 1000 lux
Water temperature: 21.0 – 22 °C
Dissolved oxygen: 62 – 98 % of air saturation
pH range: 6.9 - 7.6

Experimental dates: 19 November to 10 December 2019

Pre-exposure phase

On pre-exposure day 0, three pairs of adult male and female *X. laevis* from the Smithers in-house culture were induced to breed. Each frog was provided a primary injection of human chorionic gonadotropin (hCG, 250 International Units (I.U.) for both males and females) and a secondary

injection in the afternoon (250 I.U. for males and 750 I.U. for females). Each pair was placed into a separate 20 L breeding aquarium containing a plastic false bottom, which allowed the egg masses to fall to the bottom of the aquarium. The embryos remained in their respective breeding aquaria until all viable embryos had hatched and all tadpoles had reached feeding stage (6 days post-fertilization). The spawn selected for use in the exposure yielded approximately 2500 embryos (minimum number of embryos criterion = 1500), and the embryo survival from this spawn was estimated at >90 %. One hundred tadpoles were transferred to each rearing tank for a larval density of 10 tadpoles/L. During this pre-exposure period, tadpoles were maintained under conditions similar to that of the actual exposure.

Definitive exposure phase

On pre-exposure day 14 (day 14 post-fertilization), most tadpoles had reached NF developmental stage 51 (Nieuwkoop and Faber, 1994). For selection, normal, healthy looking tadpoles were observed under a binocular dissection microscope to determine the developmental stage. Staging was conducted on each individual tadpole according to the normal table of *Xenopus laevis* (Nieuwkoop and Faber, 1994). Once an appropriate number of stage 51 tadpoles were collected, the tadpoles were randomly distributed to assigned test vessels.

Analytics:

Prior to the start of the definitive exposure, samples from replicates of each treatment level and the control, as well as the diluter stock solution were collected and analysed for cinmethylin. Results of the pre-test analyses were used to determine whether sufficient quantities of cinmethylin were being delivered and maintained in the exposure aquaria to initiate the assay.

During the in-life phase of the definitive study, exposure solution samples were removed at exposure initiation and weekly thereafter. On test day 0 (exposure initiation), analytical samples were removed from replicates A and B of each treatment level and the control. The analytical recovery in replicate A of the 0.019 mg/L treatment level at the day 0 interval was out of specification (280 % of targeted). Replicate A was replaced on day 2 by the Study Director in the following fashion: a clean, new replacement aquarium was filled with approximately 2 L of solution from the replicate B aquarium and the replicate A tadpoles were then carefully transferred to the new aquarium. On test day 7, analytical samples were removed from all replicates (A through D) of the 0.019 mg/L treatment level as a conservative measure to verify that the replicate A aquarium replacement on test day 2 provided acceptable recoveries. Recoveries of cinmethylin were within expectations on day 7. Analytical samples were removed from replicates C and D of the 0.19 and 1.9 mg/L treatment levels and the control.

Biological measurements

Day 7 measurements

On day 7 of the exposure, five tadpoles were randomly selected from each test vessel for growth and development metrics.

Tadpoles were euthanized with buffered MS-222 solution, rinsed with water, and gently blotted dry. Developmental stage (Nieuwkoop and Faber, 1994) was then determined for each tadpole using a binocular dissection microscope. Digital images were then taken of each tadpole for snout-vent length and hind limb length measurements. Whole body wet weight was then determined. Developmental stage and images were obtained using a Zeiss Stemi-2000 microscope and a Zeiss AxioCam ICc 5 camera. Calibrated Carl Zeiss Zen 2011 Blue Edition image analysing software Version 1.0.1.0 was used to measure hind limb and snout-vent length.

Day 21 (test termination) measurements

At test termination (day 21), the remaining tadpoles were removed from the test vessels and euthanized with buffered MS-222. Tadpoles were then removed from the euthanizing solution, rinsed with water, and gently blotted dry. Developmental stage (Nieuwkoop and Faber, 1994) was determined using a binocular dissection microscope. Digital images were taken of each tadpole for snout-vent length and

hind limb length measurements using the equipment and procedures described above. Each tadpole was then weighed to the nearest 0.1 mg.

Each tadpole was transferred to individually labelled storage containers labelled for identification. Each container was filled Davidson's fixative. Tadpoles remained submerged for approximately 72 hours. Samples were then rinsed with 70 % reagent grade ethanol and stored in 10 % neutral buffered formalin.

Thyroid Gland Histology

For histological analyses, five tadpoles were selected from each replicate at day 21 (20 per test concentration). Five tadpoles were selected, preferentially from NF stage 59. If five tadpoles were not at NF stage 59 in a replicate, NF stage 60 was selected. The histological processing and analyses were performed by Experimental Pathology Laboratories (EPL), Sterling, Virginia. The OECD Guidance Document on Amphibian Thyroid Histopathology (OECD, 2007) was used as the reference document for these analyses.

The following table depicts the observation timepoints for the primary endpoints.

Endpoints	Daily	Day 7	Day 21
Mortality	X		
Developmental Stage		X	X
Snout-Vent Length		X	X
Hind Limb Length		X	X
Whole Body Wet Weight		X	X
Thyroid Gland Histology			X

Additional information recorded

Dead animals were removed from the test tank and recorded when observed. Observations of abnormal behaviour, such as floating on the surface, lying on the bottom of the tank, irregular swimming or differences in food consumption, visible gross malformations, or lesions were also recorded.

Statistics:

The approaches taken by the study author for statistical analysis are provided in full below.

All statistical conclusions were made at the greater than 95 % level of rejection of the null hypothesis (H0: No difference from the control) except in the case of the basic assumption tests, e.g., Shapiro-Wilks' Test (normal distribution) and Bartlett's Test (homogeneity of variance) in which the 99 % level of rejection of the null hypothesis (H0: The observed distributions are normal and homogeneous) was applied. CETIS Version 1.9 (Tidepool Scientific Software, McKinleyville, California, USA) was used to perform statistical computations for survival, developmental stage, day 7 snout-vent length, whole body wet weight, and hind limb length normalized by snout-vent length. The following procedures were used for the above endpoints:

1. Monotonicity was empirically assessed from the treatment means for all apical endpoints. Reduced tadpole size and slight developmental retardation was observed during the 7-day preliminary range-finding exposure. Therefore, since reduced development and growth was expected in the definitive exposure, all endpoints were analysed using a one-tailed (C > T) test.

2. Developmental stage was analysed at day 7 and day 21 using a one-tailed (C > T) Jonckheere-Terpstra's Step-Down Test on the replicate medians.
   a. Developmental stage was also analysed at day 7 and day 21 using a one-tailed (C > T) multi-quantal Jonckheere-Terpstra's Step-Down Test from the 20^{th} to 80^{th} percentile to evaluate developmental stage effects among the distribution profile.

3. Day 7 hind limb length normalized by snout-vent length did not resemble a monotonic concentration response. Therefore, these endpoints were analysed using a one-tailed (C > T) Dunnett's

Multiple Comparison Test, a parametric procedure, on the replicate means. This endpoint data met the assumptions of normal distribution and homogeneity of variance using the assumption tests described above.

4. Day 21 survival did not resemble a monotonic concentration response. Therefore, the replicate means were analysed with a one-tailed (C > T) Fisher's Exact Test with Bonferroni-Holm Adjustment.

After NF stage 60, tadpoles show a reduction in size and weight due to tissue resorption and reduction of absolute water content. Therefore, day 21 snout-vent length, whole body wet weight, and hind-limb length normalized by snout-vent length measurements from NF stage 61 or 62 tadpoles cannot appropriately be used in statistical analyses for differences in growth rates. There were an increased number of tadpoles that showed development beyond NF stage 60 (i.e., ≥ 20 %) in the 0.21 mg/L treatment level (23 %). There were also an appreciable number of late stage (i.e., >NF stage 60) tadpoles in the control (18.3 %). Therefore, a two-factor analysis of variance (ANOVA) with a nested variance structure was used to assess the above endpoints while taking the effect of late stage development into account (OECD, 2009). Statistical Analysis Software Version 9.4 (SAS, 2012) was used to perform statistical computations on day 21 snout-vent length, whole body wet weight, and hind-limb length normalized by snout-vent length endpoints. The following procedures were used:

1. Shapiro-Wilks' Test for normality was conducted to evaluate the distribution of the data. For this study, snout-vent length, wet weight, and hind limb length normalized by snout-vent length data were not normally distributed. Therefore, a Blom normalized rank-order transformation of the data was used when treatment data were evaluated based on the recommendation in Annex 3 of OECD 231 (OECD, 2009; Blom, 1958).

a. The Blom normalized rank-order transformation of the wet weight data caused the transformed weight data to fail homogeneity of variance. Therefore, the two-factor ANOVA with nested variance structure was determined not to be appropriate. Therefore, the day 21 whole body wet weight data were analysed using CETIS. Late stage and non-late stage treatment whole body wet weights were compared to the respective control data separately.

2. Levene's Equality of Variance Test was conducted to evaluate the homogeneity of the data. For this study, transformed snout-vent length and hind limb length normalized by snout-vent length data met the assumption of homogeneity of variance.

3. The results from the SLICE procedure were evaluated for concentration-related effects within the late stage and non-late stage groups. Where the corresponding F-slice was not significant ($p > 0.05$), the Bonferroni-Holm's corrected p-value was used to evaluate treatment effects. Normalized hind limb length of late stage tadpoles and snout-vent length of both late and non-late stage tadpoles utilized this procedure. If the F-slice was significant ($p < 0.05$), the uncorrected p-value from the individual contrasts was used to evaluate treatment effects. Normalized hind limb length of non-late stage tadpoles utilized this procedure.

4. Individual contrasts were performed to compare the growth response in each concentration against the control within the individual groups (i.e., late stage and non-late stage).

Histopathology results were also statistically analysed using CETIS Version 1.9 (Appendix 9). The following procedures were used:

- Thyroid follicular cell hyperplasia and follicular cell hypertrophy findings were analysed using the Rao-Scott Cochran-Armitage by slices (RSCABS; Green et al., 2014).

- Thyroid hypoplasia/atrophy findings exhibited zero variance in at least one treatment or control group. Therefore, thyroid hypoplasia/atrophy was analysed using the Cochran Armitage Trend Test.

- Increases in incidence/severity are typically associated with increasing test substance concentrations. Therefore, a one-tailed test (C < T) was used for thyroid follicular cell hyperplasia and thyroid hypoplasia/atrophy. However, based on the biological link of the

hypoplasia/atrophy findings to the follicular cell hypertrophy findings, a one-tailed test (C > T) was used for thyroid follicular cell hypertrophy.

RESULTS

Biological results:

Tadpoles in all treatment levels and the control exhibited what is characterized as normal behaviour throughout the exposure period. No noticeable differences in food consumption between treatments were observed. Also, no gross malformations or lesions were observed.

Spinal deformities

On day 7 spinal deformities (i.e., scoliosis, bent tail) were observed in 50 % of control animals and in 55, 55, and 10 % of tadpoles exposed to the 0.023, 0.21, and 1.9 mg/L treatment levels, respectively. On day 21 spinal deformities were observed in 27 % of control animals and in 26, 15, and 8 % of tadpoles exposed to the 0.023, 0.21, and 1.9 mg/L treatment levels, respectively. For the entire exposure spinal deformities were observed for 33 % of control animals and for 33, 25, and 9 % of tadpoles exposed to the 0.023, 0.21, and 1.9 mg/L treatment levels, respectively. The study author stated that the spinal deformities did not impact any endpoint collected for this assay or growth/survival of tadpoles and was not attributed to cinmethylin exposure.

Larval survival (indicative of a non-specific toxic effect)

Following 21 days of exposure, survival averaged 100 % for controls. The average percent survival for tadpoles exposed to the 0.023, 0.21, and 1.9 mg a.s./L treatment levels was 100, 98, and 100 %. There was no significant reduction in day 21 larval survival among tadpoles exposed to any of the treatment levels tested compared to the control.

Endpoints Sensitive to, but Not Diagnostic of, the Thyroid Modality

Snout-Vent Length (SVL) and Whole-Body Wet Weight

The statistical analysis of SVL and whole-body wet weight data suggest that *Xenopus laevis* tadpole growth is not impacted by cinmethylin exposure although non-significantly reduced at the highest test concentration (see Table B.9.2.3-16 below).

Endpoints Indicative of the Thyroid-Mediated Modality

Developmental Stage

The day 21 developmental stage distribution profile was analysed by applying the multi-quantal Jonckheere-Terpstra's Step-Down Test to the 20^{th} through the 80^{th} percentiles for all treatment levels compared to the control. The overall multi-quantal procedure determined a significant reduction in day 21 percentile developmental stage at 1.9 mg/L and corroborated the standard comparison test result for day 7 developmental stage. The 10^{th} and 90^{th} percentile of day 21 developmental stage distribution was 3.1 stages (maximum allowable spread $\leq$ 4 stages).

Hind Limb Length Normalized by Snout-Vent Length

No significant reduction in day 7 or day 21, Nieuwkoop and Faber (NF) stage >60 tadpoles was shown for hind limb length normalized by SVL among tadpoles exposed to any of the treatment levels tested compared to the control. A significant difference in day 21 hind limb length normalized by SVL for NF stage $\leq$ 60 tadpoles exposed to the 1.9 mg/L treatment level compared to the control as indicated in the tables below.

Table B.9.2.3-15: Bioassay for *Xenopus laevis* tadpoles after flow-through exposure to cinmethylin

Parameters		Targeted concentration [mg a.s./L] Control	0.019	0.19	1.9
		Mean measured concentration [mg/L] Control	0.023	0.21	1.9
No of replicates		4	4	4	4
Survival [%]	Day 21	100	100	98	100
Median developmental stage Range shown in brackets	Day 7	54 (53-54)	54 (53-54)	54 (53-54)	54 (53-54)
	Day 21	60 (57-61)	59 (57-62)	59 (57-61)	59 (57-61)

Table B.9.2.3-16: Endpoints for *Xenopus laevis* tadpoles after flow-through exposure to cinmethylin

Endpoints

Parameters			Targeted concentration [mg a.s./L] Control	0.019	0.19	1.9
			Mean measured concentration [mg a.s./L] Control	0.023	0.21	1.9
Mean hind limb length [mm] a)	Day 7		0.127	0.123	0.127	0.124
	Day 21	NF ≤ 60	0.693	0.697	0.647	0.537*
		NF ≥ 61	1.048	1.050	1.040	0.880
Mean snout-vent length [mm]	Day 7		16.86	16.94	17.38	16.27
	Day 21	NF ≤ 60	24.01	23.63	23.17	22.55
		NF ≥ 61	19.44	18.53	17.57	17.88
Mean whole body wet weight [g]	Day 7		0.3803	0.3812	0.4051	0.3308
	Day 21	NF ≤ 60	1.0612	1.0225	0.9519	0.8381
		NF ≥ 61	0.7907	0.7791	0.6661	0.6416

a) Normalized by Snout-Vent Length (SVL)
***** statistically significant difference between controls and treatments determined, using a two-factor ANOVA with nested variance structure

Based on statistical analysis there was a significant effect on hind limb length at day 21 for NF ≤ 60 tadpoles, noting lengths were normalized by SVL. It is unclear that normalisation by SVL is required in OECD 231. Therefore, HSE has provided the raw data for replicates (Hind Limb Length and whole wet body weight) in the table below.

Table B.9.2.3-17: Hind Limb Length and whole body wet weight following 21-day flow-through exposure to cinmethylin of tadpoles NF stage ≤ 60

Mean Measured Concentration (mg a.s./L)	Replicate ID	Hind Limb Length (mm)			Whole Body Wet Weight (g)		
		Mean	Median	SD	Mean	Median	SD

Mean Measured Concentration (mg a.s./L)	Replicate ID	Hind Limb Length (mm)			Whole Body Wet Weight (g)		
		Mean	Median	SD	Mean	Median	SD
Control	A	15.01	15.39	3.41	0.9951	0.9877	0.1620
	B	18.44	18.24	2.52	1.1532	1.0996	0.3033
	C	15.72	15.53	2.31	1.2121	1.1918	0.2190
	D	16.64	16.51	1.94	0.8843	0.8691	0.1911
	Mean	**16.45**	**16.42**	**1.483**	**1.0612**	**1.0370**	**0.1493**
0.023	A	16.72	16.61	3.06	1.0596	1.1508	0.2169
	B	15.94	15.73	3.00	0.9251	0.9751	0.1223
	C	14.36	14.23	3.05	0.8354	0.7954	0.1519
	D	18.01	18.09	2.09	1.2699	1.3055	0.2702
	Mean	**16.26**	**16.16**	**1.526**	**1.0225**	**1.0567**	**0.1889**
0.21	A	13.87	14.24	2.17	0.8340	0.7973	0.1558
	B	15.45	14.35	3.55	1.1538	1.1849	0.1449
	C	13.00	13.20	3.40	0.5453	0.5119	0.1036
	D	17.24	17.01	1.68	1.2744	1.2210	0.2235
	Mean	**14.89**	**14.70**	**1.865**	**0.9519**	**0.9288**	**0.3286**
1.9	A	9.41	9.23	2.41	0.4984	0.5146	0.0718
	B	13.43	13.04	2.84	1.0203	1.0333	0.1533
	C	11.68	12.63	2.99	0.6418	0.6575	0.1347
	D	14.00	14.35	2.17	1.1920	1.1712	0.1889
	Mean	**12.129**	**12.313**	**2.067**	**0.8381**	**0.8442**	**0.3227**

SD = Standard Deviation
CV = Coefficient of Variation

Thyroid gland histology

The histopathology analysis was conducted in-line with OECD 231 guideline (and separate guideline for histopathology OECD 2007). Samples should be graded according to 4 levels with 0 being no remarkable effects in accordance with guidance. In this study remarkable effects were graded in-line with guidance i.e. Grade 1 ('mild' with 30 – 50 % of tissue affected), Grade 2 ('moderate' with 60 – 80 % of tissue affected) and Grade 3 ('severe' with > 80 % of tissue affected).

Histopathologic findings in the thyroid glands are shown in the table below.

Table B.9.2.3-18: Thyroid Gland Histology for *Xenopus laevis* tadpoles after 21-day flow-through exposure to cinmethylin

Cinmethylin Treatment Group (mg a.s./L)		0.0 (control)					0.023					0.21					1.9				
Replicate		A	B	C	D	T[a]	A	B	C	D	T	A	B	C	D	T	A	B	C	D	T
Number examined		5	5	5	5	**20**	5	5	5	5	**20**	5	5	5	5	**20**	5	5	5	5	**20**
Follicular cell hyperplasia	Mild	3	2	2	1	**8**	3	3	0	3	**9**	1	2	0	4	**7**	0	3	2	3	**8**
Follicular cell hypertrophy	Mild	4	4	3	2	**13**	5	4	1	4	**14**	1	4	4	3	**12**	0	4	0	4	**8**
	Moderate	0	1	0	1	**2**	0	0	0	1	**1**	0	1	0	2	**3**	0	1	0	1	**2**
Thyroid hypoplasia/atrophy	Mild	0	0	0	0	**0**	0	0	0	0	**0**	0	0	0	0	**0**	2	0	0	0	**2**
Follicular lumen area (previously colloid area)		0	0	0	0	**0**	0	0	0	0	**0**	0	0	0	0	**0**	0	0	0	0	**0**

Colloid quality	0 0 0 0 **0**	0 0 0 0 **0**	0 0 0 0 **0**	0 0 0 0 **0**

[a] T = Total

Analytical results

Table B.9.2.3-19: Analytical verification of test item during the study

Target Concentration (mg/L)	Measured Concentration (mg/L)				Mean Measured Concentration (SD)[e]	Percent of Target (%)[e]	% CV[e]
	Day 0[a]	Day 7[b]	Day 14[c]	Day 21[d]			
Control	<0.0040[f] <0.0040	<0.0013 <0.0013	<0.0013 <0.0013	<0.0013 <0.0013	NA[g] (NA)	NA	NA
0.019	0.053 0.019 NA NA	0.021 0.020 0.020 0.019	0.018 0.018 NA NA	0.019 0.018 NA NA	0.023 (0.011)	120	48
Mean	**0.034**	**0.020**	**0.018**	**0.019**			
0.19	0.21 0.20	0.21 0.21	0.20 0.19	0.22 0.19	0.21 (0.011)	110	5.6
Mean	**0.20**	**0.21**	**0.20**	**0.21**			
1.9	1.7 1.8	2.0 1.9	1.8 1.7	2.2 1.9	1.9 (0.17)	98	9.3
Mean	**1.7**	**2.0**	**1.7**	**2.1**			

[a] Analytical samples were removed from replicates A and B of each treatment level and the control. Analytical recovery in replicate A of the 0.019 mg/L treatment level at the day 0 interval was out of specification (>200% of target).
[b] Analytical samples were removed from all replicates (A through D) of the 0.019 mg/L treatment level to verify that the aquarium replacement on test day 2 provided acceptable recoveries. Analytical samples were removed from replicates C and D of the 0.19 and 1.9 mg/L treatment levels and the control.
[c] Analytical samples were removed from replicates A and B of each treatment level and the control.
[d] Analytical samples were removed from replicates C and D of each treatment level and the control.
[e] Mean measured concentration, standard deviations (SD), percent of target, and % coefficient of variation (CV) were calculated using the actual (unrounded) analytical results and not the rounded values (two significant figures) presented in this table.

HSE evaluator Comments

The study was well reported and conducted in line with OECD 231 (2009). The performance criteria outlined in the guideline have been evaluated with regards to acceptability and are presented below.

Performance criteria according to OECD 231 (2009)

Criterion	**Acceptable limits**	**Study Performance**	**Criterion Met (Yes/No)**
Test concentrations	Maintained at ≤ 20 % CV (variability of measured test concentration) over the 21-day test	CV values ranged from 5.6 to 41%.	No[a]
Mortality in controls ≤10 % - mortality in any one replicate in the controls should	≤10% - mortality in any one replicate in the controls should not exceed 2 tadpoles	No mortality was observed in any of the control replicates.	Yes

Minimum median developmental stage of controls at end of test 57	57	At test termination, the median developmental stage in the control was 60.	Yes
Spread of development stage in control group	The 10th and the 90th percentile of the development stage distribution should not differ by more than 4 stages	The difference between the 10th and 90th percentile of the developmental stage in the controls was 3.1 stages.	Yes
Dissolved Oxygen	≥ 40 % air saturation*	Dissolved oxygen was maintained between 62 to 98 % of air saturation during the study.	Yes
pH	pH should be maintained between 6.5-8.5. The inter-replicate/ inter-treatment differentials should not exceed 0.5.	6.9 to 7.6; inter-replicate and inter-treatment differentials were maintained at <0.5 pH units between days for the majority of the exposure. On test day 16, the pH inter-replicate differential for replicate D of the control group slightly exceeded 0.5 with a differential of 0.6 pH units. There were no sub-lethal effects or indications of stress observed in any tadpole on the days these slight pH fluctuations occurred.	No[b]
Water temperature	22 ± 1 ºC - the inter-replicate/inter-treatment differentials should not exceed 0.5 ºC	Water temperature was maintained between 20 and 22 °C; inter-replicate/inter-treatment did not exceed a difference of 0.5 °C.	Yes
Test concentrations without overt toxicity	≥ 2	No test concentrations had overt toxicity.	Yes
Replicate performance	≤ 2 replicates across the test can be compromised	No mortality was not observed in any of the treatments or control replicates.	Yes

[a] Analytical recovery in replicate A of the 0.019 mg/L treatment level at the day 0 interval was out of specification (>200% of target). Based on the acceptable recovery in the adjacent aquarium (0.019 mg/L replicate B), the replicate A aquarium was replaced as a conservative measure on test day 2 by the Study Director.

[b] The pH of the exposure solutions was maintained within the 6.5 to 8.5 criterion in all aquaria on all exposure days. The slight pH deviation is not considered to have been detrimental to the outcome of the study since it was short lived.

Validity Criteria according to OECD 231 (2009)

Criterion	Acceptable Limits	Study Performance	Criterion Met (Yes/No)
Treatment/Control Mortality	For any given treatment (including controls), mortality should not exceed 10 %. For any given replicate, mortality should not exceed three tadpoles	All replicate mortality <10 % and ≤ 1 tadpole	Yes
Treatment levels analysed	At least two treatment levels, with four uncompromised replicates, will be used	All replicates uncompromised; no abnormal behaviour or gross malformations in	Yes

	for analysis	any replicate	
Test concentrations (non-control) without overt toxicity	≥2	No test concentrations had overt toxicity	Yes

The feeding regime recommended in the guideline was not adhered to for the 0.023 mg/L treatment. For replicate C in this treatment, since a non-toxicant related mortality occurred on day 3 reduced daily food rations were provided. The study reports that this reduction is based on the laboratory's experience with using the species when performing this study which is considered to be an acceptable deviation by HSE and animals in this replicate continued to develop as expected within close ranges of the remaining three replicates with unaltered diets. In addition, the food recommended in the guideline was not used in this study (Sera Micron ®). Instead, *Xenopus* Express tadpole food (Brooksville, Florida) was used. This is not considered to have had a detrimental effect on the study and results since validity criteria were all met and control organisms performed as required.

It was noted that the analytical recovery in replicate A of the 0.019 mg/L treatment level at the day 0 interval was out of specification (>200% of target). Based on the acceptable recovery in the adjacent aquarium (0.019 mg/L replicate B), the replicate A aquarium was replaced as a conservative measure on test day 2 by the Study Director. HSE considers this acceptable as it occurred at test initiation and therefore the study organisms were exposed to the concentration with acceptable recovery (within 20 % of nominal) for the majority of the study. In addition, growth stages of this replicate did not deviate from other replicates in this test concentration throughout the study and all measured output variables were within the ranges of other replicates in this test concentration.

Statistical analysis

The statistical analysis outlined in the summary above appear to be mostly in line with the methods recommended in OECD 231 (2009) with deviations being thoroughly justified and considered to be based on experience by the laboratory in data analysis and therefore acceptable. Transformation of data was performed where data was not normally distributed, and variances were not homogeneous.

Analytical verification

Concentrations of the test substance were not maintained within 20 % of the nominal values and mean measured values were calculated which is considered to be the correct approach.

The Chemistry specialist evaluated the method of analysis used in this study (full method evaluation at KCA 4.1.2/39) and concluded the following: *The method was considered fully validated in accordance with SANCO/3029/99 rev. 4 in tap water and M4-medium, under study CA 4.1.2/40, and is shown be acceptable in support of the current study with an LOQ of 1.0 µg/L.*

This study and results have been considered further in the endocrine hazard assessment for cinmethylin (start of section B.9.2.3.).

B.9.2.4. Acute toxicity to aquatic invertebrates

B.9.2.4.1 Active substance: Cinmethylin:

Report: CA 8.2.4.1/1
Forbis A. *et al*., 1983 c
Acute toxicity of SD95481 to *Daphnia magna*
CI-521-001
Guidelines: None referenced
GLP: No

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Cinmethylin (SD 95481, Reg.no.: 900 202), batch no. 5-4-0-0, purity: 92 %.

B. STUDY DESIGN

Test species: Water flea (*Daphnia magna*), neonates collected from in house culture; first instar, < 24 h old at test initiation.

Test design: Static system (48 hours), 5 test item concentrations plus control and solvent control (acetone); 2 replicates with 10 daphnids each; assessment of immobility and adverse effects after 24 and 48 hours.

Endpoints: EC_{50} based on mobility of daphnids.

Test concentrations: Control (0), solvent control (0), 1.0, 1.8, 3.2, 5.6, 10 mg a.s./L, corresponding to mean measured concentration of 0 (control), 0 (solvent control), 1.0, 1.8, 3.0, 5.2 and 9.5 mg a.s./L.

Test conditions: 250 mL glass beakers, test volume 200 mL, dilution water: ABC well water; pH 8.0 – 8.6; oxygen concentration: 8.6 – 9.0 mg/L; temperature: 19 °C; total hardness: 255 ppm (dilution water); photoperiod: 16 h light: 8 h dark; light intensity: 50 – 70 fc (foot candles, determined by HSE evaluator to be approximately equivalent to 500 – 700 lux);

Analytics: Analytical verification of test item concentrations was conducted using an HPLC-method with UV detection.

Statistics: Descriptive statistics; Probit, binomial and moving average angle analysis for determination of EC_{50} values.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each test item concentration at the beginning of the test and at the end of the test. The concentration of cinmethylin in the freshly prepared test solutions ranged from 87 to 102 % of nominal. After 48 h the concentration of cinmethylin in the test solutions ranged between 95 and 107 % of nominal concentrations. It was not stated in the study report whether precipitation of the test item was assessed.

The results are shown in the table below:

Table B.9.2.4.1-1: Measured concentrations during study

Nominal (mg a.s./L)	0 hours		48 hours	
	Measured concentration (mg a.s./L)	% of nominal	Measured concentration (mg a.s./L)	% of nominal
0	na	na	na	na
1.0	1.02	102.0	1.07	107.0
1.8	1.69	93.9	1.85	102.8
3.2	2.79	87.2	3.13	97.8
5.6	4.89	87.3	5.58	99.6
10	9.44	94.4	9.52	95.2

na = not applicable

Validity criteria:

This study was conducted before the most recent OECD guideline. Nonetheless in order to determine whether the study is valid the criteria in OECD 202 (2004) have been considered below:

- In the control, including the control containing the solubilising agent, not more that 10 percent of the daphnids should have been immobilised. Obtained: 0 %
- The dissolved oxygen concentration at the end of the test should be ≥ 3 mg/l in control and test vessels. Obtained: minimum of 8.6 mg/l.

The above validity criteria were met during the study.

Biological results: At 24 hours, 75 % of the *D. magna* exposed to 9.5 mg a.s./L, the highest concentration tested, were immobilised. At 48 hours, 5 and 90 % were dead in test item concentrations of 5.2 and 9.5 mg a.s./L, respectively. Results are summarised in Table B.9.2.4.1-2 below.

Table B.9.2.4.1-2: Effects of cinmethylin on *Daphnia magna* mobility

Concentration (nominal) [mg a.s./L]	**Control**	**Solvent control**	**1.0**	**1.8**	**3.2**	**5.6**	**10**
Concentration (mean measured) [mg a.s./L]	**--**	**--**	**1.0**	**1.8**	**3.0**	**5.2**	**9.5**
Mortality (24 h) [%]	0	0	0	0	0	0	75
Mortality (48 h) [%]	0	0	0	0	0	5	90
Behavioural observations	N	N	N	N	4OB	2OB	2OB
Endpoints [mg a.s./L] (mean measured)							
EC_{50} (48 h)	**7.2 (95 % confidence interval: 6.5 – 8.0)**						
$NOEC_{mortality}$ (48 h)	3.0						

N = Normal, OB = On bottom of test vessel during observations.

III. CONCLUSION

In a 48-hour static acute toxicity study with *Daphnia magna*, the EC_{50} of cinmethylin was determined to be 7.2 mg a.s./L based on mean measured concentrations. The NOEC was determined to be 3.0 mg a.s./L.

HSE evaluator comments:

It was noted that the test concentration was maintained within ± 20 % of nominal concentrations. Therefore, the endpoints could have been based on nominal concentrations. However, the HSE evaluator considers the use of mean measured concentrations acceptable, noting they are marginally more conservative than nominal values.

The study was not conducted to GLP and the analytical method was not sufficiently validated in accordance with SANCO/3029/99 (see volume 3, CA, section B5 for full details). The following deficiencies were noted:

i) It is not possible to accept the linearity of the method as the example chromatograms for the highest concentration of the linear range show overloading of the sample, with the detector being saturated giving a broad flat top peak.
ii) To assess accuracy and precision, at least 5 recoveries should be provided for at least 2 fortification levels.
iii) The LOQ is not supported by 5 recovery determinations.
iv) Procedural recoveries have not been completed.

Therefore, this study has not been considered further in the risk assessment section.

Report: CA 8.2.4.1/2
Haerthe N., 2016 a
Acute toxicity of BAS 684 H (Cinmethylin) to *Daphnia magna* STRAUS in a 48-hour static test
2016/1001943
Guidelines: OECD 202, EPA 850.1010 draft April 1996
GLP: Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Cinmethylin (Reg. no. 900 202), batch no. COD - 002038; purity: 93 ± 1.0 %.

B. STUDY DESIGN

Test species: Water flea (*Daphnia magna* STRAUS), neonates collected from in house culture; >2 < 24 hours old at test initiation.

Test design: Static system (48 hours), 5 test item concentrations plus control, 4 replicates with 5 daphnids in each; assessment of immobility after 24 and 48 hours.

Endpoints: NOEC and EC_{50} based on immobility of daphnids.

Test concentrations: Control, 1.0, 1.8, 3.2, 5.6 and 10 mg a.s./L (nominal).

Test conditions: Glass vessels, test volume 50 mL, dilution water "M4" (Elendt medium); pH 7.99 – 8.08; oxygen concentration: 8.19 – 8.51 mg/L; hardness: 2.58 mmol/L (test initiation); conductivity: 651 µS/cm (test initiation); temperature: 20 – 21 °C; photoperiod: 16 h light: 8 h dark; light intensity: 480 – 510 lux; no feeding, no aeration.

Analytics: Analytical verification of test item concentrations was conducted using a UHPLC-method with MS detection.

Statistics: Descriptive statistics, Fisher's Exact Binominal Test with Bonferroni Correction for determination of the NOEC ($\alpha = 0.05$, one-sided greater). Probit analysis for determination of the EC_{50} value

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of cinmethylin was carried out of all test concentrations at the beginning and at the end of the test. Measured values at test initiation ranged from 84 – 91 % and at test termination 81 – 86 % for cinmethylin of nominal. It was not stated in the study report whether precipitation of the test item was assessed.

The results are shown in the table below:

Table B.9.2.4.1-3: Measured concentrations during study

Nominal (mg a.s./L)	0 hours		48 hours	
	Measured concentration (mg a.s./L)	% of nominal	Measured concentration (mg a.s./L)	% of nominal
0	< LoD	na	< LoD	na
1.0	0.907	91	0.822	82
1.8	1.59	88	1.53	85
3.2	2.82	88	2.68	84
5.6	5.15	92	4.82	86
10	8.41	84	8.06	81

na = not applicable, LoD = 0.002 mg a.s./L

Validity criteria:

In order to determine whether the study is valid the criteria in OECD 202 (2004) have been considered below:

- In the control, including the control containing the solubilising agent, not more that 10 percent of the daphnids should have been immobilised. Obtained: 0 %
- The dissolved oxygen concentration at the end of the test should be ≥ 3 mg/l in control and test vessels. Obtained: minimum of 8.19 mg/l.

The above validity criteria were met during the study.

Biological results: After 48 h of exposure, no immobility of daphnids was observed in the control and at test item concentrations of up to and including 3.2 mg a.s./L, whereas, 15% immobility was observed at the test item concentrations of 5.6 mg a.s./L. At the highest test item concentration, 90% of the daphnids were immobile after 48 hours of exposure. Statistically significant effects on mobility of daphnids were detected at the highest test item concentration. (Fisher's Exact Binomial Test with Bonferroni Correction, $\alpha = 0.05$, one-sided greater). For results see Table B.9.2.4.1-4. Behavioural observations were not recorded during the study.

Table B.9.2.4.1-4: Effects of BAS 684 H on *Daphnia magna* mobility

Concentration (nominal) [mg a.s./L]	**Control**	**1.0**	**1.8**	**3.2**	**5.6**	**10**
Immobility (24 h) [%]	0	0	0	0	0	80*
Immobility (48 h) [%]	0	0	0	0	15	90*
	Endpoints [mg a.s./L] (nominal)					
EC_{50} (48 h)	7.26 (95 % confidence limits: 6.34 – 8.34)					
NOEC (48 h)	3.2#					

* Statistically significant differences compared to control (Fisher's Exact Binomial Test with Bonferroni Correction, $\alpha = 0.05$, one-sided greater).

\# NOEC value was set to 3.2 mg a.s./L based on expert judgement.

Reference item test: A reference study was conducted with potassium dichromate. The 24-hour EC_{50} was calculated as 1.23 mg reference item/L which is within the range stated in OECD 202 i.e. 0.6 to 2.1 mg/L.

III. CONCLUSION

In a 48-hour static acute toxicity study with *Daphnia magna*, the EC_{50} of cinmethylin was determined to be 7.26 mg a.s./L based on nominal concentrations. The NOEC was determined to be 3.2 mg a.s./L (nominal).

HSE evaluator comments:

The above study was conducted to GLP and considered valid. Furthermore, the analytical method was sufficiently validated in accordance with SANCO/3029/99, LOQ of 0.01 mg/L (see volume 3, CA, section B5 for full details).

The following endpoint will be considered in the risk assessment:

- 48-hour EC_{50} = **7.26 mg a.s./L** (based on nominal)

The following study (Pearson and Stephenson, 1987a) tested a total of four invertebrate species. The study summary has been divided into four detailing each test species separately below.

Report:	CA 8.2.4.2/1 Pearson N.,Stephenson R.R., 1987 a WL95481: Acute toxicity to *Gammarus pulex*, *Lymnaea stagnalis*, *Tubifex* and *Chironomus lugubris* CI-521-006
Guidelines:	EPA 540/9-82-024
GLP:	Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test item:	Cinmethylin (WL95481, Reg. No. 900 202), WRC Tox. Sample No.: 513F; purity: 91.9 – 93.1 %.

B. STUDY DESIGN

Test species:	Amphipod crustacean *Gammarus pulex* (Linnaeus); > 1 < 2 mm; maintained in-house; wild catch (stream in Hollingbourne, Kent).
Test design:	Static system (96 hours), 5 test item concentrations plus a control; solvent (acetone: 1.0 ml/L) in each concentration plus control; 3 replicates with 10 gammarids in each; assessment of mortality after 24, 48, 72 and 96 hours.
Endpoints:	LC_{50} based on mortality of gammarids.
Test concentrations:	Control, 1, 2, 5, 10 and 20 mg a.s./L (nominal).
Test conditions:	350 mL glass crystallizing dished, test volume 300 mL, dilution water dechlorinated mains water; pH 8.0 – 8.4; oxygen concentration: 8.2 – 9.7 mg/L; temperature: 17.3 – 18.1 °C; total hardness of dilution water: 142 mg $CaCO_3$/L.
Analytics:	Analytical verification of test item concentrations was conducted using an GC-method with MS detection.
Statistics:	Descriptive statistics; Probit analysis or moving average angle method for determination of LC_{50} values.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each test item concentration at the beginning of the test and at the end of the test. The concentrations of cinmethylin determined in samples collected at exposure initiation were between 115 and 120 % of nominal concentration. The concentrations of test item determined in samples collected at exposure

termination were in the range of 82 to 95 % of nominal concentrations. The following biological results are, therefore, based on nominal concentrations. It was not stated in the study report whether precipitation of the test item was assessed.

The results are summarised in the table below.

Table B.9.2.4.1-5: Measured concentrations during study

Nominal (mg a.s./L)	0 hours		96 hours	
	Mean measured (mg a.s./L)	% of nominal	Mean measured (mg a.s./L)	% of nominal
0.0	< 0.01	--	< 0.01	--
1	1.2	120	0.89	89
2	2.3	115	1.8	90
5	5.9	118	4.1	82
10	12	120	9.8	98
20	23	115	19	95

Validity criteria:

In OCSPP 850.1020 (2016) if one or more of the following criteria are met the study is considered invalid:

- All test vessels were not identical. In this study test vessels were identical; 350 ml glass crystallising dishes.
- Treatments were not randomly or indiscriminately assigned to individual test vessel locations, or individual test organisms were not randomly or indiscriminately assigned to test vessels. It was not clear based on the study report whether treatments were randomised, or individual test organisms randomly assigned. Whilst this adds uncertainty the HSE evaluator does not consider this point alone enough to invalidate the study.
- A dilution water control (and vehicle (solvent) control, if a vehicle was used) was not included in the test. In this study a control was included with acetone (0.1 ml l^{-1}) however a control without solvent was not. However, control mortality was acceptable in the control and a common solvent tested at an appropriate rate based on OECD 23 (guidance for aquatic toxicity testing of difficult substances) i.e. 0.1 ml/l^{-1} was used. Therefore, the HSE evaluator does not consider this point alone enough to invalidate the study.
- More than 10 % of the organisms in either the dilution water or vehicle (solvent) controls showed signs of disease, stress (*e.g.*, discoloration, unusual behaviour, immobilization), and/or death. In this study 3.3 % mortality was observed at the end of the test period in control and there were not observations reported.
- Gammarids were fed during the test. In this study the test organisms were not fed.
- A surfactant or dispersant was used in the preparation of a stock or test solution. (However, adjuvants may be used when testing pesticide typical end-use products). In this study neither surfactants or dispersants were used.

Based on the above this study is considered valid by the HSE evaluator.

Biological results: After 96 hours of exposure, cumulative mortality was 3, 29 and 30 dead gammarids in the concentrations of 5, 10 and 20 mg a.s./L, respectively. One dead gammarid was observed in the control at the first 24-hours observation interval. For results see Table B.9.2.4.1-6.

Table B.9.2.4.1-6: Effects of cinmethylin on *Gammarus pulex* mortality

Concentration [mg a.s./L] (nominal)	**Solvent Control**	**1**	**2**	**5**	**10**	**20**
Cumulative mortality (24 h) [number in pooled replicates]	1	0	0	0	2	26
Cumulative mortality (48 h) [number in pooled replicates]	1	0	0	0	10	30
Cumulative mortality (72 h) [number in pooled replicates]	1	0	0	1	18	30
Cumulative mortality (96 h) [number in pooled replicates]	1	0	0	3	29	30
% mortality at end of study (96 hours)	3.3	0	0	10	96.7	100
Endpoint [mg a.s./L] (nominal)						
LC_{50} (96 h)	6.6 (95 % confidence limits 5.9 – 7.6)					

In total 30 test organisms were exposed for both the control and treatment groups.

III. CONCLUSION

In a 96-hour static acute toxicity study with *Gammarus pulex*, the LC_{50} of cinmethylin was determined to be 6.6 mg a.s./L based on nominal concentrations.

HSE evaluator comments:

As detailed above there was some uncertainty regarding validity criteria, specifically the lack of control without solvent and whether test vessels/organisms were randomly assigned. Despite this the HSE evaluator considers these points alone insufficient to invalidate the study and it can be considered as supporting information in the risk assessment.

The analytical method was not sufficiently validated in accordance with SANCO/3029/99 (see volume 3, CA, section B5 for full details). The following deficiencies were noted:

i) No specificity data have been provided and no chromatograms have been submitted to check interferences.
ii) No linearity data have been provided.
iii) To assess accuracy and precision, at least 5 recoveries should be provided for at least 2 fortification levels.
iv) The LOQ is not supported by 5 recovery determinations.
v) Matrix effects have not been investigated.
vi) Procedural recoveries have not been completed.

Whilst the analytical method was not sufficiently validated as the study assessed an additional invertebrate species the HSE evaluator has considered this study further in the risk assessment section as supporting information.. Hence it can be used to potentially identify whether another species may be more sensitive than the accepted standard species (*Daphnia*) endpoint.

The above study was conducted to GLP and the following endpoint will be considered in the risk assessment:

- Cinmethylin 96-hour LC_{50} = **6.6 mg a.s./L** (based on nominal), for use as **supporting information only**, noting uncertainty detailed above regarding validity criteria and analytical method.

Report:	CA 8.2.4.2/1 Pearson N.,Stephenson R.R., 1987 a WL95481: Acute toxicity to *Gammarus pulex*, *Lymnaea stagnalis*, *Tubifex tubifex* and *Chironomus lugubris* CI-521-006
Guidelines:	EPA 540/9-82-024
GLP:	Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test item:	Cinmethylin (WL95481, Reg. No. 900 202), WRC Tox. Sample No.: 513F; purity: 91.9 – 93.1 %.

B. STUDY DESIGN

Test species:	Fresh water snail (*Lymnaea stagnalis*) (Linnaeus)*;* 3 – 10 days old; maintained in-house; juveniles of wild catches (ponds in Headcorn, Kent).
Test design:	Static system (96 hours), 5 test item concentrations plus a control; solvent (acetone: 1.0 ml/L) in each concentration plus control; 3 replicates with 10 snails each; assessment of mortality after 24, 48, 72 and 96 hours.
Endpoints:	LC_{50} based on mortality of snails.
Test concentrations:	Control, 1, 2, 5, 10 and 20 mg a.s./L (nominal).
Test conditions:	150 mL glass crystallizing dished with glass plates, test volume 150 mL, dilution water dechlorinated mains water; pH 7.7 – 8.4; oxygen concentration: 6.6 – 9.7 mg/L; temperature: 17.3 – 18.1 °C; total hardness of dilution water: 142 mg $CaCO_3$/L.
Analytics:	Analytical verification of test item concentrations was conducted using an GC-method with MS detection.
Statistics:	Descriptive statistics; Probit analysis or moving average angle method for determination of LC_{50} values.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each test item concentration at the beginning of the test and at the end of the test. The concentrations of cinmethylin determined in samples collected at exposure initiation were between 115 and 120 % of nominal concentration. The concentrations of test item determined in samples collected at exposure termination were in the range of 90 to 105 % of nominal concentrations. The following biological results are, therefore, based on nominal concentrations. It was not stated in the study report whether precipitation of the test item was assessed.

The results are summarised in the table below.

Table B.9.2.4.1-7: Measured concentrations during study

Nominal (mg a.s./L)	0 hours		96 hours	
	Mean measured (mg a.s./L)	% of nominal	Mean measured (mg a.s./L)	% of nominal
0.0	< 0.01	--	< 0.01	--
1	1.2	120	1.0	100
2	2.3	115	2.1	105
5	5.9	118	5.2	104
10	12	120	9.6	96
20	23	115	18	90

Validity criteria:

It was not possible to compare this study to validity criteria. However, the HSE evaluator notes 3.3 % mortality in control occurred which is within 10 %, a typical criterion for acute studies testing aquatic invertebrates.

Biological results: After 96 hours of exposure, cumulative mortality was 6, 24 and 30 dead *L. stagnalis* in the concentrations of 5, 10 and 20 mg a.s./L, respectively. One dead snail was observed in the control at the first 24-hours observation interval. For results see Table B.9.2.4.1-8.

Table B.9.2.4.1-8: Effects of cinmethylin on *L. stagnalis* mortality

Concentration [mg a.s./L] (nominal)	**Control**	**1**	**2**	**5**	**10**	**20**
Cumulative mortality (24 h) [number in pooled replicates]	1	0	0	0	0	6
Cumulative mortality (48 h) [number in pooled replicates]	1	0	0	0	4	30
Cumulative mortality (72 h) [number in pooled replicates]	1	0	0	1	15	30
Cumulative mortality (96 h) [number in pooled replicates]	1	0	0	6	24	30
% mortality at end of study (96 hours)	3.3	0	0	20	80	100
Endpoint [mg a.s./L] (nominal)						
LC_{50} (96 h)	7.0 (95 % confidence limits 6.0 – 8.2)					

In total 30 test organisms were exposed for both the control and treatment groups.

III. CONCLUSION

In a 96-hour static acute toxicity study with *Lymnaea stagnalis*, the LC_{50} of cinmethylin was determined to be 7.0 mg a.s./L based on nominal concentrations.

HSE evaluator comments:

Due to the lack of a validated guideline for this species it was not possible to confirm whether this study is valid. Nonetheless the HSE evaluator considers this study can be used as supporting information.

The analytical method was not sufficiently validated in accordance with SANCO/3029/99 (see volume 3, CA, section B5 for full details). The following deficiencies were noted:

i) No specificity data have been provided and no chromatograms have been submitted to check interferences.
ii) No linearity data have been provided.
iii) To assess accuracy and precision, at least 5 recoveries should be provided for at least 2 fortification levels.
iv) The LOQ is not supported by 5 recovery determinations.
v) Matrix effects have not been investigated.
vi) Procedural recoveries have not been completed.

Whilst the analytical method was not sufficiently validated as the study assessed an additional invertebrate species the HSE evaluator has considered this study further in the risk assessment section as supporting information.. Hence it can be used to potentially identify whether another species may be more sensitive than the accepted standard species (*Daphnia*) endpoint.

The above study was conducted to GLP and the following endpoint will be considered in the risk assessment:

- Cinmethylin 48-hour LC_{50} = **7.0 mg a.s./L** (based on nominal), for use as **supporting information only**, noting uncertainty detailed above regarding validity criteria and analytical method.

Report:	CA 8.2.4.2/1 Pearson N.,Stephenson R.R., 1987 a WL95481: Acute toxicity to *Gammarus pulex*, *Lymnaea stagnalis*, *Tubifex tubifex* and *Chironomus lugubris* CI-521-006
Guidelines:	EPA 540/9-82-024
GLP:	yes

I. MATERIAL AND METHODS

A. MATERIALS

Test item:	Cinmethylin (WL95481, Reg. No. 900 202), WRC Tox. Sample No.: 513F; purity: 91.9 – 93.1 %.

B. STUDY DESIGN

Test species:	Tubificid annelid (*Tubifex tubifex*) (Muller)*;* obtained from "aquatic supplies department", Norton Ash Garden Centre, Teynham, Kent.
Test design:	Static system (96 hours), 5 test item concentrations plus a control; solvent (acetone: 1.0 ml/L) in each concentration plus control; 3 replicates with 10 *tubifex* in each; assessment of mortality after 24, 48, 72 and 96 hours.
Endpoints:	LC_{50} based on mortality of tubificids.
Test concentrations:	Control, 1, 2, 5, 10 and 20 mg a.s./L (nominal)
Test conditions:	150 mL glass crystallizing dished, test volume 100 mL, dilution water dechlorinated mains water with 2 mm washed silver sand; pH 8.1 – 8.3;

	oxygen concentration: 9.5 – 9.8 mg/L; temperature: 17.1 – 17.7 °C; total hardness of dilution water: 185 mg $CaCO_3$/L; no feeding
Analytics:	Analytical verification of test item concentrations was conducted using an GC-method with MS detection.
Statistics:	Descriptive statistics; Probit analysis or moving average angle method for determination of LC_{50} values.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each test item concentration at the beginning of the test and at the end of the test. The concentrations of cinmethylin determined in samples collected at exposure initiation were between 100 and 125 % of nominal concentration. The concentrations of test item determined in samples collected at exposure termination were in the range of 95 to 105 % of nominal concentrations. The study author calculated endpoints based on nominal concentrations. It was not stated in the study report whether precipitation of the test item was assessed.

The results are summarised in the table below.

Table B.9.2.4.1-9: Measured concentrations during study

Nominal (mg a.s./L)	0 hours		96 hours	
	Mean measured (mg a.s./L)	% of nominal	Mean measured (mg a.s./L)	% of nominal
0.0	< 0.1	--	0.03	--
1	1.2	120	1.0	100
2	2.5	125	2.1	105
5	5.1	102	5.0	100
10	10	100	9.9	99
20	20	100	19	95

Validity criteria:

It was not possible to compare this study to validity criteria. However, the HSE evaluator notes 3.3 % mortality in control occurred which is within 10 %, a typical criterion for acute studies testing aquatic invertebrates.

Biological results: After 96 hours of exposure, cumulative mortality was 5, 5, 7, 25 and 29 dead *tufifex* in the concentrations of 1, 2, 5, 10 and 20 mg a.s./L, respectively. One dead snail was observed in the control at the 48-hours observation interval. For results see Table B.9.2.4.1-10.

