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 (PPP) – BAS 684 03 H

Ecotoxicology

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Version History

When	What
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B.9. ECOTOXICOLOGY DATA AND ASSESSMENT OF RISKS FOR NON-TARGET SPECIES

B.9.1. EFFECTS ON BIRDS AND OTHER TERRESTRIAL VERTEBRATES

Background information

BAS 684 03 H is the representative formulation for the approval of the new herbicidal active substance BAS 684 H (also referred to as cinmethylin in this dossier). BAS 684 03 H is an EC (emulsifiable concentrate) formulation, containing 750 g BAS 684 H/L intended for the use in winter wheat and winter oilseed rape.

Enantiomeric ratios of cinmethylin and batches tested

All batches tested in the ecotoxicology studies in terms of impurities were considered comparable to the reference specification based on the technical equivalence check (see section C.1.5.2 of volume 4 for full details). The active substance cinmethyin is manufactured and placed on the market as a 50:50 racemic enantiomer mixture consisting of (-)-cinmethylin (Reg.No. 5925581) and (+)-cinmethylin (Reg.No. 5925632).

Where ratios were reported all batches used in the ecotoxicology studies and subsequently used in the risk assessment sections had a racemic composition of approx. 50:50 (see table 1.5-3, volume 4). Only one batch did not report the ratio, this was tested in a single study that was considered in the aquatic invertebrate risk assessment (Pearson & Stephenson, 1987a). It is unclear whether the ratios in this batch were representative of the active being placed on the market. However, this study has only been used as supporting information due to the analytical method not being sufficiently validated.

For each non-target organism group the change of ratios has been considered in volume 1, section 2.12.7. Sufficient information was provided to demonstrate an acceptable risk when considering enantiomers for proposed uses.

Environmentally significant metabolites

The following table provides a summary of the environmentally significant metabolites as identified in Section B.8 of Volume 3.

Table B.9-1 significant cinmethylin metabolites

Environmental Compartment	Metabolite(s)
Soil	None
Groundwater	None
Surface water	M684H001, M684H003
Sediment	None
Air	None

Uses

The following table outlines the intended uses of BAS 684 03 H.

Table B.9-2 Proposed use pattern of 'BAS 684 03 H'

Crop	Crop group	Application time (BBCH growth stage)	Number of applications	Interval [d]	Application rate per treatment	
					BAS 684 H [kg a.s./ha]	BAS 684 03 H [L/ha]
Winter wheat/barley	Bare soil	00-09	1	--	0.50	0.666
Winter wheat/barley		00-09	1	--	0.25	0.333
Winter oilseed rape		00-09	1	--	0.25	0.333
Winter wheat/barley	Cereals	10-29	1	--	0.50	0.666
Winter wheat/barley	Cereals	10-29	1	--	0.25	0.333
Winter oilseed rape	Oilseed rape	10-18	1	--	0.25	0.333

B.9.1.1. Effects on birds

Summary of endpoints

Table B.9.1.1-1: Toxicity endpoints for the risk assessment for birds for BAS 684 H

Species	Substance	Exposure System	Results	Reference (BASF DocID)
Acute toxicity				
Colinus virginianus	BAS 684 H	Oral, 1 d Acute	LD_{50} > 2000 mg a.s./kg b.w. **$LD_{50\ extrapolated}$ > 3776 mg a.s./kg b.w.**[1]	[redacted] (2016a) (2016/7005980)
Chronic toxicity				
Colinus virginianus	BAS 684 H	Dietary Reproductive toxicity	**NOEL = 99.1 mg a.s./kg b.w./d** NOEC = 1200 mg a.s./kg diet	[redacted], (2016a) (2016/7009945)
Anas platyrhynchos	BAS 684 H	Dietary Reproductive toxicity	NOEL = 174 mg a.s./kg b.w./d NOEC = 1200 mg a.s./kg diet	[redacted], (2018c) (2017/7016288)

Bold indicates endpoints used in risk assessment.

[1] Extrapolation according to EFSA (2009) Chapter 2.1.2. has been applied to the acute endpoint LD_{50} >2000 mg a.s./kg bw ([redacted], 2016a) since 10 animals were tested and there were no mortalities at the limit dose (extrapolation factor = 1.888).

Choice of acute avian endpoint for use in the risk assessment

Active substance

For birds one acute oral and two short-term dietary toxicity studies with the active substance are available. In the acute oral study with the bobwhite quail no mortality or sublethal effects occurred, resulting in a LD_{50} > 2000 mg a.s./kg b.w. ([redacted], 2016a). Short-term dietary toxicity studies have been conducted using both the bobwhite quail ([redacted], 2018a) and the mallard duck ([redacted], 2018b) to meet regulatory requirements outside Great Britain and the European Union. The results of these two studies do not indicate a higher toxicity compared to the acute route of dosing (gavage). As such these will not be relied upon for the risk assessment

The endpoints from the acute oral and the short-term dietary studies are above the highest doses or concentrations tested, indicating a low acute toxicity via the oral gavage and dietary route of exposure. As no increase in toxicity compared to the acute oral LD_{50} was seen in the short-term dietary studies, the results of the acute oral LD_{50} study are used to assess the acute risk from exposure to BAS 684 H. The endpoint (LD_{50} > 2000 mg a.s./kg b.w.) was extrapolated to LD_{50} = 3776 mg a.s. /kg b.w. according to EFSA/2009/1438 for use in the TER_A calculations. This is considered justified due to the absence of any mortality up to the highest dose with 10 tested individuals, and no signs of toxicity in surviving individuals.

The extrapolated LD_{50} of 3776 mg a.s./kg b.w. will be used in the acute risk assessment.

Formulation

An acute bird study was submitted on the representative formulation BAS 684 03 H (BASF Doc I.D. 2017/7016204). However this study is considered to be superfluous and has not been evaluated as the risk assessment can be undertaken using active substance data. This approach is supported by the fact that mammalian testing does not suggest that the formulation is of greater toxicity than the active substance alone (see B.9.1.3. Effects on vertebrates other than birds).

<u>Choice of chronic avian endpoints for use in the risk assessment</u>

Active substance

There are two 1-generation bird reproduction studies available for BAS 684 H. The study with the bobwhite quail resulted in a reproductive endpoint of NOAEL = 99.1 mg a.s./kg b.w./d ([redacted], 2016a) and the study with the mallard duck resulted in a NOAEL = 174 mg a.s./kg b.w./d ([redacted], 2018c) with no effects up to the top dose.

The lowest chronic endpoint, NOEL 99.1 mg a.s./kg b.w./d from the quail reproduction study is used in the chronic risk assessment.

Commission Regulation 283/2013 and 284/2013 require estimates of EC_X (e.g. EC_{10}, EC_{20}) together with the NOEL for chronic studies. The applicant provided the following reasoning why this could not be undertaken for the chronic bird studies which is accepted by the evaluator:

Since only three widely spaced dietary concentrations were tested, this study design is not suitable for calculating EC_x values. Additionally, the risk assessment and trigger values in EFSA/2009/1438 are based on the use of NOEL values. In both the mallard and the quail studies with BAS 684 H no effects were detected up to the highest tested dose. Thus, independent of the wide dose spacing an EC_x calculation is not possible.

B.9.1.3. Effects on terrestrial vertebrates other than birds

Summary of endpoints

The tables below provide acute and chronic mammalian toxicity endpoints from studies evaluated in Vol.3 B6.

Table B.9.1.3-1: Acute toxicity endpoints for the risk assessment for mammals

Species	Substance	Exposure System	Results	Reference [BASF Doc ID]
Acute active substance				
Rat (Female)	**BAS 684 H**	**Acute Oral**	**LD_{50} > 2000 mg a.s./kg b.w**	[redacted] **(2016) [2016/1273410] B.6.2.1 1)**
Rat (Male and Female)	SD 95481	Acute Oral	LD_{50} = 4550 mg/kg bw	[redacted] (1982) [CI-411-001] B.6.2.1 2)
Mouse (Male and Female)	BAS 684 H	Acute Oral	LD_{50} > 5000 mg/kg bw	[redacted] (1982) [CI-411-002] B.6.2.1 3)
Acute formulation				
Rat (Female)	BAS 684 03 H	Acute Oral	LD_{50} > 2000 mg formulation/kg b.w. (a.s. content: 741.0 g/L)	[redacted], 2017a 2017/1156822 B.6.1.1

Bold indicates endpoints to be used in the risk assessment.

Table B.9.1.3-2: Reproductive toxicity endpoints for the risk assessment for mammals

Endpoint	NOAEL (mg a.s./kg bw/d)	Reference	Studies to check
Body weight change[1], behavioural effects and systemic toxicity[2]	80 based on a decrease in body weight gain in females.	2-generation study (dietary) in the rat. [redacted], 2018a 2017/1094504 and [redacted], 2018 2018/1099151	Repeated dose 28-day oral toxicity study in rodents (OECD 407) Sub-chronic oral toxicity study-rodent 90 day study (OECD 408) Multi-generation study (OECD 416) Developmental studies (OECD 414)
	30 based on a decrease in body weight gain in females.	Pre-natal developmental toxicity study (oral gavage) in the rat. [redacted], 1984 CI-432-001	
	80 based on a decrease in body weight	Pre-natal developmental toxicity study (oral gavage) in the rabbit. [redacted], 2018 b 2015/1158053	

	gain in females.		
	Males: 201 **Females: 58 based on decreased body weight gain.**	**90-day oral (dietary) in the mouse.** [redacted]**, 2018b (2015/1005983)**	
Indices of gestation, litter size, pup and litter weight[3]	80 based on a decrease in fœtal weight.	Pre-natal developmental toxicity study (oral gavage) in the rabbit. [redacted], 2018 b 2015/1158053	Multi-generation study (OECD 416) Developmental studies (OECD 414)
Indices of viability, pre- and post-implantation loss	2000 based on post-implantation loss.	Pre-natal developmental toxicity study (oral gavage) in the rat. [redacted], 1984 CI-432-001	Multi-generation study (OECD 416) Developmental studies (OECD 414)
Embryo/foetal toxicity including teratological effects	300 based on increased incidence of anomalies (predominantly variations) and hydrocephaly at 2000)	Pre-natal developmental toxicity study (oral gavage) in the rat. [redacted], 1984 CI-432-001	Multi-generation study (OECD 416) Developmental studies (OECD 414)
Number aborting and number delivering early	No increase with treatment.	N/A	Multi-generation study (OECD 416) Developmental studies (OECD 414)
Systemic toxicity and effects on adult body weight	See above for 'Body weight change, behavioural effects and systemic toxicity'	N/A	Multi-generation study (OECD 416) Developmental studies (OECD 414)
Indices of post-natal growth[4], indices of lactation and data on physical landmarks	N/A	N/A	Multi-generation study (OECD 416) Developmental studies (OECD 414)
Survival and general toxicity up to sexual maturity	N/A	N/A	Multi-generation study (OECD 416) Developmental studies (OECD 414)

[1] Included as an indicator for parental effects which may disrupt reproduction.
[2] Effects derived from absorption of the substance that causes modification of an organ or an apparatus (biochemical, physiological and/or morphological). Examples include behavioural or physiological impairment (e.g. reduced locomotive activity, altered reflexes).
[3]Any effects in foetal body weight should be evaluated in the context of all pertinent data including other developmental effects as well as maternal toxicity.
[4] For example body weight gain, ear and eye opening, tooth eruption, hair growth and effects on sexual maturation such as age and body-weight at vaginal opening or balano-preputial separation.
Bold indicates endpoints to be used in the risk assessment.

Choice of acute mammalian endpoints for use in the risk assessment

Active substance

There is one active substance study and one formulation study, both with valid endpoints. As the endpoint from the formulation study does not indicate that the formulation is more toxic, the active substance endpoint of >2000 mg a.s./kg bw will be used in the risk assessment as opposed to that of the formulation expressed as active substance. Although it would technically be a lower endpoint, no mortalities occurred in the formulation study and the endpoint for this study is unbound. The higher active substance endpoint demonstrates that the LD_{50} exceeds the highest dose tested in the study i.e. 2000 mg a.s./kg bw.

The acute endpoint for use in the risk assessment is >2000 mg a.s./kg bw.

Choice of chronic mammalian endpoint for use in the risk assessment

Active substance

Mammalian toxicity studies have been evaluated by the toxicology specialist (see Volume 3 B.6. for full evaluations). and have been considered below in order to derive the chronic mammalian endpoint (see Table B.9.1.3-2 for a summary).

The applicant proposed that the 2-generation dietary study in the rat derived from [redacted] (2018) (2017/1094504) and [redacted] (2018) (2017/1094504) was the appropriate study to set the endpoint for reproductive toxicity in mammals. The NOAEL from this study was 80 mg a.s./kg bw/d based on a reduction of body weight in F0 females. It is agreed that this is an ecotoxicologically relevant affect that could have potential impact on competition, fitness and hence the ability to reproduce. This endpoint was based more specifically on effects seen at the top dose tested of 394 mg/kg bw/d; up to a 5% reduction in F0 female body weight during the gestation period and a 6% reduction during the lactation phase, both statistically significant reductions. With regards to F0 female body weight gain, at the same top dose during the first 7 days of exposure there was a 20% reduction and overall during the gestation period there was a 10% reduction. Conversely, during the lactation phase bodyweight gain was increased at the top dose (to 123.8% of the control which was statistically significant) during days 4-7, however overall the increase was 116.3% of the control and not statistically significant. It was noted that these effects were seen only in the F0 female generation and not the F1 and males were not considered in the study.

The NOAEL from the pre-natal developmental toxicity study (oral gavage) in the rabbit [redacted] 2018; 2015/1158053) was 80 mg/kg bw/d both for maternal and developmental toxicity. With regards to maternal effects, this endpoint was selected based on changes in bodyweight gain and systemic toxicity. At the dose above the NOAEL (250 mg/kg bw/d) bodyweight gain was reduced by 40.9% (from days 6-28 i.e. over the whole dosing period). During days 6-9 (initial dosing period) and from days 16-19 the percentage of reduction in bodyweight gain was 68.8% and 200% respectively; both statistically significant reductions. Over whole study body weight gain was reduced by 22.3% at this dose. With regards to systemic toxicity, effects on maternal liver weight were observed at 250 mg/kg bw/d namely 18% absolute increase and 21% relative increase (considering animal body weight). Both were considered statistically significant. At the NOAEL concentration of 80 mg/kg bw/d there was a 13% increase in total weight and 12 % increase in relative weight; both statistically significant effects. However in the toxicology assessment a 15% effect trigger is used and hence this was not considered to be a strong enough effect to push the NOAEL down past 80 mg/kg bw/d. The ecological impact of increased liver weight on wild mammals is uncertain. The only known impact would be an overall weight increase of the animal which would be expected to be minimal in this instance when only one organ is increased to this degree. Therefore for the purpose of the ecotoxicology assessment, this effect is not considered to impact the NOAEL based on systemic effects. Finally with regards to developmental toxicity, a decrease in mean foetal weight at 250 mg/kg bw/d and 320 mg/kg bw/d of 14.4% and 11.2% respectively resulted in the NOAEL of 80 mg/kg bw/d where the decrease was 0.3%.

The two NOAEL endpoints derived from the 90-day oral (dietary) in the mouse [redacted] 2018b) were 58 mg/kg bw/d for females and 43 mg/kg bw/d for males based on decreased body weight gain (in females) and liver toxicity effects including increased liver weight observed at the mid dose (201 mg/kg bw/d for males and 285 mg/kg bw/d for females). The NOAEL for body weight effects in males was 201 mg/kg bw/d based on a reduction of body weight gain at the top dose of 1200 mg/kg bw/d for the three periods of time (days 0-77, 0-84 and 0-91) – during all of these the percentage reduction ranged from 26.5-27.9% and was statistically significant. For female body weight gain, the endpoint was set at 58 mg/kg bw/d due to effects at the mid dose of 285 mg/kg bw/d. During the first 4 weeks there was a statistically significant decrease in body weight gain of 43.5% (from days 0-21) and when a longer period in the study was considered (days 0-28) this effect lessened to 32.6% but remained statistically significant. With regards to effects on the liver, there was a statistically significant increase absolute and relative weight seen in males at the mid dose of 1000 ppm (201 mg/kg bw/d) ; respectively 8.8% and 9.6%. At the top dose of 5000 ppm (1200 mg/kg bw/d) the same respective increases were 21.1% and 30.5%. In females at the mid dose of 1000 ppm (285 mg/kg bw/d) there was a statistically significant increase in relative liver weight (8.6%) and at the top dose of 5000 ppm (1304 mg/kg bw/d) there was a statistically significant increase in absolute and relative liver weight (20% and 24.2% respectively). In males there were also significant changes in clinical chemistry parameters indicative of liver toxicity at the mid dose of 1000 ppm (201 mg/kg bw/d).

The endpoint from the pre-natal developmental toxicity study in the rat [redacted], 1984) resulted in a NOAEL for human health assessment that was the lowest relevant endpoint provided in Table B.9.1.3-2; 30 mg/kg bw/d. In this study pregnant females were administered the active substance by oral gavage on days 6-15 of gestation. The endpoint was based on a statistically significant reduction in body weight gain (24.7%) during gestation days 6-12 at 300 mg/kg bw/d, however the effect is reduced and not statistically significant in the same dose group later on in the study (when considering days 6-16; 13.2% and days 6-20; 6.8%) which indicates a recovery of this effect and it is also reduced when the entire gestation period of days 0-20 is considered (6.5% reduction in body weight gain and not statistically significant). A similar pattern is observed in the dose above of 1000 mg/kg/d at the same time points. No recovery in the reduction of body weight change is observed at the top dose of 2000 mg/kg bw/d and it is apparent throughout the gestation period. A reduction in body weight was only present at the top dose on days 12, 16 and 20. A NOEL of 2000 mg/kg bw/d for post-implantation loss referenced in Table B.9.1.3-2 was also derived from this study as well as a NOAEL of 300 mg/kg bw/d for embryo/foetal toxicity including teterological effects due to increased incidence of anomalies (predominantly variations) and hydrocephaly at the 2000 mg/kg bw/d dose. In conclusion therefore HSE proposes an ecotoxicologically relevant NOAEL from this study of 300 mg a.s./kg.bw/day due to the bodyweight reduction seen at 300 mg/kg/day in females being temporally limited and when considering the entire gestation length the extent of effect is not considered relevant to adversely impact population performance.

As well as the standard studies used in the derivation of the chronic mammal endpoint, carcinogenicity studies on the rat and mouse also indicated that bodyweight effects was a sensitive parameter.

The chronic endpoint proposed for use in the risk assessment is 58 mg/kw bw/d based on adverse effects on body weight gain from the 90-day oral (dietary) in the mouse ([redacted], 2018b).

B.9.2. RISK ASSESSMENT FOR BIRDS AND OTHER TERRESTRIAL VERTEBRATES

B.9.2.1. Risk assessment for birds

Acute toxicity to birds

Table B.9.2.1-1: BAS 684 H: Screening step of the acute risk for birds due to the use of BAS 684 03 H for the crop group "bare soil" at 1 x 250 g a.s./ha

	Crop	**Application rate (Kg a.s./ha)**	**Number of applications**	**Application Interval**	**DT_{50}**	**LD_{50}**	
	Bare soil	0.25	1	365	10.0	>3776	
Screening step:							
Acute risk assessment screening step	**Indicator species**	**Shortcut value**	**Daily Dietary Dose (single)**	**MAF (90)**	**Daily Dietary Dose (Multiple)**	**TER**	**No refinement step required**
	Small granivorous bird	25.3	6.325	1.0	6.325	>597	

Table B.9.2.1-2: BAS 684 H: Screening step of the acute risk for birds due to the use of BAS 684 03 H for the crop group "bare soil" at 1 x 500 g a.s./ha

	Crop	**Application rate (Kg a.s./ha)**	**Number of applications**	**Application Interval**	**DT_{50}**	**LD_{50}**	
	Bare soil	0.5	1	365	10.0	>3776	
Screening step:							
Acute risk assessment screening step	**Indicator species**	**Shortcut value**	**Daily Dietary Dose (single)**	**MAF (90)**	**Daily Dietary Dose (Multiple)**	**TER**	**No refinement step required**
	Small granivorous bird	25.3	12.65	1.0	12.65	>298.5	

Table B.9.2.1-3: BAS 684 H: Screening step of the acute risk for birds due to the use of BAS 684 03 H for the crop group "cereals" at 1 x 250 g a.s./ha

	Crop	**Application rate (Kg a.s./ha)**	**Number of applications**	**Application Interval**	**DT_{50}**	**LD_{50}**	
	Cereals	0.25	1	365	10.0	>3776	
Screening step:							
Acute risk assessment screening step	**Indicator species**	**Shortcut value**	**Daily Dietary Dose (single)**	**MAF (90)**	**Daily Dietary Dose (Multiple)**	**TER**	**No refinement step required**
	Small omnivorous bird	158.8	39.7	1.0	39.7	>95.1	

Table B.9.2.1-4: BAS 684 H: Screening step of the acute risk for birds due to the use of BAS 684 03 H for the crop group "cereals" at 1 x 500 g a.s./ha

	Crop	**Application rate (Kg a.s./ha)**	**Number of applications**	**Application Interval**	**DT_{50}**	**LD_{50}**	
	Cereals	0.5	1	365	10.0	>3776	
Screening step:							
Acute risk assessment screening step	**Indicator species**	**Shortcut value**	**Daily Dietary Dose (single)**	**MAF (90)**	**Daily Dietary Dose (Multiple)**	**TER**	**No refinement step required**
	Small omnivorous bird	158.8	79.40	1.0	79.40	>47.6	

Table B.9.2.1-5: BAS 684 H: Screening step of the acute risk for birds due to the use of BAS 684 03 H for the crop group "oilseed rape" at 1 x 250 g a.s./ha

	Crop	**Application rate (Kg a.s./ha)**	**Number of applications**	**Application Interval**	**DT_{50}**	**LD_{50}**	
	Oilseed rape	0.25	1	365	10.0	>3776	
Screening step:							
Acute risk assessment screening step	**Indicator species**	**Shortcut value**	**Daily Dietary Dose (single)**	**MAF (90)**	**Daily Dietary Dose (Multiple)**	**TER**	**No refinement step required**
	Small omnivorous bird	158.8	39.70	1.0	39.70	>95.1	

All TERs at the screening step exceed the trigger value of 10 demonstrating acceptable acute risk to birds from the active substance according to the GAP. No further consideration is required.

Long-term/reproductive toxicity to birds

Table B.9.2.1-6: BAS 684 H: Screening step of the long-term/reproductive risk for birds due to the use of BAS 684 03 H for the crop group "bare soil" at 1 x 250 g a.s./ha

	Crop	**Application rate (Kg a.s./ha)**	**Number of applications**	**Application Interval**	**DT_{50}**	**Reproductive End Point (mg/kg b.w./d)**	**Time weighted average (TWA)**
	Bare soil	0.25	1	365	10	99.1	0.53
Screening step:							
Reproductive risk assessment screening step	**Indicator species**	**Shortcut value**	**Daily Dietary Dose (single)**	**MAF mean**	**Daily Dietary Dose (Multiple)**	**TER**	**No refinement step required**
	Small granivorous bird	11.4	1.51	1.0	1.51	65.61	

Table B.9.2.1-7: BAS 684 H: Screening step of the long-term/reproductive risk for birds due to the use of BAS 684 03 H for the crop group "bare soil" at 1 x 500 g a.s./ha

	Crop	**Application rate (Kg a.s./ha)**	**Number of applications**	**Application Interval**	**DT_{50}**	**Reproductive End Point (mg/kg b.w./d)**	**Time weighted average (TWA)**
	Bare soil	0.5	1	365	10	99.1	0.53
Screening step:							
Reproductive risk assessment screening step	**Indicator species**	**Shortcut value**	**Daily Dietary Dose (single)**	**MAF mean**	**Daily Dietary Dose (Multiple)**	**TER**	**No refinement step required**
	Small granivorous bird	11.4	3.02	1.0	3.02	32.8	

Table B.9.2.1-8: BAS 684 H: Screening step of the long-term/reproductive risk for birds due to the use of BAS 684 03 H for the crop group "cereals" at 1 x 250 g a.s./ha

	Crop	**Application rate (Kg a.s./ha)**	**Number of applications**	**Application Interval**	**DT_{50}**	**Reproductive End Point (mg/kg b.w./d)**	**Time weighted average (TWA)**
	Cereals	0.25	1	365	10	99.1	0.53
Screening step:							
Reproductive risk assessment screening step	**Indicator species**	**Shortcut value**	**Daily Dietary Dose (single)**	**MAF mean**	**Daily Dietary Dose (Multiple)**	**TER**	**No refinement step required**
	Small omnivorous bird	64.8	8.59	1.0	8.59	11.54	

Table B.9.2.1-9: BAS 684 H: Screening step of the long-term/reproductive risk for birds due to the use of BAS 684 03 H for the crop group "cereals" at 1 x 500 g a.s./ha

	Crop	**Application rate (Kg a.s./ha)**	**Number of applications**	**Application Interval**	**DT_{50}**	**Reproductive End Point (mg/kg b.w./d)**	**Time weighted average (TWA)**
	Cereals	0.5	1	365	10	99.1	0.53
Screening step:							
Reproductive risk assessment screening step	**Indicator species**	**Shortcut value**	**Daily Dietary Dose (single)**	**MAF mean**	**Daily Dietary Dose (Multiple)**	**TER**	**No refinement step required**
	Small omnivorous bird	64.8	17.17	1.0	17.17	5.8	

Table B.9.2.1-10: BAS 684 H: Screening step of the long-term/reproductive risk for birds due to the use of BAS 684 03 H for the crop group "oilseed rape" at 1 x 250 g a.s./ha

	Crop	**Application rate (Kg a.s./ha)**	**Number of applications**	**Application Interval**	**DT_{50}**	**Reproductive End Point (mg/kg b.w./d)**	**Time weighted average (TWA)**
	Oilseed rape	0.25	1	365	10	99.1	0.53
Screening step:							
Reproductive risk assessment screening step	**Indicator species**	**Shortcut value**	**Daily Dietary Dose (single)**	**MAF mean**	**Daily Dietary Dose (Multiple)**	**TER**	**No refinement step required**
	Small omnivorous bird	64.8	8.59	1.0	8.59	11.54	

All TERs at the screening step exceed the trigger value of 5 demonstrating acceptable chronic risk to birds from the active substance according to the GAP. No further consideration is required.

Effects of secondary poisoning on birds

According to EFSA/2009/1438 an assessment of the potential risk of secondary poisoning is triggered with a log Kow of greater than 3. The log Kow of the active substance BAS 684 H is 4.5 at 20°C and pH=7 (see Volume 3 CA B.2.). Hence, the risk of secondary poisoning will be assessed to fish- and earthworm-eating birds.

Food chain from fish to fish-eating birds

The risk assessment for fish-eating birds is based on the worst-case PEC_{sw} derived from the Environmental Fate section (see section Volume 3 B.8.). The BCF value confirmed to be correct was 707 which will be used in the risk assessment below.

The calculations and the resulting TER_{LT} values are presented in Table B.9.2.1-11.

Table B.9.2.1-11: Risk assessment for the active substance BAS 684 H concerning fish-eating birds

Parameter	BAS 684 H	Reference
PEC_{sw} [mg/L] [1]	0.026923	Volume 3 CA B.8.
BCF_{fish} [5]	707	Volume 3 CP B.9.3.2.
PEC_{fish} [mg/kg] [2]	19.03	--
Daily dose [mg/kg b.w./d] [3]	3.03	--
NOEL [mg/kg b.w./d]	99.1	See above
TER_{LT} [4]	32.74	--

1 Highest PEC_{sw} value resulting from Tier 1 drainflow calculations which was worst case when considering spray drift and drainflow. For details see Volume 3 CA B.8.

2 $PEC_{fish} = PEC_{sw} \times BCF$

3 Daily dose = 0.159 x PEC_{fish}

4 TER_{LT} = NOEL / Daily dose

5 Highest BCF_{fish} used as worst-case as selected from the results of two studies (BASF DocID CI-690-004 + CI-705-001; 2017/1156422 and 2017/1208842).

The TER_{LT} exceeds the trigger of 5 demonstrating acceptable risk to fish-eating birds. No further consideration is required.

Food chain from earthworm to earthworm-eating birds

The risk assessment for earthworm-eating birds will be based on the worst case 21 day PEC_{soil} twa values derived from the environmental fate section (see Volume 3 B.8.). The calculations and the resulting TER_{LT} values are summarized in Table B.9.2.1-12.

Table B.9.2.1-12: Risk assessment for the active substance BAS 684 H concerning earthworm-eating birds

Parameter	Bare soil and Cereal	Bare soil and Cereal	Oilseed rape	Reference (Section or BASF DocID)
	Application rate			
	500 g a.s./ha	250 g a.s./ha	250 g a.s./ha	
PEC_{soil} [mg/kg soil]	0.662 [1]	0.331 [2] [8]	0.331 [2]	Volume 3 CP B.8.
K_{ow}	31623	31623	31623	Volume 3 CP B.2.[3]
K_{oc} (geometric mean, n = 5)	282.39	282.39	282.39	Volume 3 CP B.8.[7]
f_{oc} (default)	0.02	0.02	0.02	EFSA/2009/1438
BCF [4]	67.34	67.34	67.34	--
PEC_{worm} [5]	44.58	22.29	22.29	--
Daily dose [mg/kg b.w./day] [6]	46.81	23.40	23.40	--
NOEL [mg/kg b.w./day]	99.1	99.1	99.1	See above
TER_{LT}	**2.12**	**4.24**	**4.24**	--

Bold indicates a TER that fails the risk assessment (<5)

1 Worst-case 21 day twa PEC_{soil} twa from applications to winter cereals at pre-emergence with a single application rate of 500 g a.s./ha. For details see section Volume 3 CP B.8.

2 Worst-case 21 day twa PEC_{soil} value calculated from applications to winter cereals at pre-emergence as worst-case scenario with application rate of 250 g a.s./ha. For details see section Volume 3 CP B.8.

3 K_{ow} recalculated from $logK_{ow}$ of 4.5 (see Volume 3 CP B.2.)

4 Bioconcentration factor (BCF) = $(0.84 + 0.012 * K_{ow}) / f_{oc} * K_{oc}$

5 $PEC_{worm} = PEC_{soil} * BCF$

6 Daily dose = 1.05 (default value for birds) * PEC_{worm}

7 K_{oc} is a geometric mean from 5 soils (see Volume 3 CP B.8.)

8 This covers the GAP for bare soil and cereals at the application rate of 250 g a.s./ha.

All TER_{LT} values for all application scenarios are below the trigger of 5 and therefore need further consideration to be refined.

Higher tier risk assessment for earthworm-eating birds

The applicant proposed higher tier refinements to address the outstanding risk to earthworm-eating birds. It should be noted that in the applicant's dossier only the cereals use failed the risk assessment at tier 1, however due to PECsoil values differing between the applicant's dossier and HSE's assessment, the risk fails for all three GAP uses.

The proposed higher tier refinements comprised of firstly a qualitative statement summarising data from animal metabolism and fish bioconcentration studies. The applicant proposed to extrapolate metabolism data on vertebrates to earthworms which is not agreed with by the HSE evaluator since there is no data to indicate that the metabolisms of vertebrates mentioned (rats, hens and goats) are comparable to that of an earthworm. In addition, the applicant has compared the bioconcentration in earthworms to bioconcentration in fish which is not considered to be acceptable. The former is dependent of the availability of the substance in the soil. This availability is dependent on the Foc and Koc, parameters that are not involved in the uptake phase for fish. Furthermore, the lipid content of earthworms is different than the lipid content of fish. The qualitative assessment provided by the applicant is therefore not accepted by the HSE evaluator.

The second aspect of the applicant's proposed higher tier refinement is two quantitative refinements; one based on the proposal of applying a correction factor of 5.6 to the BCF value of the mechanistic model by Jager (1998); the model used in EFSA/2009/1438 for the calculation of BCFs in earthworms. The second presents a further refined risk assessment using bird focal species in arable fields and assuming a conservative proportion of earthworms in their diet.

The proposal of using the correction factor (dividing the earthworm BCF by a factor of 5.6) outlined in Jager (1998)[1] is not accepted by the HSE evaluator. The study states "*this estimate should be regarded as a maximum BCF that is not always reached in soil situations. The theoretical model also seems sufficiently protective to cover most field situations but special care must be taken in case of pesticide spraying. The heterogeneous vertical distribution of chemical in soil or the specific contamination of food sources may result in high exposure for specific species. As an example, L. terrestris a litter feeder that constructs semipermanent burrows is more susceptible to chemicals present in litter and granular pesticide formulations, but less susceptible to chemicals incorporated in the top soil layer, compared to shallow-living soil feeders such as A. caliginosa. The ecology of individual species can thus be a dominant factor influencing body residues in field*". Based on this information the factor of 5.6 cannot be applied as proposed since the BCF will likely then be underestimated for litter feeders. In addition the EFSA Bird and Mammal Guidance Document (2009) does not specify that this correction factor can be applied as a refinement option. Refinement options in addition to those presented in section 6 of the guidance could be "*to carry out a BCF study with earthworms or modelling the internal body burden of earthworms by using information on uptake and elimination kinetics in earthworms as well as information on dissipation kinetics in soil*". Based on this information, the above proposed quantitative refinement for earthworm eating birds is not acceptable.

The applicant proposed to use a paper (Dietzen *et al*., 2017) to refine the proportion of food (i.e. earthworms) in a bird's diet through the identification of focal species for the specified GAP and then by looking at data from the study on birds observed diets. HSE considered this document and decided to use elements of this assessment as well as information in Crocker and Irving, 1999[2]. The reason for using Crocker and Irving (1999) is two-fold, firstly it is relevant for Great Britain and has been previously used in UK higher tier risk assessment. Secondly, whilst Dietzen is a very comprehensive review, it covers a wide range of species, some of which are not relevant to Great Britain. Furthermore, it references many texts which are currently not available to HSE, hence it was felt more appropriate to use both sources of information to try to determine what species that consume earthworms could occur on either cereal or oilseed rape fields in Great Britain.

Identifying focal Species

Crocker and Irving (1999) describes a project in which bird surveys were conducted on more than 200 arable fields in the UK in order to identify specific bird species likely to be present in different crops. The arable crops surveyed were oilseed rape, sugar beet, winter barley and winter wheat with approximately 20 fields of each type selected. Each received an average of five surveys between November 1998 and December 1999. Bird presence was expressed as the average number (abundance) of birds counted on a particular field type and the average percentage presence (prevalence).

Earthworm-eating focal species for winter wheat for bare soil (BBCH 00-09) and BBCH 10-29

The table below provides bird species observed in winter wheat during each season that were most prevalent and abundant according to Crocker and Irving (1999).

[1] Jager, T. 1998. Mechanistic approach for estimating bioconcentration of organic chemicals in earthworms (Oligochaeta). *Environ. Toxicol. Chem*. 17:2080–2090.

[2] Crocker, D.R. & Irving P.V. Contract PN0915 : Improving Estimates of Wildlife Exposure to Pesticides in Arable Crops – Milestone report 02/01 Variation of bird numbers on arable crops.

Table B.9.2.1-13: Data from Crocker and Irving (1999) on species observed in winter wheat

Season	Small bird focal species ranked in order of prevalence and abundance combined	Bird weight (g)
Autumn	Skylark	33-45 [b]
Winter	Fieldfare	80-130 [a]
	Skylark	32-47 [b]
	Starling	79.9-84.7 [b]
Spring	Skylark	33-45 [b]
	Swallow	51.8-16.2 [a]
	Yellow wagtail	16-22 [a]
	Fieldfare	80-130 [a]
	Meadow pipit	15-22 [b]
Summer	Skylark	33-45 [b]
	Swallow	16-25 [a]
	Yellow hammer	25-36 [a]
	House sparrow	24-38 [a]
	House martin	19 [b]

[a] Bird weight from [3]Buxton *et al*. (1998)
[b] Bird weight from www.rspb.org.uk

It should be noted that as Crocker and Irving (1999) involved collecting data year- round, it is expected that pre-emergence crops have been included as part of surveying and therefore the GAP use of 'bare soil' in winter wheat is also covered by the ranked species in Table 1 above.

Several studies referenced in Buxton *et al*. (1998) and Dietzen *et al*. (2017) indicate that earthworms have been observed in the diet of skylarks; likewise Dietzen *et al*. (2017) indicates that from Table 2 of the study, not only skylark but also fieldfare and starling consume earthworms.

With regards to the skylark specifically, regulatory studies conducted on cereals referenced in Table 3 of Dietzen *et al*. (2017) (i.e. Moosmayer, 2008 and Barfknecht, 2006 and Sadowski *et al.*, 2014) are stated to contain information with regards to the proportion of earthworms present in the skylark diet. HSE is of the view that the crop and growth stages in Moosmayer (2008) of spring cereals BBCH 0-10 could be of relevance for part of the cinmethylin GAP (bare soil in winter cereals). Sadowski *et al*. (2014) was conducted in spring cereal fields at BBCH<10 and therefore could also be considered potentially relevant to the cinmethylin 'bare soil cereals' GAP. Finally Barfknecht (2006) referenced in Dietzen *et al*. (2017) was conducted on freshly drilled winter cereals and hence could be of relevance to the bare soil scenario given additional information regarding BBCH growth stages in the study could be provided. *The Applicant was asked to provide details regarding derivation of the proportion of earthworms in the skylark in these studies and also where needed provide justification as to how crop growth stages in the studies can be extrapolated to the proposed GAP of cinmethylin.*

The swallow and house martin are known to feed when in flight and hence are not relevant.

Of the remaining species, i.e. yellow wagtail, meadow pipit, house sparrow and yellowhammer, there is a lack of information regarding whether they consume earthworms. It is, however, assumed that given their size, earthworms are not considered to form a significant part of its diet, if at all. *The Applicant was asked to confirm this.*

From the work presented in both Dietzen *et al.* (2017) and Crocker and Irving (1999), birds that potentially consume earthworms are: skylark, starling and fieldfare. *The Applicant is asked to submit copies of the relevant papers that provide evidence of the proportion of earthworms in the diets of these species during the Applicant's proposed GAP that has been requested along with a critical summary of the papers. The papers according to Table 2 of Dietzen et al. (2017) are:*

[3] Buxton, J. M., Crocker, D. R. and Pascual, J. A. 1998. Update CONTRACT PN0919 MILESTONE REPORT Birds and farming: information for risk assessment.

Skylark: Bosenberg (1969), Collinge (1924-27), Jeromin (2002) and Sikora (1980).

Fieldfare: Christensen *et al*. (1996), Lubcke (1975), Meidell (1937), Christensen (1996) and Otto (1979).

Starling: Bruns and Haberkorn (1960), Christensen *et al*. (1996), Havlinn (1977), Kluijver (1933), Gromadzki (1969), Eble (1963), Havlin (1977) and Havlin and Folk (1965).

The Applicant was asked to provide clarification regarding the relevance of yellowhammer, meadow pipit, yellow wagtail and house sparrow.

Focal species for winter oil seed rape for bare soil (BBCH 00-09) and BBCH 10-18

The table below provides bird species observed in winter oil seed rape during each season that were most prevalent and abundant according to Crocker and Irving (1999). Bird weight has been included in order to determine the most appropriately conservative species for use in the refined risk assessment.

Table B.9.2.1-14: Data from Crocker and Irving (1999) on species in winter oil seed rape

Season	Small bird focal species in order of prevalence and abundance combined	Bird weight (g)
Winter	Skylark	32-47 [a]
	Meadow pipit	15-22 [b]
	Linnet	14.5-21 [a]
	Wren	7.5-10.5 [a]
	Starling	79.9-84.7 [a]
Autumn	Skylark	32-47 [a]
	Meadow pipit	15-22 [b]
	Dunnock	19.7 [a]
	Song thrush	66.6-68.9 [a]
	Blackbird	113 [a]
Spring	Dunnock	19.7 [a]
	Blackbird	113 [a]
	Skylark	32-47 [a]
	Linnet	14.5-21 [a]
	Yellowhammer	26.5 [a]
Summer	Blackbird	113 [a]
	Linnet	14.5-21 [a]
	Dunnock	19.7 [a]
	Greenfinch	27.8 [a]
	Song thrush	66.6-68.9 [a]

[a] Bird weight from Buxton *et al*. (1998)
[b] Bird weight from www.rspb.org.uk

From data in Crocker and Irving (1999) in the table above the skylark has been ranked highest for winter oil seed rape in the winter and autumn when considering abundance and prevalence combined and as discussed above earthworms do form part of their diet suggesting it to be a potentially appropriate focal species for the higher tier assessment.

Of the species listed in Table 2 above, Dietzen *et al.* (2017) indicates that blackbird and song thrush consume earthworms.

From data presented in Dietzen *et al.* (2017) and Buxton *et al.* (1998) it is clear that linnet and greenfinch are not relevant species.

Of the remaining species, i.e. meadow pipit, wren, dunnock and yellowhammer, there is a lack of information regarding whether they consume earthworms. It is, however, assumed that given their

size, earthworms are not considered to form a significant part of its diet, if at all. *The Applicant is asked to confirm this.*

From the work presented in both Dietzen *et al.* (2017) and Crocker and Irving (1999), birds that potentially consume earthworms are: skylark, blackbird and song thrush.