Table B.9.2.4.1-10: Effects of cinmethylin on *Tubifex tubifex* mortality

Concentration [mg a.s./L] (nominal)	**Control**	**1**	**2**	**5**	**10**	**20**
Cumulative mortality (24 h) [number in pooled replicates]	0	1	1	1	19	27
Cumulative mortality (48 h) [number in pooled replicates]	1	2	4	4	22	28
Cumulative mortality (72 h) [number in pooled replicates]	1	3	5	6	23	29
Cumulative mortality (96 h) [number in pooled replicates]	1	5	5	7	25	29
% mortality at end of study (96 hours)	3.3	16.7	16.7	23.3	83.3	96.7
Endpoint [mg a.s./L] (nominal)						
LC_{50} (96 h)	5.4 (95 % confidence limits 3.3 – 8.8)					

In total 30 test organisms were exposed for both the control and treatment groups.

III. CONCLUSION

In a 96-hour static acute toxicity study with *Tubifex tubifex*, the LC_{50} of cinmethylin was determined to be 5.4 mg a.s./L based on nominal concentrations.

HSE evaluator comments:

It was noted that the measured concentrations were not within ± 20 % of nominals for all test concentrations at study initiation. However, as shown in table B.9.2.4.1-9 there is only one occasion where this occurred at a test concentration of 2 mg/L (nominal) where 125 % of nominal was recovered. The exceedance is relatively low and implies that nominal values are more conservative. Therefore, the HSE evaluator considers the use of nominal concentrations acceptable to derive endpoints.

Due to the lack of a validated guideline for this species it was not possible to confirm whether this study is valid. Nonetheless the HSE evaluator considers this study can be used as supporting information.

The analytical method was not sufficiently validated in accordance with SANCO/3029/99 (see volume 3, CA, section B5 for full details). The following deficiencies were noted:

i) No specificity data have been provided and no chromatograms have been submitted to check interferences.
ii) No linearity data have been provided.
iii) To assess accuracy and precision, at least 5 recoveries should be provided for at least 2 fortification levels.
iv) The LOQ is not supported by 5 recovery determinations.
v) Matrix effects have not been investigated.
vi) Procedural recoveries have not been completed.

Whilst the analytical method was not sufficiently validated as the study assessed an additional invertebrate species the HSE evaluator has considered this study further in the risk assessment section as supporting information.. Hence it can be used to potentially identify whether another species may be more sensitive than the accepted standard species (*Daphnia*) endpoint.

The above study was conducted to GLP and the following endpoint will be considered in the risk assessment:

- Cinmethylin 48-hour LC_{50} = **5.4 mg a.s./L** (based on nominal), for use as **supporting information only**, noting uncertainty detailed above regarding validity criteria and analytical method.

Report:	CA 8.2.4.2/1 Pearson N.,Stephenson R.R., 1987 a WL95481: Acute toxicity to *Gammarus pulex*, *Lymnaea stagnalis*, *Tubifex tubifex* and *Chironomus lugubris* CI-521-006
Guidelines:	EPA 540/9-82-024
GLP:	Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test item:	Cinmethylin (WL95481, Reg. No. 900 202), WRC Tox. Sample No.: 513F; purity: 91.9 – 93.1 %.

B. STUDY DESIGN

Test species:	Nonbiting midge species (*Chironomus lugubris*) (Zetterstedt)*;* larvae, < 26 days old; mean length: 5.9 mm (SD ± 0.7 mm); wild catch from a rainwater tank, South Green, Kent.
Test design:	Static system (48 hours), 5 test item concentrations plus a control; solvent (acetone: 1.0 ml/L) in each concentration plus control; 3 replicates with 10 chironomids in each; assessment of mortality after 24 and 48 hours.
Endpoints:	LC_{50} based on mortality of chironomids.
Test concentrations:	Control, 1, 2, 5, 10 and 20 mg a.s./L (nominal)
Test conditions:	150 mL glass crystallizing dished, test volume 100 mL, dilution water dechlorinated mains water; pH 8.1 – 8.3; oxygen concentration: 9.7 – 9.8 mg/L; temperature: 17.1 – 17.7 °C; total hardness of dilution water: 185 mg $CaCO_3$/L; no feeding.
Analytics:	Analytical verification of test item concentrations was conducted using a GC-method with MS detection.
Statistics:	Descriptive statistics; Probit analysis or moving average angle method for determination of LC_{50} values.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each test item concentration at the beginning of the test and at the end of the test. The concentrations of cinmethylin determined in samples collected at exposure initiation were between 100 and 125 % of nominal concentration. The concentrations of test item determined in samples collected at exposure termination were in the range of 70 to 85 % of nominal concentrations. The study author calculated endpoints based on nominal concentrations. However, given concentrations were not maintained within ± 20 % of nominals during study the HSE evaluator has, where possible, calculated the geometric mean measured concentrations. It was not stated in the study report whether precipitation of the test item was assessed.

The results are summarised in the table below.

Table B.9.2.4.1-11: Measured concentrations during study

Nominal (mg a.s./L)	0 hours		48 hours		Geometric mean (mg a.s./L)*
	Mean measured (mg a.s./L)	% of nominal	Mean measured (mg a.s./L)	% of nominal	
0.0	<0.1	--	<0.01	--	--
1	1.2	120	0.7	70	**0.92**
2	2.5	125	1.7	85	**2.06**
5	5.1	102	--	--	--
10	10	100	--	--	--
20	20	100	--	--	--

-- Not tested or not applicable.
* Calculated by HSE evaluator.

Validity criteria:

In OECD 235 (2011), *Chironomus* sp acute immobilisation test, the following criteria are stated:

- In the control, including the solvent control if appropriate, not more than 15 percent of the larvae should show immobilisation or other signs of disease or other stress (e.g. abnormal appearance or unusual behaviour, such as trapping at the water surface) at the end of the test. Obtained: 10 %.
- The dissolved oxygen concentration at the end of the test should be ≥ 3 mg/L in control and test vessels. Obtained: minimum of 9.7 mg/L.

During the study the above criteria were met.

Biological results: After 48 hours of exposure, cumulative mortality was 6, 9, 28, 30 and 30 dead chironomids in the concentrations of 1, 2, 5, 10 and 20 mg a.s./L, respectively. Three dead chironomids were observed in the control at the 48-hours observation interval. For results see Table B.9.2.4.1-12.

Table B.9.2.4.1-12: Effects of cinmethylin on *Chironomus lugubris* mortality

Concentration [mg a.s./L] (nominal)	**Control**	**1**	**2**	**5**	**10**	**20**
Concentration [mg a.s./L] (geometric mean)	**Control**	**0.92**	**2.06**	**n.c**	**n.c**	**n.c**
Cumulative mortality (24 h) [number in pooled replicates]	0	1	1	1	3	16
Average number dead per replicate at 48 hours (± s.d.)	1 (± 0)	2 (± 2.6)	3 (± 1)	9 (± 0.6)	10 (± 0)	10 (± 0)
Cumulative mortality (48 h) [number in pooled replicates]	3	6	9	28	30	30
% mortality at end of study (48 hours)	10	20	30	93.3	100	100

n.c = Not possible to calculate as analytical measurement only conducted at study initiation.
In total 30 test organisms were exposed for both the control and treatment groups.

III. CONCLUSION

In a 48-hour static acute toxicity study with *Chironomus lugubris*, the LC_{50} of cinmethylin was determined to be 2.5 mg a.s./L based on nominal concentrations.

HSE evaluator comments:

It was noted that the measured concentrations were not within ± 20 % of nominals hence the HSE evaluator has calculated the geometric mean measured concentrations rather than nominal values used by study author.

The analytical method was not sufficiently validated in accordance with SANCO/3029/99 (see volume 3, CA, section B5 for full details). The following deficiencies were noted:

i) No specificity data have been provided and no chromatograms have been submitted to check interferences.
ii) No linearity data have been provided.
iii) To assess accuracy and precision, at least 5 recoveries should be provided for at least 2 fortification levels.
iv) The LOQ is not supported by 5 recovery determinations.
v) Matrix effects have not been investigated.
vi) Procedural recoveries have not been completed.

Whilst the analytical method was not sufficiently validated as the study assessed an additional invertebrate species the HSE evaluator has considered this study further in the risk assessment section as supporting information.. Hence it can be used to potentially identify whether another species may be more sensitive than the accepted standard species (*Daphnia*) endpoint.

The above study was conducted to GLP and the following endpoint will be considered in the risk assessment:

- Cinmethylin 48-hour LC_{50} = **> 2.06 mg a.s./L** (based on geometric mean measured), for use as **supporting information only**, noting uncertainty detailed above regarding validity criteria and analytical method.

Report: CA 8.2.4.2/2
Ward G.S., 1983 b
Acute toxicity of technical SD 95481 to mysid shrimp (*Mysidopsis bahia*)
CI-521-002
Guidelines: None reported
GLP: No

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Technical SD 95481 (cinmethylin, Reg. no. 900 202), Code 5-4-0-0; purity: 92 %.

B. STUDY DESIGN

Test species: Saltwater mysid (*Mysidopsis bahia* Syn. *Americamysis bahia*), age: 3 days old; source: in-house cultures (BMRL).

Test design: Static system (96 hours); 5 test item concentrations plus a control and a solvent control (0.001% acetone), 2 replicates per treatment; 10 mysids per replicate; daily assessment of mortality.

Endpoints: LC_{50} (96 h), mortality.

Test concentrations: Control (seawater), 0.625, 1.25, 2.5, 5.0 and 10.0 mg a.s./L (nominal).

Test conditions:	1.6 L glass bowls, test volume approx. 1 L; dilution water: natural saltwater, filtered (5 µm pore size); salinity: 20 ‰; temperature: 21.0 - 23.0 °C; pH 7.6 - 8.0; oxygen content: 4.8 – 8.4 mg/L.
Analytics:	Analytical verification of test item concentrations was conducted in highest test concentration and where 100 % mortality occurred (not reported).
Statistics:	Descriptive statistics; for calculation of LC_{50} values the moving average angle method was used.

II. RESULTS AND DISCUSSION

Analytical measurements: not stated.

Validity criteria:

In OCSPP 850.1035 (2016) if one or more of the following criteria are met the study is considered invalid:

- All test vessels (and retention chambers) were not identical. In this study test vessels were simply described as 'bowls', hence it is unclear whether test vessels were identical.
- Treatments were not randomly or indiscriminately assigned to individual test vessel locations, or individual test organisms were not randomly or indiscriminately assigned to test vessels (or retention chambers). It was not clear based on the study report whether treatments were randomised, or individual test organisms randomly assigned.
- A dilution water control (and vehicle (solvent) control, if a vehicle was used) was not included in the test. In this study both a control and solvent control were included.
- More than 10 % of the organisms in either the dilution water or vehicle (solvent) controls showed signs of disease, stress (*e.g.*, discoloration, unusual behaviour, immobilization), and/or death. In this study 5 % mortality was observed at the end of the test period in solvent control and there were not observations reported.
- A surfactant or dispersant was used in the preparation of a stock or test solution. (However, adjuvants may be used when testing pesticide typical end-use products). In this study neither surfactants or dispersants were used.

Based on the above it was not possible to confirm whether validity criteria were met, resulting in uncertainty. In general, the methodology was briefly reported and did not state whether precipitation of the test item was assessed/observed.

Biological results: After 96 hours of exposure, mortality ranged from 5 % in the 0.625 and 1.25 mg a.s./L test concentrations to 90 % in the 5.0 mg a.s./L test concentration. At the highest test item concentration of 10.0 mg a.s./L, 100 % mortality occurred. There was no mortality in the seawater control and 5 % mortality in the solvent control. The 96-hour LC_{50} was 2.5 mg a.s./L with 95 % confidence limits of 2.0 - 3.2 mg a.s./L.

The results are summarised in table B.9.2.4.1-13.

Table B.9.2.4.1-13: Acute toxicity (96h) of technical SD 95481 (cinmethylin) to saltwater mysids (*Mysidopsis bahia* Syn. *Americamysis bahia*)

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**0.625**	**1.25**	**2.5**	**5**	**10**
Mortality [%] (96 h)	0	5	5	5	50	90	100
Symptoms after 96 h [#]	none	none	none	none	none	2 L	n.d.
Endpoints [mg a.s./L] (nominal)							
LC_{50} (96 h)	2.5 (95 % confidence limits: 2.0 – 3.2)						

[#] Symptoms after 96 h: L = lethargy
n.d. = not determined; all animals dead

III. CONCLUSION

In a static acute toxicity study with saltwater mysids (*Mysidopsis bahia* Syn. *Americamysis bahia*) the LC_{50} (96 h) for technical SD 95481 (cinmethylin) was determined to be 2.5 mg a.s./L.

HSE evaluator comments:

The applicant provided the following text (shown in italics): '*The study was not conducted according to the GLP principles. In addition, the verification of the test item has been conducted at test initiation and termination for the highest concentration only. Moreover, the measured concentrations are not reported in the study report. According to the current guideline the oxygen saturation was lower than recommended and the amount of the used solvent was too high. The age of test organisms was not in the specified range as recommended by the respective current guidelines. Temperature was below the recommended limit of 25°C. Thus, the study is invalid; however, it is reported for the sake of completeness.'*

We agree with the comments provided by the applicant and the HSE evaluator has concluded that this study should not be considered further in the risk assessment section. It should be noted that based on the reported information it is not possible for the chemistry specialist to confirm whether the analytical method is sufficiently validated.

Report: CA 8.2.4.2/3
Ward G.S., 1983 c
Acute toxicity of technical SD 95481 to fiddler crabs (*Uca pugilaton*)
CI-521-003
Guidelines: None reported
GLP: No

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Technical SD 95481 (cinmethylin, Reg. no. 900 202), Code 5-4-0-0; purity: 92 %.

B. STUDY DESIGN

Test species: Fiddler crabs (*Uca pugilator*), size: 13-15 millimeters carapace width, (wet) weight: 1.0 - 1.6 g; source: commercial supplier.

Test design: Static system (96 hours); 1 test item concentration plus an untreated control and a solvent control, 4 replicates per treatment and control group; 5 crabs per replicate (loading: 0.4 g/L); daily assessment of mortality.

Endpoints:	LC_{50} (96 h), mortality.
Test concentrations:	Control (seawater), solvent control (0.066 %), 1 000 mg a.s./L (nominal).
Test conditions:	19 L glass jars, test volume 15 L; dilution water: natural saltwater, filtered (5 µm pore size); salinity: 20 ‰; temperature: 21.0 - 22.0 °C; pH 7.2 - 7.9; oxygen content: 2.5 – 8.1 mg/L.
Analytics:	Analytical verification of test item concentration was conducted (not reported).
Statistics:	Descriptive statistics.

II. RESULTS AND DISCUSSION

Analytical measurements: not stated. It was not stated in the study report whether precipitation of the test item was assessed.

Validity criteria:

It was not possible to compare this study to validity criteria. However, the HSE evaluator notes 3.3 % mortality in control occurred which is within 10 %, a typical criterion for acute studies testing aquatic invertebrates.

Biological results: After 96 hours of exposure, mortality was 5 % in the 1 000 mg a.s./L test concentration. There was 5 % mortality in both the seawater and solvent control. The results are summarised in Table B.9.2.4.1-14.

Table B.9.2.4.1-14: Acute toxicity (96h) of technical SD 95481 (cinmethylin) to fiddler crab (*Uca pugilator*)

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**1000**
Mortality [%] (96 h)	5	5	5
Symptoms after 96 h	none	none	none
Endpoints [mg a.s./L] (nominal)			
LC_{50} (96 h)	>1000		

III. CONCLUSION

In a static acute toxicity study with saltwater fiddler crab (*Uca pugilator*) the LC_{50} (96 h) for technical SD 95481 (cinmethylin) was determined to be >1000 mg a.s./L (nominal).

HSE evaluator comments:

Due to the lack of validated guideline for this species it was not possible to confirm whether this study is valid. In addition, the study was not conducted to GLP and analytical data was not reported. Therefore, the HSE evaluator has concluded that this study should not be considered further in the risk assessment section.

Report:	CA 8.2.4.2/4 Ward G., 1983 a Acute toxicity of technical SD 95481 to embryos-larvae of eastern oysters (*Crassostrea virginica*) CI-521-004
Guidelines:	None reported

GLP: No

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Technical SD 95481 (cinmethylin, Reg. no. 900 202), Code 5-4-0-0, purity: 92 %.

B. STUDY DESIGN

Test species: Eastern oyster (*Crassostrea virginica*), embryos (1-hour post fertilization); source: commercial supplier.

Test design: Static system (48 hours); 6 test item concentrations plus a control and a solvent control, 3 replicates for each test item concentration and the controls with an estimated 26 619 embryos animals per treatment; assessment of the normal developed larvae after 48 hours of exposure.

Endpoints: EC_{50} for the number of normal developed larvae.

Test concentrations: Control (dilution water), solvent control (0.11% acetone), 0.6, 1.2, 2.5, 5, 10 and 20 mg a.s./L (nominal).

Test conditions: Glass jars (950 mL), test volume approx. 900 mL; dilution water: natural saltwater, filtered (5 µm pore size); salinity: 25 ‰; temperature: 21 ± 1 °C; pH 8.1; oxygen content: 6.0 - 6.7 mg/L; light intensity: ambient room lighting.

Analytics: Analytical verification of the highest test item concentration was conducted (not reported).

Statistics: Descriptive statistics; moving average angle method with William's Test of multiple comparison ($p \leq 0.05$).

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in the highest test concentration at test initiation and at test termination. No results are presented in the report and it was not stated whether precipitation of the test item was assessed/observed.

Validity criteria:

In OCSPP 850.1055 (2016) if one or more of the following criteria are met the study is considered invalid:

- All test vessels were not identical. In this study test vessels were identical; 950 ml glass jars.
- Treatments were not randomly or indiscriminately assigned to individual test vessel locations, or individual test organisms were not randomly or indiscriminately assigned to test vessels. It was not clear based on the study report whether treatments were randomised, or individual test organisms randomly assigned.
- A dilution water control (and vehicle (solvent) control, if a vehicle was used) was not included in the test. In this study both a control and solvent control were included.
- Less than 70 % of oyster embryos in either the dilution water control or vehicle (solvent) control resulted in normal larvae at test termination. Not possible to determine for this study as only ‘normal’ embryos were reported for controls.
- A surfactant or dispersant was used in the preparation of a stock or test solution. (However, adjuvants may be used when testing pesticide typical end-use products). In this study neither surfactants or dispersants were used.

Based on the above it was not possible to confirm whether validity criteria were met, resulting in uncertainty. In general, the methodology was briefly reported.

Biological results: The calculated 48-hour EC_{50} for embryos-larvae of eastern oysters exposed to technical SD 95481 in static, unaerated seawater was 3.2 mg a.s./L with 95 % confidential limits of 2.3 - 4.9 mg a.s./L. No significant reduction of embryos-larvae which developed normally to the straight-hinged veliger stage occurred in the l.2 mg a.s/L test concentration; but significant reduction of numbers of normal larvae did occur in test concentrations ≥ 2.5 mg a.s./L. Normally developed larvae were statistically significantly reduced at the four highest test item concentrations compared to the control (William's Test, $p \leq 0.05$). The results are summarised in Table B.9.2.4.1-15.

Table B.9.2.4.1-15: Acute toxicity (48 h) of technical SD 95481 (cinmethylin) to eastern oysters (*Crassostrea virginica*)

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**0.6**	**1.2**	**2.5**	**5**	**10**	**20**
Reduction of normal 48-h larvae [%]	-	-	n.r.	-10	-20 *	-86 *	-99 *	-99 *
Endpoints [mg a.s./L] (nominal)								
EC_{50} (48 h)	3.2 (95 % confidence limits: 2.3 – 4.9)							

n.r.= not reported
* Statistically significant difference compared to the control (William's Test, $p \leq 0.05$).

III. CONCLUSION

In a static, unaerated acute toxicity study with eastern oysters (*Crassostrea virginica*), the EC_{50} (48 h) was 3.2 mg a.s./L based on nominal concentrations of technical SD 95481 (cinmethylin).

HSE evaluator comments:

Due to the lack of reported information it was not possible to confirm whether this study is valid. In addition, the study was not conducted to GLP and analytical data was not reported. Therefore, the HSE evaluator has concluded that this study should not be considered further in the risk assessment section.

B.9.2.4.2 Metabolite (M684H001):

Report: CA 8.2.4.1/4
Turek T., 2018 a
Reg. No. 6055521 (Metabolite of BAS 684 H, M684H001) *Daphnia magna*, acute immobilisation test
2017/1069818
Guidelines: OECD 202 (2004)
GLP: Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test item: M684H001 (Metabolite of BAS 684 H, Reg. no. 6 055 521), batch no. L87-226; purity: 99.9 %.

B. STUDY DESIGN

Test species: Water flea (*Daphnia magna* STRAUS), neonates collected from in house culture; < 24 h old at test initiation, not first brood progeny.

Test design:	Static system (48 hours), 1 test item concentrations plus control, 4 replicates with 5 daphnids in each; assessment of immobility after 24 and 48 hours.
Endpoints:	EC_{50} based on immobility of daphnids.
Test concentrations:	100 mg metabolite/L plus control.
Test conditions:	150 mL glass beakers, test volume 100 mL, dilution water "M7" (Elendt medium); pH 6.80 – 7.98; oxygen concentration: 8.0 – 8.6 mg/L; temperature: 20.7 – 21.6 °C; photoperiod: 16 h light: 8 h dark; fluorescent light source, no feeding, no aeration.
Analytics:	Analytical verification of test item concentrations was conducted using a HPLC-method with DAD (Diode Array Detector).
Statistics:	Descriptive statistics- study was a limit test.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of metabolite M684H001 was carried out in the test concentrations at the beginning and at the end of the test. Measured value at test initiation was 92.3 % and at test termination 95.2 % of nominal. Therefore, the following biological results are based on nominal test item concentrations. It was reported that visually test solutions were clear.

Validity criteria:

In order to determine whether the study is valid the criteria in OECD 202 (2004) have been considered below:

- In the control, including the control containing the solubilising agent, not more that 10 percent of the daphnids should have been immobilised. Obtained: 0 %
- The dissolved oxygen concentration at the end of the test should be ≥ 3 mg/l in control and test vessels. Obtained: minimum of 8.0 mg/l.

The above validity criteria were met during the study.

Biological results: After 48 h of exposure, no immobility of daphnids was observed in the control, whereas 5 % immobile daphnia where observed at the tested concentrations of 100 mg metabolite/L. For results see Table 9.2.4.2-1.

Table 9.2.4.2-1: Effects of M684H001 on *Daphnia magna* mobility

Concentration (nominal) [mg metabolite/L]	**Control**	**100**
Immobility (24 h) [%]	0	0
Immobility (48 h) [%]	0	5
Endpoint [mg metabolite/L] (nominal)		
EC_{50} (48 h)	> 100	

Reference item test: A reference study was conducted with potassium dichromate. The 24-hour EC_{50} was calculated as 0.61 mg reference item/L which is within the range stated in OECD 202 i.e. 0.6 to 2.1 mg/L.

III. CONCLUSION

In a 48-hour static acute toxicity study with *Daphnia magna*, the EC_{50} of M684H001 was determined to be > 100 mg metabolite/L based on nominal concentrations.

HSE evaluator comments:

The above study was conducted to GLP and considered valid. The analytical method was sufficiently validated in accordance with SANCO/3029/99 with an LoQ of 0.002 mg metabolite/L (see volume 3, CA, section B5 for full details). The following endpoint will be considered in the risk assessment:

- Metabolite 'M684H001' 48-hour EC_{50} = **> 100 mg metabolite/L** (based on nominal)

B.9.2.4.3 Metabolite Cineole alcohol (M684H003):

Report:	CA 8.2.4.1/10 [redacted], 1988 a Cineole alcohol: Acute toxicity to rainbow trout *Salmo gairdneri* and *Daphnia magna* CI-570-001
Guidelines:	EEC 79/831 A V C, EEC 79/831 A V C 2
GLP:	Yes

It should be noted this study reports data for both an acute fish and invertebrate study. The following summary details the invertebrate study. The fish study has been summarised in section B.9.2.1.1, acute toxicity to fish.

I. MATERIAL AND METHODS

A. MATERIALS

Test item:	Cineole alcohol (Metabolite of cinmethylin, CAS: 87172-89-2), Code no. SD93853, purity: 98 ± 2.0 %.

B. STUDY DESIGN

Test species:	Water flea (*Daphnia magna* STRAUS), neonates collected from in house culture; < 24 h old at test initiation, originally obtained from [redacted].
Test design:	Static system (48 hours), 6 test item concentrations plus control; 2 replicates with 10 daphnids each; 1 replicate without daphnia for analytical measurements; assessment of immobility after 24 and 48 hours.
Endpoints:	EC_{50} based on immobility of daphnids.
Test concentrations:	Control (0), 53, 95, 170, 310, 556, 1000 mg cineol alcohol/L, corresponding to mean measured concentration of < 0.01, 56, 91, 170, 295, 515, 925 mg metabolite/L
Test conditions:	150 mL glass beakers, test volume 100 mL, dilution water: reconstituted water; pH 8.0 – 8.2; oxygen concentration: 8.6 – 9.2 mg/L; temperature: 20.3 - 21.2 °C; total hardness: 172 mg $CaCO_3$/L (dilution water); photoperiod: 16 h light: 8 h dark; no feeding, no aeration.
Analytics:	Analytical verification of test item concentrations was conducted using an GC-method with MS detection.
Statistics:	Descriptive statistics; Probit analysis after log transformation for determination of EC_{50} values.

II. RESULTS AND DISCUSSION

Analytical measurements: The concentration of cineole alcohol ranged from 83 to 126 % of nominal test concentrations. It was not stated in the study report whether precipitation of the test item was assessed.

The results are shown in the table below:

Table B.9.2.4.3-1: Measured concentrations during study

Nominal (mg metabolite/L)	0 hours	24 hours	48 hours
	% of nominal	% of nominal	% of nominal
0	na	na	na
53	106	104	98
95	94	98	108
170	97	100	109
310	87	106	120
556	83	109	126
1000	85	107	121

na = not applicable

Validity criteria:

This study was conducted before the most recent OECD guideline. Nonetheless in order to determine whether the study is valid the criteria in OECD 202 (2004) have been considered below:

- In the control, including the control containing the solubilising agent, not more that 10 percent of the daphnids should have been immobilised. Obtained: 0 %
- The dissolved oxygen concentration at the end of the test should be ≥ 3 mg/l in control and test vessels. Obtained: minimum of 8.6 mg/l.

The above validity criteria were met during the study.

Biological results: At 24 hours, 7 dead daphnids were observed in test item concentration of 1000 mg/L, the highest concentration tested. After 48 hours of exposure, 1, 7 and 11 dead daphnids were observed at test item concentrations of 310, 556 and 1000 mg metabolite/L (nominal), respectively (total of 20 daphnids per replicate). Results are summarised in Table B.9.2.4.3-2.

Table B.9.2.4.3-2: Effects of cineol alcohol (M684H003, metabolite of BAS 684 H) on *Daphnia magna* mobility

Concentration (nominal) [mg metabolite/L]	Control	53	95	170	310	556	1000
% Immobility (48 h)	0	0	0	0	5	35	55
Endpoints [mg metabolite/L] (nominal)							
EC_{50} (48 h)	840 (95 % confidence interval: 660 – 1300)						
NOEC	n.d.						

n.d. = not determined

III. CONCLUSION

In a 48-hour static acute toxicity study with *Daphnia magna*, the EC_{50} of the metabolite cineol alcohol (M684H003, metabolite of cinmethylin) was determined to be 840 mg/L based on nominal concentrations.

HSE evaluator comments:

It was noted that the test concentration increased during the study and resulted in measured concentrations > 20 % above nominal concentrations, noting the maximum recovery was 126 % which is relatively low compared to OECD 202 criteria of ± 20 % of nominals, hence the HSE evaluator considers the use of nominal concentrations is acceptable.

The analytical method is not considered validated in accordance with SANCO/3029/99 rev. 4 due to insufficient information being provided (see volume 3, CA, section B5 for full details). The following deficiencies have been noted:

i) Missing data on the specificity (no chromatograms were provided)
ii) Linearity not fully addresses as no calibration curve, equation or standards were provided

Given the analytical method was not sufficiently validated this study is not suitable for quantitative use in the risk assessment. However, as the study tested a relevant metabolite and due to the lack of other invertebrate studies submitted the HSE evaluator has considered the derived endpoint as supporting information in the risk assessment section:

- Cineole alcohol (M684H003) 48-hour EC_{50} = **840 mg metabolite/L** (based on nominal) used as **supporting information only**

Report:	CA 8.2.4.1/5 Turek T., 2018 b Reg.No. 4539586 (Metabolite of BAS 684 H, M684H003) - *Daphnia magna*, acute Immobilisation test 2017/1069817
Guidelines:	OECD 202 (2004)
GLP:	Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test item:	M684H003 (Metabolite of BAS 684 H, Reg. no. 4 539 586), batch no. L87-86; purity: 99.7 %.

B. STUDY DESIGN

Test species:	Water flea (*Daphnia magna* STRAUS), neonates collected from in house culture; < 24 h old at test initiation, not first brood progeny.
Test design:	Static system (48 hours), 1 test item concentrations plus control, 4 replicates with 5 daphnids in each; assessment of immobility after 24 and 48 hours.
Endpoints:	EC_{50} based on immobility of daphnids.
Test concentrations:	100 mg/L plus control.
Test conditions:	150 mL glass beakers, test volume 100 mL, dilution water "M7" (Elendt medium); pH 7.90 – 8.05 (control); oxygen concentration: 7.7 – 8.5 mg/L; temperature: 19.0 – 20.0 °C; photoperiod: 16 h light: 8 h dark; fluorescent light source, no feeding, no aeration.
Analytics:	Analytical verification of test item concentrations was conducted using a GC-method with FID (Flame Ionization Detector).
Statistics:	Descriptive statistics- study was a limit test.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of metabolite M684H003 was carried out in the test concentrations at the beginning and at the end of the test. Measured value at test initiation was 98.26 % and at test termination 101.32 % of nominal. Therefore, the following biological results are based on nominal test item concentrations. Test solutions were reported as visually homogeneous and transparent.

Validity criteria:

In order to determine whether the study is valid the criteria in OECD 202 (2004) have been considered below:

- In the control, including the control containing the solubilising agent, not more that 10 percent of the daphnids should have been immobilised. Obtained: 0 %
- The dissolved oxygen concentration at the end of the test should be ≥ 3 mg/l in control and test vessels. Obtained: minimum of 7.9 mg/l.

Biological results: After 48 h of exposure, no immobility of daphnids was observed in the control and at the tested concentrations of 100 mg/L. For results see Table B.9.2.4.3-3.

Table B.9.2.4.3-3: Effects of M684H003 on *Daphnia magna* mobility

Concentration (nominal) [mg/L]	**Control**	**100**
Immobility (24 h) [%]	0	0
Immobility (48 h) [%]	0	0
Endpoint [mg metabolite/L] (nominal)		
EC_{50} (48 h)	> 100	

Reference item test: A reference study was conducted with potassium dichromate. The 24-hour EC_{50} was calculated as 0.98 mg reference item/L which is within the range stated in OECD 202 i.e. 0.6 to 2.1 mg/L.

III. CONCLUSION

In a 48-hour static acute toxicity study with *Daphnia magna*, the EC_{50} of M684H003 was determined to be > 100 mg/L based on nominal concentrations.

HSE evaluator comments:

The above study was conducted to GLP and considered valid. The analytical method was sufficiently validated in accordance with SANCO/3029/99 rev. 4 with an LOQ of 0.002 mg metabolite/L (see volume 3, CA, section B5 for full details) and the following endpoint will be considered in the risk assessment:

- Metabolite 'M684H003' 48-hour EC_{50} = **> 100 mg metabolite/L** (based on nominal)

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

B.9.2.5.1 Active substance: Cinmethylin:

Report: CA 8.2.5.1/1
Pearson N.,Girling A., 1989 a
WL95481: Chronic toxicity to *Daphnia magna*
CI-523-001

Guidelines: Not reported
GLP: Yes

I. MATERIAL AND METHODS

Test item: Cinmethylin (WL 95481, Reg. no.: 900 202), WRC Tox. sample no. 513F, purity: 92 % (+1.1 – 0.1 %; analysed).

A. MATERIALS

B. STUDY DESIGN

Test species: Water flea (*Daphnia magna* STRAUS) neonates, < 24 h old at exposure initiation, from in-house culture; originally obtained from "Institut National de Recherche Applique (I. R. Ch. A.) ", France.

Test design: Static-renewal system (21 days), water renewal in 3-day intervals, 6 test concentrations plus control, 4 replicates per treatment and control; 10 animals per test vessel; daily assessment of parent mortality and reproduction; determination of body length at test termination.

Endpoints: NOEC based on parent mortality, reproduction and growth (parent length).

Test concentrations: Control (0), 0.1, 0.2, 0.5, 1.0, 2.0 and 5.0 mg a.s./L (nominal).

Test conditions: 600 mL glass beakers; test volume: 500 mL; dilution water: reconstituted hard water; temperature: 18 – 22 °C; pH 7.8 – 8.1; oxygen content: 7.8 – 9.8 mg/L; total hardness: 100 – 196 mg $CaCO_3$/L; photoperiod 16 hours light: 8 hours dark; feeding: in dilution water (0.15 x 10^6 cells/ml *Chlorella vulgaris*); no aeration.

Analytics: Analytical verification of test item concentrations was conducted using an GC-method with MS-detection.

Statistics: Descriptive statistics; Dunnett's test for determination of the NOEC values ($p < 0.05$; $p < 0.01$).

II. RESULTS AND DISCUSSION

Analytical results: The concentrations of cinmethylin were chemically determined in samples of all test item concentrations and the control in fresh test media and before water exchange every 72 hours. Concentrations of cinmethylin in the samples of fresh test media ranged between 60 to 150 % of nominal and 60 to 167 % for aged solutions. The results are shown in the table below. The HSE evaluator has calculated the geometric mean concentrations as the results are not within ± 20 % of nominals.
It was not stated in the study report whether precipitation of the test item was assessed.

Table B.9.2.5.1-1: Measured concentrations during study

N (mg a.s./L)	Day 0	Day 3		Day 6		Day 9		Day 12		Day 15		Day 18		Day 21	GM* (mg a.s./L)
	'Fresh' %	'Aged' %	'Fresh' %	'Aged' %	'Fresh' %	'Aged' %	'Fresh' %	'Aged' %	'Fresh' %	'Aged' %	'Fresh' %	'Aged' %	'Fresh' %	'Aged' %	
0	na	na	na	na	na	na	na	na	na	na	na	na	na	na	0
0.1	72	80	60	64	86	100	100	98	75	72	88	72	80	86	0.1
0.2	70	70	65	60	95	95	95	95	75	75	100	70	70	90	0.2
0.5	76	78	68	64	102	86	86	90	74	70	82	76	78	84	0.4
1	73	80	69	64	100	78	91	96	70	74	87	73	80	84	0.8
2	80	70	70	60	110	85	80	95	75	65	85	80	70	85	1.6
3	137	120	117	107	163	150	133	167	127	113	150	137	120	ND	4.0

N = nominal concentration, GM = Geometric mean concentration, % = Percentage of nominal concentration, * calculated by HSE evaluator
na = not applicable, ND = not determined

Validity criteria:

In order to determine whether the study is valid the criteria in OECD 211 (2012) have been considered below:

- The mortality of the parent animals (female *Daphnia*) does not exceed 20 % at the end of the test. Obtained: 22 % therefore criteria not met
- The mean number of living offspring produced per parent animal surviving at the end of the test is > 60. Obtained: 33.8 therefore criteria not met.

The above validity criteria were not met during the study.

Biological results: After 21 days of exposure, parent mortality occurred in the control groups leading to a 78 % survival of adult *daphnia*. *D. magna* exposed to cinmethylin at a nominal concentration of 5 mg a.s./L showed increased mortality after 14 days exposure and did not produce any live young. The total number of live young produced by *D. magna* exposed to cinmethylin at a nominal concentration of 2 mg a.s./L was significantly reduced after 8 days exposure (Dunnett's test, $p > 0.01$). The total number of live young produced by *D. magna* exposed to cinmethylin at a nominal concentration of 1 mg a.s./L was significantly reduced after 18 to 20 days exposure (Dunnett's test; $p > 0.05$). No treatment-related effects were detected in *D. magna* exposed for 21 days to cinmethylin at a nominal concentration of 0.5 mg a.s./L.
The results are summarised in Table B.9.2.5.1-2.

Table B.9.2.5.1-2: Effects of cinmethylin on *Daphnia magna* parent mortality, reproduction and growth after 21 days of exposure

Concentration [mg a.s./L] (nominal)	**Control**	**0.1**	**0.2**	**0.5**	**1.0**	**2.0**	**5.0**
Concentration [mg a.s./L] (geometric mean)	**Control**	**0.1**	**0.2**	**0.4**	**0.8**	**1.6**	**4.0**
Parent survival [%]	78	80	73	78	78	93	0**
Mean cumulative offspring / parent	33.8	31.0	34.0	32.7	31.8	24.0**	0**
Mean cumulative offspring / replicates (n=10) [1)]	305	286	297	297	274*	226**	0**
Mean cumulative offspring / replicates (n=10) [2)]	317	293	311	310	293	236**	0**
Body length [mm]	3.45	3.39	3.46	3.37	3.48	3.40	--
Endpoint [mg a.s./L] (nominal test item)							
NOEC $_{overall}$ (21 d) [3)]	0.5 (equivalent to 0.4 based on geometric mean concentration)						

* Statistically significant effects compared to the control (Dunnett`s test; $p < 0.05$).
** Statistically significant effects compared to the control (Dunnett`s test; $p < 0.01$).
1) Mean cumulative offspring per replicate on day 20.
2) Mean cumulative offspring per replicate on day 21.
3) NOEC value is based on mean cumulative offspring per replicate; Values from days 18 – 20 were significantly different compared to control.

III. CONCLUSION

In a 21-day static-renewal toxicity study with *Daphnia magna* the NOEC of cinmethylin was determined to be 0.5 mg a.s./L based on nominal concentration equivalent to 0.4 mg a.s./L based on geometric mean concentration.

HSE evaluator comments:

The above study was conducted to GLP but not considered valid by the HSE evaluator. As detailed above the validity criteria were not met in particular there was relatively high control mortality (22 %) and reproductive rate was low (criteria: > 60 per surviving adult, obtained: 33.8). Therefore, the study has not been considered further in the risk assessment section. It is noted that the temperature deviated by more than 2℃ during the study, noting this is considered a minor deviation by the HSE evaluator compared to the failed validity criteria.

Report: CA 8.2.5.1/2
Rzodeczko H., 2017 b
BAS 684 H - *Daphnia magna* reproduction test
2017/1000684
Guidelines: OECD 211 (2012), EPA 850.1300, EPA 712-C-16-005
GLP: Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Cinmethylin (Reg. no.: 900 202), batch no. COD-002038, purity: 93.0 % (tolerance ± 1 %).

B. STUDY DESIGN

Test species:	Water flea (*Daphnia magna* STRAUS), < 24 h old at exposure initiation, not first brood progenity, parent age 21 – 25 days, from in-house culture;
Test design:	Semi-static system (21 days, water renewal 3 times per week), 5 test concentrations plus control, 10 replicates per treatment; one animal per test vessel; daily assessment of parent mortality and reproduction; determination of body length and dry weight at test termination.
Endpoints:	EC_{10}; NOEC and LOEC based on parent mortality, reproduction, growth (parent length and dry weight) and population growth rate.
Test concentrations:	Control (dilution water), 0.31, 0.63, 1.25, 2.5 and 5.0 mg a.s./L (nominal test item); corresponding to mean measured concentrations of 0.288, 0.586, 1.163, 2.325 and 4.65 mg a.s./L, respectively.
Test conditions:	Glass beakers; test volume: 50 mL; dilution water "M7" (Elendt medium); temperature: 18.8 – 21.5 °C; pH 7.47 – 7.95; oxygen content: 72 – 99 %; total hardness: 171 – 195 mg $CaCO_3$/L; alkalinity: 47.6 – 82.1 mg $NaHCO_3$/L; conductivity: 472 - 539 µS/cm; photoperiod 16 hours light: 8 hours dark; light intensity: 1009 - 1248 lux; feeding: daily with algae (2:1 (v/v) mixture of *Pseudokirchneriella subcapitata* and *Desmodesmus subspicatus*); no aeration.
Analytics:	Analytical verification of test item concentrations was conducted using an LC-method with DAD-detection.
Statistics:	Descriptive statistics; Probit method calculations and analysis by Williams Multiple Sequential t-test Procedure and Multiple Sequentially-rejective Welsh t-test after Bonferroni-Holm for determination of the NOEC values ($\alpha = 0.05$, one-sided smaller).

II. RESULTS AND DISCUSSION

Analytical results:

The concentrations of cinmethylin determined in samples collected from freshly prepared samples (when measured) ranged from 86.5 – 119.8 % of nominal concentrations. For aged samples the concentrations of cinmethylin determined (when measured) ranged from 73.5 – 109.6 % of nominal concentrations. The HSE evaluator notes that concentrations were not maintained within ± 20 % of nominal concentrations during the study based on the aged samples at day 21. Therefore the geometric mean measured concentrations have been calculated. It was unclear in the study report whether precipitation of the test item was assessed. The results are shown in the table below:

Table B.9.2.5.1-3: Measured concentrations during study

N (mg a.s./L)	**Day 0**	**Day 2**		**Day 4**		**Day 7**		**Day 9**		**Day 11**		**Day 14**		**Day 16**		**Day 18**		**Day 21**	**TW mean* (mg a.s./L)**
	F mg a.s./L	**A mg a.s./L**	**F mg a.s./L**	**A mg a.s./L**	**F mg a.s./L**	**A mg a.s./L**	**F mg a.s./L**	**A mg a.s./L**	**F mg a.s./L**	**A mg a.s./L**	**F mg a.s./L**	**A mg a.s./L**	**F mg a.s./L**	**A mg a.s./L**	**F mg a.s./L**	**A mg a.s./L**	**F mg a.s./L**	**A mg a.s./L**	
0	0	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	**0**
0.31	0.37 119 %	0.33 107 %	0.32 103 %	0.30 97 %	0.29 94 %	0.25 81 %	0.34 110 %	0.25 81 %	0.30 97 %	0.26 84 %	0.32 103 %	0.26 84 %	0.34 110 %	0.26 84 %	0.30 97 %	0.26 84 %	0.27 87 %	0.23 74 %	**0.29**
0.63	0.74 118 %	0.62 98 %	ND	ND	ND	ND	0.64 102 %	0.51 81 %	ND	ND	ND	ND	0.66 104 %	0.53 84 %	ND	ND	ND	0.47 75 %	**ND**
1.25	1.45 116 %	1.37 110 %	ND	ND	ND	ND	1.26 101 %	1.06 85 %	ND	ND	ND	ND	1.38 110 %	1.04 83 %	ND	ND	ND	0.94 75 %	**ND**
2.5	2.95 118 %	2.48 99 %	ND	ND	ND	ND	2.59 104 %	2.11 84 %	ND	ND	ND	ND	2.43 97 %	2.01 80 %	ND	ND	ND	1.90 76 %	**ND**
5	5.99 120 %	5.48 110 %	5.79 116 %	5.20 104 %	5.26 105 %	4.12 82 %	4.98 100 %	4.22 84 %	4.65 93 %	4.14 83 %	5.78 116 %	4.80 96 %	4.72 94 %	4.30 86 %	4.76 95 %	4.11 82 %	4.43 89 %	4.00 80 %	**4.79**

N = nominal concentration, TW* = Time-weighted mean concentration calculated according to OECD 211 due to an incomplete sample set this has been calculated for the lowest and highest test concentrations by the HSE evaluator, F = 'fresh' media, A = 'Aged' media, % = Percentage of nominal concentration, na = not applicable, ND = Not determined, LoQ = 0.002 mg a.s./L, LoD = 0.001 mg a.s./L

Validity criteria:

In order to determine whether the study is valid the criteria in OECD 211 (2016) have been considered below:

- The mortality of the parent animals (female *Daphnia*) does not exceed 20 % at the end of the test. Obtained: 0 %
- The mean number of living offspring produced per parent animal surviving at the end of the test is > 60. Obtained: 212.6

The above validity criteria were met during the study.

Biological results:

After 21 days of exposure, no parent mortality occurred in the control groups and at the test item concentrations of up to and including the highest concentration tested. Statistically significant differences in the number of offspring per parent were observed at the two highest test item concentrations (Multiple Sequentially-rejected Welsh test after Bonferroni-Holm; α = 0.05, one-sided smaller).

The intrinsic rate of increase and day of first brood were significantly affected at the three highest test item concentrations (Williams Multiple Sequential t-test; α = 0.05, one-sided smaller).

Body length of the parent animals was significantly affected at the three highest test item concentration (Williams Multiple Sequential t-test; α = 0.05, one-sided smaller).

The results are summarised in Table B.9.2.5.1-4 and endpoints in table B.9.2.5.1-5.

Table B.9.2.5.1-4: Effects of cinmethylin on *Daphnia magna* parent mortality, reproduction and growth after 21 days of exposure

Concentration [mg test item/L] (nominal test item)	Control	0.31	0.63	1.25	2.5	5
Concentration [mg a.s./L] (nominal content of a.s.) [2)]	--	0.288	0.586	1.163	2.325	4.65
Time weighted mean measured concentration [mg a.s./L]	**Control**	**0.29**	-	-	-	**4.797**
Parent Mortality [%]	0	0	0	0	0	0
Cumulative offspring / parent	212.6	213.3	209.7	199.1	185.2*	34.4*
% reproductive effects to control based on cumulative offspring/parent	n.a.	-0.3	+1.4	+6.3	+12.9	+83.8
Body dry weight / parent [mg]	0.72	0.71	0.69	0.66	0.64	0.61
Mean dry weight of parent as compared with control [%]	n.a.	+1.4	+4.2	+8.3	+11.1	+15.3
Body length [mm]	3.82	3.78	3.71	3.58**	3.53**	3.17**
Mean body length of parent as compared with control [%]	n.a.	+1.0	+2.9	+6.3	+7.6	+17.0
Mean Intrinsic rate of increase (population growth rate)	0.412	0.405	0.400	0.392**	0.386**	0.220**
Mean intrinsic rate as compared with control [%]	n.a.	+1.7	+2.9	+4.9	+6.3	+46.6

* Statistically significant effects compared to the control (Multiple Sequentially-rejected Welsh test after Bonferroni-Holm; α = 0.05, one-sided smaller).

** Statistically significant effects compared to the control (Williams Multiple Sequential t-test; α = 0.05, one-sided smaller).

n.a. = not applicable, negative value indicates increase compared to control and positive value is decrease.

Table B.9.2.5.1-5: Calculated endpoints for chronic *Daphnia* study (based on 21 day exposure period)

Parameter	Endpoints [mg a.s./L] (nominal test item) [2)]
EC_{10} (21 d)[1)]	2.21 (95 % confidence limits: 1.40 – 3.49) equivalent to > 0.29 based on time weighted mean measured concentrations
EC_{20} (21 d)[1)]	2.55 (95 % confidence limits: 1.76 – 3.70) equivalent to > 0.29 based on time weighted mean measured concentrations
$LOEC_{overall}$ (21 d)	1.16
NOEC (21 d based on reproductive effects)	1.16
$NOEC_{overall}$ (21 d based on most conservative parameters- body length of parent and population growth rate)	0.59

1) $EC_{10/20}$ values based on mean cumulative offspring per female data (most conservative parameter for statistically derived endpoints).
2) Concentration is corrected for the purity of the test item (i.e. purity 93 %)

III. CONCLUSION

In a 21-day semi-static toxicity study with *Daphnia magna* the NOEC of cinmethylin was determined to be 0.59 mg a.s./L based on nominal concentrations. When considering reproductive effects, the NOEC is 1.16 mg a.s./L based on nominal concentrations.

The EC_{10} and EC_{20} values based on nominal concentrations were 2.21 and 2.55 mg a.s./L respectively.

HSE evaluator comments:

It was noted that analytical confirmation was not conducted for all test concentrations at each renewal (three times per week) for both 'fresh' and 'aged' solutions. In accordance with OECD 211 if the measured concentrations are not maintained within ± 20 % of nominals then all test concentrations should be measured during the study. As the concentrations were not within ± 20 % of nominals results should be expressed as time-weighted means. The HSE evaluator has calculated time weighted means but this is only possible for the lowest and highest test concentrations due to not all test concentrations being analysed during the study. This means the derived endpoints below are conservative. This is because the NOEC based on nominal concentration was 0.59 mg a.s./L, however due to the lack of analytical measurements it is not possible to derive a TWA concentration for this treatment group. Therefore the next lowest concentration which is possible to determine is 0.29 mg a.s./L (TWA concentration).

The analytical method is sufficiently validated in accordance with SANCO/3029/99 rev. 4 with an LOQ of 0.002 mg a.s./L (see volume 3, CA, section B5 for full details).

The above study was conducted to GLP and considered valid.

- Cinmethylin NOEC = **0.29 mg a.s./L** (time-weighted mean measured concentrations based on reproductive effects), noting as described above this endpoint is conservative.
- Cinmethylin EC_{10} = **> 0.29 mg a.s./L** (time-weighted mean measured concentrations based on reproductive effects)
- Cinmethylin EC_{20} = **> 0.29 mg a.s./L** (time-weighted mean measured concentrations based on reproductive effects)

B.9.2.6. Effects on algal growth

Report:	CA 8.2.6.1/1 Pearson N.,Stephenson R.R., 1987 b WL95481: Acute toxicity to *Selenastrum capricornutum* CI-521-005
Guidelines:	EPA
GLP:	Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test item:	Cinmethylin (WL 95481, Reg. no.: 900 202), WRC Tox. sample no. 513F, purity: 92.3 % (+0.77 – 0.4 %; analyzed).

B. STUDY DESIGN

Test species:	Unicellular fresh water green alga, *Pseudokirchneriella subcapitata*, (Reinsch) Korshikov (syn. *Selenastrum capricornutum* Prinz) ATCC 22662; in-house culture; stock originally obtained from "The American Type Culture Collection", Maryland, USA.
Test design:	Static system; test duration 96 hours; 7 test concentrations, each with 1 replicate per treatment plus a control with 6 replicates; acetone (0.1 ml/L) was used in each test item concentrations and the control; Additional replicates with and without algal cells for analytical determination of test item concentrations at test initiation (without algae) and test termination (with algae); daily assessment of growth.
Endpoints:	E_bC_{50} based on cell counts over 96 hours.
Test concentrations:	Control, 0.5, 0.82, 1.4, 2.2, 3.7, 6.1 and 10 mg a.s./L (nominal)
Test conditions:	Erlenmeyer flasks; test volume 50 mL; test medium: conditioned deionized water; pH 7.2 – 8.3; temperature: 22 - 26 °C; initial cell density 500 cells/mL; continuous light at approx. 3000 lux; constant shaking (100 rpm).
Analytics:	Analytical verification of test item concentrations was conducted using an GC-method with MS detection.
Statistics:	Descriptive statistics; Probit analysis for determination of EC_x value.

II. RESULTS AND DISCUSSION

Analytical results: Analytical verification of test item concentrations was conducted in each concentration at the beginning and at the end of the test in flasks without and with algae, respectively. Measured concentrations of cinmethylin were, with one exception, within ± 20 % of nominal values. The study author based the calculated endpoints on nominal concentrations. It was unclear in the study report whether precipitation of the test item was assessed.
The results are shown in the table below:

Table B.9.2.6-1: Measured concentrations during study

Nominal concentration (mg a.s./L)	0 hours		96 hours	
	Measured concentration (mg a.s./L)	% of nominal	Measured concentration (mg a.s./L)	% of nominal
0	<0.001	n.a.	< 0.004	n.a.
0.5	0.65	130	0.49	98
0.82	0.98	120	0.68	83
1.4	1.5	107	1.3	93
2.2	2.4	109	2.1	95
3.7	4.4	119	3.9	105
6.1	6.8	111	6.0	98
10	9.7	97	9.9	99

n.a. = not applicable

Validity criteria:

The criteria in OECD 201 (2011) have been considered below, it should be noted the HSE evaluator has used the raw data in the study report to calculate CV values based on 72-hour values:

- The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period. This corresponds to a specific growth rate of 0.92 day^{-1}. Obtained: 540.
- The mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3, for 72-hour tests) in the control cultures must not exceed 35 %. Obtained: 53 % therefore criteria were not met.
- The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures must not exceed 7% in tests with *Pseudokirchneriella subcapitata*. Obtained: 1.52 %.

During the study the above criteria were not met.

Biological results: After 96 h of exposure, results show a gradual reduction in cell numbers up to the highest test item concentration of 10 mg/a.s./L. The results are summarized in Table B.9.2.6-2.

Table B.9.2.6-2: Effect of cinmethylin on the growth of the green alga *Selenastrum capricornutum*

Concentration [mg a.s./L] (nominal)	**Control**	**0.5**	**0.82**	**1.4**	**2.2**	**3.7**	**6.1**	**10**
Cell number at 96 h as % of mean control cell number	--	46	97	69	56	51	23	6
Endpoints [mg a.s./L] (nominal)								
E_bC_{50} (96 h)	2.9 (95 % confidence intervals 2.1 – 4.1)							

III. CONCLUSION

In a 96-hour algae test with *Selenastrum capricornutum* (syn. *Pseudokirchneriella subcapitata*), the E_bC_{50} for cinmethylin was determined to be 2.9 mg a.s./L, based on nominal concentration.

HSE evaluator comments:

The above study was conducted to GLP but is not considered valid by the HSE evaluator. The following observations were noted:

- As stated above the validity criteria detailed in OECD 201 were not met.
- Only a single replicate for each test concentration was used generating uncertainty, noting six replicates were used for the control.
- The initial cell density was 500 cells/ml rather than the recommended 5 $x10^3$ – 1 $x10^4$ cells/ml based on OECD 201.
- Considering the dose response is interrupted the confidence limits are reasonably close together raising some uncertainty regarding the statistical analysis.
- Based on analytical data concentrations were within ± 20 % of nominals with one exception at the lowest test concentration (130 %).
- Study was conducted for 96 hours instead of the recommended 72 hours, noting this is not a major limitation as measurements were made at 72 hours.
- Endpoints were calculated based on biomass not growth rate. As stated in EFSA 2013 aquatic guidance, algal study endpoints used in the risk assessment should be based on growth rate.
- EC_{10} and EC_{20} values were not calculated. These are required under current data requirements i.e. 283/2013.

Given the above the HSE evaluator has not considered this study further in the risk assessment.

Report:	CA 8.2.6.1/2 Kauf A., 2017 a Effect of BAS 684 H (Reg.No.: 900202) on the growth of the green alga *Pseudokirchneriella subcapitata* 2016/1001944
Guidelines:	OECD 201, EPA 850.4500, OECD-ENV/JM/MONO(2002)/9
GLP:	Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Cinmethylin (Reg. no.: 900 202), batch no. COD-002038, purity: 93 %.

B. STUDY DESIGN

Test species: Unicellular fresh water green alga, *Pseudokirchneriella subcapitata*, (Reinsch) Korshikov (syn. *Selenastrum capricornutum* Prinz), SAG 61.81; stock obtained from "The Culture Collection of Algae", Göttingen University, Germany.

Test design: Static system; test duration 96 hours; 7 test concentrations, each with 5 replicates per treatment plus a control with 10 replicates; daily assessment of growth.

Endpoints: EC_{50} with respect to growth rate and yield after exposure over 96 hours.

Test concentrations: Control, 0.32, 0.85, 2.24, 5.90, 15.52, 40.9 and 100 mg a.s./L corresponding to geometric mean concentration: control (0); 0.270, 0.651, 1.765, 4.294, 11.447, 36.748 and 63.440 mg a.s./L

Test conditions: 100 mL Erlenmeyer dimple flasks; test volume 60 mL; test medium: OECD medium; pH 7.68 – 7.79; temperature: 22 ± 1 °C; initial cell densities 1 x 10^4 cells/mL; continuous light at about 7800 lux; constant shaking (130 rpm).

Analytics: Analytical verification of test item concentrations was conducted using an LC-method with MS/MS detection.

Statistics: Descriptive statistics; Probit analysis for determination of EC_x values (α = 0.05). Multiple Sequentially-rejective U-test After Bonferroni-Holm and

Williams multiple Sequential t-test for determination of NOEC (α = 0.05, one-sided smaller).

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration at the beginning and at the end of the test. Results of the cinmethylin analysis showed recovery values of 63.0 to 90.9 % of the nominal concentration at day 0. At 96 hours values of 63.9 to 88.8 % of the nominal were recorded. Therefore, the following biological results are based on geometric mean measured test item concentrations. It was unclear in the study report whether precipitation of the test item was assessed.

The results are shown in the table below:

Table B.9.2.6-3: Measured concentrations during study

Nominal concentration (mg a.s./L)	0 hours		96 hours		Geometric mean measured (mg a.s./L)
	Measured concentration (mg a.s./L)	% of nominal	Measured concentration (mg a.s./L)	% of nominal	
0	< LoD	n.a.	< LoD	n.a.	0
0.32	0.281	87.9	0.26	81.2	0.27
0.85	0.765	90.1	0.55	65.2	0.651
2.24	1.96	87.6	1.59	70.8	1.765
5.90	4.68	79.3	3.94	66.8	4.294
15.52	12.1	78.2	10.8	69.6	11.447
40.9	37.2	90.9	36.3	88.8	36.748
100	63.0	63.0	63.9	63.9	63.44

n.a. = not applicable, LoD = 0.01 mg a.s./L

Validity criteria:

The criteria in OECD 201 (2011) have been considered below:

- The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period. This corresponds to a specific growth rate of 0.92 day^{-1}. Obtained: 48.4.
- The mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3, for 72-hour tests) in the control cultures must not exceed 35 %. Obtained: 7.3 %.
- The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures must not exceed 7% in tests with *Pseudokirchneriella subcapitata*. Obtained: 1.3 %.

During the study the above criteria were met.

Biological results: No morphological effects on algae were observed in the control and at all test item concentrations. After 72 h of exposure, statistically significant effects compared to the control were detected at all test item concentrations for yield and at concentrations of 1.765 mg a.s./L and above based on growth rate (α = 0.05, one-sided smaller).

The results are summarised in Table B.9.2.6-4.

Table B.9.2.6-4: Effect of cinmethylin on the growth of the green alga *Pseudokirchneriella subcapitata*

Concentration [mg a.s./L] (nominal)	**Control**	**0.32**	**0.85**	**2.24**	**5.90**	**15.52**	**40.9**	**100**
Concentration [mg a.s./L] (geometric mean)	**--**	**0.27**	**0.651**	**1.765**	**4.294**	**11.447**	**36.748**	**63.44**
Inhibition in 72 h (growth rate) [%]#	--	-0.4	1.1	5.6*	16.1*	22.0*	67.0*	79.2*
Inhibition in 72 h (yield) [%]#	--	-1.5*	4.2*	20.0*	47.4*	58.4*	94.1*	97.3*
Endpoints [mg a.s./L] (geometric mean)								
E_rC_{50} (72 h)	23.04 mg/L (95 % confidence intervals 20.648 – 25.648)							
E_rC_{20} (72 h)	7.87 mg/L (95 % confidence intervals 6.460 – 9.252)							
E_rC_{10} (72 h)	4.49 mg/L (95 % confidence intervals 4.34 – 5.560)							
E_yC_{50} (72 h)	5.96 mg/L (95 % confidence intervals 5.326 – 6.672)							
E_yC_{20} (72 h)	1.76 mg/L (95 % confidence intervals 1.460 – 2.060)							
E_yC_{10} (72 h)	0.93 mg/L (95 % confidence intervals 0.723 – 1.144)							
NOEC growth rate (72 h)	0.651							
NOEC yield (72 h)	0.27**							

Negative values indicate stimulated growth compared to the control.
* Significant difference to control ($\alpha = 0.05$, one-sided smaller).
** Noting a significant increase compared to control at this concentration.

Reference item test: A reference study was conducted with potassium dichromate. The 72-hour E_rC_{50} was calculated as 1.0 mg reference item/L which is within the range stated in OECD 201 i.e. 0.6 – 1.03 mg reference item/L.

III. CONCLUSION

In a 72-hour algae test with *Pseudokirchneriella subcapitata,* the E_rC_{50} and the E_yC_{50} for cinmethylin were determined to be 23.04 and 5.96 mg a.s./L (geometric mean).

HSE evaluator comments:

The above study was conducted for 96 hours. However, as the standard duration for algal studies under data requirements 283/2013 is 72 hours only these endpoints have been considered.

It was noted the E_rC_{10} based on growth rate (both mean and lower confidence interval) is not in-line with the experimental data generating some uncertainty i.e. E_rC_{10} (growth rate) is 4.49 mg a.s./L (geometric mean) but there was 16.1 % inhibition compared to control at 4.29 mg a.s./L (geometric mean). This generates some uncertainty as the E_rC_{10} may not be protective of observed toxicity. Therefore, the HSE evaluator proposes setting the E_rC_{10} as > 1.765 mg a.s./L (geometric mean) to allow for the uncertainty.

The analytical method is sufficiently validated in accordance with SANCO/3029/99 rev. 4 with an LOQ of 0.135 mg a.s./L (see volume 3, CA, section B5 for full details).

This study was conducted to GLP and considered valid. The following endpoints were derived:

- Cinmethylin E_rC_{50} (72 hours) = **23.04** mg a.s./L (based on geometric mean measured)
- Cinmethylin E_rC_{20} (72 hours) = 7.87 mg a.s./L (based on geometric mean measured)
- Cinmethylin E_rC_{10} (72 hours) = > 1.765 mg a.s./L (based on geometric mean measured), not statistically derived, see above.

- Cinmethylin E_yC_{50} (72 hours) = 5.96 mg a.s./L (based on geometric mean measured)
- Cinmethylin E_yC_{20} (72 hours) = 1.76 mg a.s./L (based on geometric mean measured)
- Cinmethylin E_yC_{10} (72 hours) = 0.93 mg a.s./L (based on geometric mean measured)
- Cinmethylin NOEC (72 hours growth rate) = 0.651 mg a.s./L (based on geometric mean)
- Cinmethylin NOEC (72 hours yield) = 0.27 mg a.s./L (based on geometric mean)

Report:	CA 8.2.6.2/1 Kauf A., 2017 b Effect of BAS 684 H (Reg.No.: 900202) on the growth of the blue alga *Anabaena flos-aquae* 2016/1001945
Guidelines:	EPA 850.4500, EPA 850.4550, OECD 201, OECD-ENV/JM/MONO(2002)/9
GLP:	Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Cinmethylin (Reg. no.: 900 202), batch no. COD-002038, purity: 93 %.

B. STUDY DESIGN

Test species: Filamentous fresh water blue alga, *Anabaena flos-aquae*, (Reinsch) (Lyngbye) de Brébisson, UTEX B 1444; stock obtained from the "The Culture Collection of Algae", University of Texas, Austin, USA.

Test design: Static system; test duration 96 hours; 5 test concentrations, each with 5 replicates per treatment plus a control with 10 replicates; daily assessment of growth.

Endpoints: EC_{50} with respect to growth rate and yield after exposure over 72 and 96 hours.

Test concentrations: Control, nominal concentrations of 2.56, 6.4, 16, 40, 100 mg a.s./L corresponding to geometric mean concentrations of 1.36, 3.24, 8.22, 21.09, 53.17 mg a.s./L)

Test conditions: 100 mL Erlenmeyer dimple flasks; test volume 60 mL; test medium: OECD medium and OCSPP 850.4550; pH 7.56 – 7.67; temperature: 24 ± 1°C; initial cell densities 1 x 10^4 cells/mL; continuous light at about 3000 lux; constant shaking (130 rpm).

Analytics: Analytical verification of test item concentrations was conducted using an HPLC-method with MS detection. Measurements were taken at test initiation and termination.

Statistics: Descriptive statistics; probit analysis for determination of EC_x values (α = 0.05). Multiple Sequentially-rejective U-test After Bonferroni-Holm and Williams multiple Sequential t-test for determination of NOEC (α = 0.05, one-sided smaller).

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration at the beginning and at the end of the test. At test initiation, the mean measured values of cinmethylin were found to be in a range of 50 - 59 % of the nominal concentrations (average of 54.7 %) and 46 - 55 % of the nominal concentrations (average of 49.7 %) at the end of the study. Therefore, the following biological results are based on geometric mean measured test item concentrations. It was unclear in the study report whether precipitation of the test item was assessed.

Table B.9.2.6-5: Measured concentrations during study

Nominal concentration (mg a.s./L)	0 hours		96 hours		Geometric mean measured* (mg a.s./L)
	Measured concentration (mg a.s./L)	% of nominal	Measured concentration (mg a.s./L)	% of nominal	
0	< 0.2	n.a.	< 0.2	n.a.	0
2.56	1.44	56	1.31	51	1.36
6.4	3.17	50	3.08	48	3.24
16	9.49	59	7.93	50	8.22
40	21.78	54	20.04	50	21.09
100	56.12	56	49.88	50	53.17

n.a. = not applicable, * The HSE evaluator calculated different geometric mean values in some cases i.e. 1.37, 3.12, 8.68, 20.89 and 52.91 mg a.s./L. However, the difference is relatively low and most likely due to rounding, therefore the geometric mean measured values reported by study author are considered acceptable.

Validity criteria:

The criteria in OECD 201 (2011) have been considered below:

- The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period. This corresponds to a specific growth rate of 0.92 day^{-1}. Obtained: 35.1.
- The mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3, for 72-hour tests) in the control cultures must not exceed 35 %. Obtained: 32.5 %.
- The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures must not exceed 7 % in tests with *Pseudokirchneriella subcapitata*. For other less frequently tested species, the value should not exceed 10 %. Obtained: 4.0 %.

During the study the above criteria were met.

Biological results: No morphological effects on algae were observed in the control and at all test item concentrations. After 72 h of exposure, statistically significant effects compared to the control were detected at the three highest test item concentrations (8.22, 21.09 and 53.17 mg a.s./L based on geometric mean) for yield and at concentrations of 21.09 mg a.s./L (geometric mean) and above based on growth rate ($\alpha = 0.05$, one-sided smaller).

The results are summarised in Table B.9.2.6-6.

Table B.9.2.6-6: Effect of cinmethylin on the growth of the blue algae *Anabaena flos-aquae*

Concentration [mg a.s./L] (nominal)	**Control**	**2.56**	**6.4**	**16**	**40**	**100**
Concentration [mg a.s./L] (geometric mean)	**--**	**1.36**	**3.24**	**8.22**	**21.09**	**53.17**
Inhibition in 72 h (growth rate) [%] #	--	-4.3	-3.2	-0.3	6.1*	52.4*
	Endpoints [mg a.s./L] (geometric mean)					
E_rC_{50} (72 h)	51.335 mg/L (95 % confidence intervals 50.416 – 52.292)					
E_rC_{20} (72 h)	31.627 mg/L (95 % confidence intervals 30.446 – 32.711)					
E_rC_{10} (72 h)	24.553 mg/L (95 % confidence intervals 23.229 – 25.775)					
E_yC_{50} (72 h)	31.098 mg/L (95 % confidence intervals 29.841 – 32.443)					
E_yC_{20} (72 h)	Not reported and unclear whether calculated based on study report.					
E_yC_{10} (72 h)	16.864 mg/L (95 % confidence intervals 15.708 – 17.932)					
NOEC growth rate and yield (72 h)	8.22					

Negative values indicate stimulated growth compared to the control. It should be noted the study report did not state inhibition at 72 hours based on yield and it was not possible to determine as the raw data for yield was not clearly scanned into the study report.

* Significant difference to control ($\alpha = 0.05$, one-sided smaller).

Reference item test: A reference study was conducted with 3,5-DCP. The 96-hour E_rC_{50} was reported as 4.738 mg reference item/L noting an acceptable range for this species is not stated in OECD 201.

III. CONCLUSION

In an 72-hour algae test with *Anabaena flos-aquae,* the 72-E_rC_{50} and E_yC_{50} values for cinmethylin were determined to be 51.335 and 31.098 mg a.s./L (geometric mean).

HSE evaluator comments:

The above study was conducted for 96 hours. However, as the standard duration for algal studies under data requirements 283/2013 is 72 hours only these endpoints have been considered.

It was unclear based on the study report whether an EC_{20} value based on yield was generated for 72-hour data. Therefore, this endpoint could not be determined, noting the inhibition values based on yield at 72-hours were not clearly reported. However, given this is a HSE evaluation and the EFSA aquatic guidance 2013 recommends the use of growth rate values in the risk assessment further information from the applicant was not requested. In addition, EC_{20} values are currently not considered in the risk assessment or classification and the data requirements (283/2013) do not specify that EC_{20} values based on yield are required.

The analytical method is sufficiently validated in accordance with SANCO/3029/99 rev. 4 with an LOQ of 0.2 mg a.s./L (see volume 3, CA, section B5 for full details).

This study was conducted to GLP and considered valid. The following endpoints were derived:

- Cinmethylin E_rC_{50} (72 hours) = 51.34 mg a.s./L (based on geometric mean measured)
- Cinmethylin E_rC_{20} (72 hours) = 31.63 mg a.s./L (based on geometric mean measured)
- Cinmethylin E_rC_{10} (72 hours) = 24.55 mg a.s./L (based on geometric mean measured)
- Cinmethylin E_yC_{50} (72 hours) = 31.10 mg a.s./L (based on geometric mean measured)
- Cinmethylin E_yC_{10} (72 hours) = 16.86 mg a.s./L (based on geometric mean measured)

- Cinmethylin NOEC (growth rate/yield, 72 hours) = 8.22 mg a.s./L (based on geometric mean)

B.9.2.7. Effects on aquatic macrophytes

B.9.2.7.1 Active substance: Cinmethylin:

Report:	CA 8.2.7/1 Vlechev S., 2017 a Effect of BAS 684 H on the growth of *Lemna gibba* 2015/1029521 (certified by Landesamt fuer Umwelt, Mainz, Germany)
Guidelines:	OECD 221, EPA 850.4400, ASTM E 1415-91
GLP:	Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Cinmethylin (Reg. no.: 900 202), batch no. COD-002038, purity: 93 %.

B. STUDY DESIGN

Test species: Duckweed (*Lemna gibba* G3), inocula from 7 – 10 days old cultures; cultures maintained in-house; stock obtained from, Friedrich-Schiller University. Jena, Germany.

Test design: Static system (7 days); 7 test item concentrations with 4 replicates plus a control with 6 replicates, the purity of the test substance (93%) was taken into account of the stock solution; 2 plants with 4 fronds and 1 plant with 3 three fronds in each vessel, total number of fronds at test initiation: 11 per replicate; assessment of growth and other effects on days 2, 5 and at test termination. The yield based on the dry weight was determined at test beginning from a sample of the inoculum culture and at test termination with the plant material from each test item concentration and control.

Endpoints: $EC_{10,}$ EC_{50} and NOEC with respect to growth rate and yield after exposure over 7 days.

Test concentrations: Control (0), 1.0, 2.6, 6.7, 17.3, 45, 116 and 300 µg a.s./L, corresponding to mean measured concentrations of 0 (control), 0.9, 2.3, 6.0, 14.2, 38, 99 and 258 µg a.s./L.

Test conditions: 400 mL glass beakers, test volume 160 mL covered by a glass plate, 20x-AAP nutrient medium, pH 7.45 - 7.51 at test initiation and pH 8.47 – 8.57 at test termination; temperature: approx. 25 °C, continuous light, light intensity: approx. 6990 lux.

Analytics: Analytical verification of the test item was conducted using an LC-method with MS/MS detection.

Statistics: Descriptive statistics; Probit analysis for determination of the EC_x values, Williams Multiple Sequential t-test Procedure (NOEC Yield based on frond number/dry weight and growth rate based on dry weight, $\alpha = 0.05$, one-sided smaller) and Welch-t test for Inhomogeneous Variances with Bonferroni-Holm Adjustment (Growth rate based on frond number, $\alpha = 0.05$, one-sided smaller).

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each test concentration at the beginning and at the end of the test. The mean measured recoveries for cinmethylin were 100 – 112 % of nominal at test initiation and 66 – 82 % of nominal at test termination. The following biological results are based on geometric mean measured concentrations of the analytically determined concentrations of the test item. It was unclear in the study report whether precipitation of the test item was assessed.

The results are shown in the table below:

Table B.9.2.7.1-1: Measured concentrations during study

Nominal concentration (µg a.s./L)	Day 0		Day 7		Geometric mean measured (µg a.s./L)
	Measured concentration (µg a.s./L)	% of nominal	Measured concentration (µg a.s./L)	% of nominal	
0	< LoD	n.a.	< LoD	n.a.	0
1	1.095	110	0.822	82	0.9
2.6	2.736	105	1.977	76	2.3
6.7	7.527	112	4.845	72	6.0
17.3	17.3	100	11.583	67	14.2
45	47.9	106	30.240	67	38
116	124.7	108	78.750	68	99
300	334.9	112	199.267	66	258

n.a. = not applicable, LoD = 0.04 µg a.s./L

Validity criteria:

The criteria in OECD 221 (2006) have been considered below:

- For the test to be valid, the doubling time of frond number in the control must be less than 2.5 days (60 h), corresponding to approximately a seven-fold increase in seven days and an average specific growth rate of 0.275 d^{-1}. Obtained: doubling time was 2 days and the multiplication factor over 7 days was 10.9.

During the study the above criteria were met.

Biological results: Shorter roots were observed in test item concentration of 6.0 and 14.2 µg a.s./L (geometric mean), very short roots were observed at the three highest test item concentrations (38, 99 and 258 µg a.s./L based on geometric mean), whereas, chlorosis was observed in the two highest test item concentrations at the end of the study. Significant effects compared to control were observed (for at least one measured parameter) at the four highest test concentrations (14.2, 38, 99 and 258 µg a.s./L based on geometric mean).

Effects on growth rate and yield are summarised in Table B.9.2.7.1-2.

Table B.9.2.7.1-2: Effect of cinmethylin on the growth of duckweed *Lemna gibba*

Concentration (nominal) [µg a.s./L]	**Control**	**1.0**	**2.6**	**6.7**	**17.3**	**45**	**116**	**300**
Concentration (geometric mean measured) [µg a.s./L]	**--**	**0.9**	**2.3**	**6.0**	**14.2**	**38**	**99**	**258**
Inhibition after 7 d [%] [#] (growth rate based on frond no.)	--	-0.88	0.39	0.46	2.56	15.1*	57.12*	86.33*
Inhibition after 7 d [%] [#] (growth rate based on dry weight)	0.0	-0.53	-1.07	0.95	3.66**	12.46**	26.01**	40.13**
Inhibition after 7 d [%] [#] (yield based on frond no.)	0.0	-2.37	0.84	1.07	6.56	33.36***	81.68***	96.11***
Inhibition after 7 d [%] [#] (yield based on dry weight)	0.0	-1.73	-3.92	2.84	11.11***	32.53***	57.33***	72.31***
	Endpoints [µg a.s./L] (geometric mean measured)							
E_rC_{50} (7 d) based on frond no.	88.77 (95 % limits: 82.42 – 95.61)							
E_rC_{20} (7 d) based on frond no.	42.07 (95 % limits: 37.02 – 46.78)							
E_rC_{10} (7 d) based on frond no.	28.48 (95 % limits: 23.94 – 32.76)							
E_yC_{50} (7 d) based on frond no.	51.51 (95 % limits: 48.11 – 55.15)							
E_yC_{20} (7 d) based on frond no.	26.97 (95 % limits: 24.00 – 29.70)							
E_yC_{10} (7 d) based on frond no.	19.23 (95 % limits: 16.43 – 21.83)							
E_rC_{50} (7 d) based on dry weight	> 258							
E_rC_{20} (7 d) based on dry weight	73.53 (95 % limits: 63.74 – 83.22)							
E_rC_{10} (7 d) based on dry weight	30.02 (95 % limits: 23.44 – 36.56)							
E_yC_{50} (7 d) based on dry weight	84.05 (95 % limits: 71.11 – 100.39)							
E_yC_{20} (7 d) based on dry weight	21.97 (95 % limits: 16.27 – 27.67)							
E_yC_{10} (7 d) based on dry weight	10.89 (95 % limits: 7.13 – 14.89)							
NOEC overall [1)]	2.3							

[#] Negative values indicate stimulated growth compared to the control, * Statistically different compared to control (Welch t-test with Bonferroni-Holm adjustment, α = 0.05, one-sided smaller), ** Statistically different compared to control (Multiple sequentially-rejective U-test after Bonferroni-Holm, α = 0.05, one-sided smaller), *** Statistically different compared to control (Williams multiple sequential t-test, α = 0.05, one-sided smaller), §nominal values are based on stock solutions which are corrected for purity of the active substance (93 %)

[1)] based on visual observation: shorter root's were observed.

Reference item test: A reference study was conducted with 3,5-DCP. The following endpoints were derived:

- E_rC_{50} (frond number): 12.4 mg reference item/L
- E_yC_{50} (frond number): 8.7 mg reference item/L
- E_rC_{50} (dry weight): 11.1 mg reference item/L
- E_yC_{50} (frond number): 8.3 mg reference item/L

The guidance document OECD 221 references a ring test report for appropriate ranges. Whilst the range for *Lemna gibba* using 3,5-DCP was 2.7 to 3.4 mg/L for EC_{50} values it is not clear whether these were based on growth rate. In addition, it is stated that further work is needed for this species and reference item before appropriate ranges can be derived. Therefore, it is not possible to confirm whether the sensitivity was appropriate following OECD 221. The study author did not state in the study report whether the result was within historical limits.

III. CONCLUSION

In a 7-day aquatic plant test with *Lemna gibba,* the E_rC_{50} and the E_yC_{50} for cinmethylin were determined to be 88.77 and 51.51 µg a.s./L based on frond number and 408.1 and 84.05 µg a.s./L for dry weight, respectively (based on geometric mean measured concentrations).

HSE evaluator comments:

As stated above there is some uncertainty regarding the reference test result i.e. it is not possible to confirm adequate sensitivity based on OECD 221 or the study report. Nonetheless it is noted that the validity criteria were met and that it is recommended to conduct a reference test based on OECD 221. Therefore, the HSE evaluator does not consider this point sufficient to invalidate the study.

Whilst observations were made it was not reported how many plants demonstrated phytotoxicity. The observations detailed in the appendix mention shorter roots observed at geometric mean concentrations of 6.0 and 14.2 µg a.s./L. 'Very short' roots were observed at the three highest concentrations and 'slight chlorosis' occurred at the two highest test concentrations (99 and 258 µg a.s./L based on geometric mean). Given the lack of detail regarding effects it is not possible to derive an EC_{50} endpoint based on phytotoxicity. However, with the exception of the two highest concentrations (99 and 258 µg a.s./L based on geometric mean) only effects on roots were observed at concentrations of 6.0 µg a.s./L (geometric mean) and above. This will be discussed further in the risk assessment section.

The analytical method is sufficiently validated in accordance with SANCO/3029/99 rev. 4 with an LOQ of 0.2 µg a.s./L (see volume 3, CA, section B5 for full details).

This study was conducted to GLP and considered valid. The following endpoints were derived (all are based on geometric mean measured concentrations), note the equivalent endpoint in mg a.s./L has also been presented:

- Cinmethylin E_rC_{50} = **88.77** µg a.s./L equivalent to 0.0888 mg a.s./L (frond number)
- Cinmethylin E_rC_{20} = 42.07 µg a.s./L equivalent to 0.0421 mg a.s./L (frond number)
- Cinmethylin E_rC_{10} = 28.48 µg a.s./L equivalent to 0.0285 mg a.s./L (frond number)

- Cinmethylin E_yC_{50} = 51.51 µg a.s./L equivalent to 0.0515 mg a.s./L (frond number)
- Cinmethylin E_yC_{20} = 26.97 µg a.s./L equivalent to 0.0270 mg a.s./L (frond number)
- Cinmethylin E_yC_{10} = 19.23 µg a.s./L equivalent to 0.0192 mg a.s./L (frond number)

- Cinmethylin E_rC_{50} = > 258 µg a.s./L equivalent to > 0.2580 mg a.s./L (dry weight)
- Cinmethylin E_rC_{20} = 73.53 µg a.s./L equivalent to 0.0735 mg a.s./L (dry weight)
- Cinmethylin E_rC_{10} = 30.02 µg a.s./L equivalent to 0.0300 mg a.s./L (dry weight)

- Cinmethylin E_yC_{50} = 84.05 µg a.s./L equivalent to 0.0841 mg a.s./L (dry weight)
- Cinmethylin E_yC_{20} = 21.97 µg a.s./L equivalent to 0.0220 mg a.s./L (dry weight)
- Cinmethylin E_yC_{10} = 10.89 µg a.s./L equivalent to 0.0109 mg a.s./L (dry weight)
- Cinmethylin NOEC (overall) = 2.3 µg a.s./L equivalent to 0.0023 mg a.s./L (based on phytotoxicity)

Report: CA 8.2.7/2
Vlechev S., 2017 b
Effects of BAS 684 H on the growth of the aquatic plant *Glyceria maxima*
2015/1029520

Guidelines: OECD 221, OECD 219, OECD 239 (2016), ASTM E 1913-04
GLP: Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Cinmethylin (Reg. no.: 900 202), batch no. COD-002038, purity: 93 %.

B. STUDY DESIGN

Test species: Reed Sweet-grass (*Glyceria maxima*), a monocotyledonous aquatic plant species from the poacea family was cultivated in house (non-GLP) after purchase from the plant nursery. The plants were placed in aquaria with sediment and Smart & Barko medium for the acclimatization in a climate-controlled room 6 days prior to the study.

Test design: Static renewal system (14 days); 6 test item concentrations with 5 replicates plus a control with 10 replicates and test concentrations application of test item via water phase; the purity of the test substance was taken into account in the calculations of the stock solution; water renewal at day 7; 1 plant with 2 blades per pot/replicate at test start; assessment of growth and other effects on days 7, 10 and at test termination. Dry and wet weight on day 0 was determined with 10 visually comparable plants from the acclimatization phase. At test initiation and day 7 (new solution), the water chemistry (such as O_2, temperature, conductivity and pH) were determined in the bulk solution and on day 7 (old solution) and at the end of the study in each replicate of each test concentration.

Endpoints: EC_{50} and EC_{10} with respect to growth rate and yield after exposure over 14 days.

Test concentrations: Control, 0.01, 0.03, 0.1, 0.3, 1 and 3 mg a.s./L, corresponding to mean measured concentration of 0.008, 0.026, 0.086, 0.247, 0.813 and 2.64 mg a.s./L.

Test conditions: 2 L glass beakers (tall form), test volume 750 mL, standard artificial sediment after OECD 219, Smart & Barko medium; sediment-pH 7.3; water-pH 7.84 – 7.87; water temperature: 19.8 – 21.9 °C, photo period: 16: 8 hours; light intensity: 10.21 – 11.88 klux.

Analytics: Analytical verification of the test item in water was conducted using an UHPLC-method with MS detection.

Statistics: Descriptive statistics; Probit analysis for determination of the EC_x values, The NOEC was determined statistically by Dunnett's test (dry weight yield and growth rate, $\alpha = 0.05$, one-sided smaller) and Welch-t Test (wet weight yield and growth rate, total length yield and growth rate, $\alpha = 0.05$, one-sided smaller).

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of the test item was carried out in each test concentration at the beginning, at day 7 and at the end of the test. At test initiation and day 7 (new solution) the analytical samples were taken from the respective bulk solutions, on day 7 (old solution) and at the end of the test from mixed samples (pooled replicates of each treatment). The mean measured values determined in the bulk solutions at test initiation and day 7 (new solution) were between 96 and 111 % of nominal. At day 7 (old solution) and test termination concentrations were between 64 and 81 % of nominal. Therefore, the following biological results based on geometric mean measured test item concentrations. Stock solutions were reported as visibly clear.

The results are shown in the table below:

Table B.9.2.7.1-3: Measured concentrations during study

Nominal concentration (mg a.s./L)	Day 0 'new'		Day 7 'old'		Day 7 'new'		Day 14 'aged'		Geometric mean measured* (mg a.s./L)
	MC (mg a.s./L)	% of nominal	MC (mg a.s./L)	% of nominal	MC (mg a.s./L)	% of nominal	MC (mg a.s./L)	% of nominal	
0	<LoD	n.a.	<LoD	n.a.	<LoD	n.a.	<LoD	n.a.	0.0
0.01	0.010	102	0.006	64	0.010	109	0.007	75	0.008
0.029	0.030	105	0.020	68	0.032	110	0.022	76	0.026
0.098	0.106	108	0.064	65	0.109	111	0.073	74	0.086
0.294	0.299	102	0.209	71	0.283	96	0.211	72	0.247
0.980	0.982	100	0.645	66	0.964	98	0.714	73	0.813
2.939	3.14	107	2.13	72	3.060	104	2.390	81	2.640

MC = Measured Concentration, n.a. = not applicable, LoD = 1.0 µg a.s./L. * The HSE evaluator calculated different geometric mean values in some cases i.e. 0.008, 0.025, 0.086, 0.247, 0.813 and 2.645 mg a.s./L. However, the difference is relatively low and most likely due to rounding, therefore the geometric mean measured values reported by study author are considered acceptable.

Validity criteria:

The criteria in OECD 239 (2014) have been considered below, noting these guidelines were developed for a different species i.e. *Myriophyllum spicatum*:

- For the test results to be valid, the mean total shoot length and mean total shoot fresh weight in control plants at least double during the exposure phase of the test. In addition, control plants must not show any visual symptoms of chlorosis and should be visibly free from contamination by other organisms such as algae and/or bacterial films on the plants, at the surface of the sediment and in the test medium. Observed: 300 % increase. However, algae growth was observed in the control replicates.
- The mean coefficient of variation for yield based on measurements of shoot fresh weight (i.e. from test initiation to test termination) in the control cultures does not exceed 35 % between replicates. Observed: 20 % (based on yield of wet weight)

During the study some of the above criteria were met, the one exception being algal growth in the control cultures.

Biological results: At day 7 growth of algae was observed in all test concentrations and the control (the higher the concentration, the slighter the algae growth); however, this was deemed by the study author to be at levels not effecting performance of the study. From day 10 on, a total necrosis of the plant was observed in all replicates at the highest test concentration of 2.939 mg a.s./L (nominal). At day 14 in the test concentration of 0.980 mg a.s./L (nominal) in four replicates single blades show some necrosis and in one replicate a complete necrosis of the plant was observed, phytotoxicity was not reported in concentrations of 0.294 mg a.s./L (nominal) and below. Statistically significant inhibition of yield based on total length, wet weight and dry weight was observed at the four highest test item concentrations (Welch t-test with Bonferroni-Holm adjustment for total length and wet weight and Dunnett's multiple t-test for dry weight, $\alpha = 0.05$, one-sided smaller). Statistically significant inhibition of growth rate compared to control was observed at the three highest test item concentrations based on wet weight and dry weight (Welch t-test with Bonferroni-Holm adjustment for total length and wet weight and Dunnett's multiple t-test for dry weight, $\alpha = 0.05$, one-sided smaller) and at the four highest test item concentrations based on total length (Welch t-test with Bonferroni-Holm adjustment, $\alpha = 0.05$, one-sided smaller). Effects on growth rate and yield are summarized in Table B.9.2.7.1-4.

Table B.9.2.7.1-4: Effect of cinmethylin on the growth of *Glyceria maxima*

Concentration (nominal) [mg a.s./L]	**Control**	**0.010**	**0.029**	**0.098**	**0.294**	**0.980**	**2.939**
Concentration (geometric mean measured) [mg a.s./L]	**--**	**0.008**	**0.026**	**0.086**	**0.247**	**0.813**	**2.64**
Inhibition after 14 d [%] # (growth rate based on total length)	--	-5.0	11.4	37.7* 1)	65.3* 1)	92.0* 1)	93.3* 1)
Inhibition after 14 d [%] # (growth rate based on wet weight)	--	-2.6	6.3	26.0	67.0* 1)	95.9* 1)	98.7* 1)
Inhibition after 14 d [%] (growth rate based on dry weight)	--	0.1	0.7	15.7	40.8* 2)	60.4* 2)	65.9* 2)
Inhibition after 14 d [%] # (yield based on total length)	--	-11.6	4.9	44.4* 1)	74.1* 1)	95.9* 1)	96.6* 1)
Inhibition after 14 d [%] # (yield based on wet weight)	--	-4.3	8.3	40.9* 1)	78.2* 1)	97.9* 1)	98.8* 1)
Inhibition after 14 d [%] # (yield based on dry weight)	--	-0.3	0.3	29.4* 2)	60.9* 2)	78.4* 2)	82.6* 2)

	Endpoints [mg a.s./L] (geometric mean measured)
E_rC_{50} based on total length	0.137 (95 % limits: 0.112 – 0.168)
E_rC_{20} based on total length	0.043 (95 % limits: 0.029 – 0.056)
E_rC_{10} based on total length	0.023 (95 % limits: 0.014 – 0.033)
E_yC_{50} based on total length	0.112 (95 % limits: 0.090 – 0.138)
E_yC_{20} based on total length	0.044 (95 % limits: 0.029 – 0.058)
E_yC_{10} based on total length	0.027 (95 % limits: 0.015 – 0.039)
E_rC_{50} based on wet weight	0.159 (95 % limits: 0.145 – 0.147)

E_rC_{20} based on wet weight	0.068 (95 % limits: 0.058 – 0.078)
E_rC_{10} based on wet weight	0.044 (95 % limits: 0.035 – 0.052)
E_yC_{50} based on wet weight	0.109 (95 % limits: 0.105 – 0.113)
E_yC_{20} based on wet weight	0.046 (95 % limits: 0.043 – 0.048)
E_yC_{10} based on wet weight	0.029 (95 % limits: 0.027 – 0.031)
E_rC_{50} based on dry weight	0.621 (95 % limits: 0.319 – 1.470)
E_rC_{20} based on dry weight	0.095 (95 % limits: 0.017 – 0.201)
E_rC_{10} based on dry weight	0.035 (95 % limits: 0.003 – 0.096)
E_yC_{50} based on dry weight	0.215 (95 % limits: 0.108 – 0.438)
E_yC_{20} based on dry weight	0.055 (95 % limits: 0.010 – 0.109)
E_yC_{10} based on dry weight	0.027 (95 % limits: 0.002 – 0.064)
NOEC overall	0.026 [3)]

[#] Negative values indicate stimulated growth compared to the control.
* Statistically significant difference compared to control.
$ Nominal values are based on stock solutions which are corrected for purity of the active ingredient (93%)
1) Statistically different compared to control (Welch t-test with Bonferroni-Holm adjustment, α = 0.05, one-sided smaller).
2) Statistically different compared to control (Dunnett's multiple t-test, α = 0.05, one-sided smaller).
3) based on NOEC calculations for nominal concentrations

Reference item test: A reference study was not conducted.

III. CONCLUSION

In a 14-day aquatic plant test with *Glyceria maxima,* the E_rC_{50} and the E_yC_{50} for cinmethylin were determined to be 0.137 and 0.112 mg a.s./L based on total length, 0.159 and 0.109 mg a.s./L based on wet weight and 0.621 and 0.215 mg a.s./L based on dry weight, respectively (geometric mean measured concentrations).

HSE evaluator comments:

It was noted a reference item study was not conducted, however as the validity criteria were met and the species tested was different to OECD guideline 239 this is considered acceptable by the HSE evaluator. In addition, algae growth was observed in the control but not considered sufficient by the study author to invalidate the study. When considering the analytical measurements, whilst application was made via water exposure no measurements in the sediment were taken. The reason for the lack of measurements was not clear.

When considering dry weight it was not clear from the study report how wet and dry weight at test initiation was measured i.e. whether plant material above the sediment or whole plant including roots. However, as long as the same approach was taken at both initiation and termination the HSE evaluator does not consider this point sufficient to invalidate the study. In addition, these parameters were not the most sensitive based on the endpoints derived from this study.

For the highest test concentration (nominal concentration of 2.939 mg a.s./L) there was total necrosis of all plants reported however length, wet/dry weight were still recorded at this test concentration, suggesting dead plant material was included in these measurements. This generates uncertainty hence the HSE evaluator has considered further both the endpoints generated and phytoxicity recorded below.

The most conservative E_rC_{50} value was 0.137 mg a.s./L based on total length. When considering the observed phytotoxicity the lowest concentration where effects were observed was 0.980 mg a.s./L where necrosis occurred in five replicates. Therefore, the most conservative E_rC_{50} endpoint appears to be protective of 50 % phytotoxicity and necrosis did not occur at test concentrations above the E_rC_{50}.

The analytical method is sufficiently validated in accordance with SANCO/3029/99 rev. 4 with an LOQ of 0.005 mg a.s./L (see volume 3, CA, section B5 for full details).

This study was conducted to GLP and considered valid. The following endpoints were derived (all are based on geometric mean measured concentrations):

- Cinmethylin E_rC_{50} = **0.137** mg a.s./L (based on total length)
- Cinmethylin E_rC_{20} = 0.043 mg a.s./L (based on total length)
- Cinmethylin E_rC_{10} = 0.023 mg a.s./L (based on total length)

- Cinmethylin E_yC_{50} = 0.112 mg a.s./L (based on total length)
- Cinmethylin E_yC_{20} = 0.044 mg a.s./L (based on total length)
- Cinmethylin E_yC_{10} = 0.027 mg a.s./L (based on total length)

- Cinmethylin E_rC_{50} = 0.159 mg a.s./L (based on wet weight)
- Cinmethylin E_rC_{20} = 0.068 mg a.s./L (based on wet weight)
- Cinmethylin E_rC_{10} = 0.044 mg a.s./L (based on wet weight)

- Cinmethylin E_yC_{50} = 0.109 mg a.s./L (based on wet weight)
- Cinmethylin E_yC_{20} = 0.046 mg a.s./L (based on wet weight)
- Cinmethylin E_yC_{10} = 0.029 mg a.s./L (based on wet weight)

- Cinmethylin E_rC_{50} = 0.621 mg a.s./L (based on dry weight)
- Cinmethylin E_rC_{20} = 0.095 mg a.s./L (based on dry weight)
- Cinmethylin E_rC_{10} = 0.035 mg a.s./L (based on dry weight)

- Cinmethylin E_yC_{50} = 0.215 mg a.s./L (based on dry weight)
- Cinmethylin E_yC_{20} = 0.055 mg a.s./L (based on dry weight)
- Cinmethylin E_yC_{10} = 0.027 mg a.s./L (based on dry weight)
- Cinmethylin NOEC (overall) = 0.026 mg a.s./L

Report: CA 8.2.7/3
Rzodeczko H., 2017 c
BAS 684 H - Water-sediment *Myriophyllum spicatum* toxicity test
2017/1000221
Guidelines: OECD 239 (2014)
GLP: Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Cinmethylin (Reg. no.: 900 202), batch no. COD-002038, purity: 93 ± 1.0 %

B. STUDY DESIGN

Test species: Watermilfoil (*Myriophyllum spicatum*) (Linné), dicotyledonous freshwater submerged plant, macrophyte, in-house culture.

Test design: Static water-sediment system (14 days); 6 test item concentrations with 4 replicates plus a control with 6 replicates; three plants per replicate; rooting phase of 7 days; during the rooting five plants per replicate; exposure phase of 14 days; application of test item via water phase; weight ratio of wet sediment to overlaid aqueous phase was 1 : 4.5; analytical determination of test item concentration was conducted for samples from test initiation (without plant), day 7 and test termination (with plant) for water phase and sediment.

Endpoints: EC_{50} and NOEC with respect to growth rate and yield after exposure over 14 days.

Test concentrations: Control, 0.0179, 0.0572, 0.183, 0.586, 1.88 and 6 mg/L a.s./L, corresponding to 0.01665, 0.0532, 0.1702, 0.545, 1.748 and 5.58 mg a.s./L (corrected for purity).

Test conditions: Glass beakers (11 x 24 cm), test volume 2 L, test medium Smart and Barko (91.7 % calcium chloride dihydrate, 69 % magnesium sulphate heptahydrate, 58.4 % sodium hydrogen carbonate, 15.4 % potassium hydrogen carbonate) and wet sediment (75 – 76 % quartz sand, 20 % caolin clay, 4 – 5 % peat, 0.05 – 0.1 % calcium carbonate, 0.01 – 0.015 % ammonium chloride, 0.01 – 0.015 % sodium phosphate, 30 – 50 % deionized water); temperature: 20.1 – 24.5 ºC, pH: 7.80 – 9.48, dissolved oxygen concentration in the control: 85 – 109 % ASV, light intensity: 9.62 – 11.85 klux in a

daily cycle of 16 h day and 8 h night. It was noted both the sediment and test medium used are in-line with OECD 239 recommendations.

Analytics: Analytical verification of the test item was conducted using an LC-method with DAD detection.

Statistics: Descriptive statistics; Probit analysis for determination of the EC_x values, The NOEC was determined statistically Multiple Sequentially rejective U-test After Bonferroni-Holm, Williams Multiple Sequential t-test Procedure (one-sided smaller, $\alpha = 0.05$).

II. RESULTS AND DISCUSSION

Analytical measurements:
At exposure initiation, the determined concentration of cinmethylin in aqueous phase was in the range of 85.5 – 106.6 % of nominal concentration.

In samples of the test item nominal concentrations 6, 1.88 and 0.0179 mg a.s./L collected on day 7 the determined concentration of cinmethylin in aqueous phase was 61.2, 60.6 and 53.6 % of nominal concentration, respectively. In samples of the test item concentration 6, 1.88 and 0.0179 mg a.s./L (nominal) collected at exposure termination the determined concentration of cinmethylin in aqueous phase was 51.3, 42.4 and 45.3 % of nominal concentration, respectively. In samples of the test item concentration 6, 1.88 and 0.0179 mg a.s./L (nominal) collected on day 7 the determined concentration of cinmethylin in sediment was 1.62, 0.44 mg a.s./kg and below the LoD value, respectively. In samples of the test item concentration 6, 1.88 and 0.0179 mg a.s./L (nominal) collected at exposure termination the determined concentration of cinmethylin in sediment was 0.969 and 0.400 mg/kg and below the LoD value, respectively. It was unclear in the study report whether precipitation of the test item was assessed.

Analytical results are shown in the tables below:

Table B.9.2.7.1-5: Measured concentrations during study **(in aqueous phase)**

Nominal concentration (mg a.s./L)	Day 0		Day 7		Day 14	
	MC (mg a.s./L)	% of nominal	MC (mg a.s./L)	% of nominal	MC (mg a.s./L)	% of nominal
0	< LoD	n.a.	< LoD	n.a.	< LoD	n.a.
0.0179	0.0166	92.7	0.0096	53.6	0.0081	45.3
0.0572	0.0566	99.0	--	--	--	--
0.183	0.195	106.6	--	--	--	--
0.586	0.570	97.3	--	--	--	--
1.88	1.84	97.9	1.14	60.6	0.797	42.4
6	5.13	85.5	3.67	61.2	3.08	51.3

MC = measured concentration, n.a. = not applicable, LoD = 0.001 mg a.s./L, -- = not tested, NP = Not possible to determine

Table B.9.2.7.1-6: Measured concentrations during study aqueous **(in sediment)**

Nominal concentration (mg a.s./kg)	Day 7	Day 14
	MC (mg a.s./kg)	MC (mg a.s./kg)
0	< LoD	< LoD
0.0179	< LoD	< LoD
0.0572	--	--
0.183	--	--
0.586	--	--
1.88	0.44	0.38
6	1.62	0.95

MC = measured concentration, n.a. = not applicable, LoD = 0.02 mg a.s./kg, -- = not tested

The study author calculated endpoints based on nominal concentrations. However, as concentrations were not maintained within ± 20 % of nominal values (noting only highest two test concentrations and lowest were monitored during study) in accordance with OECD 239 then endpoints should be based on geometric mean measured concentrations. Following a request from the HSE evaluator a separate report (BASF DocID: 2017/1000221) was submitted, which calculated geometric mean concentrations. Given not all test concentrations were analysed the applicant extrapolated values using the following method:

Single First Order (SFO) kinetics (equation 1):

$c(t) = c(0) \times e^{kt}$

c(t): concentration at time t
c(0): initial concentration
k: rate constant

To simplify further analysis the SFO is linearized (equation 2):

$\tilde{c}(t) = \tilde{c}(0) + k \times t$

$\tilde{c}(t)$: natural logarithm of concentration at time t
$\tilde{c}(0)$: natural logarithm of initial concentration
k: rate constant

For each of the concentration levels at which the concentration was determined at all three-time steps, the linearized SFO was calibrated (using the "lm" function in R 3.5.2). From those calibration results the estimated rate constant (i.e. slope in a linear model) that indicates the strongest decline (equivalent to shortest DT_{50}) was selected as a 'worst-case' representative.

This worst-case rate constant was used to predict the concentrations of the concentration levels where only the initial concentration had been determined. For this the 'worst-case' rate constant was used in Equation 1 as k, the initial concentration as $\tilde{c}(0)$, and 7 and 14 days as t to yield the predicted concentrations after 7 and 14 days.

Finally, the geometric mean is calculated from the determined and extrapolated (which is based on the 'worst-case' slope) concentrations. The concentrations calculated in BASF DocID: 2017/1000221 are shown below:

Table B.9.2.7.1-7: Analytical measurements and extrapolated values calculated by applicant in study report BASF DocID, 2017/1000221

Nominal concentration (mg a.s./L)	**Day**			**Geometric mean (mg a.s./L)**
	0	**7**	**14**	
0.0179	0.0166	0.0096	0.0081	**0.0109**
0.0572	0.0566	0.0373*	0.0245*	**0.0373***
0.183	0.195	0.1283*	0.0845*	**0.1283***
0.586	0.570	0.3751*	0.2469*	**0.3751***
1.88	1.84	1.14	0.797	**1.1868**
6	5.13	3.67	3.08	**3.8706**

* Extrapolated value based on method described above.

Validity criteria:

The criteria in OECD 239 (2014) have been considered below:

- For the test results to be valid, the mean total shoot length and mean total shoot fresh weight in control plants at least double during the exposure phase of the test. In addition, control plants must not show any visual symptoms of chlorosis and should be visibly free from contamination by other organisms such as algae and/or bacterial films on the plants, at the surface of the sediment and in the test medium. Observed: mean total shoot length in the control increased 2.1-fold. Mean fresh weight in the control increased 2.3-fold. No visual symptoms were reported for the control group.

- The mean coefficient of variation for yield based on measurements of shoot fresh weight (i.e. from test initiation to test termination) in the control cultures does not exceed 35 % between replicates. Observed: 12.9 % (based on yield fresh weight)

During the study the above criteria were met.