The Applicant was asked to submit copies of the relevant papers along with a critical summary of the papers. The papers according to Table 2 in Dietzen *et al.* (2017) are:

Skylark: Bosenberg (1969), Collinnge (1924-27), Jeromin (2002), Sikora (1980), Donald *et al.* (2001), Geiger *et al.* (2014)

Blackbird: Collinge (1941), Havlin (1977), Vauk and Wittig (1971), Dyrcz (1969), Torok (1985), Iglesias *et al.* (1993) and Schnack (1991).

Song thrush: Collinge (1913), Davies and Snow (1965), Dyrcz (1969), Korodi-Gal (1969), Raiss (1976), Siivonen (1939), Totok (1985), Gruar *et al.* (2003), Schnack (1991), Dyrcz (1969) and Korodi-Gal (1969).

The Applicant was asked to provide clarification regarding the relevance of yellowhammer, meadow pipit, dunnock and wren.

Applicant response and further higher tier refinement

In response to the above request and in line with suggestions in the EFSA Bird and Mammals Guidance document (2009), the Applicant provided a earthworm bioconcentration study (Simon, 2019; BASF DocID 2019/1059201). The study was evaluated and deemed to have produced a valid **bioconcentration factor (BCF) endpoint of 1.12.** This has been used in a refined higher tier secondary poisoning risk assessment presented below.

Table B.9.2.1-15: Higher tier assessment for the active substance BAS 684 H concerning earthworm-eating birds

Parameter	Bare soil and Cereal	Bare soil and Cereal	Oilseed rape	Reference (Section or BASF DocID)
	Application rate			
	500 g a.s./ha	250 g a.s./ha	250 g a.s./ha	
PEC_{soil} [mg/kg soil]	0.662 [1)]	0.331 [2) 8)]	0.331 [2)]	Volume 3 CP B.8.
K_{ow}	31623	31623	31623	Volume 3 CP B.2.[3)]
K_{oc} (geometric mean, n = 5)	282.39	282.39	282.39	Volume 3 CP B.8.[7)]
f_{oc} (default)	0.02	0.02	0.02	EFSA/2009/1438
BCF [4)]	1.12	1.12	1.12	New study : Simon, 2019; BASF DocID 2019/1059201
PEC_{worm} [5)]	0.741	0.371	0.371	--
Daily dose [mg/kg b.w./day] [6)]	0.778	0.390	0.390	--
NOEL [mg/kg b.w./day]	99.1	99.1	99.1	See above
TER_{LT}	127.38	254.10	254.10	--

Bold indicates a TER that fails the risk assessment (<5)
[1] Worst-case 21 day twa PEC_{soil} twa from applications to winter cereals at pre-emergence with a single application rate of 500 g a.s./ha. For details see section Volume 3 CP B.8.
[2] Worst-case 21 day twa PEC_{soil} value calculated from applications to winter cereals at pre-emergence as worst-case scenario with application rate of 250 g a.s./ha. For details see section Volume 3 CP B.8.
[3] K_{ow} recalculated from $logK_{ow}$ of 4.5 (see Volume 3 CP B.2.)
[4] Bioconcentration factor (BCF) from Simon, 2019; BASF DocID 2019/1059201
[5] $PEC_{worm} = PEC_{soil} * BCF$
[6] Daily dose = 1.05 (default value for birds) * PEC_{worm}
[7] K_{oc} is a geometric mean from 5 soils (see Volume 3 CP B.8.)
[8] This covers the GAP for bare soil and cereals at the application rate of 250 g a.s./ha.

All TER_{LT} values for all application scenarios exceed the trigger of 5 and therefore there is no need for further consideration and the risk is acceptable.

Metabolite assessment

In accordance with the guidance of EFSA (2009) it must be identified if any metabolites are likely to be formed in avian food items which may then be consumed by relevant focal species.

From the available plant metabolism data, it was identified that two plant metabolites, M684H005 and M684H006, were formed at 10% or greater total radioactive residues in wheat straw, wheat forage (edible yield) and oilseed rape straw (for full details on studies see Vol.3 B.7 studies CA 6.2.1/001 and 002). The measured concentrations are provided in Table Table B.9.2.1-16 below. It was noted that this data does not provide a specific indication of plant metabolites present at the time birds would be in the field and therefore potentially exposed. What it does provide however is an indication that these metabolites are formed at significant levels in plant material as part of the plant metabolism of the active substance. Therefore, there is the potential for them to be present in food items consumed by birds and their further consideration is required with respect to the bird dietary risk assessment.

Table B.9.2.1-16: Maximum metabolite percentage formation of total radioactive residue (TRR) in plant metabolism studies (where >10% formed)

Crop: Wheat			**BBCH at sampling**
Matrix	Wheat Forage		
Metabolite	**mg/kg**	**%TRR**	BBCH59
M684H005	0.281	9.78	
M684H006	0.770	26.76	
Matrix	Wheat Forage		
Metabolite	**mg/kg**	**%TRR**	BBCH59
M684H005	0.396	14.70	
M684H006	0.796	29.56	
Matrix	Wheat Straw		
Metabolite	**mg/kg**	**%TRR**	BBCH89
M684H005	0.852	14.92	
M684H006	0.720	12.61	
Matrix	Wheat Straw		
Metabolite	**mg/kg**	**%TRR**	
M684H005	1.120	11.46	BBCH89
M684H006	1.798	18.39	
Matrix	Wheat Straw		
Metabolite	**mg/kg**	**%TRR**	BBCH89
M684H005	0.852	14.92	
M684H006	0.720	12.61	
Crop: Oilseed rape			
Matrix	Oilseed Rape Straw		
Metabolite	**mg/kg**	**%TRR**	BBCH89
M684H005	0.393	10.33	
M684H006	0.439	11.54	
Matrix	Oilseed Rape Straw		
Metabolite	**mg/kg**	**%TRR**	BBCH89
M684H005	0.712	18.78	

In order to determine whether a dietary risk assessment is necessary for these metabolites for birds, metabolism studies conducted on poultry have been checked since a risk assessment will not be required if the metabolites in question are formed at sufficient levels in birds as the risk will be covered by the risk assessment for the active substance.

Metabolism studies in Vol 3 Section B.7.2. indicate that metabolites M684H005 and M684H006 were not found in hen metabolism studies. As such a dietary avian risk assessment should be conducted for these plant metabolites.

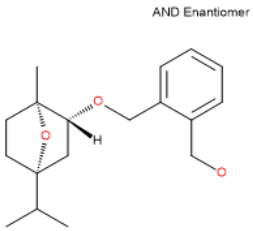
The Applicant submitted additional documentation (namely BASF Doc ID 2020/2003799 and BASF DocID 2020/2079734) for HSE to consider with respect to the metabolite dietary risk assessment and plant metabolites M684H005 and M684H006 specifically which is discussed below.

The Applicant argues that metabolites M684H005 and M684H006 do not occur in birds or mammals since conjugation with glycosides is not a typical reaction in animal xenobiotic metabolism. Within the HSE Toxicology assessment of cinmethylin, these two metabolites were not identified as rat metabolites in toxicokinetic data provided by the Applicant, nor have they been found in the hen or goat studies evaluated by HSE Residues specialists. In addition it was also confirmed by an HSE Toxicologist that the conjugation with glycosides is not one of the main biotransformation steps proposed in the studies conducted on rats, and the HSE Residues specialist confirmed the same with regards to hens or goats.

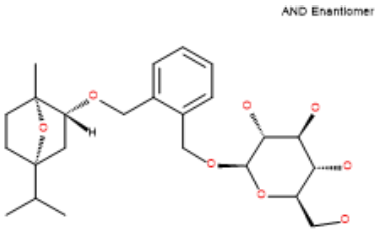
The Applicant proposed that when M684H005 and M684H006 are consumed they would be hydrolysed to M684H002 which is considered to be chemically similar to the two plant metabolites in

question which HSE agreed with. Diagrams below depict the chemical structure and hence similarity between these metabolites (from Vol.3 CA B6 Part II Section B.6.8.1.).

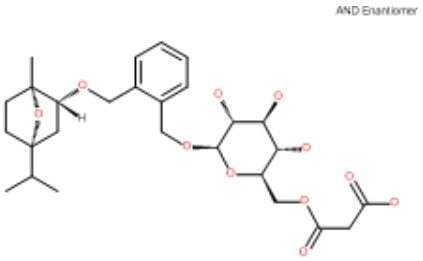
M684H002 (the aglycon)



M684H005 (the sugar conjugate)



M684H006 (the sugar conjugate)



The Applicant further argues that M684H002 (i.e. the aglycon of M684H005 and M684H006) is an intermediate metabolite common to birds and mammals and will be subject to further metabolic transformation as observed in the hen and rat metabolism studies. With regards to the rat, this was confirmed by the Toxicology specialist. With regards to the available data on goat metabolism, M684H002 was found in excreta at low levels which could highlight it is further metabolised. This metabolite was not identified in the hen study, however excreta is not tested for as part of the hen studies.

The Applicant proposes that metabolite M684H002 is considered to be covered by the active substance data. HSE Toxicologists agreed with this proposal due to the structural similarity between M684H002 and metabolite M684H012 which is a major rat metabolite in bile. In addition and specifically with respect to hen metabolism, the HSE Residues Specialist agreed with the proposal put forward by the Applicant which stated that metabolites considered to be M684H002-related are present in hen metabolic pathways. These are namely M684H001, M684H010, M684H059, M684H011, M684H021 and M684H027; all found in hen egg yolk, egg white, hen muscle, fat and liver.

Therefore, although M684H002 has not been specifically detected in the hen metabolism studies there is evidence to support its presence in hens dosed with the active substance and also that it is metabolised to other downstream metabolites that have been detected. Therefore, the toxicity of M684H002 is potentially covered by active substance toxicity data in birds, and on the basis of this evidence, it is proposed to use the same toxicity endpoint as for the active substance when assessing the risk from these metabolites.

The Applicant referred to residue trials conducted on the GAP crops wheat and oilseed rape which have been evaluated by HSE specialists and confirmed to be valid (for full details on studies see Vol.3 B.7.). The trials took place in locations in Northern and Southern Europe and there are data from 24 trials for wheat and 16 trials for oilseed rape. Plant material was sampled from the crops at 0 days after application and two or three time points after application ranging from 14 to 43 days depending on the trial. Samples were analysed for the presence of residues of active substance and the combined concentration of metabolites M684H005 and M684H006. Residue values at day 0 after application for wheat were taken at BBCH 27-29 and for oilseed rape at BBCH 18-21. The BBCH codes for sampling timepoints after day 0 of the wheat trials were at BBCH 49-59 and at BBCH 65 for 8 trials, BBCH 49 and BBCH65 for 8 trials and finally at BBCH 49 and BBCH 65-71 for the remaining 8 trials. With regards to oilseed rape, sampling timepoints after day 0 were at BBCH 51 and BBCH 65 for 8 trials and at BBCH 51-53 and BBCH 65 for the remaining 8 trials.

Sampling points are limited in these studies and it is not clear whether the maximum formed amount of the metabolites has been measured and hence can be estimated in the risk assessment. However, noting the uncertainty highlighted, HSE is of the view that incorporating the maximum measured metabolite value for each crop is considered to provide a worst case estimate of dietary exposure to these metabolites. A summary of the relevant trial data is provided in the table below.

Table B.9.2.1-17: Measured concentrations of M684H005 and M684H006 in residue trials

Residues of M684H005 and M684H006 summed (mg/kg)	
Wheat (n=24) applied at 500g a.s./ha	
Min- Max	0.16- **4.40**
Oilseed rape (n=16) applied at 250 g a.s./ha	
Min- Max	0.25-**1.50**

Bold values are maximums and will be used in the risk assessment.

A dietary risk assessment for birds is presented below which uses the active substance acute and chronic endpoints.

Risk assessment

Both of these identified metabolites have a calculated log Pow of < 3: (see Vol.3 B.2.). As such a low risk to birds from these metabolites via secondary poisoning would be expected and no further assessment of the risk is required.

Acute risk to birds from plant metabolites

The screening step acute risk assessment for these metabolites is presented in the table below which covers the whole GAP. This is due to the data from residue trials being incorporated in the risk assessment which involved the maximum application rate proposed for cereals (500g a.s./ha) and oilseed rape (250g a.s./ha). Since there is no available residue data for bare soils, the value for the trials on cereals (wheat) will be used as the maximum application rate for bare soils is in line with that used in these trials (i.e. 500g a.s./ha).

Table B.9.2.1-18: Screening step acute risk assessment for birds for – metabolites in plant food items

Crop + scenario	**Generic focal species**	**DDD**		**DDD[5] [mg/kg bw/d]**	**LD_{50} [mg a.s./kg bw] [3]**	**TER**	**Trigger**
		Residue value [mg/kg]	**FIR/bw[4]**				
M684H005 and M684H006							
Bare soil[1] BBCH 00-09	Small granivorous bird	4.4	0.28	1.232	>3776	>3064	10
Cereals[1] BBCH 10-29	Small omnivorous bird	4.4	2.26	9.944		>379.7	
OSR[2] BBCH 10-18	Small omnivorous bird	1.5	2.26	3.39		>1113.9	

[1] Maximum residue value for the sum of M684H005 and M684H006 from residue trial data on wheat.
[2] Maximum residue value for the sum of M684H005 and M684H006 from residue trial data on oilseed rape.
[3] Active substance endpoint used as considered to cover toxicity of plant metabolites M684H005 and M684H006.
[4] From Appendix A of EFSA Bird and Mammal Guidance Document (2009).
[5] DDD = FIR/bw x Residue value.

Acute TERs exceed the trigger value of 10 at the screening step demonstrating acceptable acute risk to birds from plant metabolites M684H005 and M684H006 for the GAP application rates of 250 g a.s./ha and 500 g a.s./ha assuming worst-case toxicity of the metabolites.

Long-term/reproductive risk to birds from plant metabolites

The screening step of the reproductive risk assessment for these metabolites is presented in the table below using maximum residue concentration values from residue trials.

Table B.9.2.1-19: Long-term/reproductive screening step risk assessment for birds – metabolites in plant food items

Crop + scenario	Generic focal species	DDD		DDD[5]	NOEL [mg a.s./kg bw] [3]	TER	Trigger
		Residue value [mg/kg]	FIR/bw[4]				
M684H005 and M684H006							
Bare soil[1] BBCH 00-09	Small granivorous bird	4.4	0.28	1.232	99.1	80.4	5
Cereals[1] BBCH 10-29	Small omnivorous bird	4.4	2.26	9.944		10	
OSR[2] BBCH 10-18	Small omnivorous bird	1.5	2.26	3.39		29.2	

[1] Maximum residue value for the sum of M684H005 and M684H006 from residue trial data on wheat.
[2] Maximum residue value for the sum of M684H005 and M684H006 from residue trial data on oilseed rape.
[3] Active substance endpoint used as considered to cover toxicity of plant metabolites M684H005 and M684H006.
[4] From Appendix A of EFSA Bird and Mammal Guidance Document (2009).
[5] DDD = FIR/bw x Residue value.

Long-term/reproductive TERs exceed the trigger value of 5 at the screening step for the bare soil, cereals and oilseed rape uses demonstrating acceptable risk to birds from plant metabolites M684H005 and M684H006 for the GAP application rates of 250 g a.s./ha and 500 g a.s./ha for the proposed uses.

Risk for birds through drinking water

Of the two drinking water risk assessment scenarios for birds in EFSA/2009/1438, *i.e.* the leaf and the puddle scenario, the leaf scenario is not relevant for use in cereals and oilseed rape. Consequently, the 'puddle scenario' will be considered for the application of BAS 684 03 H in cereals and oilseed rape.

Puddle scenario
According to EFSA/2009/1438, no specific calculations of exposure and TER values are necessary when the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg b.w./d) does not exceed 50 in the case of less sorptive substances (K_{oc} < 500) or 3000 in the case of more sorptive substances (K_{oc} ≥ 500). The ratio for acute and reproductive endpoints for BAS 684 H (0.13 and 5.05, respectively) do not exceed the threshold value of 50 as given by EFSA/2009/1438 for less sorptive substances (K_{oc} < 500), thus no specific calculations of exposure for birds through drinking water for the puddle scenario are necessary.

B.9.2.2. Risk assessment for terrestrial verbetrates other than birds

Acute toxicity to mammals

The risk from the formulation is considered to be covered by the active substance risk assessment as previously discussed. The acute active stubstance risk assessment for mammals is presented below.

Table B.9.2.2-1: BAS 684 H: Screening step of the acute risk for mammals due to the use of BAS 684 03 H for the crop group "bare soil" at 1 x 250 g a.s./ha

	Crop	**Application rate (Kg a.s./ha)**	**Number of applications**	**Application Interval**	**DT_{50}**	**LD_{50}**	
	Bare soil	0.25	1	365	10.0	>2000	
Screening step:							
Acute risk assessment screening step	**Indicator species**	**Shortcut value**	**Daily Dietary Dose (single)**	**MAF (90)**	**Daily Dietary Dose (Multiple)**	**TER**	**No refinement step required**
	Small granivorous mammal	14.4	3.6	1.0	3.6	>555.6	

Table B.9.2.2-2: BAS 684 H: Screening step of the acute risk for mammals due to the use of BAS 684 03 H for the crop group "bare soil" at 1 x 500 g a.s./ha

	Crop	**Application rate (Kg a.s./ha)**	**Number of applications**	**Application Interval**	**DT_{50}**	**LD_{50}**	
	Bare soil	0.5	1	365	10.0	>2000	
Screening step:							
Acute risk assessment screening step	**Indicator species**	**Shortcut value**	**Daily Dietary Dose (single)**	**MAF (90)**	**Daily Dietary Dose (Multiple)**	**TER**	**No refinement step required**
	Small granivorous mammal	14.4	7.20	1.0	7.20	>277.8	

Table B.9.2.2-3: BAS 684 H: Screening step of the acute risk for mammals due to the use of BAS 684 03 H for the crop group "cereals" at 1 x 250 g a.s./ha

	Crop	**Application rate (Kg a.s./ha)**	**Number of applications**	**Application Interval**	**DT_{50}**	**LD_{50}**	
	Cereals	0.25	1	365	10.0	>2000	
Screening step:							
Acute risk assessment screening step	**Indicator species**	**Shortcut value**	**Daily Dietary Dose (single)**	**MAF (90)**	**Daily Dietary Dose (Multiple)**	**TER**	**No refinement step required**
	Small herbivorous mammal	118.4	29.6	1.0	29.6	>67.6	

Table B.9.2.2-4: BAS 684 H: Screening step of the acute risk for mammals due to the use of BAS 684 03 H for the crop group "cereals" at 1 x 500 g a.s./ha

	Crop	**Application rate (Kg a.s./ha)**	**Number of applications**	**Application Interval**	**DT_{50}**	**LD_{50}**	
	Cereals	0.5	1	365	10.0	>2000	
Screening step:							
Acute risk assessment screening step	**Indicator species**	**Shortcut value**	**Daily Dietary Dose (single)**	**MAF (90)**	**Daily Dietary Dose (Multiple)**	**TER**	**No refinement step required**
	Small herbivorous mammal	118.4	59.20	1.0	59.20	>33.8	

Table B.9.2.2-5: BAS 684 H: Screening step of the acute risk for mammals due to the use of BAS 684 03 H for the crop group "oilseed rape" at 1 x 250 g a.s./ha

	Crop	**Application rate (Kg a.s./ha)**	**Number of applications**	**Application Interval**	**DT_{50}**	**LD_{50}**	
	Oilseed rape	0.25	1	365	10.0	>2000	
Screening step:							
Acute risk assessment screening step	**Indicator species**	**Shortcut value**	**Daily Dietary Dose (single)**	**MAF (90)**	**Daily Dietary Dose (Multiple)**	**TER**	**No refinement step required**
	Small herbivorous mammal	118.4	29.60	1.0	29.60	>67.6	

All acute TERs exceed the trigger value of 10 demontrating acceptable acute risk to mammals.

Long-term/reproductive toxicity to mammals

The long-term/reproduction active stubstance risk assessment for mammals is presented below.