Biological results:

At exposure termination, no visible morphological changes were observed in the control plants and in test item concentrations 0.0179, 0.0572 and 0.183 mg a.s./L (nominal). The observations recorded are shown in the table below.

The inhibition of growth rate for total shoot length ranged from 2.1 to 57.2 %, for fresh weight 1.3 to 68.3 % and for dry weight -4.2 to 75.0 %, compared to control. The inhibition of yield for total shoot length ranged from 3.5 to 65.8 %, for fresh weight 2.2 to 75.8 % and for dry weight -5.5 to 81.8 % compared to control.

Statistically significant changes compared to control were observed for each endpoint parameter in the five highest test item concentrations (Williams Multiple Sequential t-test and Multiple Sequentially-rejective U-test after Bonferroni-Holm, one-sided smaller, $\alpha = 0.05$) except for growth rate and yield based on dry weight in which statistically significant differences compared to control were observed at the 3 highest test item concentrations (Williams Multiple Sequential t-test, $p = 0.05$). Effects on growth rate and yield are summarised in Table B.9.2.7.1-8.

Table B.9.2.7.1-8: Effect of Cinmethylin on the growth of *Myriophyllum spicatum*

Concentration (nominal) [mg a.s./L]	Control	0.0179	0.0572	0.183	0.586	1.88	6.0
Nominal concentration (corrected for purity) [mg a.s./L]	--	0.0167	0.0532	0.170	0.545	1.748	5.58
Geometric mean (mg a.s./L)[3)]	--	**0.0109**	**0.0373[#]**	**0.1283[#]**	**0.3751[#]**	**1.1868**	**3.8706**
Inhibition after 14 d [%] (growth rate based on total shoot length)	--	2.1	8.7 [*1)]	27.4 [*1)]	38.1 [*1)]	49.6 [*1)]	57.2 [*1)]
Inhibition after 14 d [%] (growth rate based on fresh weight)	--	1.3	12.8 [*1)]	35.6 [*1)]	50.7 [*1)]	64.6 [*1)]	68.3 [*1)]
Inhibition after 14 d [%] [##] (growth rate based on dry weight)	--	-4.2	-4.1	0.8	30.5 [*1)]	65.1 [*1)]	75.0 [*1)]
Inhibition after 14 d [%] (yield based on total shoot length)	--	3.5	14.0 [*2)]	36.6 [*2)]	47.6 [*2)]	59.2 [*2)]	65.8 [*2)]
Inhibition after 14 d [%] (yield based on fresh weight)	--	2.2	17.7 [*1)]	45.2 [*1)]	60.7 [*1)]	73.1 [*1)]	75.8 [*1)]
Inhibition after 14 d [%] [##] (yield based on dry weight)	--	-5.5	-5.3	1.9	39.5 [*1)]	73.5 [*1)]	81.8 [*1)]
Phytotoxicity	N V	N V	N V	N V	Br- 58 % M- 50 %	B- 75 % F- 75 %	B- 100 % F- 50 %

Paramter	**Endpoints [mg a.s./L] (geometric mean measured) [3)]**
E_rC_{50} (14 d) based on total shoot length	1.219 (95 % limits: 0.835 – 1.612)
E_rC_{20} (14 d) based on total shoot length	0.058 (95 % limits: 0.028 – 0.099)
E_rC_{10} (14 d) based on total shoot length	0.010 (95 % limits: 0.003 – 0.021)
E_yC_{50} (14 d) based on total shoot length	0.558 (95 % limits: 0.369 - 0.751)
E_yC_{20} (14 d) based on total shoot length	0.035 (95 % limits: 0.017 – 0.058)
E_yC_{10} (14 d) based on total shoot length	0.007 (95 % limits: 0.003 – 0.014)
E_rC_{50} (14 d) based on fresh weight	**0.414** (95 % limits: 0.195 – 0.632)
E_rC_{20} (14 d) based on fresh weight	0.048 (95 % limits: 0.013 – 0.083)
E_rC_{10} (14 d) based on fresh weight	0.019 (95 % limits: 0.002 – 0.036)
E_yC_{50} (14 d) based on fresh weight	**0.231** (95 % limits: 0.111 – 0.353)
E_yC_{20} (14 d) based on fresh weight	0.026 (95 % limits: 0.008 – 0.049)
E_yC_{10} (14 d) based on fresh weight	0.007 (95 % limits: 0.002 – 0.017)
E_rC_{50} (14 d) based on dry weight	0.810 (95 % limits: 0.338 – 1.907)
E_rC_{20} (14 d) based on dry weight	0.220 (95 % limits: 0.108 – 0.448)
E_rC_{10} (14 d) based on dry weight	0.111 (95 % limits: 0.053 – 0.232)
E_yC_{50} (14 d) based on dry weight	0.556 (95 % limits: 0.376 - 0.740)
E_yC_{20} (14 d) based on dry weight	0.188 (95 % limits: 0.092 – 0.278)
E_yC_{10} (14 d) based on dry weight	0.100 (95 % limits: 0.037 – 0.169)
NOEC overall	0.0109

N = Normal/no changes to plants above sediment, V = Very good development of roots, Br = Brown-colored tip of shoot and easily damaged young top leaves, B = Black-colored tip of shoot and easily damaged top leaves/visibly shorter stem/easily damaged apical part of stem, M = Moderate development of roots, F = Few short roots or fragmentation of roots

[#] Extrapolated values as only initial measured concentrations were determined.

[##] Negative values indicate stimulated growth compared to the control

-- = not applicable or possible to determine.

* Statistically significant difference compared to control.

[1)] Statistically different compared to control (Williams Multiple Sequential t-test, one-sided smaller, $\alpha = 0.05$).

[2)] Statistically different compared to control (Multiple Sequentially-rejective U-test after Bonferroni-Holm, one-sided smaller, $\alpha = 0.05$).

[3] Endpoint results based on geometric mean concentrations noting extrapolation was required for the following nominal concentrations; 0.0572, 0.183 and 0.586 mg a.s./L.

Reference item test: A reference study was conducted with 3,5-DCP. The following endpoints were derived:

- E_rC_{50} (total shoot length): 5.42 mg reference item/L
- E_yC_{50} (total shoot length): 5.30 mg reference item/L
- E_rC_{50} (fresh weight): 5.60 mg reference item/L
- E_yC_{50} (fresh weight): 5.37 mg reference item/L
- E_rC_{50} (dry weight): 5.82 mg reference item/L
- E_yC_{50} (dry weight): 5.47 mg reference item/L

The guidance document OECD 239 states that endpoints for measured parameters should be between 4.7 and 6.1 mg reference item/L. Therefore, the reference test results are considered acceptable.

III. CONCLUSION

In a 14-day aquatic plant test with *Myriophyllum spicatum,* the most sensitive E_rC_{50} and E_yC_{50} values for cinmethylin were determined to be 0.414 and 0.231 mg a.s./L based on fresh weight (based on geometric mean measured concentrations, noting extrapolation of test concentrations was used to derive endpoints).

HSE evaluator comments:

This study was conducted to GLP and considered valid. In addition, it was concluded that the analytical method is sufficiently validated in accordance with SANCO/3029/99 rev. 4 with LOQs of 0.002 mg a.s./L in water and 0.05 mg a.s./kg in sediment (see volume 3, CA, section B5 for full details).

The HSE evaluator does not consider this study suitable for quantitative use in the risk assessment. This is due to insufficient analytical samples being analysed throughout the study for all test concentrations. Specifically, the nominal concentrations; 0.0572, 0.183 and 0.586 mg a.s./L were only measured at study initiation. For the missing values the applicant extrapolated likely decline based on other concentrations that were measured during the study. This generates uncertainty around the endpoints derived. Therefore, the HSE evaluator has considered the endpoints as supporting information only and discussed the results further in the risk assessment section.

When considering phytotoxicity approximately 50 % effects were observed at 0.3751 mg a.s./L (geometric mean measured concentration, noting endpoints are to be used as supporting information). Therefore, the most sensitive E_rC_{50} value of 0.414 mg a.s./L is above but the concentration with 50 % phytotoxicity effects. Whilst this generates uncertainty as to whether the proposed endpoints are protective of phytotoxicity the endpoints are broadly comparable as the difference is relatively low. In addittion, this study is being considered as supporting information in the risk assessment section.

The following endpoints were derived (all are based on geometric mean measured concentrations, noting due to concerns regarding analytical confirmation these endpoints will be considered as supporting information only):

- Cinmethylin E_rC_{50} = 1.219 mg a.s./L (total shoot length)- supporting information
- Cinmethylin E_yC_{50} = 0.558 mg a.s./L (total shoot length)- supporting information
- Cinmethylin E_rC_{50} = **0.414** mg a.s./L (fresh weight)- supporting information
- Cinmethylin E_yC_{50} = **0.231** mg a.s./L (fresh weight)- supporting information
- Cinmethylin E_rC_{50} = 0.810 mg a.s./L (dry weight)- supporting information
- Cinmethylin E_yC_{50} = 0.556 mg a.s./L (dry weight)- supporting information

Report:	CA 8.2.7/4 Rzodeczko H., 2018 a BAS 684 H, water-sediment *Elodea canadensis* toxicity test 2017/1000222
Guidelines:	OECD 239 (2014)
GLP:	Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test item:	Cinmethylin (Reg. No. 900 202), batch no. COD-002038, Purity: 93.0 % (tolerance ± 1.0 %).

B. STUDY DESIGN

Test species:	Canadian waterweed *Elodea canadensis* (Michx), monocotyledonous freshwater submerged plant, macrophyte, maintained in culture at the Institute of Industrial Organic Chemistry Branch Pszczyna, Department of Ecotoxicology, Laboratory of Aquatic Toxicology. At test start: total shoot length: 6.0 cm; shoot length above sediment: approx. 4.5 cm
Test design:	Static water-sediment system (14 days); application into aqueous phase; no rooting phase of plants; 7 treatment groups (6 test item concentrations plus control); 3 plants per replicate; 4 replicates for each test item concentration and 6 replicates for control; assessment of growth and other effects on days 7 and 14. At test termination, fresh weight and dry weight of each plant were measured.
Endpoints:	EC_{10}, EC_{50} and NOEC with respect to growth rate and yield after exposure over 14 days.
Test concentrations:	Control; 0.0179, 0.0572, 0.183, 0.586, 1.88 and 6.00 mg a.s./L corresponding to 0.01665, 0.0532, 0.1702, 0.545. 1.748 and 5.58 mg a.s./L (corrected for purity).
Test conditions:	Glass beakers (11 x 24 cm), test volume 2 L and 453.2 g sediment (min. 441 g, maximum 466 g), sediment: water ratio was 1: 4.4; test medium accord. to Smart and Barko, wet sediment accord. OECD TG 219; temperature: 17.6 – 26.4 ºC, pH: 7.81 – 9.31, dissolved oxygen concentration: 74 – 103 % ASV, light intensity: 9.28 – 11.75 klux; 16 h day: 8 h night,
Analytics:	Analytical verification of the test item in aqueous phase and in sediment was performed with HPLC method with DAD detection.
Statistics:	Descriptive statistics, EC_x-calculations by Probit analysis with Williams Multiple Sequential t-test Procedure (one-sided smaller, $\alpha = 0.05$).

II. RESULTS AND DISCUSSION

Analytical measurements:

Aqueous phase: In samples collected at exposure initiation the determined concentration of cinmethylin was in the range of 101.5 – 113.4 % of nominal concentration. On day 7, the determined concentration at test concentration 6, 1.88 and 0.586 mg a.s./L was 72.3, 72.3 and 73.4 % of nominal concentration, respectively. In samples of the test item concentration 6, 1.88 and 0.586 mg a.s./L collected at exposure termination, the determined concentration of cinmethylin was 52.0, 55.3 and 50.3 % of nominal concentration, respectively.

Sediment: On day 7, the determined concentration of cinmethylin in samples of the test item concentration 6, 1.88 and 0.586 mg a.s./L (nominal) was 2.72, 1.065 and 0.23 mg a.s./kg, respectively. At exposure termination, the determined concentrations of cinmethylin in samples of the test item concentration 6, 1.88 and 0.586 mg a.s./L (nominal) was 3.09; 1.18 and 0.286 mg a.s./kg, respectively.

Analytical results are shown in the tables below. The stock solution was reported as homogeneous without visibly non-dissolved particles.

Table B.9.2.7.1-9: Measured concentrations during study **(in aqueous phase)**

Nominal concentration (mg a.s./L)	Day 0		Day 7		Day 14	
	MC (mg a.s./L)	% of nominal	MC (mg a.s./L)	% of nominal	MC (mg a.s./L)	% of nominal
0	< LoD	n.a.	< LoD	n.a.	< LoD	n.a.
0.0179	0.0203	113.4	--	--	--	--
0.0572	0.061	106.6	--	--	--	--
0.183	0.191	104.4	--	--	--	--
0.586	0.595	101.5	0.430	73.4	0.295	50.3
1.88	2.07	110.1	1.36	72.3	1.04	55.3
6	6.42	107.0	4.34	72.3	3.12	52.0

MC = measured concentration, n.a. = not applicable, LoD = 0.001 mg a.s./L, -- = not tested, NP = Not possible to determine

Table B.9.2.7.1-10: Measured concentrations during study aqueous **(in sediment)**

Nominal concentration (mg a.s./kg)	Day 7	Day 14
	MC* (mg a.s./kg)	MC* (mg a.s./kg)
0	< LoD	< LoD
0.0179	--	--
0.0572	--	--
0.183	--	--
0.586	0.230	0.286
1.88	1.065	1.180
6	2.720	3.090

MC = measured concentration, n.a. = not applicable, LoD = 0.02 mg a.s./kg, -- = not tested, * based on average of measurements taken by two different columns used for analysis.

The study author calculated endpoints based on nominal concentrations. However, as concentrations were not maintained within ± 20 % of nominal values (noting only highest two test concentrations and lowest were monitored during study) in accordance with OECD 239 then endpoints should be based on geometric mean measured concentrations. Following a request from the HSE evaluator a separate report (BASF DocID: 2017/2050445) was submitted, which calculated geometric mean concentrations. Given not all test concentrations were analysed the applicant extrapolated values using the following method:

Single First Order (SFO) kinetics (equation 1):

$c(t) = c(0) \times e^{kt}$

c(t): concentration at time t
c(0): initial concentration
k: rate constant

To simplify further analysis the SFO is linearized (equation 2):

$\tilde{c}(t) = \tilde{c}(0) + k \times t$

$\tilde{c}(t)$: natural logarithm of concentration at time t
$\tilde{c}(0)$: natural logarithm of initial concentration
k: rate constant

For each of the concentration levels at which the concentration was determined at all three-time steps, the linearized SFO was calibrated (using the "lm" function in R 3.5.2). From those calibration results the estimated rate constant (i.e. slope in a linear model) that indicates the strongest decline (equivalent to shortest DT_{50}) was selected as a 'worst-case' representative.

This worst-case rate constant was used to predict the concentrations of the concentration levels where only the initial concentration had been determined. For this the 'worst-case' rate constant was used in Equation 1 as k, the initial concentration as $\tilde{c}(0)$, and 7 and 14 days as t to yield the predicted concentrations after 7 and 14 days.

Finally, the geometric mean is calculated from the determined and extrapolated (which is based on the 'worst-case' slope) concentrations. The concentrations calculated in BASF DocID: 2017/2050445 are shown below:

Table B.9.2.7.1-11: Analytical measurements and extrapolated values calculated by applicant in study report BASF DocID, 2017/2050445

Nominal concentration (mg a.s./L)	Day			Geometric mean (mg a.s./L)
	0	7	14	
0.0179	0.0203	0.0142*	0.0099*	0.0142
0.0572	0.061	0.0425*	0.0296*	0.0425
0.183	0.191	0.1332*	0.0928*	0.1332
0.586	0.595	0.43	0.295	0.4226
1.88	2.07	1.36	1.04	1.4306
6	6.42	4.34	3.12	4.4299

* Extrapolated value based on method described above.

Validity criteria:

The criteria in OECD 239 (2014) have been considered below, noting these guidelines were developed for a different species i.e. *Myriophyllum spicatum*:

- For the test results to be valid, the mean total shoot length and mean total shoot fresh weight in control plants at least double during the exposure phase of the test. In addition, control plants must not show any visual symptoms of chlorosis and should be visibly free from contamination by other organisms such as algae and/or bacterial films on the plants, at the surface of the sediment and in the test medium. Observed: mean total shoot length in the control increased 2.1-fold. Mean fresh weight in the control increased 2.5-fold. No visual symptoms were reported for the control group.
- The mean coefficient of variation for yield based on measurements of shoot fresh weight (i.e. from test initiation to test termination) in the control cultures does not exceed 35 % between replicates. Observed: 13.7 % (based on yield fresh weight)

During the study the above criteria were met.

Biological results:

At exposure termination, control plants were healthy, with green leaves and stems, without discolorations and good development of roots. In test item concentration 0.0179 mg a.s./L (nominal) no changes of the plant part above sediment and moderately developed roots for all plants were observed in comparison with plants in the control. The observations recorded are shown in the table below.

In the tested range of test item concentrations, the inhibition of growth rate for total shoot length ranged from 0.1 to 81.2 %, for fresh weight from -5.1 to 97.1 % and for dry weight from 0.3 to 68.1 % in comparison with plants in the control. The inhibition of yield for total shoot length ranged from 0.3 to 86.4 %, for fresh weight from -7.6 to 98.1 % and for dry weight from 1.1 to 81.1 % in comparison with plants in the control.

Statistically significant changes compared to control were observed for each endpoint parameter in the five highest test item concentrations (Williams Multiple Sequential t-test, one-sided smaller, $\alpha = 0.05$) for growth rate and yield based on total shoot length. For growth rate and yield based on fresh weight and dry weight, the statistically significant differences compared to control were observed at the 4 highest test item concentrations (Williams Multiple Sequential t-test, one-sided smaller, $\alpha = 0.05$). Effects on growth rate and yield are summarised in Table B.9.2.7.1-12.

Table B.9.2.7.1-12: Effect of cinmethylin on the growth of *Elodea canadensis*

Concentration (nominal) [mg a.s./L]	Control	0.0179	0.0572	0.183	0.586	1.88	6.00
Concentration (corrected for purity) [mg a.s./L]	0	0.01665	0.0532	0.1702	0.545	1.748	5.58
Geometric mean (mg a.s./L)[2)]	**0**	**0.0142[#]**	**0.0425[#]**	**0.1332[#]**	**0.4226**	**1.4306**	**4.4299**
Inhibition after 14 d [%] (growth rate based on total shoot length)	0.0	0.1	8.3*[1)]	14.1*[1)]	43.5*[1)]	59.1*[1)]	81.2*[1)]
Inhibition after 14 d [%] [##] (growth rate based on fresh weight)	0.0	-5.1	7.2	20.2*[1)]	76.6*[1)]	90.6*[1)]	97.1*[1)]
Inhibition after 14 d [%] (growth rate based on dry weight)	0.0	0.3	4.9	18.6*[1)]	26.6*[1)]	49.0*[1)]	68.1*[1)]
Inhibition after 14 d [%] (yield based on total shoot length)	0.0	0.3	11.4*[1)]	19.0*[1)]	52.3*[1)]	67.6*[1)]	86.4*[1)]
Inhibition after 14 d [%] [##] (yield based on fresh weight)	0.0	-7.6	10.6	27.8*[1)]	83.6*[1)]	93.9*[1)]	98.1*[1)]
Inhibition after 14 d [%] (yield based on dry weight)	0.0	1.1	8.8	29.9*[1)]	40.6*[1)]	64.7*[1)]	81.1*[1)]
Phytotoxicity	N G	N M	N F	Y- 33 % VF- 33 % NR- 67 %	BiP- 58 % NR	Br- 58 % NR	S NR

Parameter	**Endpoints [mg a.s./L] (geometric mean measured) [2)]**
E_rC_{50} (14d) total shoot length	0.764 (95 % limits: 0.362 – 1.592)
E_rC_{20} (14d) total shoot length	0.140 (95 % limits: 0.076 – 0.257)
E_rC_{10} (14d) total shoot length	0.058 (95 % limits: 0.031 – 0.108)
E_yC_{50} (14d) total shoot length	0.490 (95 % limits: 0.344 – 0.638)
E_yC_{20} (14d) total shoot length	0.098 (95 % limits: 0.056 – 0.142)
E_yC_{10} (14d) total shoot length	0.038 (95 % limits: 0.017 – 0.063)
E_rC_{50} (14d) fresh weight	**0.247** (95 % limits: 0.197 – 0.297)
E_rC_{20} (14d) fresh weight	0.118 (95 % limits: 0.073 – 0.163)
E_rC_{10} (14d) fresh weight	0.076 (95 % limits: 0.036 – 0.117)
E_yC_{50} (14d) fresh weight.	**0.198** (95 % limits: 0.103 – 0.374)
E_yC_{20} (14d) fresh weight.	0.094 (95 % limits: 0.055 – 0.160)
E_yC_{10} (14d) fresh weight.	0.064 (95 % limits: 0.037 – 0.110)
E_rC_{50} (14d) dry weight	1.481 (95 % limits: 1.066 – 1.904)
E_rC_{20} (14d) dry weight	0.209 (95 % limits: 0.113 – 0.318)
E_rC_{10} (14d) dry weight	0.067 (95 % limits: 0.027 – 0.119)
E_yC_{50} (14d) dry weight.	0.592 (95 % limits: 0.365 – 0.824)
E_yC_{20} (14d) dry weight.	0.088 (95 % limits: 0.040 – 0.144)
E_yC_{10} (14d) dry weight.	0.029 (95 % limits: 0.010 – 0.057)
NOEC overall	0.0142

N = Normal/no changes to plants above sediment, G = Good development of roots, M = Moderate development of roots, F = Few short roots, VF = Very few short roots, NR = No roots, Y = Yellow inner parts of top leaves, BiP = Brown inner parts of top leaves, visibly shorter shoot, Br = Brown-colored tip of shoot and easily damaged and brown apical of shoot, visibly shorter root, S = Short short

[#] Extrapolated values as only initial measured concentrations were determined.

[##] Negative values indicate stimulated growth compared to the control

* Statistically significant difference compared to control.

[1)] Statistically different compared to control (Williams Multiple Sequential t-test, one-sided smaller, $\alpha = 0.05$).

[2] Endpoint results based on geometric mean concentrations noting extrapolation was required for the following nominal concentrations; 0.0179, 0.0572 and 0.183 mg a.s./L.

Reference item test: A reference study was not conducted.

III. CONCLUSION

In a 14-day aquatic plant test with *Elodea canadensis,* the E_rC_{50} and the E_yC_{50} for cinmethylin were determined to be 0.247 and 0.198 mg a.s./L based on fresh weight (based on geometric mean measured concentrations, noting extrapolation of test concentrations was used to derive endpoints).

HSE evaluator comments:

It was noted a reference item study was not conducted, however as the validity criteria were met and the species tested was different to OECD guideline 239 this is considered acceptable by the HSE evaluator.

It was concluded that the analytical method is sufficiently validated in accordance with SANCO/3029/99 rev. 4 with LOQs of 0.002 mg a.s./L in water and 0.05 mg a.s./kg in sediment (see volume 3, CA, section B5 for full details).

The HSE evaluator does not consider this study suitable for quantitative use in the risk assessment. This is due to insufficient analytical samples being analysed throughout the study for all test concentrations. Specifically, the nominal concentrations; 0.0179, 0.0572 and 0.183 mg a.s./L were only measured at study initiation. For the missing values the applicant extrapolated likely decline based on other concentrations that were measured during the study. This generates uncertainty around the endpoints derived. Therefore, the HSE evaluator has considered the endpoints as supporting information only and discussed the results further in the risk assessment section.

When considering phytotoxicity between 33 and 67 % effects were observed at 0.1332 mg a.s./L and < 50 % effects at 0.0425 mg a.s./L (geometric mean measured concentration, noting endpoints are to be used as supporting information). This suggests, the most sensitive E_rC_{50} value of 0.247 mg a.s./L may not be protective of 50 % phytotoxicity effects. Further consideration of phytotoxicity has been considered in the risk assessment section.

The following endpoints were derived (all are based on geometric mean measured concentrations, noting due to concerns regarding analytical confirmation these endpoints will be considered as supporting information only):

- Cinmethylin E_rC_{50} = 0.764 mg a.s./L (total shoot length)- supporting information
- Cinmethylin E_yC_{50} = 0.490 mg a.s./L (total shoot length)- supporting information
- Cinmethylin E_rC_{50} = **0.247** mg a.s./L (fresh weight)- supporting information
- Cinmethylin E_yC_{50} = **0.198** mg a.s./L (fresh weight)- supporting information
- Cinmethylin E_rC_{50} = 1.481 mg a.s./L (dry weight)- supporting information
- Cinmethylin E_yC_{50} = 0.592 mg a.s./L (dry weight)- supporting information

Report: CA 8.2.7/5
Rzodeczko H., 2017 d
BAS 684 H - Water-sediment *Egeria densa* toxicity test
2017/1000224
Guidelines: OECD 239 (2014)
GLP: Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Cinmethylin (Reg. no.: 900 202), batch no. COD-002038, purity: 93 % (tolerance ± 1.0 %)

B. STUDY DESIGN

Test species: Brazilian waterweed (*Egeria densa*) (Planch.) Casp., monocotyledonous freshwater submerged plant, macrophyte, in-house culture.

Test design:	Static water-sediment system (14 days); application of test item via water phase; ratio of wet sediment to overlaid aqueous phase was 1 : 4.3; no rooting phase; 6 test item concentrations with 4 replicates plus a control with 6 replicates; 3 plants per replicate at test start; physico-chemical characteristics were assessed at beginning, day 7 and at termination of test; assessment of growth and other effects on days 7 and at test termination; analytical determination of test item concentration was conducted for samples from test initiation (without plant), day 7 and test termination (with plant) for water phase and sediment.
Endpoints:	EC_{50} with respect to growth rate and yield after exposure over 14 days.
Test concentrations:	Control, 0.0179, 0.0572, 0.183, 0.586, 1.88 and 6 mg a.s./L, corresponding to 0.01665, 0.0532, 0.1702, 0.545, 1.748 and 5.58 mg a.s./L (corrected for purity).
Test conditions:	Glass beakers (11 x 24 cm), test volume 2 L, test medium Smart and Barko and wet sediment (according to OECD TG 239); dissolved oxygen: 71 – 111 % ASV; pH: 7.68 – 9.27; water temperature: 19.8 – 24.2 °C, photo period: 16: 8 hours; light intensity: 9.41 – 11.64 klux.
Analytics:	Analytical verification of the test item was conducted using an LC-method with DAD detection in aqueous phase and sediment.
Statistics:	Descriptive statistics; Probit analysis for determination of the EC_x values, the NOEC was determined statistically by Shapiro-Wilk's Test on Normal Distribution, Levene's Test on Variance Homogeneity, Multiple Sequentially rejective U-test After Bonferroni-Holm, Williams Multiple Sequential t-test Procedure (one-sided smaller, p = 0.05).

II. RESULTS AND DISCUSSION

Analytical measurements:

Aqueous phase: At test start, cinmethylin in aqueous phase was in the range of 91.5 – 100.2 % of nominal concentration. In samples of the test item concentration 6, 1.88 and 0.586 mg a.s./L collected on day 7 the determined concentration of cinmethylin in aqueous phase was 61.0, 62.2 and 59.2 % of nominal concentration, respectively. In samples of the test item concentration 6, 1.88 and 0.586 mg a.s./L collected at exposure termination the determined concentration of cinmethylin in aqueous phase was 53.3, 49.4 and 39.9 % of nominal concentration, respectively. The stock solution was reported as homogeneous without visibly non-dissolved particles.

Sediment: In samples of the test item concentration 6, 1.88 and 0.586 mg a.s./L collected on day 7 the determined concentration of cinmethylin in sediment was 0.700, 0.119 and 0.036 mg a.s./kg, respectively. In samples of the test item concentration 6, 1.88 and 0.586 mg a.s./L collected at exposure termination the determined concentration of cinmethylin in sediment was 1.59, 0.495 and 0.052 mg a.s./kg, respectively.

Analytical results are shown in the tables below. The stock solution was reported as homogeneous without visibly non-dissolved particles.

Table B.9.2.7.1-13: Measured concentrations during study **(in aqueous phase)**

Nominal concentration (mg a.s./L)	Day 0		Day 7		Day 14	
	MC (mg a.s./L)	% of nominal	MC (mg a.s./L)	% of nominal	MC (mg a.s./L)	% of nominal
0	< LoD	n.a.	< LoD	n.a.	< LoD	n.a.
0.0179	0.0172	96.1	--	--	--	--
0.0572	0.0573	100.2	--	--	--	--
0.183	0.178	97.3	--	--	--	--
0.586	0.557	95.1	0.347	59.2	0.234	39.9
1.88	1.72	91.5	1.17	62.2	0.928	49.4
6	5.58	93.0	3.66	61.0	3.20	53.3

MC = measured concentration, n.a. = not applicable, LoD = 0.001 mg a.s./L, -- = not tested, NP = Not possible to determine

Table B.9.2.7.1-14: Measured concentrations during study aqueous **(in sediment)**

Nominal concentration (mg a.s./kg)	Day 7	Day 14
	MC* (mg a.s./kg)	MC* (mg a.s./kg)
0	< LoD	< LoD
0.0179	--	--
0.0572	--	--
0.183	--	--
0.586	0.036	0.052
1.88	0.119	0.495
6	0.700	1.590

MC = measured concentration, n.a. = not applicable, LoD = 0.02 mg a.s./kg, -- = not tested, * based on average of measurements taken by two different columns used for analysis.

The study author calculated endpoints based on nominal concentrations. However, as concentrations were not maintained within ± 20 % of nominal values (noting only highest two test concentrations and lowest were monitored during study) in accordance with OECD 239 then endpoints should be based on geometric mean measured concentrations. Following a request from the HSE evaluator a separate report (BASF DocID: 2017/2050447) was submitted, which calculated geometric mean concentrations. Given not all test concentrations were analysed the applicant extrapolated values using the following method:

Single First Order (SFO) kinetics (equation 1):

$c(t) = c(0) \times e^{kt}$

c(t): concentration at time t
c(0): initial concentration
k: rate constant

To simplify further analysis the SFO is linearized (equation 2):

$\tilde{c}(t) = \tilde{c}(0) + k \times t$

$\tilde{c}(t)$: natural logarithm of concentration at time t
$\tilde{c}(0)$: natural logarithm of initial concentration
k: rate constant

For each of the concentration levels at which the concentration was determined at all three-time steps, the linearized SFO was calibrated (using the "lm" function in R 3.5.2). From those calibration results the estimated rate constant (i.e. slope in a linear model) that indicates the strongest decline (equivalent to shortest DT_{50}) was selected as a 'worst-case' representative.

This worst-case rate constant was used to predict the concentrations of the concentration levels where only the initial concentration had been determined. For this the 'worst-case' rate constant was used in Equation 1 as k, the initial concentration as $\tilde{c}(0)$, and 7 and 14 days as t to yield the predicted concentrations after 7 and 14 days.

Finally, the geometric mean is calculated from the determined and extrapolated (which is based on the 'worst-case' slope) concentrations. The concentrations calculated in BASF DocID: 2017/2050447 are shown below:

Table B.9.2.7.1-15: Analytical measurements and extrapolated values calculated by applicant in study report BASF DocID, 2017/2050447

Nominal concentration (mg a.s./L)	**Day**			**Geometric mean (mg a.s./L)**
	0	**7**	**14**	
0.0179	0.0172	0.0111*	0.0072*	0.0111*
0.0572	0.0573	0.0371*	0.0241*	0.0371*
0.183	0.178	0.1154*	0.0748*	0.1154*
0.586	0.557	0.347	0.234	0.3563
1.88	1.72	1.17	0.928	1.2315
6	5.58	3.66	3.2	4.0280

* Extrapolated value based on method described above.

Validity criteria:

The criteria in OECD 239 (2014) have been considered below, noting these guidelines were developed for a different species i.e. *Myriophyllum spicatum*:

- For the test results to be valid, the mean total shoot length and mean total shoot fresh weight in control plants at least double during the exposure phase of the test. In addition, control plants must not show any visual symptoms of chlorosis and should be visibly free from contamination by other organisms such as algae and/or bacterial films on the plants, at the surface of the sediment and in the test medium. Observed: mean total shoot length in the control increased 2.2-fold. Mean fresh weight in the control increased 2.9-fold. No visual symptoms were reported for the control group.
- The mean coefficient of variation for yield based on measurements of shoot fresh weight (i.e. from test initiation to test termination) in the control cultures does not exceed 35 % between replicates. Observed: 11.6 % (based on yield fresh weight)

During the study the above criteria were met.

Biological results:

At exposure termination, no visible morphological changes were observed in the control plants. The observations noted in the study report are summarised in the table below.

Table B.9.2.7.1-16. Observations of morphology at study termination (day 14).

Nominal test concentration (mg a.s./L)	Plant parts above sediment	Plant parts in sediment
0	Normal shape of plants, green color of leaves and stems, no discoloration	Good development of roots
0.0179	No changes	All plants have moderate development of roots
0.0572	No changes	All plants have few visible short roots
0.183	Approx. 67 % of plants with top leaves so close that they form thick end of shoots	Approx. 50 % of plants have few short roots and 50 % no roots
0.586	All plants with top leaves so close that they form thick end of shoots and visibly shorter stems	Approx. 25 % of plants have few short roots and 75 % no roots
1.88	Approx. 42 % of plants with top leaves so close that they form thick end of shoots and visibly shorter stems	All plants have no roots
6	All plants short stems	All plants have no roots

The inhibition of growth rate for total shoot length ranged from 2.0 to 90.1 %, for fresh weight from -3.8 to 110.1 %, for dry weight from 1.4 to 74.5 % in comparison with plants in the control. The inhibition of yield for total shoot length ranged from 3.0 to 93.3 %, for fresh weight from -6.0 to 105.3 %, for dry weight from 3.6 to 85.7 % in comparison with plants in the control.

Statistically significant changes compared to control were observed for each endpoint parameter in the four highest test item concentrations (Williams Multiple Sequential t-test, Multiple Sequentially-rejective U-test after Bonferroni-Holm and Multiple Sequentially Welch t-test after Bonferroni-Holm; $p = 0.05$, one-sided smaller) except for growth rate based on total shoot length in which statistically significant differences compared to control were observed in the five highest test item concentrations (Williams Multiple Sequential t-test; $p = 0.05$, one-sided smaller). Effects on growth rate and yield are summarised in Table B.9.2.7.1-17.

Table B.9.2.7.1-17: Effect of cinmethylin on the growth of the Brazilian waterweed *Egeria densa*

Concentration (nominal) [mg a.s./L]	Control	0.0179	0.0572	0.183	0.586	1.88	6.0
Concentration (corrected for purity) [mg a.s./L]	0	0.01665	0.0532	0.1702	0.545	1.748	5.58
Geometric mean (mg a.s./L)[4)]	**0**	**0.0111[#]**	**0.0371[#]**	**0.1154[#]**	**0.36**	**1.23**	**4.03**
Inhibition after 14 d [%] (growth rate based on total shoot length)	--	2.0	6.3 [*1)]	14.6 [*1)]	33.2 [*1)]	70.5 [*1)]	90.1 [*1)]
Inhibition after 14 d [%] [##] (growth rate based on fresh weight)	--	-3.8	-2.3	58.2 [*1)]	75.4 [*1)]	80.9 [*1)]	110.1 [*1)]
Inhibition after 14 d [%] (growth rate based on dry weight)	--	1.4	4.5	18.1 [*1)]	41.4 [*1)]	64.1 [*1)]	74.5 [*1)]
Inhibition after 14 d [%] (yield based on total shoot length)	--	3.0	9.0	20.1 [*2)]	42.5 [*2)]	78.4 [*2)]	93.3 [*2)]
Inhibition after 14 d [%][##] (yield based on fresh weight)	--	-6.0	-3.5	70.5 [*3)]	83.9 [*3)]	88.1 [*3)]	105.3 [*3)]
Inhibition after 14 d [%] (yield based on dry weight)	--	3.6	8.8	29.9 [*1)]	58.3 [*1)]	78.4 [*1)]	85.7 [*1)]

Parameters	**Endpoints [mg a.s./L] (geometric mean measured)[4)]**
E_rC_{50} based on total shoot length	0.624 (95 % limits: 0.543 – 0.704)
E_rC_{20} based on total shoot length	0.194 (95 % limits: 0.150 – 0.239)
E_rC_{10} based on total shoot length	0.098 (95 % limits: 0.068 – 0.129)
E_yC_{50} based on total shoot length	0.438 (95 % limits: 0.365 – 0.512)
E_yC_{20} based on total shoot length	0.130 (95 % limits: 0.097 – 0.161)
E_yC_{10} based on total shoot length	0.064 (95 % limits: 0.043 - 0.086)
E_rC_{50} based on fresh weight	**0.116** (95 % limits: 0.089 – 0.144)
E_rC_{20} based on fresh weight	0.052 (95 % limits: 0.037 – 0.066)
E_rC_{10} based on fresh weight	0.037 (95 % limits: 0.024 – 0.049)
E_yC_{50} based on fresh weight	**0.092** (95 % limits: 0.068 – 0.117)
E_yC_{20} based on fresh weight	0.065 (95 % limits: 0.007 – 0.097)
E_yC_{10} based on fresh weight	0.053 (95 % limits: 0.002 – 0.085)
E_rC_{50} based on dry weight	0.659 (95 % limits: 0.292 – 1.471)
E_rC_{20} based on dry weight	0.105 (95 % limits: 0.054 – 0.205)
E_rC_{10} based on dry weight	0.040 (95 % limits: 0.020 – 0.081)
E_yC_{50} based on dry weight	0.283 (95 % limits: 0.183 – 0.385)
E_yC_{20} based on dry weight	0.060 (95 % limits: 0.030 – 0.092)
E_yC_{10} based on dry weight	0.024 (95 % limits: 0.009 – 0.043)
$NOEC_{overall}$	0.0111

[#] Extrapolated values as only initial measured concentrations were determined.

[##] Negative values indicate stimulated growth compared to the control.

* Statistically significant difference compared to control.

1) Statistically different compared to control (Williams Multiple Sequential t-test, p = 0.05, one-sided smaller).

2) Statistically different compared to control (Multiple Sequentially-rejective U-test after Bonferroni-Holm, p = 0.05, one-sided smaller).

3) Statistically different compared to control (Multiple Sequentially Welch t-test after Bonferroni-Holm, p = 0.05, one-sided smaller).

4) Endpoint results based on geometric mean concentrations noting extrapolation was required for the following nominal concentrations; 0.0179, 0.0572 and 0.183 mg a.s./L.

III. CONCLUSION

In a 14-day aquatic plant test with *Egeria densa,* the E_rC_{50} and the E_yC_{50} for cinmethylin were determined to be 0.116 and 0.092 mg a.s./L based on fresh weight (based on geometric mean measured concentrations, noting extrapolation of test concentrations was used to derive endpoints).

HSE evaluator comments:

It was noted a reference item study was not conducted, however as the validity criteria were met and the species tested was different to OECD guideline 239 this is considered acceptable by the HSE evaluator.

It was concluded that the analytical method is sufficiently validated in accordance with SANCO/3029/99 rev. 4 with LOQs of 0.002 mg a.s./L in water and 0.05 mg a.s./kg in sediment (see volume 3, CA, section B5 for full details).

The HSE evaluator does not consider this study suitable for quantitative use in the risk assessment. This is due to insufficient analytical samples being analysed throughout the study for all test concentrations. Specifically, the nominal concentrations; 0.0179, 0.0572 and 0.183 mg a.s./L were only measured at study initiation. For the missing values the applicant extrapolated likely decline based on other concentrations that were measured during the study. This generates uncertainty around the endpoints derived. Therefore, the HSE evaluator has considered the endpoints as supporting information only and discussed the results further in the risk assessment section.

When considering phytotoxicity between 50 and 67 % effects were observed at 0.1154 mg a.s./L and < 50 % effects at 0.0371 mg a.s./L (geometric mean measured concentration, noting endpoints are to be used as supporting information). This suggests, the most sensitive E_rC_{50} value of 0.116 mg a.s./L may not be protective of 50 % phytotoxicity effects. Further consideration of phytotoxicity has been considered in the risk assessment section.

The following endpoints were derived (all are based on geometric mean measured concentrations, noting due to concerns regarding analytical confirmation these endpoints will be considered as supporting information only):

- Cinmethylin E_rC_{50} = 0.624 mg a.s./L (total shoot length)- supporting information
- Cinmethylin E_yC_{50} = 0.438 mg a.s./L (total shoot length)- supporting information
- Cinmethylin E_rC_{50} = **0.116** mg a.s./L (fresh weight)- supporting information
- Cinmethylin E_yC_{50} = **0.092** mg a.s./L (fresh weight)- supporting information
- Cinmethylin E_rC_{50} = 0.659 mg a.s./L (dry weight)- supporting information
- Cinmethylin E_yC_{50} = 0.283 mg a.s./L (dry weight)- supporting information

B.9.2.7.2 Metabolite (M684H001):

Report:	CA 8.2.7/6 Rzodeczko H., 2017 e Reg.No. 6055521 (metabolite of BAS 684 H, M684H001) - *Lemna gibba* CPCC 310 growth inhibition test 2016/1224989
Guidelines:	OECD 221 (2006)
GLP:	Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test item: M684H001 (Metabolite of cinmethylin, Reg. no.: 6 055 521), batch no. L87-226, purity: 99.9 %.

B. STUDY DESIGN

Test species: Duckweed (*Lemna gibba,* G3) (Linné), specification: CPCC 310, inocula from 7 days old cultures; cultures maintained in-house; stock obtained from: Canadian Phycological Culture Centre (CPCC), University of Waterloo, Ontario, Canada.

Test design:	Static system (7 days); 6 test item concentrations with 3 replicates plus a control and solvent control (N, N-dimethylformamide at 100 µL/L) with 6 replicates; 3 plants with 3 fronds, total number of fronds at test initiation: 9 per replicate; assessment of growth and other effects on days 2, 4 and at test termination. The yield based on the dry weight was determined at test beginning from a representative sample of the inoculum culture and at test termination with the plant material from each test item concentration and control.
Endpoints:	EC_{10}, EC_{20}, EC_{50}, LOEC and NOEC with respect to growth rate and yield after exposure over 7 days.
Test concentrations:	Control (0), solvent control (0), 0.3, 1.0, 3.0, 10, 30 and 100 mg metabolite/L, corresponding to geometric mean measured concentrations of 0 (control), 0 (solvent control), 0.241, 0.763, 2.43, 7.74, 24.0 and 78.3 mg metabolite/L, respectively.
Test conditions:	Glass beakers (9 cm diameter), test volume 400 mL, 20x-AAP nutrient medium, pH 7.44 - 7.52 at test initiation and pH 8.99 – 9.14 at test termination (maximum pH change during the study was 1.67 units); temperature: 21.8 – 23.5 °C, continuous light, mean light intensity: 6940 - 7730 lux.
Analytics:	Analytical verification of the test item was conducted using an HPLC-method with DAD (Diode Array Detector).
Statistics:	Descriptive statistics; Probit analysis for determination of the EC_x values, Williams Multiple Sequential t-test Procedure (NOEC yield and growth rate based on frond number and dry weight, $\alpha = 0.05$, one-sided smaller).

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each test concentration at the beginning and at the end of the test. The mean measured concentrations for metabolite M684H001 were 73.6 – 79.0 % of nominal at test initiation and 74.3 – 84.2 % of nominal at test termination. The following biological results are based on the geometric mean of the analytically determined concentrations of the test item. The test item solutions were described as visually homogeneous and transparent.

The results are shown in the table below:

Table B.9.2.7.2-1: Measured concentrations during study

Nominal concentration (mg metabolite/L)	Day 0		Day 7		Geometric mean measured (mg metabolite/L)
	Measured concentration (mg metabolite/L)	% of nominal	Measured concentration (mg metabolite/L)	% of nominal	
0 (control)	< LoD	n.a.	< LoD	n.a.	0
0 (solvent control)	< LoD	n.a.	< LoD	n.a.	0
0.3	0.237	79.0	0.245	81.7	**0.241**
1	0.783	78.3	0.743	74.3	**0.763**
3	2.333	77.8	2.526	84.2	**2.43**
10	7.657	76.6	7.818	78.2	**7.74**
30	22.943	76.5	25.095	83.7	**24.0**
100	73.614	73.6	83.297	83.3	**78.3**

n.a. = not applicable, LoD = 0.0005 mg metabolite/L

Validity criteria:

The criteria in OECD 221 (2006) have been considered below:

- For the test to be valid, the doubling time of frond number in the control must be less than 2.5 days (60 h), corresponding to approximately a seven-fold increase in seven days and an average specific growth

rate of 0.275 d^{-1}. Obtained: doubling time was 2.2 days (minimum) and the multiplication factor over 7 days was 8.9. Average specific growth rate was 0.311 d^{-1} (minimum).

During the study the above criteria were met.

Biological results: No significant difference in frond number and dry weight between the control and the solvent control were found, therefore, the solvent control was used for comparisons with every treatment containing the test item.

At exposure termination, in treatments of up to and including 30 mg metabolite/L (nominal) no changes in morphological effect was observed, whereas in treatment of 100 mg metabolite/L (nominal), colony break-up and bending down fronds were observed.

The inhibition of growth rate based on frond number was between -0.6 and 32.4 %. The inhibition of yield based on frond number was between -1.4 and 57.1 %. The inhibition of growth rate based on dry weight was between -1.8 and 31.1 %. The inhibition of yield based on dry weight was between -6.0 and 58.4 % in comparison to the solvent control.

Statistically significant differences compared to the control were observed at the four highest test item concentration for inhibition of growth rate and yield based on frond number (Williams Multiple Sequential t-test Procedure, $\alpha = 0.05$, one-sided smaller). Statistically significant differences compared to control for inhibition of growth rate and yield based on dry weight was observed at the three highest test item concentrations (Williams Multiple Sequential t-test Procedure, $\alpha = 0.05$, one-sided smaller). Effects on growth rate and yield are summarised in Table B.9.2.7.2-2.

Table B.9.2.7.2-2: Effect of metabolite M684H001 on the growth of duckweed *Lemna gibba*

Concentration (nominal) [mg metabolite/L]	**Control**	**Solvent control**	**0.3**	**1.0**	**3.0**	**10**	**30**	**100**
Concentration (geometric mean measured) [mg metabolite/L]	**0.0**	**0.0**	**0.241**	**0.763**	**2.43**	**7.74**	**24.0**	**78.3**
Inhibition after 7 d [%] # (growth rate based on frond no.)	0.0	0.0	-0.6	-0.2	4.4*	5.9*	11.7*	32.4*
Inhibition after 7 d [%] # (growth rate based on dry weight)	0.3	0.0	-1.8	-2.2	0.5	4.8*	9.3*	31.1*
Inhibition after 7 d [%] # (yield based on frond no.)	0.0	0.0	-1.4	-0.5	10.4*	13.7*	25.5*	57.1*
Inhibition after 7 d [%] # (yield based on dry weight)	0.6	0.0	-5.1	-6.0	1.2	12.3*	22.5*	58.4*
	Endpoints [mg metabolite/L] (geometric mean measured)							
E_rC_{50} (7d) based on frond no.	**> 78.3 (95 % limits: n.c.)**							
E_rC_{20} (7d) based on frond no.	38.4 (95 % limits: 34.1 – 43.3)							
E_rC_{10} (7d) based on frond no.	16.2 (95 % limits: 13.1 – 19.9)							
E_yC_{50} (7d) based on frond no.	**64.2 (95 % limits: 54.4 – 75.8)**							
E_yC_{20} (7d) based on frond no.	12.6 (95 % limits: 10.4 – 15.3)							
E_yC_{10} (7d) based on frond no.	5.4 (95 % limits: 4.03 – 7.24)							
E_rC_{50} (7d) based on dry weight	**> 78.3 (95 % limits: n.c.)**							
E_rC_{20} (7d) based on dry weight	45.6 (95 % limits: 37.7 – 55.1)							
E_rC_{10} (7d) based on dry weight	22.4 (95 % limits: 16.0 – 31.4)							
E_yC_{50} (7d) based on dry weight	**61.0 (95 % limits: 47.8 – 78.01)**							
E_yC_{20} (7d) based on dry weight	17.4 (95 % limits: 12.6 – 24.0)							
E_yC_{10} (7d) based on dry weight	8.99 (95 % limits: 5.59 – 14.5)							
$LOEC_{overall}$	2.43							
$NOEC_{overall}$	0.763							

n.c.: not calculated due to mathematical reasons

\# Negative values indicate stimulated growth compared to the solvent control.

* Statistically different compared to control (Williams Multiple Sequential t-test, $\alpha = 0.05$, one-sided smaller)

Reference item test: A reference study was conducted with 3,5-DCP. The following endpoints were derived:

- E_rC_{50} (frond number): 13.91 mg reference item/L
- E_yC_{50} (frond number): 13.05 mg reference item/L
- E_rC_{50} (dry weight): 12.06 mg reference item/L
- E_yC_{50} (frond number): 7.27 mg reference item/L

The guidance document OECD 221 references a ring test report for appropriate ranges. Whilst the range for *Lemna gibba* using 3,5-DCP was 2.7 to 3.4 mg/L for EC_{50} values it is not clear whether these were based on growth rate. In addition, it is stated that further work is needed for this species and reference item before appropriate ranges can be derived. Therefore, it is not possible to confirm whether the sensitivity was appropriate following OECD 221. The study author did not state in the study report whether the result was within historical limits.

III. CONCLUSION

In a 7-day aquatic plant test with *Lemna gibba,* the E_rC_{50} for the metabolite of cinmethylin (M684H001), were determined to be >78.3 mg metabolite/L for both frond number and dry weight, the E_yC_{50} was determined to be 64.2 and 61.0 mg metabolite/L for frond number and dry weight, respectively (based on geometric mean measured concentrations).

HSE evaluator comments:

It was noted that during the study the maximum pH change was 1.67 units, but OECD 221 recommends a change of 1.5 units as a maximum. However, the HSE evaluator agrees with the justification provided by the study author i.e. the difference is relatively small, and the validity criteria were met. Therefore, this deviation has not been considered further.

As stated above there is some uncertainty regarding the reference test result i.e. it is not possible to confirm adequate sensitivity based on OECD 221 or the study report. Nonetheless it is noted that the validity criteria were met and that it is recommended to conduct a reference test based on OECD 221. Therefore, the HSE evaluator does not consider this point sufficient to invalidate the study.

When considering the endpoints calculated several are not in-line with the experimental data, as summarised in the table below, noting relatively wide confidence limits for some endpoints.

Table B.9.2.7.2-3. Difference between endpoints calculated and experimental data (concentrations stated as mg metabolite/L based on geometric mean measured concentrations).

Parameter	Endpoint	Discrepancy in experimental data
E_yC_{10} (7d) based on frond no.	5.4 mg metabolite/L (4.03 – 7.24)	10.4 % inhibition to control at 2.43 mg metabolite/L
E_rC_{10} (7d) based on dry weight	22.4 mg metabolite/L (16.0 – 31.4)	9.3 % inhibition to control at 24 mg metabolite/L
E_yC_{10} (7d) based on dry weight	8.99 mg metabolite/L (5.59 – 14.5)	12.3 % inhibition to control at 7.74 mg metabolite/L

(95 % confidence limits)

Based on above the HSE evaluator has proposed a E_yC_{10} (7d frond number) of 2.43 mg metabolite/L based on experimental data. The E_rC_{10} (7d dry weight) statistically calculated is more conservative than the experimental data and therefore likely to be protective. For E_yC_{10} (7d dry weight) the statistically derived endpoint is less conservative than the experimental data, noting the experimental data is within the calculated confidence limits. Therefore, the HSE evaluator proposes the lowest confidence interval is a more appropriate E_yC_{10} (7d dry weight) i.e. 5.59 mg metabolite/L. It should be noted that none of these endpoints will be used in the risk assessment.

When considering phytotoxicity, effects were only observed in the highest test concentration (100 mg metabolite/L) where colony break-up/bending of fronds was observed. However, it was not reported how many plants were impacted. Therefore, it is not possible to determine a 50 % phytotoxicity value or whether the endpoints derived are protective of phytotoxicity. Whilst this generates some uncertainty it is noted the study was conducted testing a cinmethylin metabolite (M684H001) which demonstrated relatively low toxicity to aquatic plants compared to the herbicide active cinmethylin. In addition there was a wide margin of safety in the metabolite (M684H001) risk assessment for aquatic plants hence the HSE evaluator considers the lack of information regarding phytotoxicity acceptable.

The analytical method is sufficiently validated in accordance with SANCO/3029/99 rev. 4 with an LOQ of 0.002 mg metabolite/L (see volume 3, CA, section B5 for full details).

This study was conducted to GLP and considered valid. The following endpoints were derived (all are based on geometric mean measured concentrations):

- E_rC_{50} = **> 78.3** mg metabolite/L (based on frond number)
- E_rC_{20} = 38.4 mg metabolite/L (based on frond number)
- E_rC_{10} = 16.2 mg metabolite/L (based on frond number)

- E_yC_{50} = 64.2 mg metabolite/L (based on frond number)
- E_yC_{20} = 12.6 mg metabolite/L (based on frond number)
- E_yC_{10} = > 2.43 mg metabolite/L (based on frond number), noting uncertainty highlighted above for statistically derived value hence this endpoint has been based on experimental data

- E_rC_{50} = > 78.3 mg metabolite/L (based on dry weight)
- E_rC_{20} = 45.6 mg metabolite/L (based on dry weight)

- E_rC_{10} = 22.4 mg metabolite/L (based on dry weight)

- E_yC_{50} = 61.0 mg metabolite/L (based on dry weight)
- E_yC_{20} = 17.4 mg metabolite/L (based on dry weight)
- E_yC_{10} = 5.59 mg metabolite/L (based on dry weight), noting uncertainty highlighted above for statistically derived value hence this endpoint has been based on lowest 95 % confidence interval.
- NOEC (overall) = 0.763 mg metabolite/L

B.9.2.7.3 Metabolite Cineole alcohol (M684H003):

Report:	CA 8.2.7/7 Turek T., 2018 c Reg.No. 4539586 (Metabolite of BAS 684 H, M684H003) - *Lemna gibba* CPCC 310 growth inhibition test 2017/1032136
Guidelines:	OECD 221 (2006)
GLP:	Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test item: M684H003 (Metabolite of cinmethylin, Reg. no.: 4 539 586), batch no. L87-86, purity: 99.7 %.

B. STUDY DESIGN

Test species: Duckweed (*Lemna gibba,* G3) (Linné), specification: CPCC 310, inocula from 7 days old cultures; cultures maintained in-house; stock obtained from: Canadian Phycological Culture Centre (CPCC), University of Waterloo, Ontario, Canada.

Test design: Static system (7 days); 1 test item concentration with 6 replicates plus a control with 6 replicates; 3 plants with 3 fronds, total number of fronds at test initiation: 9 per replicate; assessment of growth and other effects on days 2, 4 and at test termination. Dry weight was determined at test beginning from a representative sample of the inoculum culture and at test termination with the plant material from test item concentration and control.

Endpoints: EC_{50} with respect to growth rate and yield after exposure over 7 days.

Test concentrations: Control (0), 100 mg metabolite/L (limit test)

Test conditions: 600 mL glass beakers, test volume 400 mL, 20x-AAP nutrient medium, pH 7.43 – 7.62 at test initiation and pH 8.83 – 8.86 at test termination; temperature: 23.6 – 24.1 °C, continuous light, light intensity: 8950 - 9035 lux.

Analytics: Analytical verification of the test item was conducted using an GC-method with FID detection.

Statistics: Descriptive statistics; Probit analysis for determination of the EC_x values.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each test concentration at the beginning and at the end of the test. The measured concentrations for M684H003 was 96.19 % of nominal at test initiation and 99.44 % of nominal at test termination. The following biological results are, therefore, based on the nominal concentrations of the test item. The test item solutions were described as visually homogeneous and transparent.

The results are shown in the table below:

Table B.9.2.7.3-1: Measured concentrations during study

Nominal concentration (mg metabolite/L)	Day 0		Day 7	
	Measured concentration (mg metabolite/L)	% of nominal	Measured concentration (mg metabolite/L)	% of nominal
0 (control)	< LoD	n.a.	< LoD	n.a.
100	96.187	96.19	99.438	99.44

n.a. = not applicable, LoD = 0.0005 mg metabolite/L

Validity criteria:

The criteria in OECD 221 (2006) have been considered below:

- For the test to be valid, the doubling time of frond number in the control must be less than 2.5 days (60 h), corresponding to approximately a seven-fold increase in seven days and an average specific growth rate of 0.275 d^{-1}. Obtained: doubling time was 2.2 days and the multiplication factor over 7 days was 9.5. Average specific growth rate was 0.321 d^{-1}.

During the study the above criteria were met.

Biological results: At exposure termination, in the test item concentration of 100 mg/L no distinctive changes from the normal development of plants in the control were observed.

Statistically significant differences compared to the control were not observed. Effects on growth rate and yield are summarised in Table B.9.2.7.3-2.

Table B.9.2.7.3-2: Effect of cinmethylin metabolite M684H003 on the growth of duckweed *Lemna gibba*

Concentration (nominal) [mg metabolite/L]	**Control**	**100**
Inhibition after 7 d [%] (growth rate based on frond no.)	0.0	0.5
Inhibition after 7 d [%] (growth rate based on dry weight)	0.0	0.8
Inhibition after 7 d [%] (yield based on frond no.)	0.0	1.5
Inhibition after 7 d [%] (yield based on dry weight)	0.0	2.4
Parameter	**Endpoints [mg metabolite/L] (nominal)**	
E_rC_{50} (7 d) based on frond no.	**> 100 (95 % limits: n.c.)**	
E_yC_{50} (7 d) based on frond no.	> 100 (95 % limits: n.c.)	
E_rC_{50} (7 d) based on dry weight	**> 100 (95 % limits: n.c.)**	
E_yC_{50} (7 d) based on dry weight	> 100 (95 % limits: n.c.)	

n.c.: not calculated due to mathematical reasons

Reference item test: A reference study was conducted with 3,5-DCP. The following endpoints were derived:

- E_rC_{50} (frond number): 12.52 mg reference item/L
- E_yC_{50} (frond number): 7.31 mg reference item/L
- E_rC_{50} (dry weight): 9.42 mg reference item/L
- E_yC_{50} (frond number): 3.98 mg reference item/L

The guidance document OECD 221 references a ring test report for appropriate ranges. Whilst the range for *Lemna gibba* using 3,5-DCP was 2.7 to 3.4 mg/L for EC_{50} values it is not clear whether these were based on growth rate. In addition, it is stated that further work is needed for this species and reference item before

appropriate ranges can be derived. Therefore, it is not possible to confirm whether the sensitivity was appropriate following OECD 221. The study author did not state in the study report whether the result was within historical limits.

III. CONCLUSION

In a 7-day aquatic plant test with *Lemna gibba,* the E_rC_{50} and E_yC_{50} for the metabolite of cinmethylin, M684H003, were determined to be > 100 mg metabolite/L for both frond number and dry weight (based on nominal concentrations).

HSE evaluator comments:

As stated above there is some uncertainty regarding the reference test result i.e. it is not possible to confirm adequate sensitivity based on OECD 221 or the study report. Nonetheless it is noted that the validity criteria were met and that it is recommended to conduct a reference test based on OECD 221. Therefore, the HSE evaluator does not consider this point sufficient to invalidate the study.

$EC_{10/20}$ values were not determined, however given this was a limit test where a maximum of 2.4 % inhibition compared to control occurred (for measured parameters) the omission is considered acceptable by the HSE evaluator.

In terms of phytotoxicity no effects were observed compared to the control at the limit test concentration of 100 mg metabolite/L.

The analytical method is sufficiently validated in accordance with SANCO/3029/99 rev. 4 with an LOQ of 0.002 mg metabolite/L (see volume 3, CA, section B5 for full details).

This study was conducted to GLP and considered valid. The following endpoints were derived (all are based on nominal concentrations):

- E_rC_{50} and E_yC_{50} = **>100** mg metabolite/L (based on frond number and dry weight)
- NOEC (overall) = 100 mg metabolite/L

B.9.2.7.4 Metabolite Cineole alcohol (M684H004):

Report:	CA 8.2.7/8 Rzodeczko H., 2017 f Reg.No. 6055480 (metabolite of BAS 684 H, M684H004) - *Lemna gibba* CPCC 310 growth inhibition test 2016/1224988
Guidelines:	OECD 221 (2006)
GLP:	Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test item:	M684H004 (Metabolite of BAS 684 H, Reg. no.: 6 055 480), batch no. L87-146, Cont.: 007, purity: 97.8 %.

B. STUDY DESIGN

Test species:	Duckweed (*Lemna gibba,* G3) (Linné), specification: CPCC 310, inocula from 7 days old cultures; cultures maintained in-house; stock obtained from: Canadian Phycological Culture Centre (CPCC), University of Waterloo, Ontario, Canada.
Test design:	Static system (7 days); 7 test item concentration with 3 replicates plus a control and a solvent control (N, N-dimethylformamide) with 6 replicates; 3 plants with 3 fronds, total number of fronds at test initiation: 9 per replicate; assessment of growth and other effects on days 2, 4 and at test termination. Dry weight was determined at

	test beginning from a representative sample of the inoculum culture and at test termination with the plant material from test item concentration and control.
Endpoints:	EC_{10}, EC_{50}, NOEC and LOEC with respect to growth rate and yield after exposure over 7 days.
Test concentrations:	Control (0), solvent control (0), 0.0298, 0.095, 0.305, 0.977, 3.13, 10 and 32 mg/L (nominal), corresponding to 0, 0, 0.0225, 0.0646, 0.2064, 0.7042, 2.37, 7.17 and 23.47 mg/L (geometric mean measured).
Test conditions:	Glass beakers (9 cm diameter), test volume 400 mL, 20x-AAP nutrient medium, pH 7.40 – 7.58 at test initiation and pH 8.85 – 9.11 at test termination; temperature: 21.5 – 23.3 °C, continuous light, light intensity: 7350 - 8270 lux.
Analytics:	Analytical verification of the test item was conducted using an HPLC-method with DAD detection.
Statistics:	Descriptive statistics; Probit analysis for determination of the EC_x values, Williams Multiple Sequential t-test Procedure, one-sided smaller, for determination of NOEC and LOEC values (α=0.05)

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each test concentration at the beginning and at the end of the test. The measured concentrations for M684H004 where in the range of 65.9 – 73.8 % of nominal at test initiation and between 63.2 – 79.9 % of nominal at test termination. The following biological results are, therefore, based on the geometric mean measured concentrations of the test item.

The results are shown in the table below:

Table B.9.2.7.4-1: Measured concentrations during study

Nominal concentration (mg metabolite/L)	Day 0		Day 7		Geometric mean measured (mg metabolite/L)
	Measured concentration (mg metabolite/L)	% of nominal	Measured concentration (mg metabolite/L)	% of nominal	
0 (control)	< LoD	n.a.	< LoD	n.a.	0
0 (solvent control)	< LoD	n.a.	< LoD	n.a.	0
0.0298	0.0213	71.5	0.0238	79.9	**0.0225**
0.095	0.0626	65.9	0.0666	70.1	**0.0646**
0.305	0.2209	72.4	0.1929	63.2	**0.2064**
0.977	0.6827	69.9	0.7264	74.4	**0.7042**
3.13	2.31	73.8	2.43	77.6	**2.37**
10	7.25	72.5	7.10	71.0	**7.17**
32	22.53	70.4	24.44	76.4	**23.47**

n.a. = not applicable, LoD = 0.001 mg metabolite/L

Validity criteria:

The criteria in OECD 221 (2006) have been considered below:

- For the test to be valid, the doubling time of frond number in the control must be less than 2.5 days (60 h), corresponding to approximately a seven-fold increase in seven days and an average specific growth rate of 0.275 d^{-1}. Obtained: doubling time was 2.4 days (minimum) and the multiplication factor over 7 days was 7.5. Average specific growth rate was 0.288 d^{-1} (minimum).

During the study the above criteria were met.

Biological results: No significant difference in frond number and dry weight between the control and the solvent control were found, therefore the solvent control was used for comparisons with every treatment containing the test item. At exposure termination, in the test item concentration of 0.0298 and 0.095 mg metabolite/L (nominal) no distinctive changes were observed. In treatment 0.977 mg metabolite/L (nominal) smaller fronds, shorter roots and discoloration on 43 % of fronds where observed. In treatment 3.13 mg/L overlapping and bending of fronds, smaller fronds and discoloration on 68 % of fronds were observed. In treatment 10 mg metabolite/L (nominal) short roots, colony break-up and brown parts of fronds were observed. In treatment 32 mg metabolite/L (nominal) colony break-up and necrosis were observed.

The inhibition of growth rate based on frond number was between -1.1 and 96.5 %. The inhibition of yield based on frond number was between -2.6 and 98.9 %. The inhibition of growth rate based on dry weight was between -1.8 and 34.1 %. The inhibition of yield based on dry weight was between -5.9 and 67.1 % in comparison to the solvent control.

Statistically significant differences in growth rate and yield compared to the control were observed in the five highest test item concentrations based on frond number and in the four highest test item concentrations based in dry weight (Williams multiple sequential t-test, $\alpha = 0.05$, one-sided smaller). Effects on growth rate and yield are summarised in Table B.9.2.7.4-2.

Table B.9.2.7.4-2: Effect of M684H004 on the growth of duckweed *Lemna gibba*

Concentration (nominal) [mg/L]	**Control**	**Solvent control**	**0.0298**	**0.095**	**0.305**	**0.977**	**3.13**	**10**	**32**
Concentration (geometric mean measured) [mg/L]	**0.0**	**0.0**	**0.225**	**0.046**	**0.2064**	**0.7042**	**2.37**	**7.17**	**23.47**
Inhibition after 7 d [%] (growth rate based on frond no.) #	0.0	0.0	-1.1	0.4	5.6*	7.5*	35.2*	79.3*	96.5*
Inhibition after 7 d [%] (growth rate based on dry weight) #	0.2	0.0	-1.8	0.4	1.3	7.5*	14.1*	29.5*	34.1*
Inhibition after 7 d [%] (yield based on frond no.) #	0.0	0.0	-2.6	0.9	12.3*	16.2*	58.4*	92.0*	98.9*
Inhibition after 7 d [%] (yield based on dry weight) #	0.6	0.0	-5.9	1.3	3.9	21.0*	36.0*	61.5*	67.1*
Parameter	**Endpoints [mg metabolite/L] (geometric mean)**								
E_rC_{50} (7 d) based on frond no.	3.28 (95 % limits: 3.10 – 3.47)								
E_rC_{20} (7 d) based on frond no.	1.38 (95 % limits: 1.26 – 1.51)								
E_rC_{10} (7 d) based on frond no.	0.881 (95 % limits: 0.782 – 0.992)								
E_yC_{50} (7 d) based on frond no.	1.79 (95 % limits: 1.65 – 1.94)								
E_yC_{20} (7 d) based on frond no.	0.704 (95 % limits: 0.621 – 0.798)								
E_yC_{10} (7 d) based on frond no.	0.432 (95 % limits: 0.365 – 0.510)								
E_rC_{50} (7 d) based on dry weight	> 23.47 (95 % limits: n.c.)								
E_rC_{20} (7 d) based on dry weight	4.41 (95 % limits: 4.02 – 4.84)								
E_rC_{10} (7 d) based on dry weight	1.08 (95 % limits: 0.918 – 1.27)								
E_yC_{50} (7 d) based on dry weight	5.30 (95 % limits: 4.82 – 5.83)								
E_yC_{20} (7 d) based on dry weight	0.730 (95 % limits: 0.632 – 0.845)								
E_yC_{10} (7 d) based on dry weight	0.259 (95 % limits: 0.211 – 0.318)								
$NOEC_{overall}$	0.046**								

n.c.: not calculated due to mathematical reasons

Negative values indicate stimulated growth compared to the control.

* Statistically different compared to control (Williams multiple sequential t-test, $\alpha = 0.05$, one-sided smaller)

** Determined by HSE evaluator as appears to be a typographical error in study report which stated 0.0646 mg metabolite/L (geometric mean) noting this concentration was not tested in the study, see table B.9.2.7.4-1.

Reference item test: A reference study was conducted with 3,5-DCP. The following endpoints were derived:

- E_rC_{50} (frond number): 13.91 mg reference item/L
- E_yC_{50} (frond number): 13.05 mg reference item/L

- E_rC_{50} (dry weight): 12.06 mg reference item/L
- E_yC_{50} (frond number): 7.27 mg reference item/L

The guidance document OECD 221 references a ring test report for appropriate ranges. Whilst the range for *Lemna gibba* using 3,5-DCP was 2.7 to 3.4 mg/L for EC_{50} values it is not clear whether these were based on growth rate. In addition, it is stated that further work is needed for this species and reference item before appropriate ranges can be derived. Therefore, it is not possible to confirm whether the sensitivity was appropriate following OECD 221. The study author did not state in the study report whether the result was within historical limits.

III. CONCLUSION

In a 7-day aquatic plant test with *Lemna gibba,* the E_rC_{50} and E_yC_{50} for the metabolite of cinmethylin, M684H004, were determined to be 3.28 and 1.76 mg/L based on frond number and >23.47 and 5.3 mg/L based on dry weight, respectively (based on geometric mean measured concentrations).

HSE evaluator comments:

As stated above there is some uncertainty regarding the reference test result i.e. it is not possible to confirm adequate sensitivity based on OECD 221 or the study report. Nonetheless it is noted that the validity criteria were met and that it is recommended to conduct a reference test based on OECD 221. Therefore, the HSE evaluator does not consider this point sufficient to invalidate the study.

The analytical method is sufficiently validated in accordance with SANCO/3029/99 rev. 4 with an LOQ of 0.005 mg metabolite/L (see volume 3, CA, section B5 for full details).

This study was conducted to GLP and considered valid. The following endpoints were derived (all are based on geometric mean measured concentrations):

- E_rC_{50} = **3.28** mg metabolite/L (based on frond number)
- E_rC_{20} = 1.38 mg metabolite/L (based on frond number)
- E_rC_{10} = 0.881 mg metabolite/L (based on frond number)

- E_yC_{50} = 1.79 mg metabolite/L (based on frond number)
- E_yC_{20} = 0.704 mg metabolite/L (based on frond number)
- E_yC_{10} = 0.432 mg metabolite/L (based on frond number)

- E_rC_{50} = > 23.47 mg metabolite/L (based on dry weight)
- E_rC_{20} = 4.41 mg metabolite/L (based on dry weight)
- E_rC_{10} = 1.08 mg metabolite/L (based on dry weight)

- E_yC_{50} = 5.30 mg metabolite/L (based on dry weight)
- E_yC_{20} = 0.73 mg metabolite/L (based on dry weight)
- E_yC_{10} = 0.259 mg metabolite/L (based on dry weight)
- NOEC (overall) = 0.046 mg metabolite/L

B.9.2.8. Further testing on aquatic organisms

B.9.2.8.1 Bioaccumulation /Bioconcentration studies, active substance, cinmethylin:

Report:	CA 8.2.2.3/1 [redacted], 1983 b Uptake, depuration and bioconcentration of 14C SD95481 by bluegill sunfish *Lepomis macrochirus* CI-690-004
Guidelines:	None reported
GLP:	No
Report:	CA 8.2.2.3/2 Lee P., 1984 a

Characterisation of 14C residues in fish samples from the 14c sd95481 bluegill sunfish bioconcentration study
CI-705-001

Guidelines: None reported
GLP: No

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Mixture of radiolabeled and unlabeled test item (ratio 1: 1):
Non-radiolabeled test item: cinmethylin (Reg. no.: 900 202), purity 92.0 %.
Radiolabeled test item: aromatic ring ^{14}C-cinmethylin; batch no. 8212-1; specific activity: 56.4 µc/mg, purity 99.5 %

B. STUDY DESIGN

Test species: Bluegill sunfish (*Lepomis macrochirus*); lot: #4982; mean body weight: 5.4 g; with a range of 3.1 – 9.4 g/fish (representative group); mean body length: 5.3 with a range of 4.5 – 6.4 cm (representative group); source: "Kurtz Fish Hatchery", Elverson, Pennsylvania, USA.

Test design: Flow-through system (28 days uptake, 14 days depuration); one test vessel per treatment and control with 110 fish per test vessel; test item concentrations in water and fish as well as wet weight of fish were determined throughout the study; total radioactive residues in fish were measured separately in whole fish, fillet and visceral portions; mortality and signs of toxicity were assessed daily.
Characterization of residues: Samples from fillet, viscera and whole fish were analysed for quantitative distribution of 14C residues using methanol/hexane and chloroform extracts from samples day 3, 28 (exposure phase) and day 3 (depuration phase). Additionally, samples from whole fish using combined ether extracts of days 1, 3, 7, 10, 14 ,21 and 28 (exposure phase) were analyzed to characterize 14C residues.

Endpoints: Bioconcentration potential (bioconcentration factor: BCFss); uptake rate constant (K1); depuration rate constant (K2).

Test concentrations: Control and 0.1 mg a.s./L (nominal) corresponding to mean measured concentration of 0.097 mg a.s./L (mixture of radiolabeled and unlabeled test item)

Test conditions: 100 L glass aquaria; test volume 70 L; aerated deep well water; flow rate: approx. 6-fold volume exchange/day/test vessel (approx. 1.5 ml/min); temperature: 21 °C; pH 8.0 - 8.5; oxygen content: 6.7 mg/L - 8.7 mg/L; ammonia concentration: 0.16 – 1.4 mg/L; conductivity: 50 µmhos/cm (dilution water); hardness: 255 ppm (dilution water); photoperiod 16 h light : 8 h dark; no aeration; feeding: commercial fish diet (Rangen`s ®) at approx. 3 % of the body weight per day.

Analytics: Determination of test item concentrations in water and fish was conducted by measuring total radioactivity using Liquid Scintillation Counting. Determination of non-labelled test item concentration using a HPLC method with UV-detection; Characterization of 14C-residues using a TLC method with additional GC analysis.

Statistics: Descriptive statistics; BCF values were calculated based on steady state concentration in fish (BCFss) and based on a two-compartment biokinetic model (k1, k2) using linear regression analysis on uptake and depuration curves.

II. RESULTS AND DISCUSSION

Validity criteria

This study was conducted before the most recent OECD guideline. Nonetheless in order to determine whether the study is valid the criteria in OECD 305 (2012) have been considered below:

- Water temperature variation is less than ± 2 ºC in treatment or control groups. Obtained: Reported as 21 °C throughout study.
- Concentration of dissolved oxygen does not fall below 60 % saturation. Obtained: Minimum of 6.7 mg/L i.e. > 60 % saturation considering the temperature of the study (100 % saturation would be approximately 8.6 mg/l at 24 ºC).
- The concentration of the test substance in the chambers is maintained within ± 20 % of the mean of the measured or nominal values during the uptake phase. Obtained: mean measured concentrations were not within ± 20 % of either the nominal or mean measured concentration during the study with ranges from 72 to 120 % and 74 to 123.4 % respectively.
- The mortality or other adverse effects/disease in both control and treated fish is less than 10 % at the end of the test; where the test is extended over several weeks or months, death or other adverse effects in both sets of fish should be less than 5 % per month and not exceed 30 % in all. Obtained: No mortalities or signs of toxicity were observed during the test period.

During the study the above criteria were not met as the concentration of the test substance in the chambers was not maintained within ± 20 % of the nominal or measured values.

Mortality and signs of toxicity
No mortalities or signs of toxicity were observed in the control and treatment group over the 28-day test period.

Analytical results in water and fish tissue

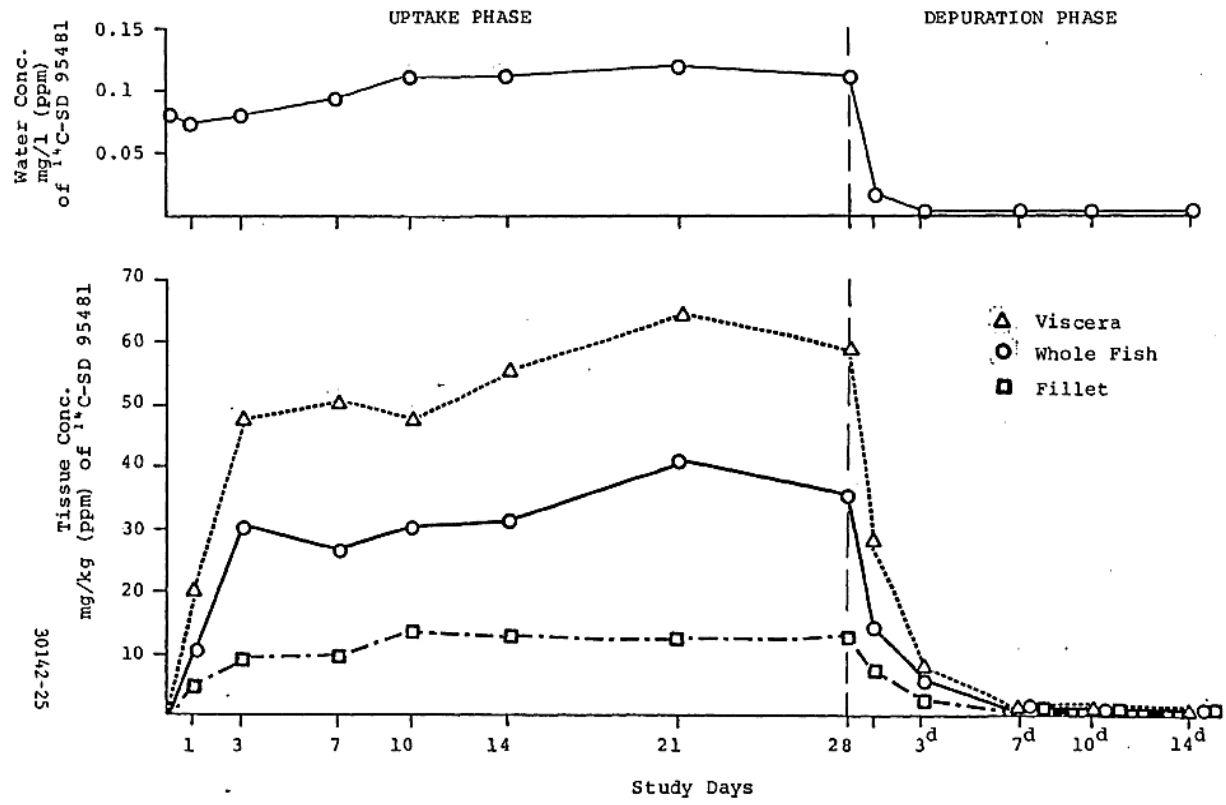
Uptake/depuration curves for ^{14}C-cinmethylin in water and the various tissue types are shown in Figure B.9.2.8.1-1. The data suggest that the compound ceased accumulating after 3 days of exposure. The mean tissue residues after 28 days of exposure were 35 ppm for whole fish, 12 ppm, for fillet and 58 ppm for viscera. These values corresponded to day 28 bioconcentration factors of 360, 120 and 600, respectively. Daily bioconcentration factors for the uptake phase of the study ranged from 130 - 430 for whole fish, 60 - 150 for fillet and 260 - 670 for viscera. An analysis of clearance rates by day 14 of the elimination period showed 99, 99 and 99 percent depuration in whole fish, fillet and viscera, respectively. The depuration data indicated a clearance of ^{14}C-cinmethylin from bluegill tissue up to day 14.

The analytical data for water concentrations are shown in the table below.

Table B.9.2.8.1-1: Measured values in water concentrations (mg a.s./L) for exposure phase, nominal concentration = 0.1 mg eq a.s./L.

Day	Measured concentration (mg eq/L)	Mean measured concentration during exposure phase	Percentage of nominal (%)	Percentage of mean measured concentration (%)
0	0.082	0.097	82.0	84.3
1	0.072		72.0	74.0
3	0.080		80.0	82.3
7	0.094		94.0	96.7
10	0.110		110.0	113.1
14	0.110		110.0	113.1
21	0.120		120.0	123.4
28	0.110		110.0	113.1

Figure B.9.2.8.1-1: Plot of the uptake and depuration curves in mg/L water, in whole fish, fillet and viscera portions.



The uptake rate constant (K_1), depuration rate constant (K_2), and steady-state bioconcentration factor (BCF) for whole fish were determined using a steady-state approach for bioconcentration. By linear regression analysis of the natural logarithm transformed tissue concentrations of uptake and depuration and a two-compartment kinetic model, these values were determined to be K_1 = 166 ppm in fish/ppm in water/day, K_2 = 0.566 /day, and BCF = 290. This latter value was 81 % of the day 28 bioconcentration factor of 360. Efficiency data for scintillation counting averaged 0.740 (±0.016 SD) for water, 0.758 (±0.018 SD) for whole fish, 0.760 (±0.017 SD) for fillet and 0.759 (±0.027 SD) for viscera. Recovery data for ^{14}C-cinmethylin in tissue sample oxidations averaged 86 % for whole fish, 84 % for fillet and 88 % for viscera. ^{14}C-Benzoic Acid instrument combustion recovery in the absence of tissue averaged 98 % for the study. The results are summarised in Table B.9.2.8.1-2.

Table B.9.2.8.1-2: Uptake and depuration rate constants and bioconcentration factors (BCF) for the whole fish based on measured and calculated data.

Parameter	**0.1 mg eq/L**
K_1 (uptake rate constant)	166
K_2, (depuration rate constant)	0.566
BCFss (steady-state BCF; L/kg) (day 3 – 28)	**290**
BCF_{day28} (whole fish)	360

Characterization of ^{14}C-residues:

Extraction and fractionation procedures recovered an estimated 87 – 95 % of the total radioactivity in the various fish tissues (whole fish = 43 %). Three major and two minor components were identified in whole fish samples: SD 95481 (cinmethylin, Product I, 11 % of the recovered radioactivity), SD 202193 (M684H001, Product II, 13 %) and SD 207574 (M684H011, Product III, 14 %) were recovered as the major radiolabeled components, whereas, SD 751 (M684H061) and SD 207856 (M684H002, 1 – 3 %) as minor components.

III. CONCLUSION

In a flow-through bioconcentration study, bluegill sunfish were exposed to ^{14}C-cinmethylin / BAS 684 F at 0.1 mg a.s./L (nominal) in water, for an uptake period of 28 days steady state was reached after 3 days. The BCF_{SS} for the whole fish was determined to be 290, by the study author, based on total radioactive residues of cinmethylin. The parent cinmethylin contributed to 11 % of the recovered ether extracts in whole fish samples.

HSE evaluator comments:

The above studies (one supporting analytical work) were not conducted to GLP or considered valid as the validity criteria were not met. In addition, lipid analysis was not conducted hence the BCF can not be normalised for lipid content as required under current data requirements. Furthermore, the fish loading rate at start of study and amount of solvent used were above recommendations in OECD 305 (0.1 – 1 g of fish/L and 0.1 ml/L) at 1.4 g fish/L and 0.4 ml/L respectively and there was no consideration of growth. Finally the analytical method was not sufficiently validated due to the following deficiencies (see volume 3, CA, section B5 for full details):

i) It is not possible to accept the linearity of the method as the example chromatograms for the highest concentration of the linear range show overloading of the sample, with the detector being saturated giving a broad flat top peak.
ii) To assess accuracy and precision, at least 5 recoveries should be provided for at least 2 fortification levels.
iii) The LOQ is not supported by 5 recovery determinations.
iv) Procedural recoveries have not been completed.

Therefore, this study is not considered valid for use in the risk assessment and has not been considered further.

Report: CA 8.2.2.3/3
[redacted], 2017 b
14C-BAS 684 H - Bioconcentration study in the bluegill sunfish (*Lepomis macrochirus*)
2017/1156422
Guidelines: OECD 305, EPA 850.1730
GLP: Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Mixture of radiolabeled and unlabeled test item (ratio 1: 1):
Non-radiolabelled test item: cinmethylin (Reg. no.: 900 202), batch no. COD-002038, purity 93.0 ± 1.0 %.
Radio-labelled test item: ^{14}C-cinmethylin (label: cyclohexane-4-C14); batch no. 1146-2001; specific activity: 76.9 MBq/g; chemical purity: 95.9 %, radiochemical purity: 97.9 %.

B. STUDY DESIGN

Test species: Bluegill sunfish (*Lepomis macrochirus*); mean body weight: 0.92 ± 0.11 g; with a range of 0.74 – 1.10 g/fish. mean body length: 4.1 ± 0.2 cm; age: approximately 14 months; source: [redacted].

Test design: Flow-through system (17 days uptake, 7 days depuration); two test vessel per treatment with 35 fish per test vessel (loading 0.14 g fish/L at start of the uptake period); test item concentrations in water and fish as well as wet weight of fish were determined throughout the study; total radioactive residues in fish were measured separately in edible (e.g. fillet) and nonedible (e.g. remaining carcass) portions; lipid content was determined in fish from control group at the start, day 10, day 17 and at the end of depuration; mortality and signs of toxicity were assessed daily.

Endpoints: Bioconcentration potential (bioconcentration factors BCFss, BCF_{SSL}, BCF_K, BCF_{Kg}, BCF_{KLg}); uptake rate; depuration rate; depuration half-life; time to 95 % steady state.

Test concentrations: Control, 0.5 and 5 µg a.s./L (nominal, mixture of radiolabeled and unlabeled test item; ratio 1: 1).

Test conditions:	Stainless steel aquaria (29.5 x 28.5 x 58 cm); overflow at approx. 27 cm; water volume: approx. 45 L; aerated non-chlorinated drinking water, charcoal filtered and diluted with deionized water; flow rate: approx. 9.4 L/h/test aquarium (= approx. 5-fold volume exchange/day/test vessel); temperature: 24 °C; pH 7.9 - 8.2; oxygen content: 7.8 mg/L - 8.6 mg/L; total organic carbon: 0.6 – 0.7 mg/L; photoperiod 16 h light : 8 h dark; no aeration; feeding: commercial fish diet (BioMar) at approx. 1 % of the body weight per day (on work days).
Analytics:	Determination of test item concentrations in water and fish was conducted by measuring total radioactivity using Liquid Scintillation Counting.
Statistics:	Descriptive statistics; BCF values were calculated based on steady state concentration in fish (BCF_{ss}) and based on the uptake and depuration curves by using a first order (one-compartment) biokinetic model (BCF_k); BCF values were further normalized to 5 % fish lipid content and corrected for growth during the experiment (according to OECD test guideline 305).

II. RESULTS AND DISCUSSION

Validity criteria

The criteria in OECD 305 (2012) have been considered below:

- Water temperature variation is less than ± 2 ºC in treatment or control groups. Obtained: minimum of 23.5 and maximum of 24.1 ºC.
- Concentration of dissolved oxygen does not fall below 60 % saturation. Obtained: 7.8 – 8.6 mg/L i.e. > 60 % saturation considering the temperature of the study (100 % saturation would be approximately 8.6 mg/l at 24 ºC).
- The concentration of the test substance in the chambers is maintained within ± 20 % of the mean of the measured or nominal values during the uptake phase. Obtained: Concentration was within ± 20 % during uptake phase.
- The mortality or other adverse effects/disease in both control and treated fish is less than 10 % at the end of the test; where the test is extended over several weeks or months, death or other adverse effects in both sets of fish should be less than 5 % per month and not exceed 30 % in all. Obtained: No mortalities in control or treatment groups.

During the study the above criteria were met.

Mortality and signs of toxicity
No mortalities or signs of toxicity were observed in the control and treatment group over the 24-day test period.

Fish growth
There was no statistically significant difference in fish growth rate between control and treatment group during the experiment, therefore data from both groups were combined to determine the overall growth rate (k_g) for "growth-corrected" calculations. The combined growth rate of both groups (k_g) was 0.0092/day.

Lipid content of fish
The mean lipid content of control fish was 3.2 % at the start of exposure, 5.4 % at the end of exposure and 7.0 % at the end of the test period. The lipid content systematically increased over the experiment. Since there was no statistically significant difference in growth between the test groups, the measured lipid content in the control fish is considered representative of the treatment groups. Therefore, for calculation of the lipid corrected BCF the mean lipid content in control fish of the uptake period (5.4 %) was used.

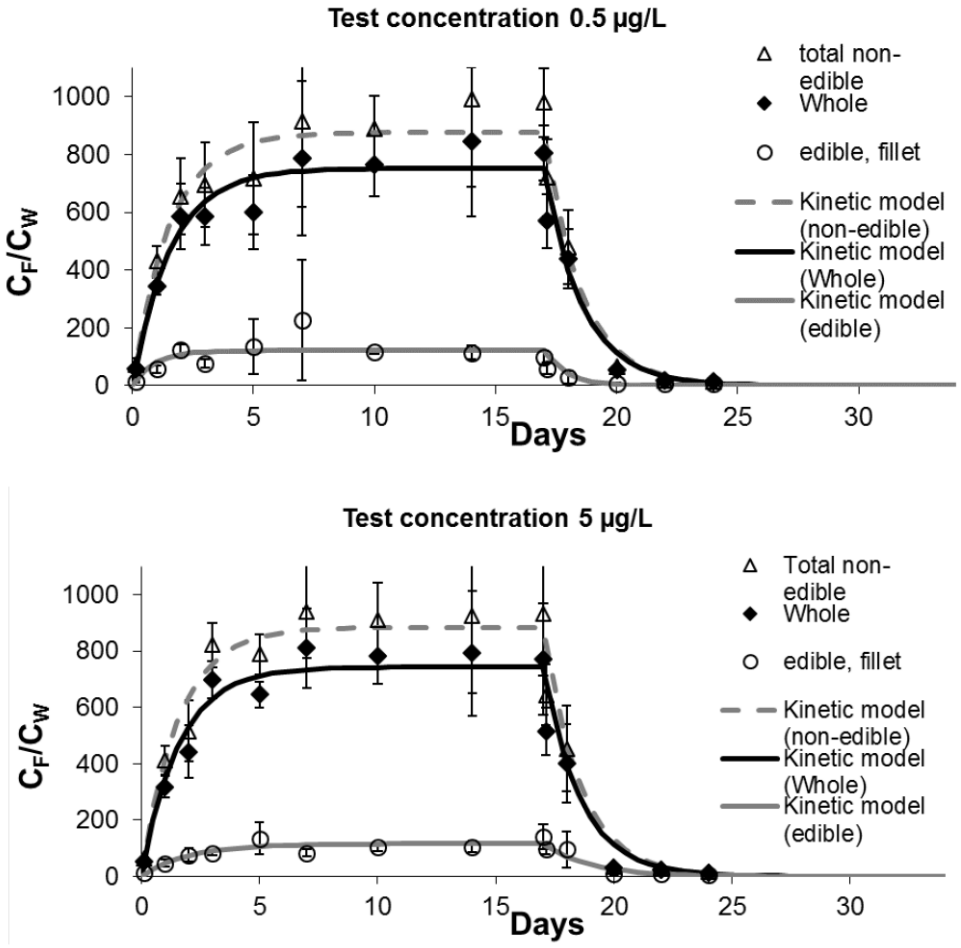
Analytical results in water and fish tissue
During the uptake period (to day 17) the concentration of the test item in water remained within ± 20 % of the nominal concentration based on radioactivity measurement. Also, the additional daily measurements indicated no deviation of > 20 % from the nominal concentration. The mean concentrations of the test substance in water during the uptake phase (through day 17) were 0.49 ± 0.03 µg a.s./L (98 % of the nominal concentration) for the

low and 4.87 ± 0.16 µg a.s./L (97 % of nominal concentration) for the high concentration. By day 3 of the depuration period, concentrations were less than 2 % of nominal.

In the concentration group 0.5 µg a.s./L the concentration of ^{14}C-cinmethylin in the whole fish on day 3 was within 20 % of the mean measured concentration in fish during the uptake period, indicating that the uptake had become approximately asymptotic with respect to time (reached plateau). The kinetic calculations indicate that 95 % of the steady state concentration was reached after 4.6 - 4.8 days. Consequently, the measured fish values from day 3 onward were considered for the calculation of the steady state bioconcentration factor (BCF_{ss}). The BCF_{ss} was 731 L/kg, determined as mean value of $C_F(t)/CW$ from days 3 – 17. When normalized to a 5 % lipid content in fish (using lipid measured at the end of uptake), the steady state bioconcentration factor (BCF_{ssL}) was 677 L/kg.
In the concentration group 5 µg a.s./L the concentration of ^{14}C-cinmethylin in the whole fish on day 3 was within 20 % of the mean measured concentration in fish during the uptake period, indicating that the uptake had become approximately asymptotic with respect to time (reached plateau). The kinetic calculations indicate that 95 % of the steady state concentration was reached after 4.6 - 4.8 days. Consequently, the measured fish values from day 3 onward were considered for the calculation of the steady state bioconcentration factor (BCF_{ss}). The BCF_{ss} was 749 L/kg, determined as mean value of $C_F(t)/CW$ from days 3 – 17. When normalized to a 5 % lipid content in fish (using lipid measured at the end of uptake), the steady state bioconcentration factor (BCF_{ssL}) was 694 L/kg. It should be noted a separate report considered the amount of active and metabolites during the study ([redacted], 2018a).

Bioconcentration kinetics
The measured data from both concentration groups from whole fish as well as edible and nonedible portions fit well to a first order kinetic model allowing an estimation of the uptake and depuration rate constants based on simultaneous curve fitting. The data illustrate that steady state was quickly reached during the uptake period, after the sampling on day 3. After the start of depuration, the concentrations in fish progressively declined. After 7 days in clean water the whole-body residues in fish from both concentration groups had declined to 2 % of the mean steady state concentration (CF_{ss}) See Figure B.9.2.8.1-2.

Figure B.9.2.8.1-2: Plot of the uptake and depuration curves as BCF ($C_F(t)/C_W$) in whole fish, fillet and non-edible portions. Not corrected for growth or lipid content.



Based on the kinetic model, the calculated uptake rate constants (k_1) were 474 and 476 day^{-1} from the low and high exposure concentrations respectively. The calculated depuration rate constants (k_2) were 0.63 and 0.65 day^{-1} from the low and high exposure concentrations respectively. The OECD 305 guideline suggests that the variation in uptake and depuration rate constants derived from exposure at two concentrations should not differ by more than 20 % between the test groups, otherwise concentration dependence may be indicated. The results are summarized in Table B.9.2.8.1-3.

Overall the measured BCF_{ss} values were very similar to the calculated BCF_K values indicating that steady state was reached, and that uptake and depuration follow first order kinetics. The most relevant BCF is the BCF_K normalized to 5 % lipid content (BCF_{KL}) because it incorporates all measurements during uptake and depuration and since it removes the influence of the test fish lipid content. In conclusion, the bioconcentration factor BCF_{KL} was 697 L/kg for the whole fish based on total radioactive residues of ^{14}C-cinmethylin.

Table B.9.2.8.1-3: Uptake and depuration rate constants and bioconcentration factors (BCF) for the whole fish based on measured and calculated data.

Parameter	**0.5 µg eq/L**	**5 µg eq/L**
k_g (growth rate constant; day^{-1}) (standard error)	0.0092 (0.0009)	0.0092 (0.0009)
k_1, (overall uptake rate constant, L/kg/day) (95% confidence interval)	474 (367 – 582)	476 (374 – 578)
k_2, (overall depuration rate constant, day^{-1}) (95% confidence interval)	0.63 (0.48 – 0.78)	0.65 (0.51 – 0.79)
k_{2g} (growth-corrected depuration rate constant, day^{-1})	0.62	0.64
$C_F$ss, (concentration in fish at steady-state, µg a.s./kg) (mean (days 2 – 14) ± standard deviation)	360 ± 54	3648 ± 310
C_W (concentration in the water, µg/L) (mean (days 0 – 14) ±standard deviation)	0.49 ± 0.03	4.78 ± 0.16
L_n (lipid normalization factor) (mean during uptake)	0.054	0.054
BCFss (steady-state BCF; L/kg) (mean (days 2 – 14) ± standard deviation)	731 ± 110	749 ± 64
BCF_{SSL} (lipid normalized steady-state BCF; L/kg)	677	694
BCF_K (kinetic BCF; L/kg)	752	732
BCF_{Kg} (growth-corrected kinetic BCF; L/kg)	764	743
BCF_{KLg} (lipid-normalized kinetic BCF_{Kg}; L/kg)	707	688
Geometric mean BCF_{KLg} [a]	**697**	
$t_{1/2}$, (depuration half-life; day)	1.10	1.07
$t_{1/2g}$ (growth-corrected half-life, day)	1.12	1.08
Time to 95 % steady state (growth-corrected, day)	4.8	4.6

[a] The most relevant BCF in this study is the growth corrected kinetic BCF normalized to 5 % lipid content, BCF_{KLG}.

III. CONCLUSION

In a flow-through bioconcentration study, bluegill sunfish were exposed to ^{14}C-cinmethylin / BAS 684 F at 0.5 and 5 µg eq/L (nominal) in water, for an uptake period of 17 days 95 % steady state was reached within 4.6 – 4.8 days. The most relevant BCF is the growth corrected kinetic BCF normalized to 5% lipid content (BCF_{KLg}) for the whole fish which was determined to be 697 L/kg (geometric mean) based on total radioactive residues of cinmethylin. The individual values were 707, 688 L/kg for 0.5 and 5 µg eq/L respectively.

HSE evaluator comments:

As this was an additional vertebrate study the following justification was provided by the applicant (required under 1107/2009): '*The above new bioconcentration study on bluegill sunfish was conducted to meet data requirements outside the European Union. On request of the RMS CTGB, the study is provided for the sake of completeness.'* The HSE evaluator considers the justification provided appropriate.

As the test item contained radio-labelled cinmethylin the HSE chemistry evaluator has not considered the analytical method further.

The growth corrected clearance of total radioactivity (t half life) values were 1.12 and 1.08 days for 0.5 and 5 µg a.s./L respectively. The time to 95 % steady state was comparable for both 0.5 and 5 µg a.s./L at 4.8 and 4.6 days respectively.

The above study was conducted to GLP and considered valid. The following endpoints will be discussed in the risk assessment:

BCF (normalised to 5 % lipid content): **707 L kg^{-1}** (whole fish) at 0.5 µg eq/L
BCF (normalised to 5 % lipid content): **688 L kg^{-1}** (whole fish) at 5 µg eq/L

It was noted an additional study report was submitted that complements the above study and has been summarised below.

Report:	CA 8.2.2.3/4 [redacted], 2018 a Metabolism of 14C-BAS 684 H in Bluegill Sunfish (bioconcentration after exposure in a flow through system 2017/1208842
Guidelines:	EPA 850.1730, OECD 305 (October 2012)
GLP:	Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test item:	Cinmethylin (mixture of radiolabeled and unlabeled test item (ratio 1: 1):
Non-radiolabeled test item:	Cinmethylin (Reg. no.: 900 202), batch no. COD-002038, purity 93.0 % ± 1.0 %; (1RS,2SR,4SR)-1,4-epoxy-p-menth-2yl 2-methylbenzyl ether

Radio-labeled test item: ^{14}C-cinmethylin (label: cyclohexane-4-C^{14}); batch no. 1146-2001; specific activity a.s.: 8.08 MBq/mg; chemical purity: 95.9 %, radiochemical purity: 97.9 %.

B. STUDY DESIGN

Test species:	Bluegill sunfish (*Lepomis macrochirus*); mean body weight: 0.92 ±0.11 g; with a range of 0.74 – 1.10 g/fish. mean body length: 4.1 ± 0.2 cm; age: approximately 14 months; source: [redacted]
Test design:	The in-life part was conducted within the BCF study [redacted], 2017b; in short: flow-through system (17 days uptake, 7 days depuration); two test vessels per treatment with 35 fish per test vessel (loading 0.14 g fish/L at start of the uptake period; continuous exposure via water phase; total radioactive residues (TRR) in fish and water were measured throughout the study, TRR in fish was measured separately in edible (e.g. fillet) and nonedible (e.g. viscera and remaining carcass) portions; lipid content was determined in fish from control group at the start, day 10, day 17 and at the end of depuration; mortality and signs of toxicity were assessed daily, samples from day 17 (end of uptake phase) were used for characterization and identification of metabolites. The formulation exposure was prepared by mixing ^{14}C-labelled, and non-radiolabeled test items in a ratio 1:1 (for details see [redacted] 2017b).
Endpoints:	Quantification and identification of metabolites in edible, non-edible portions in fish and water
Test concentrations:	Control, 0.5 and 5 µg a.s./L (nominal, mixture of radiolabeled and unlabeled test item; ratio 1: 1) for details see [redacted], 2017b.
Test conditions:	Stainless steel aquaria (29.5 x 28.5 x 58 cm); overflow at approx. 27 cm; water volume: approx. 45 L; aerated non-chlorinated drinking water, charcoal filtered and diluted with deionized water; flow rate: approx. 9.4 L/h/test aquarium (= approx. 5-fold volume exchange/day/test vessel); temperature: 24 °C; pH 7.9 - 8.2; oxygen

content: 7.8 mg/L - 8.6 mg/L; total organic carbon: 0.6 – 0.7 mg/L; photoperiod 16 h light : 8 h dark; no aeration; feeding: commercial fish diet (BioMar) at approx. 1 – 2 % of the body weight per day (on work days) for details see [redacted] 2017b.

Analytics: Aliquots from tank water as well as edible and non-edible portions of fish were analyzed using Liquid Scintillation Counting (LSC) for residues and HPLC analysis with UV detection for quantification and identification of parent and metabolites (methanol extracts from fish tissues). The residual radioactive residues after solvent extraction (RRR) were determined by combustion analysis.
Regarding HPLC-analysis, two methods were applied. In context of HPLC method LC01 (quantitation method) a RESTEK Raptor Biphenyl column was used and a binary eluent system (A: water/formic acid = 999/1, v/v; B: methanol/ACN = 60/40, v/v + formic acid = 999/1, v/v) applying gradient elution. For confirmation purposes HPLC method LC02 was used: a Phenomenex Luna PFP(2) column was used here and also a binary eluent system applying gradient elution (A: water/formic acid = 998/2, v/v; B: methanol/ACN = 30/70, v/v + formic acid = 998/2, v/v).

Statistics: Descriptive statistics

II. RESULTS AND DISCUSSION

General observations

No mortalities or signs of toxicity were observed in the control and treatment group over the test period. There was no statistically significant difference in fish growth rate between control and treatment group during the experiment (for details see [redacted], 2017b which is summarised above).