Table B.9.2.2-6: BAS 684 H: Screening step of the long-term/reproductive risk for mammals due to the use of BAS 684 03 H in the crop group "bare soil" at 1 x 250 g a.s./ha

	Crop	**Application rate (Kg a.s./ha)**	**Number of applications**	**Application Interval**	**DT_{50}**	**Reproductive End Point (mg/kg b.w./d)**	**Time weighted average (TWA)**
	Bare soil	0.25	1	365	10	58	0.53
Screening step:							
Reproductive risk assessment screening step	**Indicator species**	**Shortcut value**	**Daily Dietary Dose (single)**	**MAF mean**	**Daily Dietary Dose (Multiple)**	**TER**	**No refinement step required**
	Small granivorous mammal	6.6	0.87	1.0	0.87	66.32	

Table B.9.2.2-7: BAS 684 H: Screening step of the long-term/reproductive risk for mammals due to the use of BAS 684 03 H in the crop group "bare soil" at 1 x 500 g a.s./ha

	Crop	**Application rate (Kg a.s./ha)**	**Number of applications**	**Application Interval**	**DT_{50}**	**Reproductive End Point (mg/kg b.w./d)**	**Time weighted average (TWA)**
	Bare soil	0.5	1	365	10	58	0.53
Screening step:							
Reproductive risk assessment screening step	**Indicator species**	**Shortcut value**	**Daily Dietary Dose (single)**	**MAF mean**	**Daily Dietary Dose (Multiple)**	**TER**	**No refinement step required**
	Small granivorous mammal	6.6	1.75	1.0	1.75	33.14	

Table B.9.2.2-8: BAS 684 H: Screening step of the long-term/reproductive risk for mammals due to the use of BAS 684 03 H in the crop group "cereals" at 1 x 250 g a.s./ha

	Crop	**Application rate (Kg a.s./ha)**	**Number of applications**	**Application Interval**	**DT_{50}**	**Reproductive End Point (mg/kg b.w./d)**	**Time weighted average (TWA)**
	Cereals	0.25	1	365	10	58	0.53
Screening step:							
Reproductive risk assessment screening step	**Indicator species**	**Shortcut value**	**Daily Dietary Dose (single)**	**MAF mean**	**Daily Dietary Dose (Multiple)**	**TER**	**No refinement step required**
	Small herbivorous mammal	48.3	6.40	1.0	6.40	9.1	

Table B.9.2.2-9: BAS 684 H: Screening step of the long-term/reproductive risk for mammals due to the use of BAS 684 03 H in the crop group "cereals" at 1 x 500 g a.s./ha

	Crop	**Application rate (Kg a.s./ha)**	**Number of applications**	**Application Interval**	**DT_{50}**	**Reproductive End Point (mg/kg b.w./d)**	**Time weighted average (TWA)**
	Cereals	0.5	1	365	10	58	0.53
Screening step:							
Reproductive risk assessment screening step	**Indicator species**	**Shortcut value**	**Daily Dietary Dose (single)**	**MAF mean**	**Daily Dietary Dose (Multiple)**	**TER**	**Refinement step required**
	Small herbivorous mammal	48.3	12.8	1.0	12.8	**4.5**	

The chronic TERs for cereals use at 500 g a.s./ha is below the trigger of 5 therefore tier 1 risk assessment is required which is presented below.

Table B.9.2.2-10: BAS 684 H: Tier 1 of the long-term/reproductive risk for mammals due to the use of BAS 684 03 H in the crop group "cereals" at 1 x 500 g a.s./ha

	Crop	**Application rate (Kg a.s./ha)**	**Number of applications**	**Application Interval**	**DT_{50}**	**Reproductive End Point (mg/kg b.w./d)**	**Time weighted average (TWA)**
	Cereals	0.5	1	365	10	58	0.53
Tier 1:							
BBCH 10-19	**Generic focal species**	**Shortcut value**	**Number of applications**	**MAF mean**	**Daily Dietary Dose**	**TER**	**No refinement step required**
	Small insectivorous mammal "shrew"	4.2	1	1	1.1	52	
BBCH ≥ 20	Small insectivorous mammal "shrew"	1.9			0.5	115	**No refinement step required**
Early (shoots)	Large herbivorous mammal "lagomorph"	22.3			5.9	9.8	**No refinement step required**
BBCH 10-29	Small omnivorous mammal "mouse"	7.8			2.1	25	**No refinement step required**

Table B.9.2.2-11: BAS 684 H: Screening step of the long-term/reproductive risk for mammals due to the use of BAS 684 03 H in the crop group "oilseed rape" at 1 x 250 g a.s./ha

	Crop	**Application rate (Kg a.s./ha)**	**Number of applications**	**Application Interval**	**DT_{50}**	**Reproductive End Point (mg/kg b.w./d)**	**Time weighted average (TWA)**
	Oilseed rape	0.25	1	365	10	58	0.53
Screening step:							
Reproductive risk assessment screening step	**Indicator species**	**Shortcut value**	**Daily Dietary Dose (single)**	**MAF mean**	**Daily Dietary Dose (Multiple)**	**TER**	**No refinement step required**
	Small herbivorous mammal	48.3	6.40	1.0	6.40	9.06	

All chronic TERs exceed the trigger value of 5 demontrating acceptable chronic risk to mammals.

Effects of secondary poisoning

According to EFSA/2009/1438 an assessment of the potential risk of secondary poisoning is triggered with a log K_{ow} of greater than 3. The log K_{ow} of the active substance BAS 684 H is 4.5 at 20°C and pH=7 (see Volume 3 B.2.). Hence, the risk of secondary poisoning will be assessed to fish- and earthworm eating mammals.

Food chain from fish to fish-eating mammals

The risk assessment for fish-eating mammals is based on the maximum PEC_{sw} derived from the environmental fate section (see Volume 3 CA B.8.). The BCF value confirmed to be correct was 707 which will be used in the risk assessment below.

The calculations and the resulting TER_{LT} values are presented in Table B.9.2.2-12.

Table B.9.2.2-12: Risk assessment for the active substance BAS 684 H concerning fish-eating mammals[1)]

Parameter	BAS 684 H	Reference
PEC_{sw} [mg/L] [2)]	0.026923	Volume 3 CP B.8.
BCF fish (max. worst case)	707 [6)]	Volume 3 CP B.9.3.2.
PEC_{fish} [mg/kg] [3)]	19.03	--
Daily dose [mg/kg b.w./d] [4)]	2.70	EFSA/2009/1438
NO(A)EL [mg/kg b.w./d]	58	See above
TER_{LT} [5)]	21.46	--

1 According to EFSA/2009/1438
2 Highest PEC_{sw} value resulting from Tier 1 drainflow calculations which was worst case when considering spray drift and drainflow. For details see Volume 3 CA B.8.
3 $PEC_{fish} = PEC_{sw} \times BCF$
4 Daily dose = $0.142 \times PEC_{fish}$
5 TER_{LT} = NO(A)EL / Daily dose
6 Highest BCF_{fish} used as worst-case as selected from the results of two studies (BASF DocID CI-690-004 + CI-705-001; 2017/1156422 and 2017/1208842).

The TER_{LT} exceeds the trigger of 5 demonstrating acceptable risk to fish-eating mammals. No further consideration is required.

Food chain from earthworm to earthworm-eating mammals

The risk assessment for earthworm-eating mammals is based on the worst case PEC_{soil} (twa, 21 days) derived from the environmental fate section (Volume 3 CP B.8.). The calculations and the resulting TER_{LT} values are summarized in Table B.9.2.2.9.

Table B.9.2.2-13: Risk assessment for the active substance BAS 684 H concerning earthworm-eating mammals

Parameter	Bare soil and Cereal	Bare soil and Cereal	Oilseed rape	Reference
	Application rate			Use pattern
	500 g a.s./ha	250 g a.s./ha	250 g a.s./ha	
PEC_{soil} [mg/kg soil]	0.662 [1)]	0.331 [2) 8)]	0.331 [2)]	Volume 3 CP B.8.
K_{ow} [3)]	31623	31623	31623	Volume 3 CP B.2.
K_{oc} (geometric mean, n = 5)	282.39	282.39	282.39	Volume 3 CP B.8.
f_{oc} (default)	0.02	0.02	0.02	EFSA/2009/1438
BCF [4)]	67.34	67.34	67.34	--
PEC_{worm} [5)]	44.58	22.29	22.29	--
Daily dose [mg/kg b.w./day] [6)]	57.06	28.53	28.53	--
NOEL [mg/kg b.w./day]	58	58	58	See above
TER_{LT}	**1.02**	**2.03**	**2.03**	--

[1] Worst-case 21 day twa PEC_{soil} twa from applications to winter cereals at pre-emergence with a single application rate of 500 g a.s./ha. For details see section Volume 3 CP B.8.
[2] Worst-case 21 day twa PEC_{soil} value calculated from applications to winter cereals at pre-emergence as worst-case scenario with application rate of 250 g a.s./ha. For details see section Volume 3 CP B.8.
[3] K_{ow} recalculated from $\log K_{ow} = 4.5$
[4] Bioconcentration factor (BCF) = $(0.84 + 0.012 * K_{ow}) / f_{oc} * K_{oc}$
[5] $PEC_{worm} = PEC_{soil} * BCF$
[6] Daily dose = 1.28 x PEC_{worm}
[7] K_{oc} is a geometric mean from 5 soils (see Volume 3 CP B.8.)
[8] This covers the GAP for bare soil and cereals at the application rate of 250 g a.s./ha.

In conclusion, according to the tier 1 risk assessment for earthworm-eating mammals, the TER values for BAS 684 H are below the trigger set by Commission regulation 546/2011, i.e. < 5 for reproductive exposure. Further risk assessment is required in order to demonstrate acceptable risk.

Higher tier risk assessment for earthworm-eating mammals

The applicant proposed applying a correction factor of 5.6 from Jager (1998)[4] to the earthworm BCF. This is not accepted by the HSE evaluator for reasons outlined in Section B.9.2.7.2. Higher tier risk assessment for earthworm-eating birds.

The Applicant was informed of this outcome and given the opportunity to provide further information.

Applicant response and further higher tier refinement

In line with suggestions in the EFSA Bird and Mammals Guidance document (2009), the Applicant provided a earthworm bioconcentration study (Simon, 2019; BASF DocID 2019/1059201). The study was evaluated and deemed to have produced a valid **bioconcentration factor (BCF) endpoint of 1.12.** This has been used in a refined higher tier secondary poisoning risk assessment presented below.

[4] Jager, T. 1998. Mechanistic approach for estimating bioconcentration of organic chemicals in earthworms (Oligochaeta). *Environ. Toxicol. Chem*. 17:2080–2090.

Table B.9.2.2-14**:** Higher tier risk assessment for the active substance BAS 684 H concerning earthworm-eating mammals

Parameter	Bare soil and Cereal	Bare soil and Cereal	Oilseed rape	Reference
	Application rate			Use pattern
	500 g a.s./ha	250 g a.s./ha	250 g a.s./ha	
PEC_{soil} [mg/kg soil]	0.662 [1)]	0.331 [2) 8)]	0.331 [2)]	Volume 3 CP B.8.
K_{ow} [3)]	31623	31623	31623	Volume 3 CP B.2.
K_{oc} (geometric mean, n = 5)	282.39	282.39	282.39	Volume 3 CP B.8.
f_{oc} (default)	0.02	0.02	0.02	EFSA/2009/1438
BCF [4)]	1.12	1.12	1.12	New study : Simon, 2019; BASF DocID 2019/1059201
PEC_{worm} [5)]	0.741	0.371	0.371	--
Daily dose [mg/kg b.w./day] [6)]	0.948	0.475	0.475	--
NOEL [mg/kg b.w./day]	58	58	58	See above
TER_{LT}	61.18	122.11	122.11	--

[1] Worst-case 21 day twa PEC_{soil} twa from applications to winter cereals at pre-emergence with a single application rate of 500 g a.s./ha. For details see section Volume 3 CP B.8.
[2] Worst-case 21 day twa PEC_{soil} value calculated from applications to winter cereals at pre-emergence as worst-case scenario with application rate of 250 g a.s./ha. For details see section Volume 3 CP B.8.
[3] K_{ow} recalculated from $logK_{ow} = 4.5$
[4] Bioconcentration factor (BCF) = 1.12 from Simon, 2019; BASF DocID 2019/1059201
[5] $PEC_{worm} = PEC_{soil} * BCF$
[6] Daily dose = 1.28 x PEC_{worm}
[7] K_{oc} is a geometric mean from 5 soils (see Volume 3 CP B.8.)
[8] This covers the GAP for bare soil and cereals at the application rate of 250 g a.s./ha.

In conclusion, according to the higher tier risk assessment for earthworm-eating mammals, the TER values for BAS 684 H exceed the trigger of 5 therefore the risk is acceptable and no further consideration is required.

Risk for mammals through drinking water

Of the two drinking water risk assessment scenarios in EFSA/2009/1438, *i.e.* the leaf and the puddle scenario, the leaf scenario is not relevant for mammals. Consequently, the 'puddle scenario' will be considered for the application of BAS 684 03 H in cereals and oilseed rape.

According to EFSA/2009/1438 no specific calculations of exposure and TER values are necessary when the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg b.w./d) does not exceed 50 in the case of less sorptive substances ($K_{oc} < 500$) or 3000 in the case of more sorptive substances ($K_{oc} \geq 500$). The ratio for the acute and reproductive endpoint for BAS 684 H (0.25 and 8.6, respectively) do not exceed the threshold value of 50 as given by EFSA/2009/1438 for less sorptive substances ($K_{oc} < 500$), thus no specific calculations of exposure for mammals through drinking water for the puddle scenario are necessary.

Metabolite assessment

In accordance with the guidance of EFSA (2009) it must be identified if any metabolites are likely to be formed in mammalian food items which may then be consumed by relevant focal species.

From the available plant metabolism data, it was identified that two plant metabolites, M684H005 and M684H006, were formed at 10% or greater total radioactive residues in wheat straw, wheat forage (edible yield) and oil seed rape straw (for full details on studies see Vol.3 B.7 studies CA 6.2.1/001 and 002). The measured concentrations are provided in Table B.9.2.2-15 below. It was noted that this data does not provide a specific indication of plant metabolites present at the time mammals would be in the field and therefore potentially exposed. What it does provide however is an indication that these metabolites are formed at significant levels in plant material as part of the plant metabolism of the active substance. Therefore there is the potential for them to be present in food items consumed by mammals and their further consideration is required with respect to the mammalian dietary risk assessment.

Table B.9.2.2-15: Maximum metabolite percentage formation of total radioactive residue (TRR) in plant metabolism studies (where >10% formed)

Crop: Wheat			**BBCH at sampling**
Matrix	Wheat Forage		
Metabolite	**mg/kg**	**%TRR**	
M684H005	0.281	9.78	BBCH59
M684H006	0.770	26.76	
Matrix	Wheat Forage		
Metabolite	**mg/kg**	**%TRR**	
M684H005	0.396	14.70	BBCH59
M684H006	0.796	29.56	
Matrix	Wheat Straw		
Metabolite	**mg/kg**	**%TRR**	
M684H005	0.852	14.92	BBCH89
M684H006	0.720	12.61	
Matrix	Wheat Straw		
Metabolite	**mg/kg**	**%TRR**	
M684H005	1.120	11.46	BBCH89
M684H006	1.798	18.39	
Matrix	Wheat Straw		
Metabolite	**mg/kg**	**%TRR**	
M684H005	0.852	14.92	BBCH89
M684H006	0.720	12.61	
Crop: Oilseed rape			
Matrix	Oilseed Rape Straw		
Metabolite	**mg/kg**	**%TRR**	
M684H005	0.393	10.33	BBCH89
M684H006	0.439	11.54	
Matrix	Oilseed Rape Straw		
Metabolite	**mg/kg**	**%TRR**	BBCH89
M684H005	0.712	18.78	

In order to determine whether a dietary risk assessment is necessary for these metabolites for mammals, metabolism studies conducted on rat have been checked since a risk assessment will not be required if the metabolites in question are formed at sufficient levels in mammals as the risk will be covered by the risk assessment for the active substance.

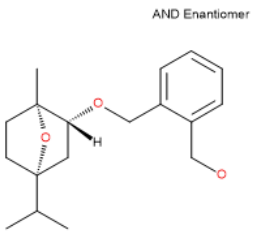
Mammalian metabolism studies in Vol 3 B.6. Part II indicate that neither M684H005 nor M684H006 were found in rat metabolism studies. As such a dietary mammal risk assessment should be conducted for these plant metabolites.

The Applicant submitted additional documentation (namely BASF Doc ID 2020/2003799 and BASF DocID 2020/2079734) for HSE to consider with respect to the metabolite dietary risk assessment and plant metabolites M684H005 and M684H006 specifically which is discussed below.

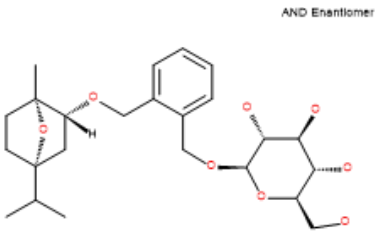
The Applicant argues that metabolites M684H005 and M684H006 do not occur in birds or mammals since conjugation with glycosides is not a typical reaction in animal xenobiotic metabolism. Within the HSE Toxicology assessment of cinmethylin, these two metabolites were not identified as rat metabolites in toxicokinetic data provided by the Applicant, nor have they been found in the hen or goat studies evaluated by HSE Residues specialists. In addition it was also confirmed by an HSE Toxicologist that the conjugation with glycosides is not one of the main biotransformation steps proposed in the studies conducted on rats, and the HSE Residues specialist confirmed the same with regards to hens or goats.

The Applicant proposed that when M684H005 and M684H006 are consumed they would be hydrolysed to M684H002 which is considered to be chemically similar to the two plant metabolites in question which HSE agreed with. Diagrams below depict the chemical structure and hence similarity between these metabolites (from Vol.3 CA B6 Part II Section B.6.8.1.).

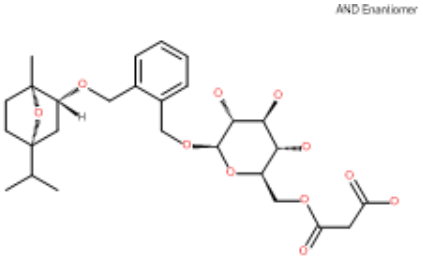
M684H002 (the aglycon)



M684H005 (the sugar conjugate)



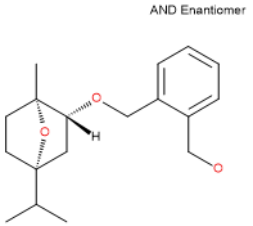
M684H006 (the sugar conjugate)



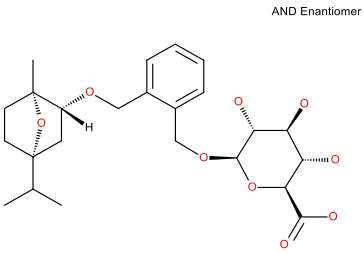
The Applicant further argues that the aglycon of M684H005 and M684H006 (i.e. M684H002) is an intermediate metabolite common to birds and mammals and will be subject to further metabolic transformation as observed in the hen and rat metabolism studies. With regards to the rat, this was confirmed by the Toxicology specialist. With regards to the available data on goat metabolism, M684H002 was found in excreta at low levels which could highlight it is further metabolised. This metabolite was not identified in the hen study, however excreta is not tested for hens.

The Applicant proposes that metabolite M684H002 is considered to be covered by the active substance data. HSE Toxicologists agreed with this proposal due to the structural similarity between M684H002 and metabolite M684H012 which is a major rat metabolite in bile. Therefore M684H002 is considered to be supported by information on M684H012 which is covered by the active substance data. Consequently this suggests that the active substance mammalian endpoints and risk assessment will cover the dietary risk to mammals from M684H005 and M684H006. Diagrams below show the structural similarity between M684H002 and M684H012 (from Vol.3 CA B6 Part II Section B.6.8.1.).

M684H002 (the aglycon)



M684H012 (glucuronide of H002)



The Applicant referred to residue trials conducted on the GAP crops wheat and oilseed rape which have been evaluated by HSE specialists and confirmed to be valid (for full details on studies see Vol.3

B.7.). The trials took place in locations in Northern and Southern Europe and there are data from 24 trials for wheat and 16 trials for oilseed rape. Plant material was sampled from the crops at 0 days after application and two or three time points after application ranging from 14 to 43 days depending on the trial. Samples were analysed for the presence of residues of active substance and the combined concentration of metabolites M684H005 and M684H006. Residue values at day 0 after application for wheat were taken at BBCH 27-29 and for oilseed rape at BBCH 18-21. The BBCH codes for sampling timepoints after day 0 of the wheat trials were at BBCH 49-59 and at BBCH 65 for 8 trials, BBCH 49 and BBCH65 for 8 trials and finally at BBCH 49 and BBCH 65-71 for the remaining 8 trials. With regards to oilseed rape, sampling timepoints after day 0 were at BBCH 51 and BBCH 65 for 8 trials and at BBCH 51-53 and BBCH 65 for the remaining 8 trials.

Sampling points are limited in these studies and it is not clear whether the maximum formed amount of the metabolites has been measured and hence can be estimated in the risk assessment However, noting the uncertainty highlighted HSE is of the view that incorporating the maximum measured metabolite value for each crop is considered to provide a worst case estimate of dietary exposure to these metabolites. A summary of the relevant trial data is provided in the table below.

Table B.9.2.2-16: Measured concentrations of M684H005 and M684H006 in residue trials

Residues of M684H005 and M684H006 summed (mg/kg)	
Wheat (n=24) applied at 500g a.s./ha	
Min- Max	0.16- **4.40**
Oilseed rape (n=16) applied at 250 g a.s./ha	
Min- Max	0.25-**1.50**

Bold values are maximums and will be used in the risk assessment.

A dietary risk assessment for mammals is presented below which uses the active substance acute and chronic endpoints which are considered to cover the toxicity of the metabolites as advised by the HSE toxicologist. The highest measured concentrations of M684H005 and M684H006 combined for each crop will be used as the application rate.

Risk assessment

Both of these identified metabolites have a calculated log Pow of < 3: (see Vol.3 B.2.). As such a low risk to mammals from these metabolites via secondary poisoning would be expected and no further assessment of the risk is required.

Acute risk to mammals from plant metabolites

The acute screening step risk assessment for these metabolites is presented in the table below which covers the whole GAP. This is due to the data from residue trials being incorporated in the risk assessment which involved the maximum application rate proposed for cereals (500g a.s./ha) and oilseed rape (250g a.s./ha). Since there is no available residue data for bare soils, the value for the trials on cereals (wheat) will be used as the maximum application rate for bare soils is in line with that used in these trials (i.e. 500g a.s./ha).

Table B.9.2.2-17: Screening step acute risk assessment for mammals for – metabolites in plant food items

Crop + scenario	Generic focal species	DDD		DDD[5] [mg/kg bw/d]	LD_{50} [mg a.s./kg bw] [3]	TER	Trigger
		Residue value [mg/kg]	FIR/bw[4]				
M684H005 and M684H006							
Bare soil[1] BBCH 00-09	Small granivorous mammal	4.4	0.17	0.748	>2000	>2673	10
Cereals[1] BBCH 10-29	Small herbivorous mammal	4.4	1.33	5.852		>341	
OSR[3] 10-18	Small herbivorous mammal	1.5	1.33	1.995		>1003	

[1] Maximum residue value for the sum of M684H005 and M684H006 from residue trial data on wheat.
[2] Maximum residue value for the sum of M684H005 and M684H006 from residue trial data on oilseed rape.
[3] Active substance endpoint which is considered to cover the toxicity of M684H005 and M684H006.
[4] From Appendix A of EFSA Bird and Mammal Guidance Document (2009).
[5] DDD = FIR/bw x Residue value.

Acute TERs exceed the trigger value of 10 demonstrating acceptable acute risk to mammals from plant metabolites M684H005 and M684H006 for the GAP application rates of 250 g a.s./ha and 500 g a.s./ha.

Long-term/reproductive risk to mammals from plant metabolites

The long-term/reproductive screening step risk assessment for these metabolites is presented in the table below using maximum residue concentration values from residue trials.

Table B.9.2.2-18: Long-term/reproductive screening step risk assessment for mammals – metabolites in plant food items

Crop + scenario	Generic focal species	DDD		DDD[5]	NOEL [mg a.s./kg bw] [3]	TER	Trigger
		Residue value [mg/kg]	FIR/bw[4]				
M684H005 and M684H006							
Bare soil[1] BBCH 00-09	Small granivorous mammal	4.4	0.17	0.748	58	77.5	5
Cereals[1] BBCH 10-29	Small herbivorous mammal	4.4	1.33	5.852		9.91	
OSR[3] 10-18	Small herbivorous mammal	1.5	1.33	1.995		29.1	

[1] Maximum residue value for the sum of M684H005 and M684H006 from residue trial data on wheat.
[2] Maximum residue value for the sum of M684H005 and M684H006 from residue trial data on oilseed rape.
[3] Active substance endpoint which is considered to cover the toxicity of M684H005 and M684H006.
[4] From Appendix A of EFSA Bird and Mammal Guidance Document (2009).
[5] DDD = FIR/bw x Residue value.

Long-term/reproductive TERs exceed the trigger value of 5 demonstrating acceptable risk to mammals from plant metabolites M684H005 and M684H006 for the GAP application rates of 250 g a.s./ha and 500 g a.s./ha.

In conclusion, the acute and long-term/reproductive risk to mammals from plant metabolites M684H005 and M684H006 is considered to be acceptable. The TER values show margins of safety with respect to the trigger values which adds confidence to this outcome given the uncertainties surrounding the relevance of residue trial data incorporated into the assessment.

B.9.3. EFFECTS ON AQUATIC ORGANISMS

B.9.3.1. Acute toxicity to fish, aquatic invertebrates, or effects on aquatic algae and macrophytes

Report:	CP 10.2.1/1 [redacted], 2017a BAS 684 03 H - Common carp, acute toxicity test 2017/1106099
Guidelines:	OECD 203 (1992)
GLP:	yes (certified by Bureau for Chemical Substances and Preparations, Lodz, Poland)

Report:	CP 10.2.1/2 [redacted], 2018 a Amendment 1: BAS 684 03 H - Common carp, acute toxicity test 2018/1018222
Guidelines:	OECD 203 (1992)
GLP:	Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test item:	BAS 684 03 H, batch no. FD-170210-0001; content of a.s.: cinmethylin (Reg. No. 900 202): 737.3 g/L (measured) 750 g/L (nominal); density: 1.001 g/cm^3.

B. STUDY DESIGN

Test species:	Common carp (*Cyprinus carpio*); age: approx. 4 months; average body length: 5.3 ± 0.2 cm; average body weight in control: 3.06 g; supplied by [redacted]
Test design:	Static system (96 hours); 1 replicate per treatment; 10 fish per replicate (loading: 0.87 g fish/L), assessment of mortality and symptoms of toxicity after 3, 6, 24, 48, 72 and 96 h after start of exposure.
Endpoints:	LC_{50}, mortality and sub-lethal effects.
Test concentrations:	Control, 1.0, 1.8, 3.2, 5.6 and 10 mg product/L (nominal) equivalent to geometric mean measured concentration recalculated on formulation of 0, 0.6, 1.216, 2.237, 4.342 and 7.904 mg product/L.
Test conditions:	Glass aquaria with glass lids, test volume: 35 L, dilution water: aerated tap water; temperature: 20.4 - 21°C; pH 7.19 – 7.91; oxygen concentration: 81 - 99% of air saturation value; total hardness: 0.42 mval/L (test medium); conductivity: 216 µS/cm (test medium); photoperiod: 16 h light: 8 h dark; no feeding.

Analytics: Analytical verification of test item concentrations was conducted using an LC-method with DAD (Diode Array Detector).

Statistics: Descriptive statistics; calculation of LC_{50} after Spearman-Karber procedure.

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. The measured concentrations of cinmethylin at test initiation ranged from 95.9 to 99.2 % of nominal concentration and from 37.5 to 64.9 % of nominal at test termination. Since the determined concentrations of cinmethylin at exposure termination were not within the range of 80 – 120 % of nominal concentration, the geometric means of determined concentrations of cinmethylin were calculated. The biological endpoints are presented based on the nominal and geometric mean measured concentrations. The test solutions were described as homogeneous and transparent in the study report.

The analytical data is shown in the table below:

Table B.9.3.1-1: Measured concentrations during study

Nominal (mg product/L)	Nominal (mg a.s./L)	0 hours		96 hours		Geometric mean (mg product/L)**
		Mean measured (mg a.s./L)	% of nominal	Mean measured (mg a.s./L)	% of nominal	
0	0.0	< LoD	--	< LoD	--	0.0
1.0	0.737	Not reported*	95.9	0.276	37.5	0.600
1.8	1.326	1.284	96.8	0.625	47.1	1.216
3.2	2.357	2.274	96.5	1.194	50.7	2.237
5.6	4.125	4.091	99.2	2.501	60.6	4.342
10	7.366	7.092	96.3	4.780	64.9	7.904

-- = not applicable LoQ = 0.002 mg a.s./L, LoD = 0.0005 mg a.s./L
* This value was not stated in the study report which appears to be in error. Based on the % of nominal recorded the value has been calculated as 0.707 mg a.s./L
** Calculated by study author following OECD 23, annex II equation. The HSE evaluator derived marginally different values for some test concentrations; 0.59, 1.214, 2.237, 4.283 and 7.912 mg product/L. However, the difference is negligible hence the calculated values are considered acceptable for the GB assessment.

Validity criteria:

In OECD 203 (1992) the following criteria are stated:

- 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. During study: 0 % mortality.
- The dissolved oxygen concentration must have been at least 60 per cent of the air saturation value throughout the test. During study: minimum of 81 %.
- 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 the four lowest test item concentrations of BAS 684 03 H, whereas 100% mortality occurred at the highest test item concentrations of 10 mg product/L.

After 96 hours of exposure, sub-lethal effects (*i.e.* loss of balance and nontypical swimming) were observed at the test item concentrations of 3.2 mg product/L and 5.6 mg/L. The results are summarized in Table B.9.3.1-2.

Table B.9.3.1-2: Acute toxicity of BAS 684 03 H on common carp (*Cyprinus carpio*)

Concentration [mg product/L] (nominal)	**Control**	**1.0**	**1.8**	**3.2**	**5.6**	**10**
Concentration [mg/L] (geometric mean) [$]	**0**	**0.6**	**1.216**	**2.237**	**4.342**	**7.904**
Mortality (96 h) [%]	0	0	0	0	0	100
Symptoms (96 h) [#]	none	none	None	B(1), N(1)	B(2), N(10)	n.d.
Endpoints [mg product/L]						
	Nominal			**Geometric mean measured** [$]		
LC_{50} (96 h)	7.48 (95 % confidence limits: n.c.)			**5.86** (95 % confidence limits: n.c.)		
NOEC	1.8			1.216		

n.c.: not calculated due to mathematical reasons (this is considered acceptable by the HSE evaluator as Trimmed Spearman-Karber was used to calculate the LC_{50});
n.d. = not determined; all animals were dead.
[#] Symptoms after 96 h: B = loss of balance, N = non-typical swimming, P = nontypical pigmentation.
[$] values are based on the measured geometric mean concentration of cinmethylin recalculated and expressed as formulation values.

III. CONCLUSION

In a static acute toxicity study with common carp, the LC_{50} (96 h) of BAS 684 03 H was 5.86 mg product/L based on geometric mean measured concentrations.

HSE evaluator comments:

It was noted that an amendment for this report (applicant reference KCP 10.2.1-002) was submitted. The key amendment to the report was the addition of endpoints based on geometric mean measured concentrations. The HSE evaluator has checked the amendment and agrees with the endpoints stated in the above study summary.

The fish length was above the recommendation in OECD 203 (4 cm), however the difference is marginal (maximum of 1.5 cm) and therefore considered acceptable by the HSE evaluator.

The above study was conducted to GLP and is considered valid. The analytical method is validated in accordance with SANCO/3029/99 rev. 4 with an LOQ of 0.002 mg/L (see volume 3, CA, section B5 for full details) and the following endpoint will be used in the risk assessment:

- Formulation '**BAS 684 03H**' 96-hour LC_{50} = **5.86 mg product/L** (based on geometric mean measured concentration) equivalent to **4.32 mg a.s./L**

Report: CP 10.2.1/3
Turek T., 2017 a
BAS 684 03 H - *Daphnia* magna, acute immobilisation test
2017/1106098
Guidelines: OECD 202 (2004)
GLP: Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test item:	BAS 684 03 H, batch no. FD-170210-0001; content of a.s.: Cinmethylin (Reg. No. 900 202): 737.3 g/L (nominal: 750 g/L); density: 1.001 g/cm^3.

B. STUDY DESIGN

Test species:	Water flea (*Daphnia magna* STRAUS), neonates collected from in-house culture, not first brood progeny, less than 24 hours old at test initiation.
Test design:	Static system (48 hours), 5 test concentrations plus control, 4 replicates with 5 daphnids in each; assessment of immobility after 24 and 48 hours.
Endpoints:	NOEC, LOEC and EC_{50} based on immobility of daphnids.
Test concentrations:	Control, 1.0, 2.0, 4.0, 8.0 and 16 mg product/L (nominal).
Test conditions:	150 mL glass beakers with transparent lids; test volume 100 mL; dilution water "M7" (Elendt medium); pH 7.59 – 7.98; oxygen content: 8.2 – 8.6 mg/L; temperature 20.7°C – 21.6°C; photoperiod: 16 h light: 8 h dark; no feeding; no aeration.
Analytics:	Analytical verification of test item concentrations was conducted using an LC-method with DAD (Diode Array Detector).
Statistics:	Descriptive statistics; Logit method calculations for determination of ECx value and analysis by Step-down Cochran-Armitage Test Procedure, one-sided greater, for NOEC and LOEC ($\alpha = 0.05$).

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. In the samples collected at exposure initiation, the determined concentrations of Cinmethylin were in the range of 93.8 % – 96.0 % of nominal concentration.

In the samples collected at exposure termination, the determined concentrations of Cinmethylin were in the range of 94.7 % – 96.2 % of nominal concentration. Therefore, the test item concentrations were stable (within ± 20 % of nominals) under test conditions and the endpoints have been expressed as nominal concentrations. The test solutions were described as homogeneous and transparent in the study report.

The analytical results are shown in the table below.

Table B.9.3.1-3: Measured concentrations during study

Nominal (mg product/L)	Nominal (mg a.s./L)	0 hours		48 hours	
		Mean measured (mg a.s./L)	% of nominal	Mean measured (mg a.s./L)	% of nominal
0	0.0	< LoD	--	< LoD	--
1.0	0.737	0.696	94.4	0.709	96.2
2.0	1.473	1.382	93.8	1.410	95.7
4.0	2.946	2.792	94.8	2.799	95.0
8.0	5.893	5.658	96.0	5.599	95.0
16.0	11.786	11.210	95.1	11.164	94.7

-- = not applicable LoQ = 0.002 mg a.s./L, LoD = 0.0005 mg a.s./L

Validity criteria:

In OECD 202 (2004) the following criteria are stated:

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

During the study the above criteria were met.

Biological results: After 24 hours of exposure no immobility of daphnids was observed in the control and at test item concentrations of up to and including 8.0 mg product/L, whereas 65% immobility was observed at the test item concentration of 16.0 mg product/L. After 48 hours of exposure, no daphnids were immobile at the four lowest tested concentrations of 1.0, 2.0, 4.0 and 8.0 mg product/L whereas 85 % daphnids were immobile at the highest test item concentration of 16.0 mg product/L. Statistically significant differences in the immobility rates compared to the control were observed at the highest test item concentration of 16.0 mg product/L after 48 h of exposure (Step-down Cochran-Armitage test, α = 0.05, one-sided greater). For results see Table B.9.3.1-4. No behavioural observations were recorded during the study.

Table B.9.3.1-4: Effect of BAS 684 03 H on *Daphnia magna* immobility

Concentration [mg product/L] (nominal)	Control	1.0	2.0	4.0	8.0	16.0
Immobility (24 h) [%]	0	0	0	0	0	65
Immobility (48 h) [%]	0	0	0	0	0	85*
Endpoints [mg product/L] (nominal)						
EC_{50} (48 h)	14.5 (95 % confidence limits: 13.5 – 15.5)					
NOEC (48 h)	8.0					

* Statistically significantly different compared to the control (Step-down Cochran-Armitage Test Procedure, α = 0.05, one-sided greater).

Reference test: A reference study was conducted with potassium dichromate. The 48-hour EC_{50} was calculated as 0.50 mg reference item/L which is within ranges of references stated in OECD 202.

III. CONCLUSION

In a 48-hour static acute toxicity study with *Daphnia magna*, the EC_{50} of BAS 684 03 H was 14.5 mg product/L based on nominal concentrations.

HSE evaluator comments:

The above study was conducted to GLP and is considered valid. The analytical method is considered fully validated in accordance with SANCO/3029/99 rev. 4 with an LOQ of 0.002 mg/L (see volume 3, CA, section B5 for full details) and the following endpoint will be used in the risk assessment:

- Formulation '**BAS 684 03H**' 48-hour EC_{50} = **14.5 mg product/L** (based on nominal concentration) equivalent to **10.68 mg a.s./L**

Report:	CP 10.2.1/4 Turek T., 2017 b BAS 684 03 H - *Pseudokirchneriella subcapitata* SAG 61.81, growth inhibition test 2017/1106097
Guidelines:	OECD 201 (2006)
GLP:	Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test item:	BAS 684 03 H, batch FD-170210-0001; content of a.s. (cinmethylin, Reg. No. 900 202): 737.3 g/L (nominal: 750 g/L); density: 1.001 g/cm³.

B. STUDY DESIGN

Test species:	Unicellular fresh water green alga, *Pseudokirchneriella subcapitata* (Reinsch) Korshikov (synonymous *Selenastrum capricornutum* Prinz), SAG 61.81; in-house culture; stock obtained from the "Algal Collection", Göttingen, Germany
Test design:	Static system; test duration 72 hours; 5 test concentrations, each with 3 replicates per treatment plus a control with 6 replicates; daily assessment of growth.
Endpoints:	NOEC, EC_{10}, EC_{20} and EC_{50} values with respect to growth rate and yield after exposure over 72 hours.
Test concentrations:	0 (control), 1.0, 2.5, 6.3, 16 and 40 mg product/L (nominal)
Test conditions:	250-mL Erlenmeyer flasks; test volume 100 mL; algal nutrient medium (AAP medium); initial cell density: 1 x 10^4 cells/mL; pH: 7.44 – 8.93; temperature: 21.9 – 22.2 °C; continuous shaking (90 rpm); continuous light at approximately 7420 - 7888 lux.
Analytics:	Analytical verification of test item concentrations was conducted using an HPLC-method with DAD (Diode Array Detector).
Statistics:	Descriptive statistics; EC_x values based on growth rate and yield were calculated via Probit analysis using linear max. likelihood regression, NOEC values were determined via Williams multiple Sequential T-test (growth rate) and Multiple Sequentially-rejective Welsh-t test after Bonferroni-Holm (yield).

II. RESULTS AND DISCUSSION

Analytical results: Samples for analytical determination of the concentration were taken at test initiation and termination. In the samples collected at exposure initiation, the determined concentrations of cinmethylin were in the range of 99.5 % - 102.6 % of nominal concentration.

In the samples collected at exposure termination, the determined concentrations of cinmethylin were in the range of 79.2 - 97.2 % of nominal concentration. Therefore, the test item concentrations were stable (within ± 20 % of nominals) under test conditions, except for the lowest test item concentration. The HSE evaluator considers the use of nominal concentrations acceptable due to the marginal difference 0.8 % below i.e. 79.2 % of nominal during study termination at lowest test concentration (80 % of nominal would be considered acceptable). Precipitation of the test item was not reported during the study.

The results are shown in the table below.

Table B.9.3.1-5: Measured concentrations during study

Nominal (mg product/L)	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	< LoD	--	< LoD	--
1.0	0.737	0.756	102.6	0.584	79.2
2.5	1.842	1.847	100.3	1.610	87.5
6.3	4.641	4.728	101.9	4.319	93.1
16	11.786	11.776	99.9	11.182	94.9
40	29.464	29.320	99.5	28.637	97.2

-- = not applicable LoQ = 0.002 mg a.s./L, LoD = 0.0005 mg a.s./L

Validity criteria:

In OECD 201 (2011) the following criteria are stated:

- 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}. In this study the control increased by a factor of 253.9.
- 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%. This criterion applies to the mean value of coefficients of variation calculated for replicate control cultures. In this study the CV was 33.6 % based on section by section.
- 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.* In this study the CV was 1.1 % over the whole test period.

During the study the above criteria were met.

Biological results: No morphological effects on the algae were observed in concentrations up to and including 6.3 mg product/L compared to control at all observation times. At 16 mg product/L 40% and at 16 mg product/L 70% of algal cells appeared deformed compared to algal cells of the control. Statistically significant inhibition was determined in the three highest test concentrations for growth rate (Williams multiple Sequential T-test, α = 0.05, one-sided smaller) and yield (Multiple Sequentially-rejective Welsh-t test after Bonferroni-Holm, α = 0.05, one-sided smaller). The results are summarized in Table B.9.3.1-6.

Table B.9.3.1-6: Effect of BAS 684 03 H on the growth of the green algae.