Total Radioactive residues (TRR) in water and fish tissues

The mean concentrations of the test substance in test solution during the uptake phase (through day 17) were 0.49 ± 0.03 µg a.s./L (98 % of the nominal concentration) for treatment group 1 and 4.87 ± 0.16 µg a.s./L (97 % of the nominal concentration) for treatment group 2. During the uptake period (to day 17) the concentration of the test substance in test solution remained within ± 20 % of the nominal concentration. By day 3 of the depuration period, the radioactivity level was below 1 % of the nominal concentration for both treatment groups. From day 5 of the depuration phase the radioactivity was too low to be determined and did not exceed the background range.

The residual values determined for the tank water samples of the treatment group (test vessel 1, nominal dose 0.5 µg a.s./L and test vessel 2, nominal dose 5.0 µg a.s./L) during the present study were slightly lower (around 0.4 µg a.s./L for test group 1 and 3.9 µg a.s./L for test group 2 compared to the measurements in the in-life part ([redacted], 2017b). The lower values found during the present study are possibly due to loss of labelled cinmethylin during sample handling for transport (e. g. adsorption to the walls of the glass bottles) or freezing and thawing of the water samples.

The total radioactive residues in fish tissues were determined separately for edible tissue (fish fillet) as well as fish carcass, and fish viscera at the sampling dates of the uptake and depuration phase. The mean values and the calculated values for inedible tissues and whole fish are presented in the summary to the BCF study [redacted], 2017b. Mean concentrations in whole fish of the treatment group 1 (0.5 µg a.s./L) reached a plateau level at approx. 360 µg a.s./kg after 3 to 17 days exposure. For the treatment group 2 (5.0 µg a.s./L), the plateau level was reached at approx. 3648 µg a.s./kg after 3 to 17 days exposure. On day 3 of the depuration phase, the radioactive residues in whole fish were below 10 % of the mean calculated concentration at steady state for both treatment groups. On day 5 and day 7 (depuration phase), the concentration of radioactive residues in whole fish had further decreased to values of approx. 3 and 2 % of the steady state concentration, respectively. After a depuration period of 7 days, the mean absolute concentration of radioactive residues in whole fish had decreased to 6.5 µg a.s./kg for treatment group 1 and to 58.4 µg a.s./kg for treatment group 2.

The total radioactive residues in fish tissues (fillet, viscera and carcass) used for investigation of the nature of the residues were calculated as the sum of extractable residues and residual residues after solvent extraction (ERR + RRR combusted, see Table 8.2.2.3-3), because of the low available sample amounts. The calculated TRR of the whole fish was based on these values.

For treatment group 1 (0.5 µg a.s./L), the highest residue levels were found in fish viscera (2.355 mg a.s./kg). The concentration of radioactive residues was considerably lower in carcass (0.107 mg/kg) and fillet samples (0.092 mg a.s./kg) of treatment group 1. For treatment group 2 (5.0 µg a.s./L), the highest residue levels were also found in fish viscera (28.168 mg a.s./kg). The concentration of radioactive residues was considerably lower in carcass (0.979 mg a.s./kg) and fillet samples (0.494 mg a.s./kg) of treatment group 2.

Extractability of radioactive residues from fish tissues

Extraction of fish tissues with methanol and water resulted in high extractabilities of 0.086 mg a.s./kg or 93.3 % TRR (fillet) to 2.312 mg a.s./kg or 98.2 % TRR (viscera) for treatment group 1 and 0.487 mg a.s./kg or 98.5 % TRR (fillet) to 27.963 mg a.s./kg or 99.3 % TRR for treatment group 2. The calculated values for whole fish accounted for 0.343 mg/kg or 96.0 % TRR for treatment group 1 and 3.616 mg/kg or 98.9 % TRR for treatment group 2 (see Table B.9.2.8.1-4). In all cases, most of the radioactive residues was extracted with methanol, water released only minor portions (below 2 % TRR). Hence, the samples were not further investigated. Due to low amounts of radioactivity in the extraction residues (RRR), ranging from 0.7 to 6.7 % TRR (viscera: 0.043 to 0.205 mg/kg, fillet and carcass: below or equal to 0.010 mg/kg), these were not further investigated. Results are summarised in Table B.9.2.8.1-4.

Table B.9.2.8.1-4: TRR and extractability of residues of 14C-cinmethylin

matrix	ERR (methanol extract)		ERR (water extract)		ERR [1)]		RRR [2)]		TRR [3)]	
	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	% TRR
Treatment group 1 (0.5 µg a.s./L)										
Edible tissue (filet)	0.085	91.8	0.001	1.6	0.086	93.3	0.006	6.7	0.092	100.0
Viscera	2.286	97.1	0.027	1.1	2.312	98.2	0.043	1.8	2.355	100.0
Carcass	0.102	95.4	0.001	1.2	0.103	96.6	0.004	3.4	0.107	100.0
Inedible tissues [4)]	0.415	95.6	0.005	1.2	0.419	96.8	0.009	3.2	0.429	100.0
Whole fish [5)]	0.339	94.7	0.004	1.3	0.343	96.0	0.009	4.0	0.351	100.0
Treatment group 2 (5 µg a.s./L)										
Edible tissue (filet)	0.486	98.3	0.001	0.2	0.487	98.5	0.007	1.5	0.494	100.0
Viscera	27.529	97.7	0.434	1.5	27.963	99.3	0.205	0.7	28.168	100.0
Carcass	0.959	98.0	0.009	0.9	0.969	98.9	0.010	1.1	0.979	100.0
Inedible tissues [4)]	4.446	98.0	0.065	1.0	4.511	99.0	0.036	1.0	4.547	100.0
Whole fish [5)]	3.565	98.0	0.051	0.8	3.616	98.9	0.030	1.1	3.646	100.0

1) ERR = Extractable Radioactive Residue (sum of combined methanol extract and combined water extract)
2) RRR = Residual Radioactive Residue after solvent extraction (with methanol and water)
3) TRR = Total Radioactive Residue, calculated as the sum of ERR + RRR

[4] The values for inedible tissues were calculated from the residue values of the individual samples carcass and viscera (considering their respective fresh weight) according to the following equation:
Residue (inedible tissues) [µg/g] = (residue (carcass) x tissue weight + residue (viscera) x tissue weight) / sum of respective tissue weights

[5] The values for whole fish were calculated from the residue values of the individual tissue samples (considering their respective fresh weight) according to the following equation: Residue (whole fish) [µg/g] = (residue (fillet) x tissue weight + residue (carcass) x tissue weight + residue (viscera) x tissue weight) / sum of tissue weights

Peak assignment

For the peak assignment of identified components sub-fractions of the methanol extracts of fish fillet, carcass and viscera were fortified with the parent compound cinmethylin. Sub-fractions of the methanol extracts of fish fillet and carcass were additionally fortified with Lab0054 (external sample Lab0364: rat urine pool 0 – 120 h from study [redacted] 2017b). Sub-fractions of the methanol extracts of fish viscera were additionally fortified with Lab0047 (external sample Lab0003: goat urine from study [redacted] 2017b).

The peak assignment for all extracts and solubilisates was based on comparison of the retention times and metabolite patterns with those of the HPLC analyses from co-chromatography experiments. Based on comparison with retention times of reference items peaks at approx. 89.1 min in quantitative and the corresponding peaks at approx. 89.6 min in confirmatory HPLC chromatograms were assigned as the parent compound cinmethylin. The peaks at approximately 13.3 min and 18.2 min were assigned to metabolite M684H026, by co-chromatography analyses with the diluted external sample "Lab0364" (rat urine pool 0 – 120 h) using HPLC methods. The peaks at approximately 62.9 min and 68.9 min were assigned to metabolite M684H012 and the peaks at approximately 34.6 min or 36.8 min and 53.8 min or 55.4 min were assigned to metabolite M684H022 (isomer 1 and isomer 2, respectively) by co-chromatography with the diluted external sample "Lab0003" (goat urine).

Peaks, which were not assigned to identified compounds, were classified as characterized by extraction behaviour and retention times. The retention times of the reference items are summarized in Table B.9.2.8.1-5.

Table B.9.2.8.1-5: Typical HPLC Retention Times

Designation	HPLC method LC01 t_R [min]	HPLC method LC02 t_R [min]
Cinmethylin	89.05 [1]	89.57 [2]
M684H012 [3]	62.87	68.87
M684H022 (isomer 1) [4]	34.63	53.80
M684H022 (isomer 2) [4]	36.80	55.37
M684H026 [4]	13.25	18.20

[1] Retention time taken from the HPLC analyses of the parent compound cinmethylin using HPLC method LC01.
[2] Retention time taken from the HPLC analyses of the methanol extract of the pooled fish fillet using HPLC method LC02
[3] Retention times taken from the HPLC analyses of the methanol extract of the pooled fish viscera
[4] Retention times taken from the HPLC analyses of the methanol extract of the pooled fish carcass

Extraction, characterization and identification of residues

Water:

Co-chromatography experiments identified the parent compound cinmethylin as the only compound in the tank water samples for both treatment groups, using HPLC methods. No degradation products of cinmethylin were detected.

Fish

Summaries of identified and characterized radioactive residues are shown in Table B.9.2.8.1-6. For inedible tissues and whole fish, the concentrations of cinmethylin and the identified metabolites were calculated from the respective concentrations in carcass, viscera and fillet. Each metabolite pattern in the extract of the respective matrix was similar for both dose groups (0.5 µg a.s./L and 5.0 µg a.s./L).

For both treatment groups (0.5 µg a.s./L and 5.0 µg a.s./L), the metabolite patterns found in the methanol extracts of fish fillet (edible tissue) and fish carcass were similar to each other except for additional peaks in fish carcass at 5.0 µg a.s./L.

Filet:

The only components in the methanol extract of fish fillet (representing edible tissue) were the unchanged parent compound cinmethylin (treatment group 1: 0.045 mg a.s./kg and 48.3 % TRR; treatment group 2: 0.208 mg a.s./kg and 42.1 % TRR) and M684H026 (treatment group 1: 0.043 mg a.s./kg and 46.0 % TRR; treatment group 2: 0.276 mg a.s./kg and 55.7 % TRR).

Carcass:

For the methanol extract of fish carcass of treatment group 1, cinmethylin (0.070 mg a.s./kg or 65.4%) and M684H026 (0.029 mg a.s./kg and 27.2 % TRR) were the only components in the methanol extract. For the methanol extract of fish carcass of treatment group 2, cinmethylin (0.242 mg a.s./kg or 24.7 % TRR) and M684H026 (0.191 mg a.s./kg or 19.5 % TRR) were the predominant components in the methanol extract. However, smaller peaks with up to 0.060 mg a.s./kg or 6.1 % TRR were additionally detected and classified as characterized.

Viscera:

For fish viscera, higher portions of cinmethylin and its biotransformation products were detected, compared to fish fillet and carcass. Metabolite M684H012 was the most abundant component in the methanol extracts of both treatment groups (treatment group 1: 0.402 mg a.s./kg or 17.1 % TRR; treatment group 2: 5.239 mg a.s./kg or 18.6 % TRR). For treatment group 1, the parent compound cinmethylin (0.257 mg a.s./kg or 10.9 % TRR) and metabolite M684H022 (isomer 1: 0.245 mg a.s./kg or 10.4 % TRR; isomer 2: 0.255 mg a.s./kg or 10.8 % TRR) accounted for similar amounts of TRR in viscera. For treatment group 2, M684H022 (isomer 2) was the second most abundant compound (3.778 mg a.s./kg or 13.4 % TRR), while the parent compound cinmethylin (1.017 mg a.s./kg or 3.6 % TRR) and the metabolite M684H0022 (isomer 1) (1.515 mg a.s./kg or 5.4 % TRR) accounted for minor amounts in viscera. For treatment group 1, additional smaller peaks with up to 0.132 mg a.s./kg or 5.6 % TRR were detected in the methanol extract of viscera and classified as characterized. For treatment group 2, additional smaller peaks with up to 2.025 mg a.s./kg or 7.2 % TRR were detected in the methanol and water extracts of fish viscera and classified as characterized.

Inedible and whole fish

Calculation of the residual concentrations for inedible fish tissues and whole fish shows the unchanged parent compound as main component in extracts of treatment group 1 (inedible fish tissue: 0.097 mg a.s./kg or 22.5 % TRR; whole fish: 0.085 mg a.s./kg or 24.1 % TRR), followed by metabolites M684H012 (inedible fish tissue: 0.058 mg a.s./kg or 13.4 % TRR; whole fish: 0.044 mg a.s./kg or 12.6 % TRR), M684H022 (isomer 1) (inedible fish tissue: 0.035 mg a.s./kg or 8.2 % TRR; whole fish: 0.027 mg/kg or 7.7% TRR), M684H022 (isomer 2) (inedible fish tissue: 0.037 mg a.s./kg or 8.5% TRR; whole fish: 0.028 mg a.s./kg or 8.0 % TRR) and M684H026 (inedible fish tissue: 0.025 mg a.s./kg or 5.8 % TRR; whole fish: 0.029 mg a.s./kg or 8.2 % TRR).

For the methanol extract of treatment group 2, the main component was metabolite M684H012 (inedible fish tissue: 0.687 mg a.s./kg or 15.1 % TRR; whole fish: 0.535 mg a.s./kg or 14.7 % TRR), followed by metabolite M684H022 (isomer 2) (inedible fish tissue: 0.496 mg a.s./kg or 10.9 % TRR; whole fish: 0.385 mg a.s./kg or 10.6 % TRR) and the unchanged parent compound cinmethylin (inedible fish tissue: 0.343 mg a.s./kg or 7.6 % TRR; whole fish: 0.313 mg a.s./kg or 8.6 % TRR). The metabolites M684H022 (isomer 1) (inedible fish tissue: 0.199 mg a.s./kg or 4.4 % TRR; whole fish: 0.155 mg/kg or 4.2 % TRR) and M684H026 (inedible fish tissue: 0.166 mg a.s./kg or 3.7 % TRR; whole fish: 0.191 mg a.s./kg or 5.2 % TRR) accounted for lower amounts for treatment group 2.

For both treatment groups, the overall total degree of identification was in the range of 94.3 to 97.8 % TRR for fish fillet, 44.2 to 92.6 % TRR for fish carcass, 41.0 to 49.2 % TRR for fish viscera and 43.3 to 60.6 % TRR for the whole fish. In addition, up to 25 further peaks were detected in the methanol extracts of fish carcass (treatment group 2) and fish viscera, which were not identified, but characterized based on their extraction with methanol or water (six peaks were in the range of 5.6- 7.2 % TRR, all others below 5 % TRR). For fish viscera

of treatment group 2, twelve additional peaks were detected in the water extract, which were not identified, but characterized by their extraction with water (the peaks accounted for up to 0.2 % TRR).

Taken together, 95.8 - 98.0 % TRR were identified and characterized for fish fillet (edible tissue), 97.6 - 97.9 % TRR for inedible tissue and 97.6 - 97.8 % TRR for the whole fish.

Table B.9.2.8.1-6: Summary of identified/characterized components in fish

Designation	Fish fillet		Fish carcass		Fish viscera		Edible tissue		Inedible tissue		Whole fish	
	mg a.s./kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
Treatment group 1 (0.5 µg a.s./L)												
Cinmethylin	0.045	48.3	0.070	65.4	0.257	10.9	0.045	48.3	0.097	22.5	0.085	24.1
M684H012	n.d.	n.d.	n.d.	n.d.	0.402	17.1	n.d.	n.d.	0.058	13.4	0.044	12.6
M684H022 (isomer 1)	n.d.	n.d.	n.d.	n.d.	0.245	10.4	n.d.	n.d.	0.035	8.2	0.027	7.7
M684H022 (isomer 2)	n.d.	n.d.	n.d.	n.d.	0.255	10.8	n.d.	n.d.	0.037	8.5	0.028	8.0
M684H026	0.043	46.0	0.029	27.2	n.d.	n.d.	0.043	46.0	0.025	5.8	0.029	8.2
Total identified	**0.087**	**94.3**	**0.099**	**92.6**	**1.159**	**49.2**	**0.087**	**94.3**	**0.251**	**58.5**	**0.213**	**60.6**
Characterized by HPLC analysis	n.d.	n.d.	n.d.	n.d.	1.148	48.8	n.d.	n.d.	0.164	38.4	0.127	36.1
Combined Water Extract (LSC)	0.001	1.6	0.001	1.2	0.027	1.1	0.001	1.6	0.005	1.1	0.004	1.2
Total Characterized	0.001	1.6	0.001	1.2	1.175	49.9	0.001	1.6	0.169	39.5	0.131	37.2
Total identified and/ or characterized	**0.089**	**95.8**	**0.100**	**93.8**	**2.334**	**99.1**	**0.089**	**95.8**	**0.420**	**98.0**	**0.344**	**97.9**
Unextracted residue (RRR, by LSC)	0.006	6.7	0.004	3.4	0.043	1.8	0.006	6.7	0.009	2.2	0.009	2.4
Total	**0.095**	**102.5**	**0.104**	**97.2**	**2.377**	**100.9**	**0.095**	**102.5**	**0.429**	**100.1**	**0.352**	**100.3**

Designation	Fish fillet		Fish carcass		Fish viscera		Edible tissue		Inedible tissue		Whole fish	
	mg a.s./kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
Treatment group 2 (5 µg a.s./L)												
Cinmethylin	0.208	42.1	0.242	24.7	1.017	3.6	0.208	42.1	0.343	7.6	0.313	8.6
M684H012	n.d.	n.d.	n.d.	n.d.	5.239	18.6	n.d.	n.d.	0.687	15.1	0.535	14.7
M684H022 (isomer 1)	n.d.	n.d.	n.d.	n.d.	1.515	5.4	n.d.	n.d.	0.199	4.4	0.155	4.2
M684H022 (isomer 2)	n.d.	n.d.	n.d.	n.d.	3.778	13.4	n.d.	n.d.	0.496	10.9	0.385	10.6
M684H026	0.276	55.7	0.191	19.5	n.d.	n.d.	0.276	55.7	0.166	3.7	0.191	5.2
Total identified	**0.484**	**98.0**	**0.433**	**44.2**	**11.548**	**41.0**	**0.484**	**97.8**	**1.892**	**41.6**	**1.578**	**43.3**
Characterized by HPLC analysis	n.d.	n.d.	0.464	47.4	16.271	57.8	n.d.	n.d.	2.539	55.8	1.974	54.1
Combined Water Extract (LSC)	0.001	0.2	0.009	0.9	n.a.	n.a.	0.001	0.2	0.008	0.2	0.006	0.2
Total characterized	**0.001**	**0.2**	**0.474**	**48.4**	**16.271**	**57.8**	0.001	0.2	2.547	56.0	1.980	54.3
Total identified and/ or characterized	0.485	98.0	0.907	92.6	27.819	98.8	**0.485**	**98.0**	**4.438**	**97.6**	**3.559**	**97.6**
Unextracted residue (RRR, by LSC)	0.007	1.5	0.010	1.1	0.205	0.7	0.007	1.5	0.036	0.8	0.030	0.8
Total	**0.492**	**99.5**	**0.917**	**93.7**	**28.024**	**99.5**	**0.492**	**99.5**	**4.474**	**98.4**	**3.588**	**98.4**

n.d. not detected

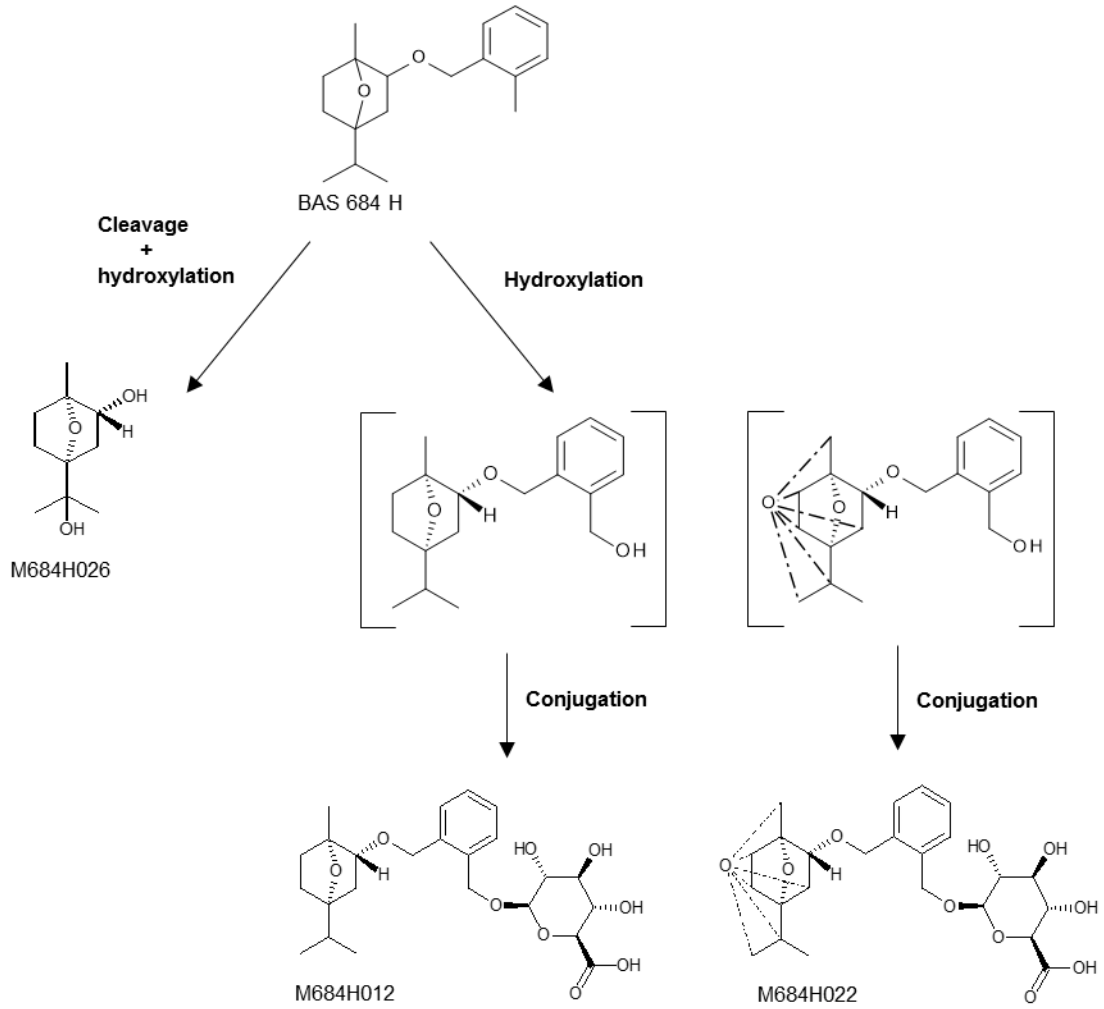
Metabolic Pathway

The proposed metabolic pathway of cinmethylin in bluegill sunfish is shown in Figure B.9.2.8.1-3. Cinmethylin is moderately metabolized in bluegill sunfish to metabolites of higher polarity after aqueous exposure in a flow-through system. The unchanged parent cinmethylin was the major component in the methanol extracts of fish fillet and carcass. For whole fish, cinmethylin accounted for 8.6 % TRR (treatment group 2) to 24.1 % TRR (treatment group 1).

The main transformation steps in the metabolic pathway of cinmethylin in bluegill sunfish are:

- Hydroxylation reactions at the phenyl and cyclohexyl moiety (M684H012, M684H026)
- Cleavage of the ether bridge (BAS684H026)
- Conjugation of hydroxylated derivatives with glucuronic acid (M684H012, M684H022)

Figure B.9.2.8.1-3: Proposed Metabolic Pathway of cinmethylin in bluegill sunfish



Storage stability
All matrices were extracted and analysed within 173 days after sacrifice of the fish (day of sacrifice: June 23, 2017, last extraction: November 29, 2017, last analysis: December 13, 2017); therefore, it was not necessary to investigate the stability of the residues in the matrix or extract

III. CONCLUSION METABOLISM IN FISH

Extraction of the radioactive residues of cinmethylin with methanol and water resulted in high extractabilities for all edible and inedible tissues of both treatment groups.

Cinmethylin and its metabolite M684H026 were the only residues detected in edible fish tissues (fillet). The unchanged parent compound represented 48.3 % TRR (treatment group 1) and 42.1 % TRR (treatment group 2) in the fillet. Its metabolite M684H026 accounted for 46.0 % TRR (treatment group 1) and 55.7 % TRR (treatment group 2) in the fillet. For fish carcass, additional compounds were detected, but cinmethylin accounted for the majority of radioactive residues (treatment group 1: 65.4% TRR; treatment group 2: 24.7 % TRR), followed by M684H026 (treatment group 1: 27.2 % TRR; treatment group 2: 19.5 % TRR). In viscera, the active substance cinmethylin was metabolized in large parts to metabolites of higher polarity. Hence, the parent compound accounted for lower amounts of TRR in fish viscera (treatment group 1: 10.9 % TRR treatment group 2: 3.6 % TRR. The metabolite M684H012 was the most abundant component for both treatment groups in viscera and accounted for 17.1 to 18.6 % TRR. The metabolites M684H022 (isomer 1 and isomer 2) accounted for 10.4 to 10.8 % TRR (treatment group 1) and 5.4 to 13.4 % TRR (treatment group 2) in viscera, respectively.

For whole fish, cinmethylin accounted for 24.1 % TRR for treatment group 1 and 8.6 % TRR for treatment group 2.

The active substance cinmethylin was observed to be metabolised mainly by cleavage of the ether bridge, hydroxylation and subsequent conjugation to glucuronic acid. The metabolite M684H026 results from cleavage of the phenyl group from the parent molecule and hydroxylation of the isopropyl moiety of the cyclohexane group.

In this study, the metabolites M684H012 and M684H022 resulted from Phase 1 conjugation of hydroxylated derivatives of the parent compound with glucuronic acid. The putative intermediate of metabolite M684H012 was generated by hydroxylation at the methyl group of the phenol ring of cinmethylin. Metabolite M684H022 shows an additional hydroxylation at the cyclohexane moiety.

The main conversion products in whole fish were metabolites M684H012 (12.6 % TRR for treatment group 1 and 14.7 % TRR for treatment group 2), M684H022 (isomer 2) (8.0 % TRR for treatment group 1 and 10.6 % TRR for treatment group 2), M684H026 (8.2 % TRR for treatment group 1 and 5.2 % TRR for treatment group 2) and M684H022 (isomer 1) (7.7 % TRR for treatment group 1 and 4.2 % TRR for treatment group 2).

Due to the metabolic activity mainly observed in viscera, the concentration of the active substance cinmethylin accounted for 0.085 mg a.s./kg or 24.1 % TRR (treatment group 1) and 0.313 mg a.s./kg or 8.6 % TRR (treatment group 2) for whole fish. For fish fillet, cinmethylin accounted for 0.045 mg a.s./kg or 48.3 % TRR (treatment group 1) and 0.208 mg a.s./kg or 42.1 % TRR (treatment group 2).

HSE evaluator comments:

The following was stated by the applicant: '*The study on metabolism of C^{14}-BAS 684 H in bluegill sunfish represents an extension of the BCF study (2017/1156422) with no additional use of further aquatic vertebrates'.*

The above study was conducted to GLP and provides supplementary information for the study [redacted], 2017b.

Only cinmethylin (BAS 684 H) was present in water samples at the end of the exposure period. When conisdering whole fish at end of exposure period (day 17) cinmethylin (BAS 684 H) accounted for 24.1 % TRR/0.085 mg a.s./kg (Total Radioactive Residue) at 0.5 µg a.s./L and 8.6 % TRR/0.045 mg a.s./kg at 5 µg a.s./L. The metabolite M684H012 accounted for 24.1 % TRR, 14.7 % TRR, M684H022 (isomer 1) for 7.7 %

TRR, 4.2 % TRR, M684H022 (isomer 2) for 8.0 % TRR, 10.6 % TRR, M684H026 for 8.2 % TRR, 5.2 % TRR at 0.5 and 5 µg a.s./L.

In terms of fish carcass cinmethylin, M684H026 were present at 65.4, 27.2 % TRR in 0.5 µg a.s./L treatment group and 24.7, 19.5 % TRR at 5 µg a.s./L. In addittion, a maximum of 25 unidentified peaks were found in cacass and viscera however, none accounted for > 7.2 % TRR.

Cinmethylin (48.3, 42.1 % TRR at 0.5 µg a.s./L and 5 µg a.s./L respectively) and the metabolite M684H026 (46.0, 55.7 % TRR at 0.5 µg a.s./L and 5 µg a.s./L respectively) were present in edible fish.

B.9.3. EFFECTS ON ARTHROPODS

B.9.3.1. Effects on bees

B.9.3.1.1. Acute oral toxicity to bees

Report:	CA 8.3.1.1.1/1 Franke M., 2016 a Acute toxicity of BAS 684 H to the honeybee *Apis mellifera* L. under laboratory conditions 2016/1044853
Guidelines:	OECD 213 (1998), OECD 214 (1998)
GLP:	yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

I. MATERIAL AND METHODS

A. MATERIALS

Test item:	BAS 684 H (Reg. No. 900 202), batch no. COD-002038, purity: 93.0% analyzed.

B. STUDY DESIGN

Test date:	Study initiation: 25 Oct 2016 Experimental start date: 7 Nov 2016 Experimental completion date: 9 Nov 2016 Study completion date: 14 Dec 2016
Test species:	*Apis mellifera* L. subspecies *iberiensis* E. (honeybee), young adult worker bees (about 3 - 5 weeks old) derived from a healthy and queen-right colony, source: beekeeper, Cazalla (Seville), Spain; collected from the top of the bee hive in the morning prior to use. The bees were taken from a hive that had not received chemical treatments for at least one month.
Test design:	In a 48-hour test, young adult worker bees of *Apis mellifera* L. were exposed orally to BAS 684 H via food (50% (w/v) aqueous sucrose solution containing 1% (v/v) acetone plus 1% (v/v) tween®80). The application volume was 200µL/cage with 10 bees for all dose groups) In total, 3 treatment groups were set up (5 dose rates of the test item, 2 untreated control groups and 4 dose rates of the reference item) with 3 replicates per dose rate and 10 bees per replicate. Assessment of bee mortality and behavioural effects were done after 4, 24 and 48 hours.
Endpoints:	Mortality (LD_{50}), behavioural impairments.
Reference item:	Dimethoate EC 400 (BAS 152 11 I, dimethoate, 420.3 g/L analyzed) Batch no. FRE-001226.

Test doses:	Control groups: 50% (w/v) sucrose solution, 50% (w/v) sucrose solution containing 1.0% (v/v) acetone and 1.0% (v/v) Tween®80. BAS 684 H: 12.5, 25.0, 50.0, 100.0 and 200.0 µg a.s./bee, resulting in an actual uptake of 12.5, 25.0, 50.0, 100.0 and 200.0 µg a.s./bee (corresponding to 13.4, 26.9, 53.8, 107.5 and 215.0 µg test item/bee). Reference item: 0.069, 0.106, 0.163 and 0.250 µg dimethoate/bee.
Test cages:	For the observation of the bees, disposable cages of cardboard with holes in the bottom for ventilation and a class plate in front were used. Cages were 95 mm x 50 mm x 65 mm and were labelled with study number and replicate number.
Test conditions:	Temperature: 23.1°C – 27.0°C; relative humidity: 50.1% - 68.7%; photoperiod: 24 h darkness; food: 50% (w/v) sucrose solution.
Feeding:	Following application, sucrose solution was provided *ad libitum*.
Statistics:	Descriptive statistics; Multiple sequentially-rejective Fisher Test after Bonferroni-Holm for mortality data (one-sided greater, $\alpha = 0.05$).

Table B.9.3.1-1 below provides details regarding the applied and consumed doses.

Table B.9.3.1-1: Applied and consumed dosages in the oral toxicity test

Treatment group	Test solution ID	Item applied	Offered dose rates		Consumed dose rates		Application volume [µL/cage with 10 bees]
Control	AC BC	- sucrose solution - sucrose solution cont. 1 % v/v acetone + 1 % v/v tween®80	--		--		200
			[µg test item/bee]	[µg a.s./ bee]*	[µg test item/bee]	[µg a.s./ bee]*	
Test item	AT BT CT DT ET	BAS 684 H*	215.0 107.5 53.8 26.9 13.4	200.0 100.0 50.0 25.0 12.5	215.0 107.5 53.8 26.9 13.4	200.0 100.0 50.0 25.0 12.5	200
			[µg product/ bee]	[µg a.s./ bee]*	[µg product/ bee]	[µg a.s./ bee]*	
Reference item	AR BR CR DR	Dimethoate EC 400	0.638 0.415 0.270 0.175	0.250 0.163 0.106 0.069	0.638 0.415 0.270 0.175	0.250 0.163 0.106 0.069	200

* based on analysed purity / content of a.s.
Calculations are performed with non-rounded values

II. RESULTS AND DISCUSSION

Validity criteria

All validity criteria were met in this study as outlined in the table below.

Table B.9.3.1-2: Validity criteria and results taken from the study report (also includes outcome of contact study as both were reported in the same study report)

Validity criterion		Occurred / calculated	Recommended
Control mortality (48 h)	Contact test: - deionised water - deionised water + wetting agent (tween solution) - pure acetone	 0.0 % 3.3 % 0.0 %	≤ 10 %
	Oral test: - sucrose solution - sucrose solution containing 1 % acetone + 1 % tween	 0.0 % 0.0 %	≤ 10 %
LD_{50} – value of the reference (24 h)	Contact toxicity test	0.179 µg a.s./bee	0.10 – 0.30 µg a.s./bee
	Oral toxicity test	0.116 µg a.s./bee	0.10 – 0.35 µg a.s./bee

After 48 hours, no mortality occurred in the control groups fed with either pure sucrose solution or sucrose solution containing 1% acetone and 1% tween.

In the test item treatment, no mortality occurred after oral consumption of 12.5, 25.0, 50.0, 100.0 and 200.0 µg a.s./bee, after 48 hours. Slight mortality of 3.3% was observed at the highest dose rate of 200.0 µg a.s./bee, whereas lower dose rates of ≤ 100.0 µg a.s./bee revealed no mortality. No test item induced behavioural effects were observed. The results are summarized in Table B.9.3.1-3.

Table B.9.3.1-3: Toxicity of BAS 684 H to *Apis mellifera* L. (honeybee) in an oral toxicity test

Treatment	**Dosage [consumed]**	**Mortality [%]**		
		4 h	**24 h**	**48 h**
Control	Sucrose solution	0.0	0.0	0.0
	Acetone-Tween sucrose solution [1)]	0.0	0.0	0.0
BAS 684 H [µg a.s./bee]	12.5	0.0	0.0	0.0
	25.0	0.0	0.0	0.0
	50.0	0.0	0.0	0.0
	100.0	0.0	0.0	0.0
	200.0	0.0	0.0	3.3
Endpoint [µg consumed a.s./bee]				
LD_{50} (48 h)	> 200.0			

[1)] Representative control for the test item.

The LD_{50} value (24 h) for the reference item was determined to be 0.116 µg dimethoate/bee (95% confidence limits: 0.104 - 0.129 µg dimethoate/bee), based on consumption.

III. CONCLUSION

In an acute oral toxicity study with BAS 684 H on honeybees, the LD_{50} value (48 h) was determined to be > 200 µg a.s./bee.

HSE evaluator comments:

The study was well reported and was conducted in line with OECD 213 (1998). Validity criteria were met since the average mortality in controls was <10% (actual = 0%) and the LD_{50} of the toxic standard dimethoate was within the range specified in the guideline i.e. LD_{50}-24h is in the range 0.10-0.35 µg a.i./bee (actual = 0.116 µg dimethoate/bee).

The endpoint for consideration in the risk assessment is:

- **Oral LD_{50} > 200 µg a.s./bee**

Report:	CA 8.3.1.1.1/2 Amsel K., 2017 a Acute toxicity of BAS 684 H to the bumblebee *Bombus terrestris* L. under laboratory conditions 2017/1140992
Guidelines:	OECD 246 (2017), OECD 247 (2017)
GLP:	yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

Report:	CA 8.3.1.1.1/3 Amsel K., 2018 a Amendment No. 1 - Acute toxicity of BAS 684 H to the bumblebee *Bombus terrestris* L. under laboratory conditions 2018/1000903
Guidelines:	OECD 246 (2017), OECD 247 (2017)
GLP:	yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

I. MATERIAL AND METHODS

A. MATERIALS

Test item:	BAS 684 H (Reg. No. 900 202); batch no.: COD-002038; analyzed purity: 93.0% ± 1.0%.

B. STUDY DESIGN

Test species:	*Bombus terrestris* L. (bumblebee), young adult worker bumblebees derived from queen-right hives; source: Biobest Belgium N.V., Westerlo, Belgium; collected in the evening prior to use. Very small and large bumblebees were excluded from the test by visual inspection.
Collection of bumblebees :	Bumblebees were collected in the evening prior to testing using red light.Bumblebees from different colonies were randomly distributed to the treatment groups and dose rates. After transfer of the bumblebees into the test units they had time for acclimatisation to the test room conditions for about 17 hours and a starving period of 4 hours prior to application of the treatments.
Pre-treatment culturing:	Each colony was held under laboratory conditions in a holding box having brood at all stages of development and a laying queen, containing about 80 bumblebee workers with sufficient food.

Test design: In a 48-hour test, adults of *Bombus terrestris* were exposed to 5 doses of BAS 684 H in treated food (50% (w/v) sucrose solution including 5% (v/v) acetone + 1% (v/v) Tween) at a volume of 47µL. In total, 8 treatment groups were set up: 2 control groups, 5 dose rates of the test item and 1 dose rate of the reference item with 30 replicates per dose and 1 bumblebee per replicate, respectively. Before the sucrose solution was filled in the feeding syringes they were weighed and then reweighed 4 hours after the test began to determine the exact quantity of test solution consumed. Following this, the syringes were replaced with ones containing untreated 50% (w/v) sucrose solution which was provided *ad libitum*. Assessments of bumblebee mortality and behavioural effects were done after 4, 24 and 48 hours. Test units were 7cm x 2cm and were part of the rearing system.

Endpoints: Mortality, behavioural impairments recorded as normal (healthy), affected (impaired locomotion) or moribund.

Reference item: Dimethoate EC 400 (BAS 152 11 I, dimethoate, 405.2 g/L analyzed).

Test doses: Sucrose control (50% (w/v) sucrose solution), sucrose control (50% (w/v) sucrose solution including 5% (v/v) acetone + 1% (v/v) Tween and filled up with 50% (w/v) sucrose solution); reference item at dose rate of 1.51 µg dimethoate/bumblebee (actual uptake: 1.50 µg dimethoate/bumblebee); test item at dose rates of 12.5, 25.0, 50.0, 100.0 and 200.0 µg BAS 684 H/bumblebee (resulting in an actual uptake of 12.3, 24.5, 48.8, 97.8 and 194.5 µg BAS 684 H/bumblebee). The table below provides details regarding applied and consumed doses.

Table B.9.3.1-4: Applied and consumed dosages in the oral toxicity test

Treatment group	Test solution ID	Item to be applied	Dose		Actual intake		Applied volume [µL/bumblebee]
Control	AC BC	sucrose solution sucrose solution + 5%(v/v) acetone + 1% (v/v)Tween	-				40
			[µg product/ bumblebee]	[µg a.s./ bumblebee]	[µg product/ bumblebee]	[µg a.s./ bumblebee]	
Test item	AT BT CT DT ET	BAS 684 H*	-	200.0 100.0 50.0 25.0 12.5	-	194.5 97.8 48.8 24.5 12.3	40
Reference item	AR	Dimethoate EC 400*	4.00	1.51	3.99	1.50	40

* based on analysed purity/analysed content of a.s.
Calculations are performed with non-rounded values.

Analytical verification: The content of active substance was analysed in the highest and lowest dose groups.

Test conditions: Temperature: 23.5 °C – 25.4 °C, relative humidity: 57% – 65%, photoperiod: 24 h darkness; food: 50% (w/v) sucrose solution. Test condition deviations of less than 2 hours per day were not reported as they were not expected to affect the integrity and outcome of the study.

Statistics: Descriptive statistics; Fisher's Exact Binominal Test with Bonferroni Correction for mortality data (one-sided greater, α = 0.05).

II. RESULTS AND DISCUSSION

Validity criteria

The table below provides the outcome of the validity criteria which were all met.

Table B.9.3.1-5: Validity criteria results (oral and contact results presented as were reported in one study)

Validity criterion		Occurred / calculated	Recommended
Mean control mortality (48 h)	Contact test: - Deionised water - 0.5% TritonX solution - Acetone	0.0% 0.0% 0.0%	≤ 10%
	Oral test: - Sucrose solution - Sucrose solution + 5% acetone + 1% Tween	0.0% 0.0%	≤ 10%
Mortality reference item (48 h)	Contact toxicity test	100.0%	≥ 50%
	Oral toxicity test	100.0%	≥ 50%

After 48 hours of oral exposure, no mortality occurred in the control group fed with 50% (w/v) sucrose solution and 50% (w/v) sucrose solution including 5% (v/v) acetone + 1% (v/v) Tween. In the test item treatment, no mortality occurred after oral consumption of 12.3, 24.5, 48.8, 97.8 and 194.5 µg a.s./bumblebee. No behavioral effects of bumblebees occurred at all tested dose rates in the oral toxicity test. The results are summarized in the table below.

Table B.9.3.1-6: Toxicity of BAS 684 H to *Bombus terrestris* (bumblebee) in an oral toxicity test

Treatment	Dosage	Mortality [%]	
		24 h	48 h
Control	Sucrose solution	0.0	0.0
	Sucrose solution + 5% (v/v) acetone + 1% (v/v) Tween	0.0	0.0
BAS 684 H [µg a.s./bumblebee]	12.3	0.0	0.0
	24.5	0.0	0.0
	48.8	0.0	0.0
	97.8	0.0	0.0
	194.5	0.0	0.0
Endpoint [µg a.s./bumblebee]			
LD_{50} (48 h)	> 194.5		

Mortality in the reference item treatment in the contact test was 100.0% after 48 hours.

Analytical results

The recovery of BAS 684H was 102% in the highest test group sample analysed and 89% in the lowest. No active substance was detected in the control sample.

III. CONCLUSION

In an acute oral toxicity study with BAS 684 H on bumblebees, the LD_{50} value (48 h) was > 194.5 µg

consumed BAS 684 H/bumblebee and the NOED after 48 hours was ≥ 194.5 µg consumed BAS 684 H/bumblebee.

HSE evaluator comments:

The study was well reported and adhered mostly to OECD Guideline 247 (2017). The study report did not note whether the bees were weighed or not, only that visual inspection was used to exclude very small and very large bees from the test. This is considered an acceptable deviation.

It was also noted that the study reported that test condition deviations of less than 2 hours per day were not reported as they were not expected to affect the integrity and outcome of the study. However the evaluator is of the view that these deviations should have been reported as raw data in order for the magnitude to be checked. Given that these deviations did not affect the validity criteria results which were all met sufficiently, it is accepted.

Validity criteria were met although it was noted that in the guideline the validity criteria outline the required results from a water control treatment. However in this study the controls used were a sucrose solution and a sucrose solution + 5% (v/v) acetone + 1% (v/v) Tween. The evaluator does not consider this to invalidate the study and results of controls available are sufficient.

It was noted that the test unit was 14cm^3; slightly smaller than the recommended 15cm^3, this was not considered to invalidate the test.

The method of analysis used in the study was validated by the chemistry specialist (see CA B5).

The endpoint for consideration in the risk assessment is:

- **LD_{50} (48 h) > 194.5 µg a.s./bumblebee**

B.9.3.1.2. Acute contact toxicity to bees

Report:	CA 8.3.1.1.2/1 Franke M., 2016 a Acute toxicity of BAS 684 H to the honeybee *Apis mellifera* L. under laboratory conditions 2016/1044853
Guidelines:	OECD 213 (1998), OECD 214 (1998)
GLP:	yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

I. MATERIAL AND METHODS

A. MATERIALS

Test item:	BAS 684 H (Reg. No. 900 202), batch no. COD-002038, purity: 93.0% analyzed.

B. STUDY DESIGN

Test date:	Study initiation: 25 Oct 2016 Experimental start date: 7 Nov 2016 Experimental completion date: 9 Nov 2016 Study completion date: 14 Dec 2016
Test species:	*Apis mellifera* L. subspecies *iberiensis* E. (honeybee), young adult worker bees (about 3 - 5 weeks old) derived from a healthy and queen-right colony, source: beekeeper, Cazalla (Seville), Spain; collected from the top of the bee hive in the morning prior to use. The bees were taken from a hive that had not received chemical treatments for at least one month.

Test design:	In a 48-hour test, young adult worker bees of *Apis mellifera* L. were exposed to 5 dose rates of BAS 684 H in an appropriate carrier (pure acetone) placed on the dorsal bee thorax. In total, 3 treatment groups were set up (5 dose rates of the test item, 3 untreated control groups and 4 dose rates of the reference item) with 3 replicates per treatment and 10 bees per replicate. Assessment of bee mortality and behavioural effects were done after 4, 24 and 48 hours.
Endpoints:	Mortality (LD_{50}), behavioural impairments.
Reference item:	Dimethoate EC 400 (BAS 152 11 I, dimethoate, 420.3 g/L analyzed).
Test doses:	Control groups: water control (deionized water), Tween control (deionized water + 1.0% v/v wetting agent (Tween®80)) and acetone control. BAS 684 H: 12.5, 25.0, 50.0, 100.0 and 200.0 µg a.s./bee (corresponding to 13.4, 26.9, 53.8, 107.5 and 215.0 µg test item/bee). Reference item: 0.106, 0.141, 0.188 and 0.250 µg dimethoate/bee.
Feeding:	Following application, sucrose solution was provided *ad libitum*.
Test conditions:	Temperature: 23.1°C – 27.0°C; relative humidity: 50.1% - 68.7%; photoperiod: 24 h darkness; food: 50% (w/v) sucrose solution.
Test cages:	For the observation of the bees, disposable cages of cardboard with holes in the bottom for ventilation and a class plate in front were used. Cages were 95 mm x 50 mm x 65 mm and were labelled with study number and replicate number.
Statistics:	Descriptive statistics; Multiple sequentially-rejective Fisher Test after Bonferroni-Holm for mortality data (one-sided greater, $\alpha = 0.05$).

Application

Before application bees in the test cage were anaesthetised with CO_2 for approximately ½ minute. They were then removed from the cage into a petri dish and turned with forceps for application. A single droplet of 2µL of test substance was placed on the dorsal bee thorax using an Eppendorf Micropipette. Tween solution was used to ensure good penetration or adhesion of the droplet on the bee body and alone Tween solution (1% v/v/) was non-toxic to honeybees at the concentration used.

Table B.9.3.1-7 below provides details regarding the applied doses.

Table B.9.3.1-7: Applied doses in contact test

Treatment group	Identifi-cation	Item applied	Dose rates		Application volume [µL/bee]
Control	AC BC CC	- deionised water - tween solution - acetone	--		2
			[µg test item/bee]	[µg a.s./bee]*	
Test item	AT BT CT DT ET	BAS 684 H	215.0 107.5 53.8 26.9 13.4	200.0 100.0 50.0 25.0 12.5	2
			[µg product/bee]	[µg a.s./bee]*	
Reference item	AR BR CR DR	Dimethoate EC 400	0.638 0.479 0.359 0.269	0.250 0.188 0.141 0.106	2

* based on analysed purity / content of a.s.
Calculations are performed with non-rounded values

II. RESULTS AND DISCUSSION

Validity criteria

All validity criteria were met in this study as outlined in the table below.

Table B.9.3.1-8: Validity criteria and results taken from the study report (also includes outcome of oral study as both were reported in the same study report)

Validity criterion		Occurred / calculated	Recommended
Control mortality (48 h)	Contact test: - deionised water - deionised water + wetting agent (tween solution) - pure acetone	 0.0 % 3.3 % 0.0 %	≤ 10 %
	Oral test: - sucrose solution - sucrose solution containing 1 % acetone + 1 % tween	 0.0 % 0.0 %	≤ 10 %
LD_{50} – value of the reference (24 h)	Contact toxicity test	0.179 µg a.s./bee	0.10 – 0.30 µg a.s./bee
	Oral toxicity test	0.116 µg a.s./bee	0.10 – 0.35 µg a.s./bee

After 48 hours of contact exposure, no mortality occurred in the control groups treated with either deionized water or acetone, whereas a slight mortality of 3.3% was observed in the tween solution control.

In the test item treatment, no statistically significant mortality occurred after thoracic application of ≤ 200.0 µg a.s./bee within 48 hours. Slight mortality of 3.3% was observed at the two highest dose rates of ≥ 100.0 µg a.s./bee, whereas the lower dose rates of ≤ 50.0 µg a.s./bee revealed no mortality. No test item induced behavioural effects were observed. The results are summarized in Table B.9.3.1-9.

Table B.9.3.1-9: Toxicity of BAS 684 H to *Apis mellifera* L. (honeybee) in a contact toxicity test

Treatment	Dosage [applied]	Mortality [%]		
		4 h	24 h	48 h
Control	Water	0.0	0.0	0.0
	Tween	0.0	0.0	3.3
	Acetone [1)]	0.0	0.0	0.0
BAS 684 H [µg a.s./bee]	12.5	0.0	0.0	0.0
	25.0	0.0	0.0	0.0
	50.0	0.0	0.0	0.0
	100.0	0.0	0.0	3.3
	200.0	0.0	0.0	3.3
Endpoint [µg a.s./bee]				
LD_{50} (48 h)	> 200.0			

[1)] Representative control for the test item.

The LD_{50} value (24 h) for the reference item was determined to be 0.179 µg dimethoate/bee (95% confidence limits: 0.164- 0.196 µg dimethoate/bee) in the contact toxicity test.

III. CONCLUSION

In an acute contact toxicity study with BAS 684 H on honeybees the LD_{50} value (48 h) was determined to be > 200.0 µg a.s./bee.

HSE evaluator comments:

The study was well reported and was conducted in line with OECD 214 (1998). Validity criteria were met since the average mortality in controls was <10% (actual = 0% for deionised water, 3.3% for deionised water and wetting agent and 0% for pure acetone) and the LD_{50} of the toxic standard dimethoate was within the range specified in the guideline i.e. contact LD_{50}-24h is in the range 0.10-0.30 µg a.i./bee µg a.i./bee (actual = 0.179 µg dimethoate/bee with 95% confidence limits: 0.164- 0.196 µg dimethoate/bee).

It was noted that the guideline suggested that the application volume of all test substances should be 1 µL unless another volume could be justified. The study uses an application volume of 2µL and states that the laboratory (BioChem agrar) has used this application volume historically with no adverse results. Since the validity criteria were met and there were no associated issues with using a larger volume, HSE considers this deviation to be acceptable.

The endpoint for consideration in the risk assessment is:

- **Contact LD_{50} > 200 µg a.s./bee**

Report:	CA 8.3.1.1.2/2 Amsel K., 2017 a Acute toxicity of BAS 684 H to the bumblebee *Bombus terrestris* L. under laboratory conditions 2017/1140992
Guidelines:	OECD 246 (2017), OECD 247 (2017)
GLP:	yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

Report:	CA 8.3.1.1.2/3 Amsel K., 2018 a Amendment No. 1 - Acute toxicity of BAS 684 H to the bumblebee Bombus terrestris L. under laboratory conditions 2018/1000903
Guidelines:	OECD 246 (2017), OECD 247 (2017)
GLP:	yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

I. MATERIAL AND METHODS

A. MATERIALS

Test item:	BAS 684 H (Reg. No. 900 202); batch no.: COD-002038; analyzed purity: 93.0% ± 1.0%.