Concentration [mg product/L] (nominal)	**Control**	**1.0**	**2.5**	**6.3**	**16**	**40**
Inhibition in 72 h (growth rate) [%]	0.0	-0.4 #	1.9	4.8*	21.0*	74.7*
Inhibition in 72 h (yield) [%]	0.0	-2.1 #	9.3	23.5**	69.0**	98.8**
	Endpoints [mg product/L] (nominal)					
E_rC_{50} (72 h)	**26.3** (95 % limits: 25.5 – 27.1)					
E_rC_{20} (72 h)	15.36 (95 % limits: 14.65 – 16.04)					
E_rC_{10} (72 h)	15.4 (95 % limits: 14.7 – 16.0)					
E_yC_{50} (72 h)	10.7 (95 % limits: 9.5 – 11.9)					
NOEC (growth rate and yield)	2.5					

Negative values indicate stimulated growth compared to the control.

* Statistically significant difference compared to control (Williams multiple Sequential T-test, α = 0.05, one-sided smaller)

** Statistically significant difference compared to control (Multiple Sequentially-rejective Welsh-t test after Bonferroni-Holm, α = 0.05, one-sided smaller)

Reference test: A reference study was conducted with 3,5 DCP. The 72-hour E_rC_{50} was calculated as 2.57 mg reference item/L which is within ranges of references stated in OECD 201.

III. CONCLUSION

In a 72-hour algae test with *Pseudokirchneriella subcapitata* the 72 h-E_rC_{50} of BAS 684 03 H was determined at 26.3 mg product/L and the 72 h E_yC_{50} was 10.7 mg product/L based on nominal concentrations.

HSE evaluator comments:

The above study was conducted to GLP and is considered valid. The analytical method is validated in accordance with SANCO/3029/99 rev. 4 with an LOQ of 0.002 mg/L (see volume 3, CA, section B5 for full details) and the following endpoints have been derived:

- '**BAS 684 03H**' 72-hour E_rC_{50} = **26.3 mg product/L** (based on nominal concentration), equivalent to **19.37 mg a.s./L**
- 'BAS 684 03H' 72-hour E_rC_{20} = 15.36 mg product/L (based on nominal concentration), noting uncertainty mentioned above, equivalent to 11.31 mg a.s./L
- 'BAS 684 03H' 72-hour E_rC_{10} = 15.4 mg product/L (based on nominal concentration), noting uncertainty mentioned above, equivalent to 11.34 mg a.s./L
- 'BAS 684 03H' 72-hour E_yC_{50} = 10.7 mg product/L (based on nominal concentration), equivalent to 7.88 mg a.s./L
- 'BAS 684 03H' NOEC (yield and growth rate) = 2.5 mg product/L (based on nominal concentration)

Report: CP 10.2.1/5
Rzodeczko H., 2017 b
BAS 684 03 H - *Lemna gibba* CPCC 310 growth inhibition test
2017/1013180
Guidelines: OECD 221 (2006)
GLP: Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test item:	BAS 684 03 H, batch FD-170210-0001; analyzed content of a.s. (Cinmethylin, Reg. no.: 900 202): 737.3 g/L (nominal: 750 g/L), density: 1.001 g/cm³

B. STUDY DESIGN

Test species:	Duckweed (*Lemna gibba* G3), specification CPCC 310, inocula from 9 days old cultures; cultures maintained in-house; stock G3 obtained from "Canadian Phycological Culture Centre (CPCC)", Department of Biology, University of Waterloo, Canada.
Test design:	Static system (7 days); 7 test item concentrations plus control; with 3 replicates for the test item treatments and 6 replicates for the control; 3 plants with 3 fronds, total number of fronds at test initiation: 9 per replicate; assessment of growth and other effects on days 2, 5 and 7.
Endpoints:	NOEC and ECx with respect to growth rate and yield after exposure over 7 days.
Test concentrations:	Control, 0.01, 0.03, 0.1, 0.3, 1.0, 3.0 and 10 mg product/L (nominal).
Test conditions:	Glass beakers (diameter 9 cm), test volume 400 mL, 20x-AAP nutrient medium, pH 7.54 - 7.75 at test initiation and pH 8.69 – 8.91 at test termination; temperature: 23.0 – 23.8 °C, continuous light, light intensity: 7840 - 8400 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 (α = 0.05, one-sided smaller) for determination of the NOEC 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. In samples collected at exposure initiation the determined concentration of Cinmethylin was in the range of 91.5 % – 102.4 % of nominal concentration. In samples collected at exposure termination the determined concentration of Cinmethylin was in the range of 62.5 % – 78.6 % of nominal concentration. The test item solutions were reported as homogeneous and without visibly non-dissolved particles.

The analytical data is shown in the table below:

Table B.9.3.1-7: Measured concentrations during study

Nominal (mg product/L)	Nominal (mg a.s./L)	Day 0		Day 7		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	< LoD	--	< LoD	--	0.0
0.01	0.007	0.00674	91.5	0.00500	67.8	0.0058
0.03	0.022	0.02198	99.5	0.01545	69.9	0.0184
0.1	0.074	0.0731	99.2	0.0505	68.5	0.0608
0.3	0.221	0.2108	95.4	0.1424	64.4	0.173
1	0.737	0.7224	98.0	0.5030	68.2	0.603
3	2.210	2.1979	99.5	1.3814	62.5	1.74
10	7.366	7.5393	102.4	5.7888	78.6	6.61

-- = not applicable LoQ = 0.002 mg a.s./L, LoD = 0.0005 mg a.s./L
* Calculated by study author following OECD 23, annex II equation. The HSE evaluator agrees with the derived values (noting they have been expressed based on a.s. content).
** The % of nominal values have been copied from the study report. It is noted there are marginal differences when using values in above table for some test concentrations which is likely to be due to rounding. As the study report would have considered raw data the HSE evaluator considers % of nominal values reported should be used.

Given that test concentrations were not maintained within ± 20 % of nominals the test concentrations should be based on geometric mean measured concentrations in accordance with OECD 221 (2006). Therefore, in response to a request from the HSE evaluator the applicant recalculated endpoints based on geometric mean measured concentrations (BASF DocID: 2017/1013180). These values are shown in the results section below.

Validity criteria:

In OECD 221 (2006) the following criteria are stated:

- 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}. During the study the doubling time was 2.2 days and a 9.4 fold increase in seven days was observed/ specific growth rate of 0.320 d^{-1}.

During the study the above criteria were met.

Biological results: The duckweed population in the control vessels showed sufficient growth. Statistically significant (inhibitory) effects on the growth of *Lemna gibba* compared to the control were observed at the five highest tested concentration for all measured parameters except for yield based on dry weight, were a significant effect occurred in each but the lowest concentration (Williams Multiple Sequential t-test Procedure, $\alpha = 0.05$, one-sided smaller). No morphological changes were observed for the plants in controls and at the lowest test item concentrations of 0.01 mg/L over the whole study duration. At the higher tested concentrations, symptoms like smaller fronds, overlapping and bending down fronds, colony break-up, fronds with short or separated roots and/or pink-yellow spots, spots of chlorosis or necrosis were observed.

Effects on growth rate and yield are summarised in Table B.9.3.1-8.

Table B.9.3.1-8: Effect of BAS 684 01 H on the growth of duckweed *Lemna gibba*

Concentration (nominal) [mg product/L]	**Control**	**0.01**	**0.03**	**0.1**	**0.3**	**1**	**3**	**10**
Geometric mean concentration [mg product/L][a]	**Control**	**0.0079**	**0.025**	**0.083**	**0.235**	**0.819**	**2.36**	**8.97**
Inhibition after 7 d [%] [#] (growth rate based on frond no.)	0.0	-2.1	1.2	20.6*	65.9*	84.8*	91.0*	98.4*
Inhibition after 7 d [%] (growth rate based on dry weight)	0.0	0.2	3.1	14.5*	17.1*	22.5*	28.8*	41.7*
Inhibition after 7 d [%] [#] (yield based on frond no.)	0.0	-5.3	3.1	41.2*	86.3*	95.1*	97.3*	99.6*
Inhibition after 7 d [%] (yield based on dry weight)	0.0	0.8	9.7*	39.1*	44.5*	54.1*	63.3*	77.4*
Observations	N	N	SF	SF, OB, 29 % PY, SR	45 % SC, OB, SR	50 % C, BF, SR	CB, SeR	CB, Ne
	Endpoints [mg product/L] (geometric mean measured)							
E_rC_{50} (7 d) based on frond no.	0.167 (95 % limits: 0.051 – 0.183)							
E_rC_{20} (7 d) based on frond no.	0.074 (95 % limits: 0.064 – 0.084)							
E_rC_{10} (7 d) based on frond no.	0.053 (95 % limits: 0.043 – 0.062)							
E_yC_{50} (7 d) based on frond no.	0.096 (95 % limits: 0.088 – 0.105)							
E_yC_{20} (7 d) based on frond no.	0.049 (95 % limits: 0.042 – 0.056)							
E_yC_{10} (7 d) based on frond no.	0.033 (95 % limits: 0.026 – 0.040)							
E_rC_{50} (7 d) based on dry weight	> 8.97 (95 % limit: n.d.)							
E_rC_{20} (7 d) based on dry weight	0.479 (95 % limits: 0.216 – 1.074)							
E_rC_{10} (7 d) based on dry weight	0.063 (95 % limits: 0.029 – 0.136)							
E_yC_{50} (7 d) based on dry weight	0.487 (95 % limits: 0.236 – 0.744 mg/L)							
E_yC_{20} (7 d) based on dry weight	0.027 (95 % limits: 0.008 – 0.053)							
E_yC_{10} (7 d) based on dry weight	0.005 (95 % limits:0.001 – 0.012)							
NOEC overall	0.0079							

N = Described as 'Normal' size, shape of colonies and roots, SF = Smaller fronds, OB = Overlapping and bending fronds, BF = Bending fronds, PY = Pink-yellow spots, SC = Spots of chlorosis, C = Chlorosis, SR = Short roots, CB = Colony break-up, SeR = Separated roots, Ne = Necrosis

n.d.: not determined due to mathematical reasons.

[#] Negative values indicate stimulated growth compared to the control.

[*] Statistically significant difference compared to control (Williams Multiple Sequential t-test, α = 0.05, one-sided smaller)

[a] Calculated by HSE evaluator based on content of active substance in formulation based on geometric mean concentrations calculated in table B.9.3.1-7, based on actual content of active substance 737.3 g/L and formulation density of 1.001 g/cm^3.

Reference test: A reference study was conducted with 3,5 DCP. The 7-day E_rC_{50} values were 13.91 and 12.06 mg/L based on frond number and dry weight respectively. 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. Following a request from the HSE evaluator historical reference test data was provided (BASF DocID: 2019/2051550). The EC_{50} values ranged from 7.45 – 17.93, 6.99 – 12.77 for frond number and dry weight respectively. Therefore, the reference test results (frond number and dry weight) for this study are within historical limits.

III. CONCLUSION

In a 7-day aquatic plant test with *Lemna gibba* the E_rC_{50} of BAS 684 03 H was determined to be 0.167 mg/L based on frond number and > 8.97 mg/L based on dry weight (geometric mean measured). The E_yC_{50} of BAS 684 03 H was determined to be 0.096 mg/L based on frond number and 0.487 mg/L based on dry weight (geometric mean measured).

HSE evaluator comments:

Phytotoxicity was also observed during the study, noting the active ingredient cinmethylin is an herbicide. Based on the observations in table B.9.3.1-8 the HSE evaluator proposes an EC_{50} based on phytotoxicity of approximately 0.819 mg product/L (based on geometric mean). Noting the lowest E_rC_{50} value reported below appears to be protective of 50 % phytotoxicity.

The above study was conducted to GLP and is considered valid (validity criteria met). The analytical method is validated in accordance with SANCO/3029/99 rev. 4 with an LOQ of 0.002 mg/L and the following endpoints have been derived:

- 'BAS 684 03H' 7-day E_rC_{50} (frond number) = **0.167** mg product/L equivalent to **0.123** mg a.s./L (based on geometric mean concentration)
- 'BAS 684 03H' 7-day E_rC_{20} (frond number) = approximately 0.074 mg product/L equivalent to 0.055 mg a.s./L (based on geometric mean concentration)
- 'BAS 684 03H' 7-day E_rC_{10} (frond number) = 0.053 mg product/L equivalent to 0.039 mg a.s./L (based on geometric mean concentration)
- 'BAS 684 03H' 7-day E_yC_{50} (frond number) = 0.096 mg product/L equivalent to 0.071 mg a.s./L (based on geometric mean concentration)
- 'BAS 684 03H' 7-day E_yC_{20} (frond number) = 0.049 mg product/L equivalent to 0.036 mg a.s./L (based on geometric mean concentration)
- 'BAS 684 03H' 7-day E_yC_{10} (frond number) = 0.033 mg product/L equivalent to 0.024 mg a.s./L (based on geometric mean concentration)
- 'BAS 684 03H' 7-day E_rC_{50} (dry weight) = > 8.97 mg product/L equivalent to > 6.607 mg a.s./L (based on geometric mean concentration)
- 'BAS 684 03H' 7-day E_rC_{20} (dry weight) = 0.479 mg product/L equivalent to 0.353 mg a.s./L (based on geometric mean concentration)
- 'BAS 684 03H' 7-day E_rC_{10} (dry weight) = 0.063 mg product/L equivalent to 0.046 mg a.s./L (based on geometric mean concentration), noting uncertainty mentioned above.
- 'BAS 684 03H' 7-day E_yC_{50} (dry weight) = 0.487 mg product/L equivalent to 0.359 mg a.s./L (based on geometric mean concentration)
- 'BAS 684 03H' 7-day E_yC_{20} (dry weight) = 0.027 mg product/L equivalent to 0.020 mg a.s./L (based on geometric mean concentration)
- 'BAS 684 03H' 7-day E_yC_{10} (dry weight) = approximately 0.005 mg product/L equivalent to 0.004 mg a.s./L (based on geometric mean concentration), noting uncertainty mentioned above.
- 'BAS 684 03H' NOEC (overall) = 0.0079 mg product/L equivalent to 0.0058 mg a.s./L (based on geometric mean concentration and phytotoxicity observations)

Report:	CP 10.2.1/6 Janson G.-M., 2017 a Effect of BAS 684 03 H on the growth of the aquatic plant *Glyceria maxima* 2017/1000861
Guidelines:	OECD 239 (2014)
GLP:	Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 684 03 H; batch FD-170210-0001; content of a.s.: cinmethylin (Reg. No. 900 202): 750 g/L (nominal) (737.3 g/L analyzed); density: 1.001 g/cm³.

B. STUDY DESIGN

Test species: *Glyceria maxima* (Reed Sweet-grass), 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 4 days prior to the study.

Test design: Static renewal system (14 days) with sediment; 4 day acclimations phase prior the exposure period, 6 test item concentrations each with 5 replicates per treatment plus a control with 10 replicates, application of test item via the water phase, 14 days exposure (after 7 days all test item concentrations plus control were renewed), a plant with two blades of grass per pot at test start; assessment of plant growth and visual effects e.g. chloroses or necrosis were conducted on day 7, day 10 and at the end of the study. Fresh weight and dry weight were determined at test termination. 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 item concentration.

Endpoints: EC_{50} and NOEC 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 product/L.

Test conditions: Glass beakers (2 L); test volume 6 cm water column; standard artificial sediment (1- 2 cm) with pH 7.30 (according to OECD 219) and Smart & Barko medium; pH 7.91 at the start of the acclimations phase and at test initiation, pH 7.90 at day 7 for the new solution; measured water temperature: 20.2 – 21.2°C; light : dark rhythm of 16 : 8 h, measured light intensity: 10.21 – 11.50 klux.

Analytics: Analytical verification of test item concentrations in water was conducted using HPLC-method with MS–detection.

Statistics: Descriptive statistics; the statistical determination of the EC_{50} was done by Probit analysis using linear max. likelihood regression. The NOEC was determined statistically by Welch-t Test for wet weight, dry weight and total length yield and growth weight (one-sided smaller, $\alpha = 0.05$) and Dunnett's test for number of blades (one-sided smaller, $\alpha = 0.05$).

II. RESULTS AND DISCUSSION

Analytical measurements: The correct application of the test item concentrations was confirmed by the analytical measurements at the beginning, 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 and day 7 (old solution) at the end 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 97.2 % and 106 % (average of 101.4 %) of nominal. At test termination and day 7 (old solution) concentrations were between 62.3 % and 76.6 % (average 69.7 %) of nominal. In addition, the test item sample of 3 mg/L (water only) was measured at DAT 7 104 % and DAT 14 100 %.

The analytical data is shown in the table below:

Table Table B.9.3.1-9: Measured concentrations during study

Nominal (mg product/L)	Nominal (mg a.s./L)	Day 0		Day 7				Day 14		GM (mg product/L)*	GM (mg product/L)**
		'Fresh' solution		'Fresh' solution		'Aged' solution		'Aged' solution			
		MM (mg a.s./L)	% of nominal[#]	MM (mg a.s./L)	% of nominal[#]	MM (mg a.s./L)	% of nominal[#]	MM (mg a.s./L)	% of nominal[#]		
0	0.0	< LoD	--	< LoD	--	< LoD	--	< LoD	--	--	--
0.01	0.007	0.00764	103	0.00777	105	0.00488	66.0	0.00566	76.5	0.0086	0.009
0.03	0.022	0.0221	100	0.0234	106	0.0146	66.3	0.0161	73.4	0.025	0.027
0.1	0.074	0.0735	99.4	0.0732	98.9	0.0461	62.3	0.0524	70.8	0.081	0.085
0.3	0.221	0.218	99.3	0.214	97.2	0.139	63.0	0.16	72.8	0.24	0.25
1.0	0.737	0.745	101	0.78	105	0.495	66.9	0.567	76.6	0.86	0.9
3.0	2.210	2.246	102	2.217	100	1.497	67.7	1.616	73.1	2.53	2.65

-- = not applicable LoQ = 0.0015 mg a.s./L, LoD = 0.0003 mg a.s./L, GM = Geometric mean, MM = Mean measured test concentration.

* Calculated by study author following OECD 23, annex II equation. The HSE evaluator agrees with the derived values (noting they have been expressed based on product content using formulation density and content of a.s. = 737 g/L).

** Geometric mean values calculated in amendment report that was used in statistical analysis to calculate endpoints based on geometric mean values rather than nominal concentrations.

[#] The % of nominal values have been copied from the study report. It is noted there are marginal differences when using values in above table for some test concentrations which is likely to be due to rounding. As the study report would have considered raw data the HSE evaluator considers % of nominal values reported should be used.

Given that test concentrations were not maintained within ± 20 % of nominals the test concentrations should be based on geometric mean measured concentrations in accordance with OECD 221 (2006). Therefore, in response to a request from the HSE evaluator the applicant recalculated endpoints based on geometric mean measured concentrations (BASF DocID: 2017/1000861). It was noted the geometric mean concentrations calculated are different from those calculated above by the original study author which may be due to rounding. The concentrations used in the reanalysis were; 0.0090, 0.027, 0.085, 0.25, 0.90 and 2.65 mg product/L. The difference between concentrations is considered minimal by the HSE evaluator therefore the recalculated geometric mean measured endpoints have been accepted by the HSE evaluator. These values are shown in the results section below.

Validity criteria:

In OECD 239 (2014) the following criteria are stated, noting this guidance document is 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. In this study the plants increased in control replicates by more than 200 % when considering the two parameters wet weight and length. It was reported that slight growth of algae was observed in the control but no other signs of phytotoxicity were observed. Whilst the prescence of algae generates uncertainty, given the rest of the validity criteria were met the HSE evaluator does not consider this deviation sufficient to invalidate the study.
- The mean coefficient of variation (CV) 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. In this study the mean CV based on yield of wet weight was 13.5 %.

During the study the above criteria were considered met by the HSE evaluator.

Biological results: On day 7 and day 10 in the test item concentration of 3 mg product/L in two replicates single blades show slight chloroses. At day 14 in the treatments 0.3 and 1.0 mg product/L partly blades tips show a slight chlorosis and necrosis. In the highest test item concentration of 3 mg product/L, in two replicates 50 % of the blade tips hang down. One replicate shows a slight necrosis. No indication of abnormality was observed in the treatments 0.01, 0.03 and 0.1 mg product/L and control.

Statistically significant inhibition of yield and growth rate based on wet weight and dry weight was observed at the five highest test item concentrations (Welch t-test, α = 0.05, one-sided smaller). Statistically significant inhibition of yield and growth rate based on total length was observed at the four highest test item concentrations (Welch t-test, α = 0.05, one-sided smaller). Statistically significant inhibition of yield based on number of blades was observed at the two highest test item concentrations (Dunnett's multiple t-test, α = 0.05, one-sided smaller). Effects on growth rate and yield are summarized in Table B.9.3.1-10.