B. STUDY DESIGN

Test species:	*Bombus terrestris* L. (bumblebee), young adult worker bumblebees derived from queen-right hives; source: Biobest Belgium N.V., Westerlo, Belgium; collected in the evening prior to use. Very small and large bumblebees were excluded from the test by visual inspection.

Collection of bumblebees : Bumblebees were collected in the evening prior to testing using red light.Bumblebees from different colonies were randomly distributed to the treatment groups and dose rates. After transfer of the bumblebees into the test units they had time for acclimatisation to the test room conditions for about 17 hours.

Test design: In a 48-hour test, adults of *Bombus terrestris* were exposed to 5 doses of BAS 684 H in an appropriate carrier (acetone) placed on the dorsal bumblebee thorax. In total, 9 treatment groups were set up: 5 dose rates of the test item, 3 control groups and 1 dose rate of the reference item with 30 replicates per dose and 1 bumblebee per replicate, respectively. Assessments of bumblebee mortality and behavioural effects were done after 4, 24 and 48 hours. Test units were 7cm x 2cm and were part of the rearing system.

Endpoints: Mortality, behavioural impairments recorded as normal (healthy), affected (impaired locomotion) or moribund.

Reference item: Dimethoate EC 400 (BAS 152 11 I, dimethoate, 405.2 g/L analyzed).

Test doses: Water control (deionized water), TritonX control (0.5% (v/v) TritonX solution), acetone control (pure acetone); reference item at a dose rate of 10.0 µg dimethoate/bumblebee (the reference item was solved in deionized water including 0.5% (v/v) TritonX.); test item at dose rates of 12.5, 25.0, 50.0, 100.0 and 200.0 µg BAS 684 H/bumblebee.

Application: Prior to application of test solution, bumblebees in the test cage were anaesthetised with CO_2 for approx. 20 seconds. They were removed from the cage to a large petri dish and turned around with forceps for application of test substance. A single droplet (2µL) of controls, test item (vehicle: acetone) and reference item.

Table B.9.3.1-10: Applied dosages in contact toxicity test

Treatment group	Test solution ID	Item to be applied	Dose		Applied volume [µL/bumblebee]
Control	AC BC CC	Deionised water 0.5% (v/v) TritonX solution Acetone	--		2
			[µg product/bumblebee]	[µg a.s./bumblebee]	
Test item	AT BT CT DT ET	BAS 684 H*	-	200.0 100.0 50.0 25.0 12.5	2
Reference item	AR	Dimethoate EC 400*	26.4	10.0	2

* based on analysed purity /analysed content of a.s.
Calculations are performed with non-rounded values.

Test conditions: Temperature: 23.5 °C – 25.4 °C, relative humidity: 57% – 65%, photoperiod: 24 h darkness; food: 50% (w/v) sucrose solution. Test condition deviations of less than 2 hours per day were not reported as they were not expected to affect the integrity and outcome of the study.

Statistics: Descriptive statistics. Fisher's Exact Binominal Test with Bonferroni Correction for mortality data (one-sided greater, α = 0.05).

Analytical verification: The content of active substance was analysed in the highest and lowest dose groups.

II. RESULTS AND DISCUSSION

Validity criteria

The table below provides the outcome of the validity criteria which were all met.

Table B.9.3.1-11: Validity criteria results (oral and contact results presented as were reported in one study)

Validity criterion		Occurred / calculated	Recommended
Mean control mortality (48 h)	Contact test: - Deionised water - 0.5% TritonX solution - Acetone	0.0% 0.0% 0.0%	≤ 10%
	Oral test: - Sucrose solution - Sucrose solution + 5% acetone + 1% Tween	0.0% 0.0%	≤ 10%
Mortality reference item (48 h)	Contact toxicity test	100.0%	≥ 50%
	Oral toxicity test	100.0%	≥ 50%

After 48 hours of contact exposure, no mortality occurred in the control groups treated either with deionized water, TritonX solution nor with acetone. In the test item treatment, no statistically significant mortality occurred after thoracic application of 12.5, 25.0, 50.0, 100.0 and 200.0 µg BAS 684 H/bumblebee within 48 hours. The dose rate of 50.0 µg BAS 684 H/bumblebee revealed a slight mortality of 3.3%, which is not statistically significant when compared with the controls (Fisher´s Exact Binomial Test (one-sided greater, α = 0.05)). Furthermore, no behavioral abnormalities of surviving bumblebees occurred throughout the contact toxicity test. The results are summarized in the table below.

Table B.9.3.1-12: Toxicity of BAS 684 H to *Bombus terrestris* (bumblebee) in a contact toxicity test

Treatment	**Dosage**	**Mortality [%]**	
		24 h	**48 h**
Control	Water control	0.0	0.0
	0.5% TritonX	0.0	0.0
	Acetone	0.0	0.0
BAS 684 H [µg a.s./bumblebee]	12.5	0.0	0.0
	25.0	0.0	0.0
	50.0	0.0	3.3
	100.0	0.0	0.0
	200.0	0.0	0.0
Endpoint [µg a.s./bumblebee]			
LD_{50} (48 h)	> 200.0		

Mortality in the reference item treatment in the contact test was 100.0% after 48 hours.

Analytical results

The recovery of BAS 684H was 81% in the highest test group sample analysed and 97% in the lowest. No active substance was detected in the control sample.

III. CONCLUSION

In an acute contact toxicity study with BAS 684 H on bumblebees, the LD_{50} value (48 h) was estimated to be > 200.0 µg BAS 684 H/bumblebee and the NOED after 48 hours was ≥ 200.0 BAS 684 H/bumblebee.

HSE evaluator comments:

The study was well reported and conducted mostly in line with OECD 246 (2017).

It was noted that the study reported that test condition deviations of less than 2 hours per day were not reported as they were not expected to affect the integrity and outcome of the study. However the evaluator is of the view that these deviations should have been reported as raw data in order for the magnitude to be checked. Given that these deviations did not affect the validity criteria results which were all met sufficiently, it is accepted.

Validity criteria outlined in the guideline were all met. It was noted that the test unit was 14cm^3; slightly smaller than the recommended minimum of 15cm^3, this was not considered to invalidate the test since control results were achieved to meet validity criteria.

The method of analysis used in the study was validated by the chemistry specialist (see CA B5).

The endpoints for consideration in the risk assessment is:

- **LD_{50} (48h) > 200.0 µg a.s./bumblebee**

B.9.3.1.2. Chronic toxicity to bees

The chronic toxicity to bees is addressed in Vol.3 CP B.9.

B.9.3.1.2. Effects on honeybee development and other honeybee life stages

Report:	CA 8.3.1.3/1 Kleebaum K., 2016 a Repeated exposure of BAS 684 H to honey bee (*Apis mellifera*) larvae under labroratory conditions (in vitro) 2016/1044854
Guidance document:	OECD 239 (2016)
GLP:	yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)
Additional report:	CA 8.3.1.3/3 Azevedo, A. B., 2018 BASF Doc ID 2018/1099616 Further statistical evaluation of the study 2016/1044854 on chronic toxicity on honey bee larvae

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 684 H (Reg. No. 900 202); batch no.: COD-002038; analyzed purity: 93.0% ±1%.

B. STUDY DESIGN

Test species: Larvae of *Apis mellifera* L. subspecies Buckfast (honeybee); synchronized first larval stage (L1); derived from at least three healthy and queen-right colonies; source: in-house colonies. The larvae were taken from hives that had not received treatments with chemical substances for at least one month. Three colonies were used in the test and each contained a seperate queen that was isolated on an empty brood comb.

Randomisation: Before application all sick or dead larvae were swapped with normal developed individuals originating from the respective colony. All plates used in the study were randomized.

Test design: 22-day repeated exposure larval toxicity test according to OECD 239 (2016).
Production of L1 larvae was done by caging each queen seperately on an empty brood comb fitted with an excluder cage on day -3. The queen laid her eggs solely on this comb and was caged for approximately 30h. in the afternoon of day -2 the queen was released from the excluder and the comb checked for freshly laid eggs. L1 honeybee larvae of *Apis mellifera* were transferred from brood combs to polystyrene grafting cells in 48-well cell culture plates 2 days before start of the treatment (on D1). Following grafting, the larvae were fed during larval development with artificial diet, containing the test item on rearing days 3, 4, 5 and 6. In total, 3 treatment groups were set up: 5 doses of the test item, 1 untreated control group, 1 solvent control and 1 dose of the reference item, each with 3 replicates and 12 larvae per replicate. Assessments of larval mortality were done 24, 48, 72, 96 and 120 hours after start of the treatment (respectively D4, D5, D6, D7, D8). Additionally, other observations such as small body size or large quantities of remaining food after 96 and 120 hours (on D7 and D8) were noted. Pupal mortality was assessed at D15 and emergence of adults was evaluated at D22.

Endpoints: Successful adult emergence (dose-effect relationship), Mortality, qualitative observations: body size, remaining food.

Reference item: Dimethoate tech. (analysed purity: 98.8% w/w).

Test doses: Control 1: untreated diet (50% aqueous yeast/sugar solution with 50% royal jelly)
Control 2: untreated diet with acetone (0.5% w/w)

Test item treatments including 0.5% w/w acetone:

Nominal dose/concentration of BAS 684 H	
Doses [µg a.s./larva]	**Concentrations [mg a.s./kg food]**
6.3	41
12.5	81
25.0	163
50.1	325
100.1	650

Reference item: treated diet with a dose of 7.4 µg dimethoate/larva (corresponding concentration: 48 mg a.s./kg food).

The test item was solved in acetone and several dilutions were prepared by adding further acetone. To ensure an even distribution of the test item in the larvae food, final diets were placed on a multitube vortexer for 5 minutes. Immediately afterwards, a

sample of each diet was taken and stored in a freezer until the analytical phase.

Final diets were first warmed to 34.5°C in a climate chamber and again carefully vortexed before the feeding event.

Test conditions: Temperature (D1-D22): 34.0°C – 35.0°C
Relative humidity:
93 - 98% (D1-D8)
75 - 80% (D8 – D15)
47 - 54% (D15 – D22)
Photoperiod: darkness (except during assessments)
Feeding scheme :

Test day	1[1]	2	3[2]	4[2]	5[2]	6[2]
Artificial diet	A	-	B	C	C	C
Volume of diet per larva	20 µL	-	20 µL	30 µL	40 µL	50 µL
Composition of diets, based on (w/w):						
Royal jelly	50 %	-	50 %		50 %	
Yeast/sugar solution	50 %		50 %		50 %	
Composition of yeast/sugar solution, based on (w/v):						
Glucose	12 %	-	15 %		18 %	
Fructose	12 %		15 %		18 %	
Yeast	2 %		3 %		4 %	

[1] day of grafting L1; [2] day of application

Statistics: Descriptive statistics; The Chi² Table Test with Bonferroni Correction (one-sided greater, $\alpha = 0.05$) for determination of NOED/NOEC (D8 and D22).

Further statistics:

BASF Doc ID 2018/1099616 was submitted to address the requirement under regulation 283/2013 for calculation of EC_{10}, EC_{20} and EC_{50} values for adult bees, where possible, and larvae together with the NOEC.

Loglogistic regression was used for the derivation of the dose-response curve based on overall survival rate on day 22 in the Rstudio software verion 1.1.447. It was noted that nominal formulation concentrations were used as opposed to active substance i.e. 6.7, 13.5, 26.9, 53.8, 107.7 µg product/larva.

Analytical verification: All final diets were sampled in duplicate as specimens for analysis and retention directly after preparation on D3, D4, D5 and D6. Samples were stored at ≤ -18°C. The determination of the active ingredient in the diets was conducted by an in-house developed method using LC-MS/MS detection.

II. RESULTS AND DISCUSSION

Validity Criteria

The validity criteria stipulated in OECD guideline 239 (2016) were met as follows:

- In the control plate(s), cumulative larval mortality from D3 to D8 was ≤15% across all replicates (actual: Control 1 with 8.3% and Control 2 with 11.1%).
- In the control plate(s), the adult emergence rate on D22 was ≥70% across all replicates (actual: Control 1 was 77.8% and Control 2 was 75%).
- Larval mortality was ≥50% on D8 across all replicates (actual = 88.9%).

Mortality

After 120 hours of repeated oral exposure (on D8) larval mortalities of 8.3 and 11.1% were observed in the controls, respectively. Pupal mortality (between D8 and D22) was 15.2% in the untreated control and 15.6% in the solvent control. The control groups showed a total mortality of 22.2% and 25.0%, respectively, at D22. In the test item group, larval mortalities at D8 ranged between 5.6 and 27.8%. Pupal mortalities ranged between

3.3 and 14.7% in the test item treatment groups. Total mortalities at D22 ranged between 19.4 and 36.1%. On D8, no remaining larva treated with BAS 684 H showed any abnormalities such as remaining food.

Emergence

In the final assessment at D22, adult emergence rates of 77.8% and 75.0 % were determined for the honeybees in the control groups. In the test item group, the adult honeybees emerged at rates ranging between 63.9 and 80.6% following an application of 6.3, 12.5, 25.0, 50.1 and 100.1 µg a.s./larva, respectively, during the larval stages. No statistically significant effect occurred in any treatment group of larvae fed with the test item (Chi² Table Test with Bonferroni Correction, one-sided greater, $\alpha = 0.05$). The results are summarized in Table B.9.3.1-13.

Table B.9.3.1-13: Toxicity of BAS 684 H to *Apis mellifera* (honeybee) in a repeated exposure larval toxicity test after 22 days

Dosage [µg a.s./larva]	**% of nominal concentration achieved**	**Concentration [mg a.s./kg food]**	**D8 mortality**		**D22 mortality [%]**		**D22 adult emergence [%]** [2]
			absolute	**corrected** [1]	**absolute**	**corrected** [1]	
Control	No active substance measured	Control	8.3	0.0	15.2	0.0	77.8
Acetone solvent control	n/a	Acetone solvent control	11.1	0.0	15.6	0.0	75.0
6.3	80-97%	41	5.6	0.0	14.7	0.0	80.6
12.5	87-99%	81	16.7	6.3	3.3	0.0	80.6
25.0	93-105%	163	25.0	15.6	7.4	0.0	69.4
50.1	88-112%	325	25.0	15.6	11.1	0.0	66.7
100.1	86-100%	650	27.8	18.8	11.5	0.0	63.9
Endpoints [D22]							
LD_{50} [µg a.s./larva] [2]			> 100.1				
$NOED_{mortality}$ [µg a.s./larva] [3]			≥ 100.1				
LC_{50} [mg a.s./kg food] [2]			> 650				
$NOEC_{mortality}$ [mg a.s./kg food] [3]			≥ 650				
ED_{50} [µg a.s./larva] [2]			> 100.1				
$NOED_{emergence}$ [µg a.s./larva] [3]			≥ 100.1				
EC_{50} [mg a.s./kg food] [2]			> 650				
$NOEC_{emergence}$ [mg a.s./kg food] [3]			≥ 650				

Negative values are set to 0.

[1] Corrected for solvent control mortality according to Schneider-Orelli (1947).

[2] Estimated value.

[3] Chi² Table Test with Bonferroni Correction, one-sided greater, $\alpha = 0.05$.

[4] Range of recovery of the active substance over D3, D4, D5 and D6.

EC_{10} and EC_{20} values

The EC_{10} was calculated to be 45.1 µg a.s./larva (95% CI = 7.8-261.5) and EC_{20} was calculated to be 100.7 µg a.s./larva (95% CI = 28.5-355.7).

III. CONCLUSION

Larval mortality

In a repeated exposure larval toxicity study with BAS 684 H, the LD_{50} (larval mortality on D8) was estimated to be > 100.1 µg a.s./larva, which is equivalent to a LC_{50} of > 650 mg a.s./kg food. The respective NOED was ≥ 100.1 µg a.s./larva and the corresponding NOEC was ≥ 650 mg a.s./kg food.

Emergence

The ED_{50} (successful adult emergence up to D22) was estimated to be > 100.1 µg a.s./larva, which is equivalent to a EC_{50} of > 650 mg a.s./kg food. The respective NOED was ≥ 100.1 µg a.s./larva and the corresponding NOEC was ≥ 650 mg a.s./kg food.

HSE evaluator comments:

It should be noted that this study will not be considered in context of the risk assessment, as no noted guidance is currently available. However results will be noted.

The study was well reported and conducted in line with OECD 239 (2016). The test conditions for each stage in the study were adhered to.

It was noted in the additional study report (BASF Doc ID 2018/1099616**)** that the reliability of the dose-response was questionable as shown in the extremely wide confidence intervals for each endpoint. This is accepted by the evaluator since the EC_{10} and EC_{20} values will not be used in the ri**sk** assessment.

The study reports that the concentration in the final diet was analytically determined for each dose concentration on days 3, 4, 5 and 6. As shown in Table B.9.3.1-13 in the study report, the recovery of each dose concentration was within 20% of the nominal values (actual range was 80-112%) which demonstrates sufficient exposure was achieved in line with the proposed nominal dose concentrations. The method of analysis used in the study was validated by the chemistry specialist (see CA B5).

The LD_{50} (larval mortality on D8) was estimated to be > 100.1 µg a.s./larva, which is equivalent to a LC_{50} of > 650 mg a.s./kg food. The respective NOED was ≥ 100.1 µg a.s./larva and the corresponding NOEC was ≥ 650 mg a.s./kg food.

The ED_{50} (successful adult emergence up to D22) was estimated to be > 100.1 µg a.s./larva, which is equivalent to a EC_{50} of > 650 mg a.s./kg food. The respective NOED was ≥ 100.1 µg a.s./larva and the corresponding NOEC was ≥ 650 mg a.s./kg food.

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

Please refer to Volume 3 CP B.9.

B.9.4. EFFECTS ON NON-TARGET SOIL MESO- AND MACROFAUNA

B.9.4.1. Earthworm – acute and sub-lethal effects

B.9.4.1.1 Acute effects:

Report:	CA 8.7/01 Friedrich S., 2016a Acute toxicity of BAS 684 H (cinmethylin) to the earthworm *Eisenia andrei* in artificial soil with 10 % peat 2016/1044851
Guidelines:	OECD 207 (1984), ISO 11268-1 (2012)

GLP: Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Cinmethylin; batch no. COD-002038; content of a.s.: cinmethylin (Reg. No. 900 202): purity 93.0 % (analysed ± 1.0 %)

B. STUDY DESIGN

Test species: *Eisenia andrei*; adult worms with clitellum and weight of 301 –495 mg, age: less than one year; source: in-house culture.

Test design: In a 14-day acute test, adults of *Eisenia andrei* were exposed to five concentrations of cinmethylin in treated artificial soil according to OECD 207 (10 % peat). In total, 6 treatment groups were set up (5 concentrations of the test item and 1 control group) with 4 replicates per treatment, 10 adult worms per replicate. The artificial soil was treated and filled into glass vessels, before the earthworms were introduced on the top of the soil. Assessment of worm mortality was done 7 and 14 days after exposure, and biomass development and behavioral effects 14 days after exposure at test termination.

Endpoints: Mortality, behavioral effects, biomass development.

Reference item: 2-Chloroacetamide. The effects of the reference item were investigated in a separate study.

Test concentrations: Solvent control, 50, 100, 200, 400 and 800 mg a.s./kg dry soil

Test conditions: Artificial soil according to OECD 207 with 10 % peat; pH 6.07 – 6.14 at test initiation, pH 5.87 – 5.97 at test termination; water content 55.3 – 55.6 % of its maximum water holding capacity (WHC) at test initiation and 54.4 – 55.0 % of WHC at test termination, temperature: 19.1 – 22.0 °C; photoperiod: continuous illumination, light intensity: 580 lux, no feeding.

Statistics: Descriptive statistics; Multiple Sequentially-rejective Fisher Test After Bonferroni-Holm for mortality ($\alpha = 0.05$, one-sided greater), Williams-t-test for weight change data ($\alpha = 0.05$, one-sided greater), Weibull analysis using linear maximum-likelihood regression for calculation of LC_{50}.

II. RESULTS AND DISCUSSION

The results are summarised in Table B.9.4.1.1-1.

Table B.9.4.1.1-1: Effects of cinmethylin on *Eisenia andrei* in a 14-day acute study

Cinmethylin [mg a.s./kg dry soil]	**Solvent Control**	**50**	**100**	**200**	**400**	**800**
Mortality (28 d) [%]	2.5	0.0	2.5	7.5	30*	100*
Weight change (14 d) [%]	-8.9	-12.1	-15.7*	-20.6*	-26.7*	--
Endpoints [mg a.s./kg dry soil]						
LC_{50}	466 (95 % confidence limits: 414 – 525)					
NOEC	50					

* Statistically significantly different compared to the control ($\alpha = 0.05$).
-- not determined as 100 % mortality occurred.

III. CONCLUSION

In a 14-day acute toxicity study with earthworms (*Eisenia andrei*), exposure to cinmethylin resulted in an LC_{50} of 466 mg a.s./kg dry soil. The NOEC was determined to be 50 mg a.s./kg dry soil.

HSE evaluator comments:

The above study has not been evaluated by HSE . It is not required under the current data requirements 283/2013. However, the endpoint generated has been considered in order to determine whether it is adverse. The mortality observed in this study is broadly comparable to the reproductive study (Friedrich, 2016b). In addittion if this endpoint was used in the risk assessment the RAC would be 466/2 (toxicity endpoint corrected as log_{pow} > 2) = 233/10 (assessment factor) = 23.3 mg a.s./kg dry soil compared to reproductive RAC of 83.5/2 (toxicity endpoint corrected as log_{pow} > 2) = 41.75/5 (assessment factor) = 8.35 mg a.s./kg dry soil. Therefore this study would not result in a more conservative risk assessment than Friedrich 2016b and has not been considered further.

B.9.4.1.2 Sub-lethal effects:

Report: CA 8.4.1/1
Friedrich S., 2016b
Sublethal toxicity of BAS 684 H to the earthworm *Eisenia andrei* in artificial soil
2016/1044852
Guidelines: OECD 222 (2004)
GLP: Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Cinmethylin (Reg. No. 900 202), batch no. COD-002038, purity: 93.0 % (analysed, ± 1.0 %).

B. STUDY DESIGN

Test species: *Eisenia andrei*; adult worms with clitellum and weight of 301 - 499 mg/worm, approximately 3 months old; source: W. Neudorff GmbH KG followed by in-house culture.

Test design: In a 56-day test, adults of *Eisenia andrei* were exposed to eight concentrations of cinmethylin in treated artificial soil according to OECD 222 (10 % peat). In total, 9 treatment groups were set up (8 concentrations of the test item and 1 solvent control group) with 4 replicates for the test item treatments and 8 replicates for the control, 10 adult worms per replicate. The artificial soil was treated and filled into vessels, before the earthworms were introduced on the top of the soil.
Assessment of worm mortality, behavioral effects and weight change was done after 28 days of exposure, after additional 28 days (56 days after application) reproduction (number of juveniles) was assessed.

Endpoints: Mortality, weight change, feeding activity, reproduction rate.

Reference item: Maypon Flow (Carbendazim, SC 500). The effects of the reference item were investigated in a separate study.

Test concentrations: Solvent control (acetone), 58.5, 87.8, 131.7, 197.5, 296.3, 444.4, 666.7 and 1000 mg a.s./kg dry soil (spacing factor: 1.5, corrected for purity).

Test conditions: Artificial soil according to OECD 222 with 10 % peat; pH 5.97 - 6.09 at test initiation, pH 5.69 – pH 5.81 at test termination; water content 55.3 - 55.6 % of its maximum water holding capacity (WHC) at test initiation and 54.5 - 55.5% of

WHC at test termination, temperature: 18.0 °C – 21.1 °C; photoperiod: 16 hours light: 8 hours dark, light intensity: 610 lux, feeding with horse manure.

Statistics: Descriptive statistics; Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm for mortality (α = 0.05, one-sided greater), Williams-t-test for biomass and reproduction (α = 0.05, one-sided smaller), Trimmed Spearman-Karber procedure for mortality, Probit analysis for reproduction.

II. RESULTS AND DISCUSSION

Validity criteria:

The criteria in OECD 222 (2016) have been considered below:

The following criteria should be satisfied in the controls for a test result to be considered valid:

- Each replicate (containing 10 adults) to have produced ≥ 30 juveniles by the end of the test. Obtained: minimum of 108 per replicate
- The coefficient of variation of reproduction to be ≤ 30 %. Obtained: 13.2 %
- Adult mortality over the initial 4 weeks of the test to be ≤ 10 %. Obtained: 0 %

Biological effects: Cinmethylin showed statistically significant mortality compared to the solvent control at 666.7 and 1000 mg a.s./kg dry soil (Multiple Sequentially-rejective Fisher Test After Bonferroni-Holm, α = 0.05, one-sided greater). The mortality of adult worms was 0 – 100 % in the treated groups and 0 % in the solvent control group. The weight change of adult worms was between -43.0 and 25.8 % in the treated groups and 24.8 % in the solvent control group. The test item caused a statistically significant change (Williams-t-test, α = 0.05, one-sided smaller) in biomass (change in fresh weight after 4 weeks relative to initial fresh weight) compared to the solvent control at concentrations of 197.5, 296.3, 444.4 and 666.7 mg a.s./kg dry soil. The biomass change at 1000 mg a.s./kg dry soil could not be determined, because all worms died at this concentration.

The reproduction rate was significantly different compared to the solvent control at concentrations of 131.7, 197.5, 296.3, 444.4, 666.7 and 1000 mg a.s./kg dry soil (Williams-t-test, α = 0.05, one-sided smaller). No pathological symptoms and no further effects on behavior of the worms were observed. The feeding activity of adult worms was reduced at 197.5, 296.3, 444.4, 666.7 and 1000 mg a.s./kg dry soil. The results are summarised Table B.9.4.1.2-1.

Table B.9.4.1.2-1: Effects of cinmethylin on *Eisenia andrei* in a 56-day reproduction study

Cinmethylin [mg a.s./kg dry soil]	**Solvent control**	**58.5**	**87.8**	**131.7**	**197.5**	**296.3**	**444.4**	**666.7**	**1000**
Mortality (28 d) [%]	0.0	0.0	0.0	2.5	0.0	0.0	10.0	60.0 *	100.0 *
Weight change (28 d) [%]	24.8	23.4	25.8	21.8	6.9 *	-7.6 *	-22.8 *	-43.0 *	-
Number of juveniles (56 d)	127.6	131.0	120.3	53.5 *	25.5 *	0.0 *	0.0 *	0.0 *	0.0 *
Reproduction (56 d) [% of control]	100	102.6	94.2	41.9	20.0	0.0	0.0	0.0	0.0
Endpoints based on reproductive effects [mg a.s./kg dry soil]									
NOEC (day 56)	87.8								
EC_{10} (day 56) [1)]	83.5 (95 % confidence limits 68.9 to 101.1)								
EC_{20} (day 56) [1)]	97.5 (95 % confidence limits 84.4 to 112.5)								
EC_{50} (day 56) [1)]	131.1 (95 % confidence limits 119.6 to 143.8)								

-: dead or not determined.

* Statistically significantly different compared to the control (Multiple Sequentially-rejective Fisher Test After Bonferroni-Holm for mortality, α = 0.05, one-sided greater, Williams-t-test for biomass and reproduction, α = 0.05, one-sided smaller).

1) Based on Probit analysis.

In a separate study the reference item Maypon Flow (Carbendazim, SC 500) had a significant effect on biomass increase and reproduction of earthworms. The reproduction rate was clearly inhibited by 39 % and 96 % compared to the control at the tested concentrations of 5 and 10 mg reference item product/kg dry soil.

III. CONCLUSION

The NOEC for reproduction was determined to be 87.8 mg a.s./kg dry soil. The EC_{10} and EC_{50} values for reproduction were calculated to be 83.5 and 131.1 mg a.s./kg dry soil, respectively.

HSE evaluator comments:

Considering there is evidence that cinmethylin is volatile (see environmental fate, volume 3 CA, section 8 dossier) in accordance with OECD 222 analytical measurements should have been taken at the start, during and end of study to confirm exposure concentration. This point has been considered in detail in the risk assessment section.

It was noted that only a solvent control was tested. Ideally there would be an additional control without solvent to ensure it did not influence the study results. Nonetheless as a common solvent (acetone) was used, the method in OECD 222 was followed (including evaporation prior to exposure) and validity criteria were met the HSE evaluator considers this acceptable.

The test vessels used were plastic and it was not stated whether the material was inert. However, given the validity criteria were met and this is unlikely to have significantly impacted the study the HSE evaluator considers the test material used appropriate.

When considering the EC_{10} value it is not in-line with the experimental data i.e. an endpoint of 83.5 mg a.s./kg despite 5.8 % reduction compared to solvent control at 87.8 mg a.s./kg. This is most likely due to the lack of partial responses at lower test concentrations, supported by the relatively wide confidence limits; 68.9 to 101.1 mg a.s./kg. Despite this as the endpoint calculated is comparable to the NOEC value and conservative it has been considered in the risk assessment section.

Using the appendix 1 of the study report it is possible to determine that the concentrations in the study have been prepared based on purity of test item i.e. the nominal test concentration of 1000 mg a.s./kg is 999.998 mg a.s./kg based on a purity of 93 % for the test item. Therefore, the endpoints derived allow for the purity of the test substance.

This study was conducted to GLP and considered valid. **However, there is uncertainty regarding volatilisation of the test item occuring during the study** which has been discussed further in the risk assessment section. The following endpoints were derived (10 % peat was tested):

- Cinmethylin NOEC (reproductive effects) = 87.8 mg a.s./kg
- Cinmethylin EC_{10} (reproductive effects) = 83.5 mg a.s./kg, noting uncertainty mentioned above
- Cinmethylin EC_{20} (reproductive effects) = 97.5 mg a.s./kg

B.9.4.2. Effects on non-target soil meso- and macrofauna (other than earthworms)

No studies submitted.

B.9.5. EFFECTS ON SOIL: CARBON AND NITROGEN TRANSFORMATION

B.9.5.1 Carbon transformation:

Report: CA 8.7/2
Schulz L., 2016 b
Effects of BAS 684 H on the activity of soil microflora (Carbon transformation test)
2016/1044848
Guidelines: OECD 217 (2000)
GLP: Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Cinmethylin (Reg. No. 900 202), batch No. COD-002038, analyzed purity: 93.0 % (tolerance ± 1.0 %).

B. STUDY DESIGN

Test soil:	Biologically active agricultural soil: loamy sand (DIN 4220) / sandy loam (USDA), pH 6.6, 1.40 % C_{org}, WHC: 36.55 g/100 g dry soil.
Test design:	Determination of carbon-transformation in soil after addition of glucose. Comparison of test item treated soil with a non-treated and a reference item treated soil. 3 replicates per concentration. A "BSB-digi" respirometer system was used to measure the O_2-consumption over a period of 12 hours at different sampling intervals. Sampling scheme: 0, 7, 14 and 28 days after treatment. Sub-samples were withdrawn from the bulk batches and subjected to measurement.
Test concentrations:	Control, 1.43 mg and 7.17 mg cinmethylin/kg dry soil.
Endpoints:	Effects on O_2 consumption after 28 days of exposure.
Reference item:	Dinoterb (purity: 98.0 % ± 0.5 % analyzed). The reference item was tested in a separate study at rates of 6.80, 16.00 and 27.00 mg/kg.
Test conditions:	Water content: 45 % of its maximum water holding capacity: measured water content: 17.18 - 18.02 g/100 g dry soil; pH 6.4 - 6.7. Soil samples were incubated at 19.8 - 21.1 °C while stored in steel vessels in the dark.
Statistics:	Descriptive statistics.

II. RESULTS AND DISCUSSION

No adverse effects of Cinmethylin on carbon transformation in soil could be observed at both test concentrations (1.43 mg/kg dry soil and 7.17 mg/kg dry soil) after 28 days. Only negligible deviations from the control of +0.4 % (test concentration 1.43 mg/kg dry soil) and -2.8 % (test concentration 7.17 mg/kg dry soil) were measured at the end of the 28-day incubation period. The results are summarized in Table B.9.5.1-1.

Table B.9.5.1-1: Effects of cinmethylin on soil micro-organisms (carbon transformation) on days 0, 7, 14 and 28 of incubation

Soil (days)	Control	1.43 mg a.s./kg dry soil		7.17 mg a.s./kg dry soil	
	O_2 consumption [mg/kg dry soil/h]	**O_2 consumption [mg/kg dry soil/h]**	**% Deviation from control [1)]**	**O_2 consumption [mg/kg dry soil/h]**	**% Deviation from control [1)]**
Loamy sand soil (0 d)	16.05	16.19	+0.9	15.99	-0.3
Loamy sand soil (7 d)	14.08	13.24	-6.0	13.49	-4.2
Loamy sand soil (14 d)	13.92	13.73	-1.3	13.83	-0.7
Loamy sand soil	11.99	12.04	+0.4	11.66	-2.8

Soil (days)	Control	1.43 mg a.s./kg dry soil		7.17 mg a.s./kg dry soil	
	O_2 consumption [mg/kg dry soil/h]	O_2 consumption [mg/kg dry soil/h]	% Deviation from control [1)]	O_2 consumption [mg/kg dry soil/h]	% Deviation from control [1)]
(28 d)					

[1)] Based on O_2-consumption; - = inhibition; + = stimulation

In a separate study, the reference item Dinoterb caused an inhibition of carbon transformation of -34.4 % and -39.2 % at 16.00 mg and 27.00 mg Dinoterb/kg dry soil, respectively, determined 28 days after application.

III. CONCLUSION

Exposure of cinmethylin in a field soil up to a test concentration of 7.17 mg a.s./kg dry soil caused no adverse effects (deviation from control < 25 %, OECD 217) on the soil carbon transformation (measured as O_2-consumption) at the end of the 28-day incubation period.

HSE evaluator comments:

The above study has not been evaluated by HSE . It is not required under the current data requirements 283/2013. In addittion, it is noted the data generated is not adverse.

B.9.5.2 Nitrogen transformation:

Report: CA 8.5/1
Schulz L., 2016 a
Effects of BAS 684 H on the activity of soil microflora (Nitrogen transformation test)
2016/1044850
Guidelines: OECD 216 (2000)
GLP: Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Cinmethylin (Reg. No. 900 202), batch No. COD-002038, analyzed purity: 93.0 % (tolerance ± 1.0 %).

B. STUDY DESIGN

Test soil: Biologically active agricultural soil: loamy sand (DIN 4220) / sandy loam (USDA), pH 6.6, 1.40 % C_{org}, WHC: 36.55 g/100 g dry soil.

Test design: Determination of the N-transformation (NO_3-N-production) in soil enriched with lucerne meal (concentration in the soil 0.5%). Comparison of test item treated soil with a non-treated soil. NH_4-nitrogen formed from organically bound nitrogen and NO_3-nitrogen formed from the nitrification process was determined using an Autoanalyzer (BRAN and LUEBBE). Sampling scheme: 0, 7, 14 and 28 days after treatment. Sub-samples (3 replicates per treatment) were withdrawn from the bulk batches and subjected to measurement. It was noted the test vessels wide-mouth glass flasks (500 ml) with screw caps that permit air exchange. The headspace was not reported and no steps were taken during sampling to minimise volatilization of the test material.

Endpoints: Effects on the NO_3-N production 0, 7, 14 and 28 days after application.

Test concentrations: Control, 1.43 mg and 7.17 mg a.s./kg dry soil.

Reference item:	Dinoterb (purity: 98.0 ± 0.5 % analyzed). The reference item was tested in a separate study at rates of 6.80, 16.00 and 27.00 mg reference item/kg dry soil.
Test conditions:	Soil moisture: approx. 45 % of maximum water holding capacity; measured water content: 16.31 - 17.20 g/100 g dry soil; pH 6.4 - 6.5. Soil samples were incubated at 19.8 - 21.1 °C while stored in glass flasks in the dark.
Statistics:	Descriptive statistics.

II. RESULTS AND DISCUSSION

Validity criteria:

In OECD 216 (2000) the following criteria are stated:

- The variation between replicate control samples should be less than ± 15 %. Obtained: maximum of 3.5 % variation.

During the study the above criteria were met.

Biological effects: Only negligible deviations from the control of -4.3 % (test concentration 1.43 mg a.s./kg dry soil) and -1.3 % (test concentration 7.17 mg a.s./kg dry soil) were measured at the end of the 28-day incubation period when considering nitrate concentration.

The results are summarised in Table B.9.5.2-1.

Table B.9.5.2-1: Effects of cinmethylin on soil micro-organisms (nitrogen transformation) on days 0, 7, 14 and 28 of incubation

Time interval (days)	**Control**	**1.43 mg a.s./kg dry soil**		**7.17 mg a.s./kg dry soil**	
	NO_3-N [mg/kg dry soil]	NO_3-N [mg/kg dry soil]	% deviation from control [1)]	NO_3-N [mg/kg dry soil]	% deviation from control [1)]
0-7	31.30	28.70	-8.3	31.80	+1.6
0-14	41.87	40.33	-3.7	45.80	+9.4
0-28	62.30	59.60	-4.3	61.47	-1.3

[1)] Based on NO_3-N production; - = inhibition, + = stimulation.

The HSE evaluator notes that the study report did not calculate nitrogen transformation rate as per OECD 216 guidance and that endpoints were instead based on nitrate concentration. Therefore, the rates have been calculated by HSE evaluator in the table below.

There was less than 25 % deviation to control during the last time period (14 – 28 days) at both test concentrations of 1.43 and 7.17 mg a.s./kg.

Table B.9.5.2-2: Effects of cinmethylin on soil micro-organisms (nitrogen transformation rate)

Time (days)	Control NO_3-N formation rate [mg/kg dry soil/day][1)]	1.43 mg a.s./kg dry soil		7.17 mg a.s./kg dry soil	
		NO_3-N formation rate [mg/kg dry soil/day][1)]	% deviation compared to control[2)]	NO_3-N formation rate [mg/kg dry soil/day][1)]	% deviation compared to control[2)]
0 -7 d	4.47	4.10	-8.31	4.54	+1.60
7 - 14 d	1.51	1.66	+10.09	2.00	+32.49
14 - 28 d	1.46	1.38	-5.71	1.12	-23.33

[1)] calculated by HSE evaluator based on raw data in study report.
[2)] calculated in excel, - = inhibition; + = stimulation.

Reference study: In a separate study, the reference item Dinoterb produced a stimulation of nitrogen transformation of +28.6 % and +40.9 % at 16.00 and 27.00 mg reference item/kg dry soil, respectively, determined 28 days after application.

III. CONCLUSION

Exposure of cinmethylin in a field soil up to a test concentration of 7.17 mg a.s./kg dry soil caused acceptable effects (deviation from control < 25 %, OECD 217) on the soil nitrogen transformation (measured as NO_3-N-production and transformation rate) at the end of the 28-day incubation period.

HSE evaluator comments:

Considering there is evidence that cinmethylin is volatile (see environmental fate, volume 3 CA, section 8 dossier) in accordance with OECD 216 several steps should be taken when dealing with volatile substances:

- When testing volatile chemicals, losses during treatment should be avoided as far as possible and an attempt should be made to ensure homogeneous distribution in the soil (e.g. the test substance should be injected into the soil at several places).
- When volatile substances are tested, sealable and gas-tight containers should be used. These should be of a size such that approximately one quarter of their volume is filled with the soil sample.
- Incubation of soil samples can be performed in two ways: as bulk samples of each treated and untreated soil or as a series of individual and equally sized subsamples of each treated and untreated soil. However, when volatile substances are tested, the test should only be performed with a series of individual subsamples.

Based on the study report it was not possible to confirm whether the above approaches had been taken during this study. This has been considered in detail in the risk assessment section.

The above study was conducted to GLP and is considered valid, noting above uncertainties regarding volatilisation of the test item. The following endpoint has been derived:

- Cinmethylin 14 – 28 day < 25 % effects = **7.17 mg a.s./kg**

Report: CA 8.7/3
Stackhouse S.C., 1983 a
The effects of sd95481 and sd96638 on microbial functions
CI-690-001
Guidelines: None stated
GLP: No

It was noted two substances were tested. However, only SD 95481 (cinmethylin) was considered in the following summary as relevant to the current assessment.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: SD 95481 (cinmethylin, Reg. no. 900 202), Code 1-3-0-0; purity: 93 %.

B. STUDY DESIGN

Test soil:	Freshly collected biologically active agricultural soil: sandy loam soil, pH 7.4, 1.22 % C_{org}.
Test design:	For nitrogen fixation tests 50 g (dry weight) amounts of SD 95481 and control soil were brought to 75 % of the 1/3 bar moisture with sterile deionized water. For nitrification tests and all substrate degradation studies, 100 g amounts of soil in 250 ml flasks were employed. Fifty-gram soil samples were extracted with 150 ml of CH_3CN by Braunsonic. Aliquots of extracts were concentrated and analyzed by HPLC using a UV detector as per MMS-R-529-1. Soil application solutions were first diluted 1:100 with aqueous 20 % CH_3CN and then analyzed by HPLC. Sampling scheme: 1, 7 and 30 days after treatment.
Endpoints:	Effects on the nitrogen fixation, nitrification and the degradation of protein, cellulose, starch, glucose and sucrose 1, 7 and 30 days after application.
Test concentrations:	Control, 1.0 ppm SD 95481.
Test conditions:	Soil moisture: approx. 75 % of 1/3 bar moisture; pH 7.4. Soil samples were incubated at 25 °C while stored in glass flasks in the dark.
Statistics:	Descriptive statistics.

II. RESULTS AND DISCUSSION

Validity criteria:

In OECD 216 (2000) the following criteria are stated:

- The variation between replicate control samples should be less than ± 15 %. Obtained: maximum of 3.26 % variation calculated by HSE evaluator.

During the study the above criteria were met.

Biological effects: 'Acceptable' effects (≤ 12 %) of SD 95481 on the degradation of protein, cellulose, starch, glucose and sucrose could be observed. However, SD 95481 exhibited an inhibitory effect upon the nitrogen functions at one or more of the early assay times. SD 95481 reduced nitrogen fixation by 17 and 16 % at 1 and 7 days, respectively, but nitrogenase levels were comparable to those of the control soil by 30 days. While not initially inhibitory to nitrification, SD 95481 reduced soil nitrate levels to 42 % of the control level at 7 days. Again, however, nitrate levels were equivalent to control soil levels at 30 days.

The results are summarised in Table B.9.5.2-3.

Table B.9.5.2-3: Effects of cinmethylin on soil micro-organisms (nitrogen fixation, nitrification, degradation of protein, cellulose, starch, glucose and sucrose) on days 1, 7 and 30 of incubation

Microbial activity	Time (days)	1.0 ppm SD 95481 % activity of control
Nitrogen fixation	1	83
	7	85
	30	96
Nitrification	1	113
	7	42
	30	96
Protein degradation	1	98
	7	97
	30	96
Starch degradation	1	101
	7	105
	30	105
Cellulose degradation	1	94
	7	88
	30	109
Glucose degradation	1	89
	7	98
	30	101
Sucrose degradation	1	92
	7	98
	30	99

The HSE evaluator has used the data provided in appendix VI of the study report to calculate nitrogen transformation rates in the table below.

Table B.9.5.2-4: Effects of cinmethylin on nitrogen transformation rate during study

Time (days)	Control NO_3-N formation rate [mg/kg dry soil/day][1)]	1.0 mg a.s./kg dry soil	
		NO_3-N formation rate [mg/kg dry soil/day][1)]	% deviation compared to control[2)]
1 – 7 d	0.36	-0.03	+108.2
7 – 30 d	-0.05	0.04	+175.4

[1)] calculated by HSE evaluator based on raw data in study report.
[2)] calculated in excel, - = inhibition; + = stimulation.

III. CONCLUSION

Exposure of SD 95481 in a field soil up to a test concentration of 1.0 ppm SD 95481 (equals 1 mg a.s./kg dry soil) showed little (≤ 12 %) or no effect upon any of the soil degradation functions after 30 days of exposure. All changes at study end are below 25 %. When considering the nitrogen transformation rate there was stimulation above 25 % for the last time period.

HSE evaluator comments:

The HSE evaluator notes the study was briefly reported and no reference study was conducted.

When considering the nitrogen transformation rates there is inhibition in the control during the last time period (1 – 7 d) compared to the first time period (7 – 30 d) adding uncertainty to the stimulation observed in the treatment rate. Therefore, it is unclear whether treatment related effects occurred. Several other parameters (protein, starch, cellulose, glucose degradation) that were measured were comparable to the control during

study. When considering nitrification effects occurred at day 7 but by the end of the study (day 30) results were comparable with the control. In addition, whilst OECD 216 validity criteria were met the study was not conducted to GLP.

Overall, given the points detailed above this study is not considered suitable for use in the risk assessment and has not been considered further.

B.9.6. EFFECTS ON TERRESTRIAL NON-TARGET HIGHER PLANTS

B.9.6.1. Summary of screening data

No studies submitted.

B.9.6.2. Testing on non-target plants

No studies submitted.

B.9.7. EFFECTS ON OTHER TERRESTRIAL ORGANISMS (FLORA AND FAUNA)

No studies submitted.

B.9.8. EFFECTS ON BIOLOGICAL METHODS FOR SEWAGE TREATMENT

Report:	CA 8.8/1 Hammer S., 2016 a BAS 684 H - Determination of the inhibition of Oxygen consumption in the activated sludge respiration inhibition test 2016/1062165
Guidelines:	(EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part C.11, OECD 209
GLP:	Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test item:	Cinmethylin (Reg. No. 900202), batch no. COD-002038, purity: 93.0 % (± 1.0 % tolerance).

B. STUDY DESIGN

Test design:	Assessment of the inhibitory effect of the test item on the oxygen consumption rate of aerobic micro-organisms (activated sludge) after short-term exposure of 3 hours; the inoculum was aerated during the contact period; 3 replicates for the test item, 2 replicates for the reference item and 6 replicates for the control, sludge concentration: 1.5 g/L Dw.
Test concentrations:	Control, 1000, 500, 250, 125 and 62.5 mg a.s./L (nominal).
Reference item:	3.5-dichlorophenol, batch no. A0330535. The reference item was applied at 1, 10 and 100 mg/L.
Test conditions:	Temperature: 18.8 – 19.4 °C; pH 7.5 – 7.6; 1 L glass beakers, 500 mL of test mixture per vessel, 16 mL/vessel of 100-fold OECD medium; oxygen concentration during aeration > 2 mg/L.
Analytics:	Not applicable.

Statistics: Descriptive statistics, probit method for calculation of EC_x values.

II. RESULTS AND DISCUSSION

Validity criteria:

In OECD 209 (2000) the following criteria are stated:

- The EC50 of 3,5-DCP was found to lie in the range 2 mg/L to 25 mg/L for total respiration. Obtained: EC_{50} of 3,5-DCP was 12.5 mg/L.
- The blank controls (without the test substance or reference substance) oxygen uptake rate should not be less than 20 mg oxygen per one gram of activated sludge (dry weight of suspended solids) in an hour. The coefficient of variation (CV) of oxygen uptake rate in control replicates should not be more than 30 % at the end of definitive test. Obtained: The average oxygen uptake in blank controls was lower than validity criteria at 15 mg/g per hour (range between 14 and 16 mg/g per hour for replicates). Therefore, this criterion was not met. However, the study author considered the study valid because the reference substance produced an acceptable result and used the same batch of activated sludge. Furthermore, the author highlighted that the activated sludge used in the test showed a maximum and minimum oxygen consumption of 49.0 and 15.0 mg/g per hour following a total of 20 measurements, noting it was unclear when these measurements were made, the report just stated '*the last 20 measurements.'* The CV for the six replicates in the blank control was 4.7 % O_2 consumption i.e. below the criteria.

During the study the above criteria were not fully met.

Biological effects: No significant inhibition of respiration was measured up to the highest tested concentration of 1000 mg a.s./L (nominal), maximum of 11 % inhibition to control based on individual replicates at highest test concentration, noting the range observed included a replicate with higher oxygen consumption than control.

The results are summarised in the table below.

Table B.9.8-1. Oxygen consumption during study.

Parameter	Test concentrations (mg a.s./l)					
	C*	62.5**	125**	250**	500**	1000**
O_2 consumption rate (mg/l x h)	22.5	21 – 23	22 – 23	23 – 24	21 – 22	20 – 23
Inhibition of respiration compared to control	-	-2 to 7 %	-2 to 2 %	-2 to -7 %	2 to 7 %	-2 to 11 %

* Based on mean of six replicates for control and three for treatment rates.
** Range across three replicates.
Negative values indicate higher Oxygen consumption rate compared to controls.

III. CONCLUSION

The EC_{50} value of cinmethylin in the activated sludge respiration inhibition test was > 1000 mg a.s./L.

HSE evaluator comments:

It was noted that some of the validity criteria were not met as summarised above. The HSE evaluator has considered the justification provided by the study author and considers the study valid, particularly given the lack of effects observed at the highest test concentration and the acceptable reference test result.

Whilst there is some uncertainty as the test concentrations are around or above the limit of solubility for cinmethylin (solubility in deionised water of 69 mg a.s./L pH 8.9 and temperature of 20 °C) the HSE evaluator considers the study sufficiently valid to conclude particularly given the lack of effects compared to control at all test concentrations.

The above study was conducted to GLP and is considered valid. The following endpoint has been derived:

- Cinmethylin EC_{50} = > 1000 **mg a.s./l**

B.9.9. MONITORING DATA

No studies submitted.

B.9.10. BIOLOGICAL ACTIVITY OF METABOLITES POTENTIALLY OCCURRING IN GROUNDWATER

No studies submitted.

Scientific peer-reviewed open literature report

Literature reviews have been carried out for the active substance cinmethylin and its' metabolites. The reviews have been conducted in accordance with Article 8(5) of Regulation No. 1107/2009 and are based on the EFSA guidance document as published in EFSA Journal 2011; 9(2):2092.

The key objective of the submitted 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 cinmethylin and its metabolites. The initial search was conducted in July 2017, followed by an update in February 2018. All available CAS numbers of stereoisomers of Cinmethylin and metabolites have been included in the search profile.

Evaluation of studies
The process of selection of relevant scientific peer-reviewed open literature was done in two steps:

The First Selection step for relevance based on summary records (e.g. titles, abstracts, index terms, keywords) was done by the Agro Information Professionals.

- Obviously irrelevant records were tagged as "Ballast". This ballast was controlled by scientific experts in the corresponding subject areas but was not further processed.
- Summary records which appear to be relevant and those of unclear relevance were tagged as "Hit" and went to the next level of evaluation.

The Second Detailed Assessment was done by the scientific experts in the corresponding areas. Records tagged as "Hit" were further evaluated in depth. To facilitate a comprehensible listing of the "Hits" in Ecotoxicology and the other sections, an Excel file was generated for each section with 3 typical registers, namely:

- "no relevant endpoint"
- "evaluated - not-relevant"
- "used for dossier"

In a first step (rapid assessment) the "Hits" were reviewed based on the information given in the title and the abstract with regard to relevance for the regulatory endpoints in the respective regulatory area. Those records which were clearly judged as not assignable to any regulatory endpoint were shifted into the register "no relevant endpoint" with an explaining reasoning.

In a second step (detailed assessment), all remaining records were assessed in detail based on the complete report by the respective expert(s) and separated into relevant reports for further discussion and those clearly not relevant.