Table B.9.3.1-10: Effect of BAS 684 03 H on the growth of *Glyceria maxima*

Concentration of BAS 684 03 H [mg product/L] (nominal)	**Control**	**0.01**	**0.03**	**0.1**	**0.3**	**1.0**	**3.0**
Geometric mean concentration [mg product/L][a]	**Control**	**0.0090**	**0.026**	**0.085**	**0.25**	**0.90**	**2.65**
Inhibition after 14 d [%] # (growth rate based on total length)	--	-1.8	5.8	19.8* 1)	26.3* 1)	51.5* 1)	60.5* 1)
Inhibition after 14 d [%] # (growth rate based on wet weight)	--	4.3	6.2* 1)	25.5* 1)	32.1* 1)	59.3* 1)	65.9* 1)
Inhibition after 14 d [%] # (growth rate based on dry weight)	--	2.5	5.5* 1)	19.6* 1)	22.5* 1)	42.4* 1)	47.7* 1)
Inhibition after 14 d [%] # (yield based on number of blades)	--	-23.1	7.7	0	15.4	46.2* 2)	61.5* 2)
Inhibition after 14 d [%] # (yield based on total length)	--	-5.8	7.7	26.6* 1)	34.1* 1)	62.9* 1)	68.0* 1)
Inhibition after 14 d [%] # (yield based on wet weight)	--	7.7	10.9* 1)	37.6* 1)	45.4* 1)	72.5* 1)	78.2* 1)
Inhibition after 14 d [%] # (yield based on dry weight)	--	5.3	11.3* 1)	30.7* 1)	39.7* 1)	62.3* 1)	68.0* 1)
Observations at end of study	3 N	3 N	2 (3 reps) 3 (2 reps) N	1 N	1 SC Sn	0 (1 rep) 1 (4 reps) SC Sn	0 (2 reps) 1 (3 reps) Sn (1 rep) 50 % BT (2 reps)

	Endpoints [mg product/L] (geometric mean measured)
E_yC_{50} (14 d) based on no. of blades	1.161 (95 % limits: 0.113-2.200)
E_yC_{20} (14 d) based on no. of blades	0.239 (95 % limits: n.d.-0.631)
E_yC_{10} (14 d) based on no. of blades	0.095 (95 % limits: n.d.-0.375)
E_rC_{50} (14 d) based on total length	0.947 (95 % limits: 0.301-1.593)
E_rC_{20} (14 d) based on total length	0.103 (95 % limits: 0.006-0.200)
E_rC_{10} (14 d) based on total length	0.040 (95 % limits: 0.0-0.090)
E_yC_{50} (14 d) based on total length	0.522 (95 % limits: 0.204-0.848)
E_yC_{20} (14 d) based on total length	0.065 (95 % limits: 0.012-0.141)
E_yC_{10} (14 d) based on total length	0.019 (95 % limits: 0.002-0.055)
E_rC_{50} (14 d) based on wet weight	0.617 (95 % limits: 0.353-0.881)
E_rC_{20} (14 d) based on wet weight	0.074 (95 % limits: 0.027-0.121)

E_rC_{10} (14 d) based on wet weight	0.030 (95 % limits: 0.006-0.054)
E_yC_{50} (14 d) based on wet weight	0.270 (95 % limits: 0.155-0.388)
E_yC_{20} (14 d) based on wet weight	0.035 (95 % limits: 0.014-0.059)
E_yC_{10} (14 d) based on wet weight	0.010 (95 % limits: 0.003-0.022)
E_rC_{50} (14 d) based on dry weight	2.218 (95 % limits: 1.213-3.224)
E_rC_{20} (14 d) based on dry weight	0.120 (95 % limits: 0.050-0.189)
E_rC_{10} (14 d) based on dry weight	0.035 (95 % limits: 0.007-0.063)
E_yC_{50} (14 d) based on dry weight	0.530 (95 % limits: 0.314-0.764)
E_yC_{20} (14 d) based on dry weight	0.035 (95 % limits: 0.011-0.074)
E_yC_{10} (14 d) based on dry weight	0.006 (95 % limits: 0.001-0.017)
NOEC (overall)	0.0090

0 = no roots, 1 = few roots, 2 = moderate roots development, 3 = very good roots development, s = roots are shorter compared to control, rep = replicate, N = No indication of abnormality, C = Chlorosis, SC = Slight chlorosis, Sn = Slight Necrosis, BT = Blade tips hang down
-- = not applicable
# Negative values indicate stimulated growth compared to the control.
n.d. = not determined
* Statistically significant difference compared to control.
1) Statistically different compared to control (Welch t-test, α = 0.05, one-sided smaller).
2) Statistically different compared to control (Dunnett's multiple t-test, α = 0.05, one-sided smaller).
[a] Calculated by applicant in re-analysis report (BASF DocID: 2017/1000861)

III. CONCLUSION

In a 14-day aquatic plant test with *Glyceria maxima,* the most sensitive E_rC_{50} for BAS 684 03 H was determined to be 0.617 mg product/L based on wet weight (equivalent to 0.454 mg a.s./L).

HSE evaluator comments:

It was noted a reference study was not conducted. However, there is not an OECD method for this species with appropriate ranges for reference items. Therefore, the omission is considered acceptable by the HSE evaluator. Similarly, there is no validity criteria for this species however the study does meet the validity criteria of OECD 239 (*Myriophyllum* water/sediment study).

Overall, the analytical method is validated in accordance with SANCO/3029/99 rev. 4. Full validation data have provided for BAS 684 H in tap water and M4-medium, in addition to a limited data set for BAS 684 H in Smart&Barko medium. The LOQ for BAS 684 H in Smart&Barko medium is 1.55 µg/L (for full details refer to volume 3 CA section B5).

It was noted lower confidence limits were not derived for the following endpoints: E_yC_{20} (blade number), E_yC_{10} (blade number) and E_rC_{10} (total length). Whilst this adds some uncertainty as to whether these values are sufficiently robust the HSE evaluator does not consider this point alone sufficient to invalidate the study. Furthermore, the most sensitive parameter was wet weight based on the available data.

Phytotoxicity was also observed during the study, noting the active ingredient cinmethylin is an herbicide. Based on the observations in table B.9.3.1-10 the HSE evaluator proposes an EC_{50} based on phytotoxicity of approximately 2.65 mg product/L (based on geometric mean). The lowest E_rC_{50} value reported below appears to be protective of 50 % phytotoxicity.

In the study report it was stated that at study termination for final fresh/dry weight measurements plants were cut above the sediment. It was unclear whether the same method was used at study initiation. The HSE evaluator does not consider this point alone sufficient to invalidate the study. In addittion, the most sensitive parameter considering growth rate was based on wet weight.

The above study was conducted to GLP and is considered valid. The following endpoints have been derived:

- 'BAS 684 03H' 14-day E_yC_{50} (number of blades) = 1.161 mg product/L equivalent to 0.855 mg a.s./L (based on geometric mean concentration)

- 'BAS 684 03H' 14-day E_yC_{20} (number of blades) = 0.239 mg product/L equivalent to 0.176 mg a.s./L (based on geometric mean concentration)
- 'BAS 684 03H' 14-day E_yC_{10} (number of blades) = 0.095 mg product/L equivalent to 0.070 mg a.s./L (based on geometric mean concentration)
- 'BAS 684 03H' 14-day E_rC_{50} (total length) = approximately 0.947 mg product/L equivalent to 0.698 mg a.s./L (based on geometric mean concentration)
- 'BAS 684 03H' 14-day E_rC_{20} (total length) = 0.103 mg product/L equivalent to 0.076 mg a.s./L (based on geometric mean concentration)
- 'BAS 684 03H' 14-day E_rC_{10} (total length) = 0.040 mg product/L equivalent to 0.029 mg a.s./L (based on geometric mean concentration)
- 'BAS 684 03H' 14-day E_yC_{50} (total length) = 0.522 mg product/L equivalent to 0.384 mg a.s./L (based on geometric mean concentration)
- 'BAS 684 03H' 14-day E_yC_{20} (total length) = 0.065 mg product/L equivalent to 0.048 mg a.s./L (based on geometric mean concentration)
- 'BAS 684 03H' 14-day E_yC_{10} (total length) = 0.019 mg product/L equivalent to 0.014 mg a.s./L (based on geometric mean concentration)
- 'BAS 684 03H' 14-day E_rC_{50} (wet weight) = **0.617 mg product/L** equivalent to **0.454** mg a.s./L (based on geometric mean concentration)
- 'BAS 684 03H' 14-day E_rC_{20} (wet weight) = 0.074 mg product/L equivalent to 0.055 mg a.s./L (based on geometric mean concentration)
- 'BAS 684 03H' 14-day E_rC_{10} (wet weight) = 0.030 mg product/L equivalent to 0.022 mg a.s./L (based on geometric mean concentration)
- 'BAS 684 03H' 14-day E_yC_{50} (wet weight) = 0.270 mg product/L equivalent to 0.199 mg a.s./L (based on geometric mean concentration)
- 'BAS 684 03H' 14-day E_yC_{20} (wet weight) = 0.035 mg product/L equivalent to 0.026 mg a.s./L (based on geometric mean concentration)
- 'BAS 684 03H' 14-day E_yC_{10} (wet weight) = 0.010 mg product/L equivalent to 0.007 mg a.s./L (based on geometric mean concentration)
- 'BAS 684 03H' 14-day E_rC_{50} (dry weight) = 2.218 mg product/L equivalent to 1.634 mg a.s./L (based on geometric mean concentration)
- 'BAS 684 03H' 14-day E_rC_{20} (dry weight) = 0.120 mg product/L equivalent to 0.088 mg a.s./L (based on geometric mean concentration)
- 'BAS 684 03H' 14-day E_rC_{10} (dry weight) = 0.035 mg product/L equivalent to 0.026 mg a.s./L (based on geometric mean concentration)
- 'BAS 684 03H' 14-day E_yC_{50} (dry weight) = 0.530 mg product/L equivalent to 0.390 mg a.s./L (based on geometric mean concentration)
- 'BAS 684 03H' 14-day E_yC_{20} (dry weight) = 0.035 mg product/L equivalent to 0.026 mg a.s./L (based on geometric mean concentration)
- 'BAS 684 03H' 14-day E_yC_{10} (dry weight) = 0.006 mg product/L equivalent to 0.004 mg a.s./L (based on geometric mean concentration)
- 'BAS 684 03H' NOEC (overall) = 0.0090 mg product/L equivalent to 0.007 mg a.s./L (based on geometric mean concentration and observations)

B.9.3.2. Additional long-term and chronic toxicity studies on fish, aquatic invertebrates and sediment dwelling organisms

No studies submitted.

B.9.3.3. Further testing on aquatic organisms

No formulation studies submitted but the risk from bioaccumulation to aquatic organisms has been considered below.

Bioaccumulation risk assessment

The log P_{ow} of the active substance cinmethylin is 4.5. Therefore, the bioaccumulation in fish has been addressed by the applicant with two BCF studies on bluegill sunfish (Forbis & Franklin, 1983b and Salinas *et al*, 2017b). It

should be noted that an additional study (Schaffert & Ufer, 2018a) complements Salinas *et al*, 2017b with a focus on the metabolism of cinmethylin. Study summaries are provided in volume 3, CA dossier, section 9.2.8.

Following evaluation by the HSE evaluator Forbis & Franklin, 1983b was not considered suitable for use in the risk assessment. Therefore, further consideration is not required. However, the other studies (Salinas *et al*, 2017b and Schaffert & Ufer, 2018a) are suitable and have been considered below.

Salinas et al, 2017b and Schaffert & Ufer, 2018a:

The BCF values from Salinas *et al*, 2017b were 707 and 688 L kg-1 at 0.5 and 5 µg a.s./L respectively when corrected for lipid content. In the study Total Radioactive Residue (TRR) was measured and clearance of total radioactivity half-life values (CT_{50}) were determined as 1.12, 1.08 days at 0.5 and 5 µg a.s./L respectively. A supporting metabolism study (Schaffert & Ufer, 2018a) was also submitted hence it is possible to ascertain the BCF for the parent (cinmethylin) and identify metabolites present. In this study at end of exposure period 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 order to account for the metabolites present using this study the HSE evaluator has re-calculated the BCF endpoints based on cinmethylin to 170 and 59 for 0.5 and 5 µg a.s./L, corresponding to a geometric mean of 100.4. As stated in EFSA aquatic guidance 2013 biomagnification must be considered for compounds where the BCF is > 1000 and the elimination of radioactivity during the 14-day depuration phase in the bioconcentration study is < 95 % and the substance is stable in water or sediment ($DegT_{90}$ > 100 days). The environmental fate section details the worst case DT_{90} in water to be 25.2 days and > 100 days in sediment meeting the 'stability' criteria for sediment. The BCF value (geomean) was 100 and based on the metabolism study (Schaffert & Ufer, 2018a) the worst case DT_{50} value considering total radioactivity was 1.12 days hence both are within the triggers detailed and further consideration is not required.

B.9.4. RISK ASSESSMENT FOR AQUATIC ORGANISMS

The following risk assessment has been conducted according to the EFSA (2013)[5] guidance document.

Exposure

All exposure estimates are reproduced from Volume 3, Section B.8 (PPP – fate dossier). Predicted Exposure Concentrations (PECs) used for risk assessment have been established by the Environmental Fate Evaluator.

Relevant metabolites for consideration in the risk assessment are outlined below in Table B.9.4-1.

Table B.9.4-1: Relevant metabolites for consideration during the aquatic risk assessment

Metabolite	Relevant environmental compartments
M684H001	Water
M684H003	Water

Toxicity

Cinmethylin toxicity

The tier-1 data available to address the toxicity of the active substance, cinmethylin, is summarised below (Table B.9.4-2).

[5] EFSA (2013). Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. EFSA Journal 2013;11(7):3290, 268 pp. doi:10.2903/j.efsa.213.3290.

Table B.9.4-2: Endpoints relevant for cinmethylin

Test substance	Test organism	Test system	Endpoint (mg a.s./L)		Reference
Acute toxicity to fish					
Cinmethylin (COD-002038)	*Oncorhynchus mykiss* (also known as *Salmo gairdneri*)	96-hours, static	LC_{50}	8.49 (m.m)[a]	Kary-Heinrich & Catchpole (2017a)
Cinmethylin (COD-002038)	*Cyprinus carpio*	96-hours, static	LC_{50}	**5.75** (g.m)	Rzodeczko (2017a) & (2018b)[#]
Cinmethylin (COD-002038)	*Pimephales promelas*	96-hours, static	LC_{50}	5.84 (m.m)[a]	Kary-Heinrich & Catchpole (2017b) & (2018a)[#]
Long-term toxicity to fish					
Cinmethylin (COD-002038)	*Pimephales promelas*	35-days, flow through, early-life-stage study	NOEC*	**0.59** (m.m)	Salinas *et al* (2017a)
			EC_{10} (bdl)*	0.92 (m.m)	
			EC_{20} (bdw)*	2.57 (m.m)	
Bioconcentration in fish					
Cinmethylin (COD-002038)	*Lepomis macrochirus*	17 days update and 7 days depuration	BCF[##]	707 L kg^{-1} (whole fish at 0.5 µg eq/L) 688 L kg^{-1} (whole fish at 5 µg eq/L) Geometric mean of 697 L kg^{-1}	Salinas *et al* (2017b)
Cinmethylin (COD-002038)	*Lepomis macrochirus*	Metabolism study supporting Salinas *et al* (2017b)	BCF (parent)	Geometric mean (whole fish 0.5 and 5 µg a.s./L) recalculated based on cinmethylin content to **100.4**** using data from Salinas *et al* 2017b	Salinas *et al* (2017b) & Schaffert (2018a)
Acute toxicity to invertebrates					
Cinmethylin (COD-002038)	*Daphnia magna*	48-hours, static	EC_{50}	**7.26** (nom.)	Haerthe (2016a)
Long-term toxicity to invertebrates					
Cinmethylin (COD-002038)	*Daphnia magna*	21-days, static renewal	NOEC	**0.29** (g.m)[###]	Rzodeczko (2017b)
			EC_{10}	> 0.29 (g.m)[###]	
			EC_{20}	> 0.29 (g.m) [###]	
Toxicity to algae					
Cinmethylin (COD-002038)	*Pseudokirchneriella subcapitata*	72-hours, static	E_rC_{50}	**23.04 (g.m)**	Kauf (2017a)
			E_rC_{20}	7.87 (g.m)	
			E_rC_{10}	> 1.765 (g.m)[b]	
			E_yC_{50}	5.96 (g.m)	
			E_yC_{20}	1.76 (g.m)	
			E_yC_{10}	0.93 (g.m)	
Cinmethylin	*Anabaena flos-*	96-hours, static	E_rC_{50}	51.34 (g.m)	Kauf (2017b)
			E_rC_{20}	31.63 (g.m)	

Test substance	Test organism	Test system	Endpoint (mg a.s./L)		Reference
(COD-002038)	*aquae*		E_rC_{10}	24.55 (g.m)	
			E_yC_{50}	31.10 (g.m)	
			E_yC_{20}	Not reported	
			E_yC_{10}	16.86	
Toxicity to aquatic macrophytes					
Cinmethylin (COD-002038)	*Lemna gibba*	7-days, static, water only	E_rC_{50}	**0.0888** g.m (f.n.) > 0.2580 g.m (d.w.)	Vlechev (2017a)
			E_rC_{20}	0.0421 g.m (f.n.) 0.0735 g.m (d.w.)	
			E_rC_{10}	0.0285 g.m (f.n.) 0.0300 g.m (d.w.)	
			E_yC_{50}	0.0515 g.m (f.n.) 0.0841 g.m (d.w.)	
			E_yC_{20}	0.0270 g.m (f.n.) 0.0220 g.m (d.w.)	
			E_yC_{10}	0.0192 g.m (f.n.) 0.0109 g.m (d.w.)	
Cinmethylin (COD-002038)	*Glyceria maxima*	14-days, static renewal, water only	E_rC_{50}	0.137 g.m (t.l.) 0.159 g.m (w.w.) 0.621 g.m (d.w.)	Vlechev (2017b)
			E_rC_{20}	0.043 g.m (t.l.) 0.068 g.m (w.w.) 0.095 g.m (d.w.)	
			E_rC_{10}	0.023 g.m (t.l.) 0.044 g.m (w.w.) 0.035 g.m (d.w.)	
			E_yC_{50}	0.112 g.m (t.l.) 0.109 g.m (w.w.) 0.215 g.m (d.w.)	
			E_yC_{20}	0.044 g.m (t.l.) 0.046 g.m (w.w.) 0.055 g.m (d.w.)	
			E_yC_{10}	0.027 g.m (t.l.) 0.029 g.m (w.w.) 0.027 g.m (d.w.)	
Other aquatic organisms					
Cinmethylin (WL95481)	*Gammarus pulex*	96-hour, static	LC_{50}	6.6 (nom.) Supporting information only	Pearson & Stephenson (1987a)[c]
Cinmethylin (WL95481)	*Lymnaea stagnalis*	96-hour, static	LC_{50}	7.0 (nom.) Supporting information only	
Cinmethylin (WL95481)	*Tubifex tubifex*	96-hour, static	LC_{50}	5.4 (nom.) Supporting information only	
Cinmethylin (WL95481)	*Chironomus lugubris*	96-hour, static	LC_{50}	> 2.06 (g.m) Supporting information only	

nom. = nominal; m.m = arithmetic mean measured; g.m. = geometric mean measured; f.n. = frond number, d.w. = dry weight, t.l. = Total shoot length, w.w. = wet weight, f.w. = fresh weight, bdl = body length, bdw = body weight

Bold *values are recommended for use in risk assessment at tier-1.*
** It should be noted the EC_{10} and EC_{20} values calculated only considered body length and body weight. The NOEC is based on survival.*
*** BCF value recalculated based on metabolism study (Schaffert 2018a). In this study at end of exposure period 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 order to account for the metabolites present using this study the HSE evaluator has re-calculated the BCF endpoints based on cinmethylin to 170 and 59 for 0.5 and 5 µg a.s./L, corresponding to a geometric mean of 100.4.*
[#] Amendment to final report also considered.
[##] BCF normalised to 5 % lipid content in accordance with OECD 305
[###] Based on time weighted average concentration. Due to lack of analytical measurements during study for three test concentrations only the lowest and highest concentrations could be calculated. Therefore, this endpoint is considered conservative.
[a] Endpoint should have been based on geometric mean measured. However, the geometric mean test concentrations calculated by the HSE evaluator are comparable to mean measured concentrations hence the study author values have been accepted, see relevant study summaries for further details.
[b] Uncertainty regarding statistically derived value hence conservative approach has been taken and a greater than value reported.
[c] Study considered suitable as supporting information only by the HSE evaluator due to uncertainties; not possible to confirm validity criteria were met and lack of control without solvent (see study summary for further details).

Toxicity to aquatic sediment dwellers

The study Pearson & Stephenson (1987a) determined an LC_{50} for *Chironomus lugubris* based on water only exposure. However, this study was only considered suitable as supporting information by the HSE evaluator and the LC_{50} value was above the highest test concentration i.e. > 2.06 mg a.s./L (g.m.).