Reliability scoring was based on Klimisch *et al*., 1997. For ecotoxicology, in addition to the Klimisch criteria, more specific criteria for reliability and relevance are given by Kase *et. al*. (2012) and have been used for the assessment of literature data. The criteria by Kase *et al*. (2012) have been developed explicitly for the assessment of ecotoxicology studies in order to assure that only high-quality data is used for the risk assessment.

Databases Searched

Only three databases have been searched (CAPLUS, BIOSIS and CABA) potentially limiting the range of studies that could be located. The reason, provided by the applicant, why each database was selected is

presented in Table B.9.11-1. There was no time limitation on the date span searched for the review, and covered 1907, 1926 and 1973 to the day of the search for CAPLUS, BIOSIS and CABA respectively. This covers the minimum requirement of 10 years prior to the date of the search.

Table B.9.11.1-1: Databases searched as part of the cinmethylin literature review (ecotoxicology)

Database:	**CAPLUS Chemical Abstracts Plus**	**BIOSIS**	**CAB Abstracts**
Provider:	STN International	STN International	STN International
Justification for choosing the source: - for STN databases referring to STN database summary sheets	The Chemical Abstracts (CA) database covers all areas of Biochemistry, Chemistry and Chemical engineering, and related sciences. Sources include over 8,000 journals, patents from 38 national patent offices and two international patent organizations, technical reports, books, conference proceedings, and dissertations. Electronic only journals and Web preprints are also covered. Bibliographic terms, indexing terms, roles, CAS Registry Numbers, International Patent Classification, and abstracts are searchable.	BIOSIS Previews® is the largest and most comprehensive life science database in the world. Amongst others, subject coverage includes Agriculture, Biochemistry, Biophysics, Botany, Environmental Biology, Physiology, Toxicology. Sources include periodicals, journals, conference proceedings, reviews, reports, patents, and short communications. Nearly 6,000 life source journals, 1,500 international meetings as well as review articles, books, and monographs are reviewed for inclusion. Bibliographic information, indexing terms, abstracts, and CAS Registry Numbers are all searchable.	The CAB Abstracts database covers worldwide literature from all areas of agriculture and related sciences including Agriculture, Agricultural chemicals, Animal sciences and production, Crop protection, Crop sciences and production, Environment, Soils and fertilizers. Sources for CABA include journals, books, reports, published theses, conference proceedings, and patents. Bibliographic information, indexing terms, abstracts, and CAS Registry Numbers are searchable.
Date span of the source:	1907 – to present	1926 – to present	1973 – to present
Date of main search:	27th July 2017 Noting search was updated 20th February 2018*	27th July 2017 Noting search was updated 20th February 2018*	27th July 2017 Noting search was updated 20th February 2018*

Search parameters

The CAS numbers searched were extensive covering the active, metabolites and mixtures containing the active substance. For completeness those for the active and metabolites have been presented in the table below. Additionally, common and trade names for the active were searched for. The HSE evaluator notes that searching for the common names of relevant metabolites and mixtures may broaden the results.

Table B.9.11.1-2: CAS numbers used as search terms for cinmethylin and metabolites.

Group searched:	CAS numbers:
Cinmethylin including isomers and isotopes	87818-31-3 or 87819-60-1 or 87818-61-9 or 112502-84-8 or 99827-45-9 or 87818-68-6
Cinmethylin metabolites	(22555-57-3 or 22621-68-7 or 38630-76-1 or 50302-07-3 or 87172-89-2 or 87819-14-5 or 96645-97-5 or 99765-53-4 or 99765-60-3 or 103834-29-3 or 110901-97-8 or 119973-51-2 or 120053-26-1 or 120053-27-2 or 130772-87-1 or 134461-72-6 or 134461-73-7) (134527-97-2 or 134527-98-3 or 134527-99-4 or 134528-00-0 or 134528-01-1 or 134528-02-2 or 152453-46-8 or 152453-51-5 or 152453-52-6 or 152453-53-7 or 152519-96-5 or 152519-97-6 or 152519-98-7 or 152519-99-8 or 1334643-80-9 or 1933681-69-6)

The chemical terms were then partnered with search terms of relevance to ecotoxicology and the environment (see table B.9.11-2) and were considered sufficiently extensive by the HSE evaluator.

The search terms used are presented in Table B.9.11-3. It should be noted that for endocrine disruption a separate literature review has been conducted and evaluated within the CA section 9 dossier.

Table B.9.11-3: Search terms used to search selected databases.

Ecotoxicology area considered:	SUBSTANCES AND search terms shown below:
Ecotox general	• (bioavail? or biotransform? or biodegrad? or bioaccumul? or bio(w)accumul? or BAF or bioconcentrat? or bio(w)concentrat? or BCF or biomagnif? or bio(w)magnif? or BMF or biomonit? or food(1a)chain# or dietary(1a)exposur?) • ((bio# or biolog?)(w)(avail? or transform? or degrad? or accumulat? or concentrat? or concn# or magnif? or monitor?)) • (ecotox? or ecolog? or ecosystem? or biosph?) • ((eco or bio?)(w)tox? or biotoxic?) • (side(w)effect#) • (fauna# or microfauna# or microflora# or macrofauna# or macroflora# or mesofauna# or mesoflora# or (micro or macro or meso)(w)(fauna# or flora#) or bacteria# or (macro or micro)(w)organism# or macroorganism# or microorganism#) • (beneficial# or non(w)target or nontarget or predator# or predac!ous or natural(w)enem###) • (NOEC or NOEL or NOER or EC50 or ER50 or LD50 or LC50 or NOAEC or NOEAEC) • (species(w)sensitiv?(w)distribution# or SSD or SSDs) • (toxic?(w)(endpoint# or threshold#)) • ((lab?)(3a)(study or studies)) • ((lab or laboratory or field)(w)(condition# or test? or bioassay# or method# or assessment#)) • (test?(1a)chemical# or (Organization(1w)Economic(w)Cooperation(1w)Development or OECD or Office(1w)Prevention(w)Pesticides(1w)Toxic(w)Substances or OPPTS)(5a)(test? or guideline#))
Ecotox Wildlife	• (wildlife#) • (bird# or AVES or avian) • (duck# or mallard# or anas) • (chicken# or gallus or chick# or pullet# or hen#) • (blackbird# or merle# or ouzel# or Turdus and merula) • (thrush## or Turdidae or Turdus) • (Blackcap# or Sylvia and atricapilla or Sylvia or sylviid(w)warbler#) • (Black(w)Redstart# or Phoenicurus and ochruros or Phoenicurus) • (Blue(w)Tit# or Bluetit# or nun# or tomtit# or (Cyanistes or Parus) and caeruleus or Parus or Paridae or Parus) • (Chaffinch## or Fringilla and coelebs or Fringilla) • (dunnock# or prunella and modularis or Prunella or Hedge(w)(Sparrow# or Accentor# or Warbler#)) • (goldfinch## or gold(w)finch## or Carduelis and carduelis or Carduelis or Carduelinae) • (linnet# or (Acanthis or Carduelis) and cannabina or Carduelis or Carduelinae) • (Partridge# or pheasant# or Phasianus or Perdix)

Ecotoxicology area considered:	SUBSTANCES AND search terms shown below:
	• (Phasianidae or Phasianinae or Odontophorinae or Perdicinae) • (Pratincole# or Greywader# or grey(w)wader# or Glareola or Stiltia or Glareolidae) • (quail# or coturnix) • (Serin or Serins or Serinus) • (wagtail# or wag(w)tail# or Motacilla and alba or Motacillidae or Motacilla) • (warbler# or phylloscopus or Phylloscopidae) • (woodlark# or Lullula and arborea or lullula) • (Zebra(w)finch## or Taeniopygia and guttata or Taeniopygia or Poephila or estrildid(w)finch## or estrildidae or estrilididae) • (OECD(5a)205 or OECD205 or OECD(5a)206 or OECD206 or OECD(5a)416 or OECD416 or OPPTS(5a)850.2100 or OPPTS850.2100 or OPPTS(5a)850.2200 or OPPTS850.2200 or OPPTS(5a)850.2300 or OPPTS850.2300) • (rat or rats or rattus) • (rodent# or rodentia) • (muridae or murinae or mouse or mice or mus) • (rabbit# or hare# or lagomorph# or leporidae or lepus) • (vole# or microtus) • (shrew# or sorex or soricidae)
****Ecotox Terrestrial (below ground)****	• (invertebrat? or earthworm# or earth(w)worm# or lumbric? or eisenia) • (soil#(5a)(organism# or microorganism# or arthropod# or mite# or fung## or function# or respiration#) or nitrogen##(w)transform?) • (Collembola# or springtail# or spring(w)tail# or Folsomia or Entomobryidae or Isotomidae or Mesostigmata or Cryptostigmata or Hypoaspis) • (OECD(5a)207 or OECD207 or OECD(5a)216 or OECD216 or OECD(5a)217 OR OECD217 or OECD(5a)222 or OECD222 or OECD(5a)226 or OECD226 or OECD(5a)232 or OECD232 or ISO(5a)11268-3 or ISO11268-3 or ISO(5a)11267? or ISO11267? or ISO(5a)17512? or ISO17512?)
****Ecotox Terrestrial (above ground)****	• (Arachnid? or mite# or acari###) • ((web or wolf)(w)spider# or Lycosidae or Pardosa or Allopecosa or Lycosa or Pirata or Oribatidae or Phytoseiidae or Typhlodromus or Amblyseius or Phytoseius) • (insect# or bee or bees or honeybee# or apis or pollinator# or Bumblebee# or bumble(w)bee# or bombus or osmia or megachile or megachilidae) • (Vanessa or Parnassius or Aglais or Inachis or Papillio) • (Pieris or Lobesia or Ostrinia or Trichoplusia or Heliothis or Spodoptera or Mamestra) • (Parasitoid# or parasitic(w)wasp# or Aphidius or Aphidiinae or Braconidae or Aphelinidae or Aphelinus or Encarsia or Trichogrammatidae or Trichogramma) • (Chrysoperla or Chrysopidae or Chrysopa or Hermerobiidae) • (Anthocoridae or Anthocoris or Orius) • (Coccinellidae or Coccinella or Adalia or Harmonia or Calvia or Propylea) • (Carabidae or Poecilus or Carabus or Calosoma or Amara or Harpalus or Pterostichus or Abax)

Ecotoxicology area considered:	SUBSTANCES AND search terms shown below:
	• (Staphylinidae or Aleochara and bilineata) • (ESCORT(w)2 or ESCORT2 or ESCORT(w)II or ESCORTII)
****Ecotox Terrestrial (above ground - Standard Test Vascular Plants)****	• ((terrestri? or non(w)target or nontarget)(w)plant#) ((allium or porrum or vulgaris) and cepa or onion#) (avena and sativa or oat#) (beta and vulgaris or beet# or sugarbeet# or fodderbeet# or mangel# or redbeet# or beetroot# or redroot# or red(a)root#) (brassica and (napus or oleifera) or rape# or rapeseed# or colza# or canola#) (brassica and oleracea or cabbage# or kale# or cauliflower# or broccoli# or calabrese# or brussel#(a)sprout# or toy or kohlrabi# or (kale or stem or hungarian or rooted or cabbage)(a)turnip# or pakchoi# or pak(w)choi#) (cucumis and (sativus or esculentus) or cucumber) (daucus and carota and sativus or carrot#) (glycine and (max or hispida or soja) or phaseolus and max or ((dolichos or hispida) and soja) or sojbean# or sojabean# or soy or soya or soybean# or soyabean#) (lactuca and sativa or lettuce# or salad# or Iceberg lettuce# or oak leaf lettuce# or lollo rosso# or Batavian#) (linum and usitatissimum or flax##(a)(common or cultivated or linen) or linseed# or (lin or linnen)(a)seed#) (lycopersic## and (esculentum or lycopersicum) or solanum and lycopersicum or tomato## or love(w)apple#) (Lolium and perrenne or ryegrass## or rye(w)grass##) (pisum and sativum or pea or peas) (zea and (mays or vulgaris) or maize or corn) ---AND --- (Seedling(3a)emerg? or vegetative(w)vigo!r# or vigo!r# or plant(w)weight# or biomass or plant(w)survival# or phytotox? or phyto(w)tox? or NOEC or ER50 or Non(w)target(w)(plant# or weed# or crop#) or adjacent(w)crop#) • (OPPTS(5a)(850.4100 or 850.4150 or 850.4200 or 850.4225 or 850.4230 or 850.4250 or 850.4300) or OPPTS850.4100 or OPPTS850.4150 or OPPTS850.4200 or OPPTS850.4225 or OPPTS850.4230 or OPPTS850.4250 or OPPTS850.4300) • (OECD(5a)(208 or 227) or OECD208 or OECD227)
****Ecotox Aquatic****	• (mesocosm## or microcosm## or macrocosm## or (meso or micro or macro)(w)cosm##) • (ELINK or HARAP or aquatic(w)(exposure# or effect# or risk(w)assessment#)) • (hazard(w)concentration# or HC1 or HC(w)1 or HC5 or HC(w)5 or lower(w)limit#) • ((freshwater# or water# or aquatic or sediment#)(3a)(organism# or animal# or plant# or invertebrate# or macroinvertebrate# or biota# or arthropod# or insect# or snail#) or aquatic(w)environment? or pelagic or benth##) • (Chironomid# or chironomidae or Chironomus and riparius or Chaoborus) • (Crustace## or phyllopoda# or cladocer? or ?daphn? or waterflea?) • (Mysid(w)shrimp# or Mysidopsis and bahia or ?mysid?) • (Tubifex or benth?(1a)(oligochaet? or macrofauna# or macro(w)fauna#)) • (Oyster# or Crassostrea and virginica) • Procambarus

Ecotoxicology area considered:	SUBSTANCES AND search terms shown below:
	• (fish## or PISCES) • (pimephales or minnow#) • (cyprinodon and variegatus or (sheepshead or sheeps(w)head)(a)minnow#) • (cyprinus and carpio or carp#) • (Oncorhynchus and mykiss or rainbow(a)trout# or trout# or salmo and (gardneri or irideus)) • (lepomis and (auritus or macrochirus or gibbosus or cyanellus) or (orangespotted or readbreast or yellowbelly or green or pumpkinseed)(w)sunfish## or bluegill#) • (brachydanio and rerio or zebrafish## or danio or rerio) • (Amphibia# or tadpole# or xenopus or frog# or toad#) • (Rana or Bufo or Lithobates or Hyla or Bombina or Anura or Caudata or Salamand### or newt#) • (Batrachochytrium or chytrid?) • (reptile# or reptilia or reptiliae) • (?plankton? or ?alga or ?algae or chlorophyt? or Selenastrum or Pseudokirchneriella or Scenedesmus or Ankistrodesmus or Desmodesmus or Chlorella) • (Dinophyt? or flagellate#) • (bacillariophyc? or diatom? or Navicula) • (cyanobacteri? or Anabaena) • (Eugleno? or Euglena) • (Skeletonema or Periphyton) • (Cryptophy? or Chroomonas or cryptomonas or Ankyra) • (macrophyt? or macro(w)phyt? or lemna## or lemnaceae or Potamogeton or pondweed# or pond(w)weed# or Chara or Ceratophyllum or hornwort# or horn(w)wort# or elodea or waterweed# or water(w)weed#) • (submer? or emergent?) • (Water(w)milfoil# or Myriophyllum) • (Alga# or OECD(5a)201 or OECD201 or OPPTS(5a)850.5400 or OPPTS850.5400) • (Amphibian(w)metamorphosis(w)assay or Xenopus or OECD(5a)231 or OECD231 or OPPTS(5a)890.1100 or OPPTS850.1100) • (Chironomus(w)acute(w)spiked(w)sediment or OECD(5a)218 or OECD218) • (Chironomus(w)acute(w)spiked(w)water or OECD(5a)219 or OECD219 or OPPTS(5a)850.1790 or OPPTS850.1790) • (Chironomus(w)chronic or OECD(5a)233 or OECD233) • (Chironomus(w)acute or OECD(5a)235 or OECD235) • (Daphni#(w)acute or OECD(5a)202 or OECD202 or OPPTS(5a)850.1010 or OPPTS850.1010) • (Daphni#(w)chronic or OECD(5a)211 or OECD211 or OPPTS(5a)850.1300 or OPPTS850.1300) • (Fish(w)acute or OECD(5a)203 or OECD203 or OPPTS(5a)850.1075 or OPPTS850.1075) • (Fish(w)assay(w)21(w)d or OECD(5a)230 or OECD230) • (Fish(w)BCF or OECD(5a)305 or OECD305 or OPPTS(5a)850.1730 or OPPTS850.1730)

Ecotoxicology area considered:	SUBSTANCES AND search terms shown below:
	• (Fish(w)ELS or OECD(5a)210 or OECD210 or OPPTS(5a)850.1400 or OPPTS850.1400) • (Fish(w)FLC or OPPTS(5a)850.1500 or OPPTS850.1500) • (Fish(w)juvenile(w)28(w)d or OECD(5a)215 or OECD215) • (Fish(w)prolonged(w)14(w)d or OECD(5a)204 or OECD204) • (Fish(w)short(w)term(w)embryo or OECD(5a)212 or OECD212) • (Fish(w)short(w)term(w)reproduction(w)assay or OECD(5a)229 or OECD229) • (Lemna or OECD(5a)221 OECD221 or OPPTS(5a)850.4400 or OPPTS850.4400) • (Microcosm(w)generic or OPPTS(5a)850.1900 or OPPTS850.1900) • (Microcosm(w)site(w)specific or OPPTS(5a)850.1925 or OPPTS850.1925) • (Mysid(w)acute or OPPTS(5a)850.1035 or OPPTS850.1035) • (Mysid(w)chronic or OPPTS(5a)850.1350 or OPPTS850.1350) • (Oyster(w)acute or OPPTS(5a)850.1025 or OPPTS850.1025)

Relevance and reliability

The relevance of literature studies has been defined as the extent to which a test is appropriate for a particular hazard or risk assessment, the way a study can be used and the framework used for evaluation hence a study may be relevant in one framework but not in another. The following criteria have been used to evaluate the relevance of the literature data:

- **Selection**: Do not set criteria too narrow.
- **Evaluate**: Title, Abstract, and Full article, if needed.
- **Test material**: The test material is the primary criterion to consider (EFSA 2011, **Appendix A**; AGES 2013, **Appendix B**), because it determines the framework of each application for registration. Applying pure logic, only when the test material is concordant or identical to the one under evaluation, a peer-reviewed article should be further evaluated. However, this is a weak point of many published schemes is that they are inconclusive on the hierarchy of the proposed categories and criteria (e.g. Kase et al. 2012, Kase (2015).
- **Data requirements**: Consider Areas (**see section 5.2.5**) and related requirements being relevant for ecotoxicology (see **Attachment D**).
- **Endpoint / Descriptive result**: High / low probability of being relevant.
- **Field studies**: European conditions (climate, species, …) are relevant.
- **Secondary literature** (e.g. review, books, etc.): To be excluded.
- **Confirmatory data** (studies without new relevant data): To be excluded.
- **Non-European species** should not be excluded.
- **Additional criteria**: To be handled flexible - Meet the needs of the active substance.

The reliability of the study has been defined as the inherent quality of a study, thus the criteria will always be the same in whatever framework reliability is evaluated, the reporting quality of methodology, experimental procedure, and results (i.e. free from bias, findings reflect true facts), and the reproducibility of the study. According to these, the reliability of the study was concluded.

It was noted by the HSE evaluator that excluding secondary literature is not in line with EFSA 2011 guidance for literature reviews i.e. '*Peer-reviewed secondary research studies (i.e. reviews) may include bibliographic references to, or summaries of, potentially relevant primary research studies that address the data requirements under assessment. Potentially relevant primary research studies identified in reviews should be assessed individually for relevance as outlined above.*' In addition, it is unclear the basis for excluding confirmatory data. Nonetheless, the HSE evaluator agrees with the exclusions during detailed analysis (table B.9.11.1-6), noting none were due to being confirmatory data.

Results

The results of the search method employed by the applicant is presented in Table B.9.11.1-4.

Table B.9.11.1-4 Literature review search results for individual databases- ecotoxicology

Database	Total	CAPLUS	BIOSIS	CABA
Total number of summary records for cinmethylin and Metabolites retrieved:	**121**	61	24*	36
Total number of summary records after removing duplicates:	**94**	61	12*	21

* The search was updated on 20th February 2018, which resulted in two additional papers being retrieved from BIOSIS database which were dismissed at the initial assessment stage.

The total numbers obtained at each stage of the process, by the applicant, are summarised in the table below. It should be noted the HSE evaluator requested further information from the applicant regarding the literature search. There appeared to be a mismatch regarding numbers of studies in the excel sheet provided and the

submitted literature search report. Therefore, where appropriate the HSE evaluator has calculated the number of publications at each step in the table below based on the lists provided by the applicant.

Table B.9.11.1-5 Literature review search results for individual databases- ecotoxicology

Number of records excluded as 'Ballast' after assessment based on Title, Abstract and full article (if needed):	70*
Number of records assessed for Relevance based on Title, Abstract and full article (if needed):	24
Records considered non-relevant after detailed assessment of full-text documents: If a record contained multiple single studies (e.g. acute and chronic test), it is considered non-relevant if none of the single studies was considered relevant.	16
Number of records not relevant and not considered in detailed assessment:	4**
Number of records being potentially relevant after detailed analysis: Note: If a record contained multiple single studies (e.g. acute and chronic test), and at least one of them was considered Relevant, the record is Relevant.	4
Number of Relevant records being Reliable or Reliable with restrictions:	1

* Calculated by HSE evaluator based on additional papers retrieved during updated search
** Calculated by HSE evaluator based on excel sheet submitted by applicant.

The studies not considered relevant by the applicant after detailed analysis are shown in the table below.

Table B.9.11.1-6 Reason for exclusion of publications at detailed analysis stage.

Year	Title	Source	Reason(s) for not including this study in the dossier provided by applicant
1994	Effects of preemergence and postemergence herbicides on urea hydrolysis and nitrification of urea nitrogen in soil.	Biol. Fert. Soils (1994), 17, 309-313	*'Not relevant because the EC formulation used for testing the transformations of urea N is not specified and cannot be similar to or identical with the representative formulation BAS 684 03 H released in 2017.'*
1997	Significance and application of microbial toxicity tests in assessing ecotoxicological risks of contaminants in soil and sediment	Chemosphere (1997), 34(3), 455-499 CODEN: CMSHAF; ISSN: 0045-6535	*'Not relevant because this record is a review article.'*
2000	Effects of twenty-four herbicides on the growth of green algae Chlorella pyrenoidosa	Huanjing Huaxue (2000), 19(6), 518-522 CODEN: HUHUDB; ISSN: 0254-6108	*'Not relevant because the EC formulation used for testing the green algae Chlorella pyrenoidosais was not specified, has a lower content of active substance than the representative formulation BAS 684 03 H, and also cannot be similar to or identical with the representative formulation BAS 684 03 H, which was released in 2017.'*
2001	Acute Toxicity of 33 Herbicides to the Green Alga Chlorella pyrenoidosa	Bulletin of Environmental Contamination and Toxicology (2001), 66(4), 536-541 CODEN: BECTA6;	*'Not relevant because the EC formulation used for testing Chlorella pyrenoidosa is not specified, has a lower content of active substance than the representative formulation BAS 684 03 H, and also cannot be similar to or*

		ISSN: 0007-4861	*identical with the representative formulation BAS 684 03 H, which was released in 2017.'*
2017	Effect-Directed Analysis of Toxicants in Sediment with Combined Passive Dosing and in Vivo Toxicity Testing	Environmental Science + Technology (2017), 51(11), 6414-6421 CODEN: ESTHAG; ISSN: 0013-936X	*'Not relevant because this record reports on the identification of contaminants responsible toxicity to Chironomus dilutus of sediments from the Pearl River in Guangzhou, South China.'*
1987	Weed control and tolerances of chinese cabbage and chinese broccoli to pre and postemergence herbicides on mineral soils.	Proceedings of the Florida State Horticultural Society (1987), Volume 100, pp. 224-226, 2 refs. ISSN: 0886-7283	*'Not relevant because this record is from conference proceedings.'*
1986	Effects of cinmethylin on weed control and soybean growth.	Proceedings, 40th annual meeting of the Northeastern Weed Science Society. (1986), 61 p. Conference: Proceedings, 40th annual meeting of the Northeastern Weed Science Society.	*'Not relevant because this record is from conference proceedings hence was not peer reviewed.'*
1993	Analysis of cell elongation as a tool to determine herbicide effects on plant growth	Target Assays Mod. Herbic. Relat. Phytotoxic Compd. (1993), 203-9. Editor(s): Boeger, Peter; Sandmann, Gerhard. Publisher: Lewis, Boca Raton, Fla. CODEN: 59RDAI	*'Not relevant because this record is a book chapter hence not peer reviewed.'*
1986	Tolerances of several cole crops to pre and postemergence herbicides on mineral soils.	Proceedings of the Florida State Horticultural Society (1986), Volume 99, pp. 362-365, 5 refs. ISSN: 0886-7283	*'Not relevant because this record is from conference proceedings. Also, the reported vigour effects are for a mixture that is not intended for the EU (i.e. cinmethylin + oxyfluorfen), and the US mineral soils may not be representative for the EU.'*
2002	A quick, simple, and accurate method of screening herbicide activity using green algae cell suspension cultures	Weed Science (2002), 50(5), 555-559 CODEN: WEESA6; ISSN: 0043-1745	*'Not relevant because the EC formulation used for testing Chlorella pyrenoidosa is not specified, has a lower content of active substance than the representative formulation BAS 684 03 H, and also cannot be similar to or identical with the representative formulation BAS 684 03 H, which was released in 2017.'*
2000	Study on microscreening method to evaluate herbicidal activity using Chlorella pyrenoidosa	Nongyaoxue Xuebao (2000), 2(2), 29-34 CODEN: NXOUAS; ISSN: 1008-7303 Note BASF: Chinese Journal of Pesticide Science.	*'Not relevant because the EC formulation used for testing Chlorella pyrenoidosa is not specified, has a lower content of active substance than the representative formulation BAS 684 03 H, and also cannot be similar to or identical with the representative formulation BAS 684 03 H, which was*

			released in 2017.'
2012	Zebrafish developmental screening of the ToxCast Phase I chemical library	Reproductive Toxicology (2012), 33(2), 174-187 CODEN: REPTED; ISSN: 0890-6238	*'Not relevant, because the study provides no endpoint results for Zebrafish (Danio rerio) that would be applicable for a risk assessment under Reg. 1107 (2009). Also, Cinmethylin was demonstrated to exhibit a low potency for malformations in zebrafish embryos and larva.'*
2010	Endocrine profiling and prioritization of environmental chemicals using ToxCast data	Environmental Health Perspectives (2010), 118(12), 1714-1720 CODEN: EVHPAZ; ISSN: 0091-6765	*'Not relevant because (1) not peer reviewed, (2) record describes the endocrine profiles of the entire US EPA ToxCast library and provides a flexible ranking system by which chemicals may be prioritized for screening. Also, Cinmethylin is not mentioned in this record.'*
1995	Effect of herbicides on nitrogen fixation (C_2H_2 reduction) associated with rice rhizosphere	Chemosphere (1995), 30(2), 339-43 CODEN: CMSHAF; ISSN: 0045-6535	*'Not relevant because the EC formulation used for testing the nitrogenase activity is not specified, has a lower content of active substance than the representative formulation BAS 684 03 H, and also cannot be similar to or identical with the representative formulation BAS 684 03 H, which was released in 2017. Furthermore, the evaluated rice rizosphere appears not representative for anticipated uses in Europe.'*
1991	Influence of soil moisture on phytotoxicity of cinmethylin to various crops	Weed Science (1991), 39(3), 402-7 CODEN: WEESA6; ISSN: 0043-1745	*'Not relevant because for none of the plant species (Glycine max, Phaseolus vulgaris, Gossypium hirsutum, Archis hypogea, Cucumis sativus) a negative influence of 50% on the parameter plant height, fresh weight or yield could be detected at all treatments (up to 900 g a.s./ha). Therefore, no EC50 with additional influence on the outcome of the risk assessment for Non-target terrestrial plants can be calculated. In addition, the trail set up will not allow to separate positive effects on plant growth by weed reduction, from negative effects directly related due to herbicide action.'*
1987	Physiological activity spectra of existing graminicides and the new herbicide 2-(2-benzothiazolyloxy)-N-methyl-N-phenylacetamide (mefenacet)	Weed Research (1987), 27(3), 221-8 CODEN: WEREAT; ISSN: 0043-1737	*'Not relevant because (1) the record compares herbicides based on their PI50 values (negative log10 of the molar I50 concentrations) for Chlamydomonas reinhardii strain 11-32a/89. As a result, no endpoint results wre reported that could be used in a deterministic risk assessment under Regulation 1107 (2009). (2) the authors state that "PI50 values below 4, which would represent I50 values in excess of 100 µM, were not estimated because these concentrations are considered to be too high for the analysis of the*

			herbicidal activity." Regarding this, the PI50 value of Cinmethylin for Chlamydomonas reinhardii strain 11-32a/89 was given a < 4.0.'

The studies considered potentially relevant after detailed assessment are shown in the table below, their reliability was then assessed by applicant, and the outcome is shown in the far-right column.

Table B.9.11.1-7: Summary of the 4 potentially relevant literature studies identified after detailed analysis.

Data Point EU	**Authors**	**Title**	**Year**	**Source**	**Reliable?**
8.2.6.1	Ma, Jianyi Xu, Ligen Wang, Shufeng Zheng, Rongquan Jin, Shuihu Huang, Songqi Huang, Youjun	Toxicity of 40 Herbicides to the Green Alga *Chlorella vulgaris*	2002	Ecotoxicology and Environmental Safety (2002), 51(2), 128-132 CODEN: EESADV; ISSN: 0147-6513	*Factors affecting Reliability: (1) Test substance source not reported. (2) Test guideline not reported. (3) Exponentially growing cells tested not reported. (4) Stock solution use of water or an organic solvent, and the amount of not specified. (5) Control number of replicates not reported. (6) Test concentrations and their number not reported. (7) Control(s) not specified (type, number of replicates, solvent). (8) Cell densities not reported for different time-points. (9) Test concentration analytical verification not reported. (10) Biomass vs. growth rate not clearly specified. (11) Validity criteria of OECD 201 not reported. (12) Non-GLP. Conclusion: Because of the multiple factors affecting reliability, the article by Ma et al. (2002) is scored as "Not reliable"*
8.2.6.1 8.2.6.2	Schrader, Kevin K. de Regt, Marjan Q. Tidwell, Paula D. Tucker, Craig S. Duke, Stephen O.	Compounds with selective toxicity towards the off-flavor metabolite-producing cyanobacterium *Oscillatoria* cf. *chalybea*	1998	Aquaculture (1998), 163(1,2), 85-99 CODEN: AQCLAL; ISSN: 0044-8486	*Factors affecting Reliability: (1) Test guideline not reported. (2) Test concentration analytical verification not reported. (3) No biological replicates. (4) No statistics. (5) Biomass vs. growth rate not clearly specified. (6) Validity criteria of*

					OECD 201 not reported. (7) Non-GLP. Conclusion: Because of the multiple factors affecting reliability, the article by Schrader et al. (1998) is scored as "Not reliable".
8.2.6.1	Ma, J. Lin, F. Wang, S. Xu, L.	Acute toxicity assessment of 20 herbicides to the green alga *Scenedesmus quadricauda* (Turp.) Breb.	2004	Bulletin of Environmental Contamination and Toxicology (2004), 72(6), 1164-1171 CODEN: BECTA6; ISSN: 0007-4861	*Factors affecting Reliability: (1) Source of test material not reported. (2) Test guideline not reported. (3) Exponentially growing cells tested not reported. (4) Test concentrations not reported. (5) Solvent and related control not reported. (6) Cell densities not reported for different time-points. (4) Biomass vs. growth rate not clearly specified. (7) Cinmethylin test concentration analytical verification not reported. (8) Validity criteria of OECD 201 not reported. (9) Non-GLP. Conclusion: Because of the multiple factors affecting reliability, the article by Ma et al. (2004) is scored as "Not reliable".*
8.2.2.3	[redacted]	Bioaccumulation of cinmethylin in bluegill sunfish	1990	Journal of Agricultural and Food Chemistry (1990), 38(1), 323-7 CODEN: JAFCAU; ISSN: 0021-8561	*Factors affecting Reliability: None. Context with other records: This record by Lee by Lee at al. (1990) bases on the following BASF-owned study: CI-690-004, Forbis AD, 1983, Uptake, depuration and bioconcentration of 14C-SD 95481 by Bluegill sunfish (Lepomis macrochirus). Because this study will be included in the dossier and is more detailed than the record, it is refered to the evaluation of study CI-690-004 in the dossier.*

HSE evaluator summary:

Overall, the literature review is considered acceptable, noting the issues previously highlighted; number of databases searched and uncertainty about some of the relevance criteria i.e. secondary literature/ confirmatory data.

Only one publication (based on two study reports; [redacted] 1983b; along with supporting study Lee, 1984a) was considered to be reliable. These study reports have been assessed in section B.9.2.8 of this dossier. After detailed consideration the HSE evaluator considered these studies were not suitable for use in the risk assessment.

B.9.11. 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
KCA 8.1.1.1 /1	[redacted]	2016 a	BAS 684 H: Acute oral toxicity test (LD50) with northern bobwhite (Colinus virginianus) [redacted] yes Unpublished	Yes	Yes	Data for first approval	BASF
KCA 8.1.1.1 /2	[redacted]	1983 a	Acute oral LD50 - bobwhite quail sd95481 CI-505-001 [redacted] no Unpublished	Yes	No	Not applicable	BASF
KCA 8.1.1.3 /1	[redacted]	2016 a	BAS 684 H: A reproduction study with the northern bobwhite 2016/7009945 [redacted] yes Unpublished	Yes	Yes	Data for first approval	BASF

KCA 8.1.1.3 /2	[redacted]	2018 c	BAS 684 H: A reproduction study witht the mallard 2017/7016288 EAG Laboratories, Easton MD, United States of America yes Unpublished	Yes	Yes	Data for first approval	BASF
KCA 8.1.3/1	Simon, M	2019	Bioaccumulation of BAS 684 H in terrestrial oligochaetes 2019/1059201	No	Yes	Data for first approval	BASF
KCA 8.2.1/1	[redacted]	1983 a	Acute toxicity of technical sd95481 to rainbow trout *Salmo gairdneri* [redacted] no Unpublished	Yes	No	Not applicable Study **not** used in risk assessment	BASF
KCA 8.2.1/2	[redacted]	2017 a	BAS 684 H (Cinmethylin) - Acute toxicity study in rainbow trout (*Oncorhynchus mykiss*) 2017/1134335 [redacted] yes Unpublished	Yes	Yes	Data for first approval- study considered in risk assessment section	BASF
KCA 8.2.1/3	[redacted]	2017 a	BAS 684 H - Carp, acute toxicity test 2016/1063240 [redacted] yes Unpublished	Yes	Yes	Data for first approval- study considered in risk assessment section	BASF
KCA 8.2.1/4	[redacted]	2018 b	Amendment No. 1 to the final report - BAS 684 H - Carp, acute toxicity test 2018/1068368 [redacted] Pszczyna, Poland	No amendment to Rzodeczko (2017a) vertebrate study	Yes	Data for first approval	BASF

			yes Unpublished				
KCA 8.2.1/5	[redacted]	2017 b	BAS 684 H (Cinmethylin) - Acute toxicity study in the fathead minnow (*Pimephales promelas*) 2017/1111618 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	Yes	Yes	Data for first approval-study considered in risk assessment section	BASF
KCA 8.2.1/6	[redacted]	2018 a	Amendment 1: BAS 684 H (Cinmethylin) - Acute toxicity study in the fathead minnow (*Pimephales promelas*) 2018/1044871 [redacted] yes Unpublished	No amendment to [redacted] (2017b) vertebrate study	Yes	Data for first approval	BASF
KCA 8.2.1/7	[redacted]	1983 a	Acute toxicity of technical SD 95481 to sheepshead minnows (*Cyprinodon variegatus*) CI-511-001 [redacted] no Unpublished	Yes	No	Not applicable Study **not** used in risk assessment	BASF
KCA 8.2.1/8	[redacted]	1983 b	Acute toxicity of technical sd95481 to bluegill sunfish *Lepomis macrochirus* CI-511-002 [redacted] no Unpublished	Yes	No	Not applicable Study **not** used in risk assessment	BASF

KCA 8.2.1/9	[redacted]	1983 a	Dynamic acute toxicity of sd95481 to bluegill sunfish *Lepomis macrochirus* CI-512-001 [redacted] no Unpublished	Yes	No	Not applicable Study **not** used in risk assessment	BASF
KCA 8.2.1/10	[redacted]	1988 a	Cineole alcohol: Acute toxicity to rainbow trout *Salmo gairdneri* and *Daphnia magna* CI-570-001 [redacted] yes Unpublished	Yes	Yes	Data for first approval It should be noted this study was used as supporting information	BASF
KCA 8.2.2.1/1	[redacted]	1990 a	WL95481 (Argold): An early life stage test with the fathead minnow (*Pimephales promelas*) RAFINESQUE CI-512-002 [redacted] yes Unpublished	Yes	No	Not applicable Study **not** used in risk assessment	BASF
KCA 8.2.2.1/1	Kubitza J.	2019	Addendum to study BASF DocID: CI-512-002 WL95481 ("ARGOLD"): An early life stage test with the fathead minnow, Pimephales promelas (Rafinesque) 2019/2050451 BASF SE Agricultural Solutions – Global	No (re-analysis of vertebrate study [redacted] 1990a	Yes	Not applicable Study **not** used in risk assessment	BASF

			Ecotoxicology Speyerer Strasse 2 67117 Limburgerhof, Germany No Unpublished				
KCA 8.2.2.1 /2	[redacted]	2017 a	BAS 684 H (Cinmethylin) - Early-Life-Stage toxicity test on the fathead minnow (*Pimephales promelas*) in a flow through system [redacted] yes Unpublished	Yes	Yes	Data for first approval-study considered in risk assessment section	BASF
KCA 8.2.2.3 /1	[redacted].	1983 b	Uptake, depuration and bioconcentration of 14c sd95481 by bluegill sunfish *Lepomis macrochirus* CI-690-004 [redacted] no Unpublished	No	No	Not applicable-study not used in risk assessment	BASF
KCA 8.2.2.3 /2	Lee P.	1984 a	Characterisation of 14c residues in fish samples from the 14c sd95481 bluegill sunfish bioconcentration study CI-705-001 Shell Development Co., Modesto CA, United States of America yes Unpublished	No	No	Not applicable-study not used in risk assessment	BASF
KCA 8.2.2.3 /3	[redacted]	2017 b	14C-BAS 684 H - Bioconcentration study in the bluegil sunfish (*Lepomis macrochirus*) 2017/1156422 [redacted]	Yes	Yes	Data for first approval-study considered in risk assessment	BASF

			[redacted]. yes Unpublished				
KCA 8.2.2.3 /4	[redacted]	2018 a	Metabolism of 14C-BAS 684 H in Bluegill Sunfish (bioconcentration after exposure in a flow through system 2017/1208842 [redacted] yes Unpublished	Yes	Yes	Data for first approval-study considered in risk assessment	BASF
KCA 8.2.3/1	[redacted]	2020a	BAS 684 H - Amphibian Metamorphosis Assay with African Clawed Frog (*Xenopus laevis*) [redacted] yes Unpublished	Yes	Yes	Data for first approval-study considered in risk assessment	BASF
KCA 8.2.3/2	[redacted]	2020	Zebrafish (*Danio rerio*) - Short term reproduction assay, Flow through conditions [redacted] yes Unpublished	Yes	Yes	Data for first approval-study considered in risk assessment	BASF
KCA 8.2.4.1 /1	Forbis A. et al.	1983 c	Acute toxicity of sd95481 to *Daphnia magna* CI-521-001 ABC - Analytical Bio-Chemistry Laboratories Inc., Columbia MO, United States of America no Unpublished	No	No	Not applicable-study **not** used in risk assessment	BASF
KCA 8.2.4.1 /2	Haerthe N.	2016 a	Acute toxicity of BAS 684 H (Cinmethylin) to *Daphnia magna* STRAUS in a 48 hour static test 2016/1001943 BASF SE, Limburgerhof,	No	Yes	Data for first approval-study considered in risk assessment section	BASF

			Germany Fed.Rep. yes Unpublished				
KCA 8.2.4.1 /3	[redacted]	1988 a	Cineole alcohol: Acute toxicity to rainbow trout *Salmo gairdneri* and *Daphnia magna* CI-570-001 Sittingbourne Research Centre, Sittingbourne Kent ME9 8AG, United Kingdom yes Unpublished	Yes	Yes	Data for first approval-note study used as supporting information in risk assessment	BASF
KCA 8.2.4.1 /4	Turek T.	2018 a	Reg. No. 6055521 (Metabolite of BAS 684 H, M684H001) *Daphnia magna*, acute immobilisation test 2017/1069818 Institute of Industrial Organic Chemistry, Pszczyna, Poland yes Unpublished	No	Yes	Data for first approval-study considered in risk assessment section	BASF
KCA 8.2.4.1 /5	Turek T.	2018 b	Reg.No. 4539586 (Metabolite of BAS 684 H, M684H003) - *Daphnia magna*, acute Immobilisation test 2017/1069817 Institute of Industrial Organic Chemistry, Pszczyna, Poland yes Unpublished	No	Yes	Data for first approval-study considered in risk assessment section	BASF
KCA 8.2.4.2 /1	Pearson N.,Stephens on R.R.	1987 a	WL95481: Acute toxicity to *Gammarus pulex*, *Lymnaea stagnalis*, *Tubifex tubifex* and *Chironomus lugubris* CI-521-006 Sittingbourne Research Centre, Sittingbourne Kent ME9 8AG, United Kingdom yes Unpublished	No	Yes	Data for first approval-this study was used as supporting information in the risk assessment	BASF
KCA	Ward G.S.	1983 b	Acute toxicity of	No	No	Not	BASF

8.2.4.2 /2			technical SD 95481 to mysid shrimp (*Mysidopsis bahia*) CI-521-002 E G & G Bionomics, Pensacola FL, United States of America no Unpublished			applicable-study **not** used in risk assessment	
KCA 8.2.4.2 /3	Ward G.S.	1983 c	Acute toxicity of technical SD 95481 to fiddler crabs (*Uca pugilaton*) CI-521-003 E G & G Bionomics, Pensacola FL, United States of America no Unpublished	No	No	Not applicable-study **not** used in risk assessment	BASF
KCA 8.2.4.2 /4	Ward G.	1983 a	Acute toxicity of technical SD 95481 to embryos-larvae of eastern oysters (*Crassostrea virginica*) CI-521-004 E G & G Bionomics, Pensacola FL, United States of America no Unpublished	No	No	Not applicable-study **not** used in risk assessment	BASF
KCA 8.2.5.1 /1	Pearson N.,Girling A.	1989 a	WL95481: Chronic toxicity to *Daphnia magna* CI-523-001 Sittingbourne Research Centre, Sittingbourne Kent ME9 8AG, United Kingdom yes Unpublished	No	No	Not applicable-study **not** used in risk assessment	BASF
KCA 8.2.5.1 /2	Rzodeczko H.	2017 b	BAS 684 H - *Daphnia magna* reproduction test 2017/1000684 Institute of Industrial Organic Chemistry, Pszczyna, Poland yes Unpublished	No	Yes	Data for first approval-study considered in risk assessment section	BASF
KCA	Pearson	1987 b	WL95481: Acute	No	No	Not	BASF

8.2.6.1 /1	N.,Stephens on R.R.		toxicity to *Selenastrum capricornutum* CI-521-005 Shell Research Ltd., Sittingbourne Kent ME9 8AG, United Kingdom yes Unpublished			applicable-study **not** used in risk assessment	
KCA 8.2.6.1 /2	Kauf A.	2017 a	Effect of BAS 684 H (Reg.No.: 900202) on the growth of the green alga *Pseudokirchneriella subcapitata* 2016/1001944 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first approval-study considered in risk assessment section	BASF
KCA 8.2.6.2 /1	Kauf A.	2017 b	Effect of BAS 684 H (Reg.No.: 900202) on the growth of the blue alga *Anabaena flos-aquae* 2016/1001945 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first approval-study considered in risk assessment section	BASF
KCA 8.2.7/1	Vlechev S.	2017 a	Effect of BAS 684 H on the growth of *Lemna gibba* 2015/1029521 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first approval-study considered in risk assessment section	BASF
KCA 8.2.7/2	Vlechev S.	2017 b	Effects of BAS 684 H on the growth of the aquatic plant *Glyceria maxima* 2015/1029520 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first approval-study considered in risk assessment section	BASF
KCA 8.2.7/3	Rzodeczko H.	2017 c	BAS 684 H - Water-sediment *Myriophyllum spicatum* toxicity test	No	Yes	Data for first approval-study considered	BASF

			2017/1000221 Institute of Industrial Organic Chemistry, Pszczyna, Poland yes Unpublished			in risk assessment section	
KCA 8.2.7/3	Kubitza J.	2019	Addendum to study BASF DocID: 2017/1000221 BAS 684 H – Water-sediment *Myriophyllum spicatum* toxicity test 2019/2050444 BASF SE Agricultural Solutions – Global Ecotoxicology Speyerer Strasse 2 67117 Limburgerhof, Germany No Unpublished	No	Yes	Data for first approval	BASF
KCA 8.2.7/4	Rzodeczko H.	2018 a	BAS 684 H, water-sediment *Elodea canadensis* toxicity test 2017/1000222 Institute of Industrial Organic Chemistry, Pszczyna, Poland yes Unpublished	No	Yes	Data for first approval- Study used as supporting information in risk assessment	BASF
KCA 8.2.7/4	Kubitza J.	2019	Addendum to study BASF DocID: 2017/1000222 BAS 684 H – Water-sediment *Elodea canadensis* toxicity test 2019/2050445 BASF SE Agricultural Solutions – Global Ecotoxicology Speyerer Strasse 2 67117 Limburgerhof, Germany No Unpublished	No	Yes	Data for first approval	BASF
KCA 8.2.7/5	Rzodeczko H.	2017 d	BAS 684 H - Water-sediment	No	Yes	Data for first	BASF

			Egeria densa toxicity test 2017/1000224 Institute of Industrial Organic Chemistry, Pszczyna, Poland yes Unpublished			approval- Study used as supporting information in risk assessment	
KCA 8.2.7/5	Kubitza J.	2019	Addendum to study BASF DocID: 2017/1000224 BAS 684 H – Water-sediment *Egeria densa* toxicity test 2019/2050447 BASF SE Agricultural Solutions – Global Ecotoxicology Speyerer Strasse 2 67117 Limburgerhof, Germany No Unpublished	No	Yes	Data for first approval	BASF
KCA 8.2.7/6	Rzodeczko H.	2017 e	Reg.No. 6055521 (metabolite of BAS 684 H, M684H001) - *Lemna gibba* CPCC 310 growth inhibition test 2016/1224989 Institute of Industrial Organic Chemistry, Pszczyna, Poland yes Unpublished	No	Yes	Data for first approval- study considered in risk assessment section	BASF
KCA 8.2.7/7	Turek T.	2018 c	Reg.No. 4539586 (Metabolite of BAS 684 H, M684H003) - *Lemna gibba* CPCC 310 growth inhibition test 2017/1032136 Institute of Industrial Organic Chemistry, Pszczyna, Poland yes Unpublished	No	Yes	Data for first approval- study considered in risk assessment section	BASF
KCA 8.2.7/8	Rzodeczko H.	2017 f	Reg.No. 6055480 (metabolite of BAS 684 H, M684H004) - *Lemna gibba* CPCC 310 growth	No	Yes	Data for first approval- study considered	BASF

			inhibition test 2016/1224988 Institute of Industrial Organic Chemistry, Pszczyna, Poland yes Unpublished			in risk assessment section	
KCA 8.3.1.1.1/1	Franke M.	2016 a	Acute toxicity of BAS 684 H to the honeybee Apis mellifera L. under laboratory conditions 2016/1044853 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first approval	BASF
KCA 8.3.1.1.1/2	Amsel K.	2017 a	Acute toxicity of BAS 684 H to the bumblebee Bombus terrestris L. under laboratory conditions 2017/1140992 BioChem agrar GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first approval	BASF
KCA 8.3.1.1.1/3	Amsel K.	2018 a	Amendment No. 1 - Acute toxicity of BAS 684 H to the bumblebee Bombus terrestris L. under laboratory conditions 2018/1000903 BioChem agrar GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first approval	BASF
KCA 8.3.1.1.2/1	Franke M.	2016 a	Acute toxicity of BAS 684 H to the honeybee Apis mellifera L. under laboratory conditions 2016/1044853 BioChem agrar	No	Yes	Data for first approval	BASF

			Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished				
KCA 8.3.1.1.2/2	Amsel K.	2017 a	Acute toxicity of BAS 684 H to the bumblebee Bombus terrestris L. under laboratory conditions 2017/1140992 BioChem agrar GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first approval	BASF
KCA 8.3.1.1.2/3	Amsel K.	2018 a	Amendment No. 1 - Acute toxicity of BAS 684 H to the bumblebee Bombus terrestris L. under laboratory conditions 2018/1000903 BioChem agrar GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first approval	BASF
KCA 8.3.1.3/1	Kleebaum K.	2016 a	Repeated exposure of BAS 684 H to honey bee (Apis mellifera) larvae under labroratory conditions (in vitro) 2016/1044854 BioChem agrar GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first approval	BASF
KCA 8.3.1.3/2	Azevedo L.B.	2018 a	Further statistical evaluation of the study 2016/1044854 on chronic toxicity on honey bee larvae 2018/1099616 BASF SE, Limburgerhof, Germany Fed.Rep. no	No	No	Data for first approval	BASF

			Unpublished				
KCA 8.4.1/1	Friedrich S.	2016 b	Sublethal toxicity of BAS 684 H to the earthworm *Eisenia andrei* in artificial soil 2016/1044852 BASF SE 67056 Ludwigshafen Germany yes Unpublished	No	Yes	Data for first approval-study considered in risk assessment	BASF
KCA 8.5/1	Schulz L.	2016 a	Effects of BAS 684 H (Cinmethylin) on the activity of soil microflora (Nitrogen transformation test) 2016/1044850 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first approval-study considered in risk assessment	BASF
KCA 8.7/1	Friedrich S.	2016 a	Acute toxicity of BAS 684 H (Cinmethylin) to the earthworm *Eisenia andrei* in artificial soil with 10% peat 2016/1044851 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	No	Not applicable-study **not** used in risk assessment	BASF PS
KCA 8.7/2	Schulz L.	2016 b	Effects of BAS 684 H (Cinmethylin) on the activity of soil microflora (Carbon transformation test) 2016/1044848 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep.	No	No	Not applicable-study **not** used in risk assessment	BASF

			yes Unpublished				
KCA 8.7/3	Stackhouse S.C.	1983 a	The effects of sd95481 and sd96638 on microbial functions CI-690-001 no Unpublished	No	No	Not applicable-study **not** used in risk assessment	BASF
KCA 8.8/1	Hammer S.	2016 a	BAS 684 H (Cinmethylin) - Determination of the inhibition of Oxygen consumption in the activated sludge respiration inhibition test 2016/1062165 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first approval-study considered in risk assessment	BASF