In accordance with EFSA aquatic guidance document 2013 consideration of toxicity to *Chironomus* sp is required if the substance accumulates in sediment (water/sediment study demonstrates > 10 % of applied radioactivity at or after day 14 present in the sediment) and the chronic *Daphnia* test shows an EC_{10}/NOEC of < 0.1 mg a.s./L. Whilst the cinmethylin levels were above 10 % after day 14, peaking at 55.9 % at 56 days the chronic *Daphnia* endpoints were above the trigger of 0.1 mg a.s./L. Therefore, further consideration is not required.

First tier RAC endpoints for cinmethylin:

Aquatic invertebrate's endpoint discussion:

When considering the acute toxicity to aquatic invertebrates only one valid GLP study is available testing *Daphnia magna* producing an EC_{50} value of 7.26 µg a.s./L. However, several other species were also assessed in the study Pearson & Stephenson (1987a) suggesting lower endpoints and that the *Daphnia* study may not be protective. The lowest LC_{50} value was unbound at > 2.06 µg a.s./L and the highest calculated endpoint was 7.0 µg a.s./L. Whilst these studies were only considered suitable as supporting information a risk assessment has been performed using the lowest unbound value to derive a separate RAC. This has been marked in the table below as illustrative but will be considered in the risk assessment.

Aquatic plant endpoint discussion:

Whilst two studies testing *Lemna gibba* and *Glyceria maxima* are considered valid for quantitative consideration by the HSE evaluator there were a further three aquatic plant studies that can be used as supporting information for the aquatic plant RAC. All five studies have been summarised in the table below and considered based on species tested and endpoints derived.

Table B.9.4-3: Summary of submitted aquatic plant studies

Test species	**Method**	**Analytical data**	**Most sensitive Endpoints**	**HSE evaluator comments on validity**
Lemna gibba (monocotyledon)	7-days, static, water only	All test concentrations analysed and analytical method validated	E_rC_{50} : 0.0888 mg a.s./L g.m. (frond number) E_yC_{50} : 0.0515 mg a.s./L g.m. (frond number) *Phytotoxicity:* Not possible to determine EC_{50} based on phytotoxicity- effects on roots (shorter and very short) observed at geometric mean concentrations of 0.006 mg a.s./L and above. Chlorosis only observed at geometric mean concentrations of 0.099 mg a.s./L (above EC_{50} values).	Valid GLP study
Glyceria maxima (monocotyledon)	14-days, static renewal, water only		E_rC_{50} : 0.137 mg a.s./L g.m. (total length) E_yC_{50} : 0.109 mg a.s./L g.m. (wet weight) *Phytotoxicity:* Above endpoints protective of 50 % phytotoxicity.	Valid GLP study
Myriophyllum spicatum (dicotyledon)	14-days, static, water-sediment system	All test concentrations analysed at study initiation. Concentrations monitored throughout study (nominals: 0.0179, 1.88 and 6 mg a.s./L). Extrapolation used for other concentrations (nominals 0.0572, 0.183 and 0.586 mg a.s./L)[#]	E_rC_{50} : 0.414 mg a.s./L g.m. (fresh weight) E_yC_{50} : 0.231 mg a.s./L g.m. (fresh weight) *Phytotoxicity:* Above endpoints protective of 50 % phytotoxicity.	Valid GLP study but only suitable as supporting information
Elodea canadensis (monocotyledon)	14-days, static, water-sediment system	All test concentrations analysed at study initiation. Concentrations monitored throughout study (nominals: 0.586, 1.88 and 6 mg a.s./L). Extrapolation used for other concentrations (nominals 0.0179, 0.0572 and 0.183 mg a.s./L)[#]	E_rC_{50} : 0.247 mg a.s./L g.m. (fresh weight) E_yC_{50} : 0.198 mg a.s./L g.m. (fresh weight) *Phytotoxicity:* 33 % (short roots and yellow inner parts of top leaves) and 67 % (no roots) phytotoxicity was observed at 0.1332 mg a.s./L (g.m.). Minor effects (< 50 %) observed at the concentration below (0.0425 mg a.s./L g.m.).	Valid GLP study but only suitable as supporting information
Egeria densa (monocotyledon)	14-days, static, water-sediment system	All test concentrations analysed at study initiation. Concentrations monitored throughout study (nominals: 0.586, 1.88 and 6 mg a.s./L). Extrapolation used for other concentrations (nominals 0.0179, 0.0572 and 0.183 mg a.s./L)[#]	E_rC_{50} : 0.116 mg a.s./L g.m. (fresh weight) E_yC_{50} : 0.092 mg a.s./L g.m. (fresh weight) *Phytotoxicity:* Between 50 (no roots) and 67 % (top leaves form thick end of shoots) phytotoxicity was observed at 0.1154 mg a.s./L g.m.)	Valid GLP study but only suitable as supporting information

Test species	Method	Analytical data	Most sensitive Endpoints	HSE evaluator comments on validity
			no effects observed at concentration below (0.0371 mg a.s./L g.m.).	

g.m. = geometric mean measured concentration, # Applicant 'extrapolated' missing analytical data based on linearized single first order kinetics using measured values for other test concentrations. Hence calculated endpoints are not considered suitable for quantitative use by the HSE evaluator.

Species tested and available studies:

Based on the terrestrial non-target plant risk assessment (see section B.9.12) it appears that monocotyledons are more sensitive to cinmethylin than dicotyledons. Therefore, the aquatic plant species tested are considered appropriate by the HSE evaluator as four of the five species are monocotyledons.

There were two valid studies submitted testing *Lemna gibba* and *Glyceria maxima* (monocots). The other three studies tested *Myriophyllum spicatum* (dicot), *Elodea canadensis* (monocot) and *Egeria densa* (monocot) but were not considered valid by HSE evaluator as analytical measurements were not determined for all test concentrations throughout the study. Instead 'extrapolated' values were used i.e. where analytical data was not available concentrations were predicted using the decline observed in concentrations that were measured. When considering all studies where analytical measurements were made the decline was broadly comparable (39.93 – 55.32 % of nominals) over the 14-day exposure period. The applicant calculated the 'extrapolated' endpoints based on the highest decline observed to predict likely concentrations by single first order kinetics. However, due to uncertainty regarding exposure the endpoints from these studies have been considered as supporting information only below.

Endpoints derived:

In-line with EFSA aquatic guidance 2013 growth rate endpoints will be considered in the risk assessment. The most sensitive valid endpoint is the *Lemna* study with an E_rC_{50} of 0.0888 mg a.s./L.

Whilst there is uncertainty regarding the endpoints derived in the other three studies (testing *Myriophyllum spicatum*, *Elodea canadensis* and *Egeria densa*) they support *Lemna* as the most sensitive species (factors of 4.7, 2.8 and 1.3 lower in sensitivity respectively). It was noted that the most sensitive endpoints for these studies were all based on fresh weight with E_rC_{50} values of 0.116 (monocot), 0.247 (monocot) and 0.414 (dicot) mg a.s./L. The results for all three are within a factor of 3.6 which could be considered broadly comparable and due to laboratory variation (factor of 3 based on EFSA aquatic guidance 2013 and 5 (WHO, 2002[#]))), noting between monocot species it was x 1.6.

As all monocotyledon studies have broadly comparable endpoints this suggests there is not wide variation between species, noting uncertainty with some studies and that only five species were tested. Overall, when considering the supporting information and valid endpoints the *Lemna* study will be used in the tier 1 RAC as the most sensitive endpoint that appears to be protective of other species based on the available data.

[#]*A factor of 5 is used to determine whether the difference is due to inter-study variability or increased toxicity. The factor is based on SANCO Sanco/10597/2003—rev. 7 final 2, 14 December 2005, which in turn references WHO/FAO (2002) Manual on development and use of FAO and WHO specifications for pesticides. First edition, FAO Plant Production and Protection Paper 173. WHO and FAO, Rome.*

Phytotoxicity:

Given cinmethylin is an herbicide the HSE evaluator has considered the phytotoxicity results that were reported in the five aquatic plant studies.

When considering the most sensitive *Lemna* E_rC_{50} endpoint of 0.0888 mg a.s./L it was not possible to confirm whether this endpoint is protective of 50 % phytotoxicity based on the data reported. Chlorosis only occurred in

concentrations above the E_rC_{50} (0.099 mg a.s./L) but roots were short at concentrations of 0.006 mg a.s./L and above. No further information was given e.g. number of plants impacted therefore there is uncertainty regarding possible phytotoxicity effects. Nonetheless the influence of root length on quantitative parameters measured in the study is unclear. When considering the other studies (including those used as supporting information) the endpoint of 0.0888 mg a.s./L is protective of 50 % phytotoxicity effects (% of plants impacted was assessed in other studies).

Overall, based on the available studies there is no conclusive indication of > 50 % phytotoxicity effects at concentrations below the lowest E_rC_{50} suggesting the endpoint is protective.

Tier 1 RACs for cinmethylin:

The tier 1 RACs are summarised in the table below.

Table B.9.4-4: Tier 1 RACs relevant for cinmethylin

Test species:	**Fish**		**Invertebrates**			**Algae**	**Aquatic plants**
	Acute *C. carpio*	**Chronic *P. promelas***	**Acute *D. magna***	**Acute *C. lugubris***	**Chronic *D. magna***	***P. subcapitata***	***L. gibba***
Endpoint [µg a.s./L]	LC_{50}	NOEC	EC_{50}	LC_{50}	NOEC	E_rC_{50}	E_rC_{50}
	5750	590	7260	>2060	290	23040	88.8
AF	100	10	100	100	10	10	10
RAC [µg a.s./L]	**57.5**	**59**	**72.6**	**20.6**	**29**	**2304**	**8.88**

AF: Assessment factor
Shaded RAC indicates study was considered as supporting information due to issues confirming validity criteria. This RAC has been included as illustrative due to the species potentially being more sensitive than the valid standard study testing *Daphnia magna*.

Metabolites:

The tier-1 data available to address the toxicity of the active substance cinmethylin metabolites are summarised below (Table B.9.4-5).

Table B.9.4-5: Endpoints relevant for metabolites of cinmethylin

Test substance	**Test organism**	**Test system**	**Endpoint (mg a.s./L)**		**Reference**
Acute toxicity to fish					
Cineole alcohol (M684H003)	*Oncorhynchus mykiss* (also known as *Salmo gairdneri*)	96-hours, static	LC_{50}	**> 1000** (nom.) Supporting information only[##]	Girling (1988a)
Acute toxicity to invertebrates					
M684H001	*Daphnia magna*	48-hours, static	EC_{50}	> 100 (nom.)	Turek (2018a)
Cineole alcohol (M684H003)		48-hours, static	EC_{50}	**840** (nom.) Supporting information only[##]	Girling (1988a)
Cineole alcohol (M684H003)		48-hours, static	EC_{50}	> 100 (nom.)	Turek (2018b)
Toxicity to aquatic macrophytes					
M684H001	*Lemna gibba*	7-days, static, water only	E_rC_{50}	> 78.3 g.m (f.n.) > 78.3 g.m (d.w.)	Rzodeczko (2017e)

Test substance	Test organism	Test system	Endpoint (mg a.s./L)		Reference
			E_rC_{20}	38.4 g.m (f.n.) 45.6 g.m (d.w.)	
			E_rC_{10}	16.2 g.m (f.n.) 22.4 g.m (d.w.)	
			E_yC_{50}	64.2 g.m (f.n.) 61.0 g.m (d.w.)	
			E_yC_{20}	12.6 g.m (f.n.) 17.4 g.m (d.w.)	
			E_yC_{10}	> 2.43 g.m (f.n.)[a] 5.59 g.m (d.w.)[a]	
Cineole alcohol (M684H003)	*Lemna gibba*	7-days, static, water only	E_rC_{50}	> 100 nom. (f.n. and d.w.)	Turek (2018c)
			E_yC_{50}	> 100 nom. (f.n. and d.w.)	
			$EC_{10/20}$	Limit study, not possible to calculate	
M684H004	*Lemna gibba*	7-days, static, water only	E_rC_{50}	3.28 g.m (f.n.) > 23.47 g.m (d.w.)	Rzodeczko (2017f)
			E_rC_{20}	1.38 g.m (f.n.) 4.41 g.m (d.w.)	
			E_rC_{10}	0.881 g.m (f.n.) 1.08 g.m (d.w.)	
			E_yC_{50}	1.79 g.m (f.n.) 5.30 g.m (d.w.)	
			E_yC_{20}	0.704 g.m (f.n.) 0.73 g.m (d.w.)	
			E_yC_{10}	0.432 g.m (f.n.)[a] 0.259 g.m (d.w.)[a]	

nom. = nominal; g.m. = geometric mean measured f.n. = frond number, d.w. = dry weight
***Bold** values are recommended for use in risk assessment at tier-1.*
[#] Amendment to final report also considered
Not considered suitable for quantitative use as insufficient information provided to confirm analytical method was validated.
[a] Uncertainty regarding statistically derived endpoint hence based on experimental data, noting these endpoints have not been used in the risk assessment.

First tier RAC endpoints for cinmethylin metabolites:

The tier 1 RACs are summarised in the table below.

Table B.9.4-6: Tier 1 RACs relevant for cinmethylin metabolites

Test species:	Fish (Acute)	**Aquatic invertebrates (Acute)**		**Aquatic plants (E_rC_{50})**	
	O. mykiss	***D. magna***		***L. gibba***	
Metabolite:	**M684H003**	**M684H001**	**M684H003**	**M684H001**	**M684H003**
Endpoint [µg metabolite/L]	>1000000	>100000	840000	>78300	>100000
AF	100	100	100	10	10
RAC [µg metabolite/L]	10000	1000	8400	7830	10000

AF: Assessment factor. It should be noted the metabolite M684H004 was not considered relevant based on the environmental fate dossier (see volume 3, CA dossier, section 8). The relevant metabolites are detailed in table B.9.4-1

Shaded RAC indicates study was considered as supporting information due to issues confirming analytical method was sufficiently validated.

Formulation:

The tier-1 data available to address the toxicity of the representative formulation 'BAS 684 03H' are summarised below (Table B.9.4-7).

Table B.9.4-7: Endpoints relevant for representative formulation 'BAS 684 03 H'

Test substance	Test organism	Test system	Endpoint mg product/L (mg a.s./L)		Reference
Acute toxicity to fish					
'BAS 684 03 H'	*Cyprinus carpio*	96-hours, static	LC_{50}	5.86 (**4.32**) g.m	[redacted] (2017a)[#]
Acute toxicity to invertebrates					
'BAS 684 03 H'	*Daphnia magna*	48-hours, static	EC_{50}	14.5 (**10.68**) nom.	Turek (2017a)
Toxicity to algae					
'BAS 684 03 H'	*Pseudokirchneriella subcapitata*	72-hours, static	E_rC_{50}	26.3 (**19.37**) nom.	Turek (2017b)
			E_rC_{20}	15.36 (11.31) nom.[a]	
			E_rC_{10}	15.4 (11.34) nom.[a]	
			E_yC_{50}	10.7 (7.88) nom.	
			E_yC_{20}	Not reported	
			E_yC_{10}	Not reported	
Toxicity to aquatic macrophytes					
'BAS 684 03 H'	*Lemna gibba*	7-days, static, water only	E_rC_{50}	**0.167** (**0.123**) g.m. f.n. > 8.97 (> 6.607) g.m. d.w.	Rzodeczko (2017b)
			E_rC_{20}	0.074 (0.055) g.m. f.n. 0.479 (0.353) g.m. d.w.	
			E_rC_{10}	0.053 (0.039) g.m. f.n. 0.063 (0.046) g.m. d.w.	
			E_yC_{50}	0.096 (0.071) g.m. f.n. 0.487 (0.359) g.m. d.w.	
			E_yC_{20}	0.049 (0.036) g.m. f.n. 0.027 (0.020) g.m. d.w.	
			E_yC_{10}	0.033 (0.024) g.m. f.n. 0.005 (0.004) g.m. d.w.	
'BAS 684 03 H'	*Glyceria maxima*	14-days, static, water/ sediment system	E_rC_{50}	0.947 (0.698) g.m. t.l. 0.617 (0.454) g.m. w.w. 2.218 (1.634) g.m. d.w.	Janson (2017a)
			E_rC_{20}	0.103 (0.076) g.m. t.l. 0.074 (0.055) g.m. w.w. 0.120 (0.088) g.m. d.w.	
			E_rC_{10}	0.040 (0.029) g.m. t.l. 0.030 (0.022) g.m. w.w. 0.035 (0.026) g.m. d.w.	

Test substance	Test organism	Test system	Endpoint mg product/L (mg a.s./L)		Reference
			E_yC_{50}	1.161 (0.855) g.m. b.n. 0.522 (0.384) g.m. t.l. 0.270 (0.199) g.m. w.w. 0.530 (0.390) g.m. d.w.	
			E_yC_{20}	0.239 (0.176) g.m. b.n. 0.065 (0.048) g.m. t.l. 0.035 (0.026) g.m. w.w. 0.035 (0.026) g.m. d.w.	
			E_yC_{10}	0.095 (0.070) g.m. b.n. 0.019 (0.014) g.m. t.l. 0.010 (0.007) g.m. w.w. 0.006 (0.004) g.m. d.w.	

nom. = nominal; m.m = arithmetic mean measured; g.m. = geometric mean measured; ini. = initial measured f.n. = frond number, d.w. = dry weight, t.l. = Total shoot length, w.w. = wet weight, f.w. = fresh weight, b.n. = blade number
***Bold** values are recommended for use in risk assessment at tier-1.*
[#] *Amendment to final report also considered.*
[a] *Uncertainty as E_rC_{10} and E_rC_{20} values are similar and confidence limits overlap, noting these endpoints are not used in the risk assessment.*

Formulation toxicity assessment:

The tier 1 RACs are summarised in the table below.

Table B.9.4-8: Tier 1 RACs relevant for formulation

Test species:	Fish (Acute)	Aquatic invertebrates (Acute)	Algae (E_rC_{50})	Aquatic plants (E_rC_{50})
	O. mykiss	***D. magna***	***P. subcapitata***	***L. gibba***
Endpoint µg product/L (µg a.s./L)	5860 (4320)	14500 (10680)	26300 (19370)	167 (123)
AF	100	100	10	10
RAC µg product/L (µg a.s./L)	**58.6 (43.2)**	**145 (106.8)**	**2630 (1937)**	**16.7 (12.3)**

AF: Assessment factor

Risk assessment:

The relevant maximum standard worst-case PEC_{SW} values for risk assessments covering the proposed use pattern and the resulting PEC/RAC ratios (ETR) for the active substance are presented in the tables below.

Table B.9.4-9: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for cinmethylin for each organism group based on standard worst-case calculations for proposed use

Group	Fish acute	Fish chronic	Invertebrate acute		Invertebrate chronic	Algae	Higher-Plant
Test species	***C. carpio***	***P. promelas***	***D. magna***	***C. lugubris***	***D. magna***	***P. subcapitata***	***L. gibba***

Group		Fish acute	Fish chronic	Invertebrate acute		Invertebrate chronic	Algae	Higher-Plant
Endpoint		LC_{50}	NOEC	EC_{50}	LC_{50}	NOEC	E_rC_{50}	E_rC_{50}
(µg a.s./L)		5750	590	7260	>2060	290	23040	88.8
AF		100	10	100	100	10	10	10
RAC (µg a.s./L)		57.5	59	72.6	20.6	29	2304	8.88
Entry pathway / Buffer zone [m] / season	**PEC $_{gl\text{-}sw\ max}$ (µg a.s./L)**	**PEC/RAC (= ETR)**						
Spray drift Standard distance (1 m)	4.617	0.080	0.078	0.0636	0.2241	0.159	0.002	0.520
Drainage	26.923	0.468	0.456	0.371	**1.307**	0.928	0.012	**3.032**

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**; Shaded RAC indicates study was considered as supporting information due to issues confirming validity criteria. This risk assessment has been included as illustrative due to the species potentially being more sensitive than the valid standard study testing *Daphnia magna*.

Conclusion at first tier:

Spray drift:

Based on the above first tier assessment an acceptable risk has been demonstrated for all groups based on spray drift, therefore mitigation in the form of buffer zones is not required.

Drain flow:

Based on the above first tier assessment an acceptable risk has been demonstrated for all groups except aquatic plants and the illustrative assessment using supporting information for aquatic invertebrates.

Higher tier assessment (cinmethylin drain flow):

The two groups where a potential risk from drain flow at first tier was identified have been considered below. It should be noted that based on the environmental fate assessment a risk envelope approach from the proposed use on oilseed rape was applied (detailed in section 8.4, fate dossier) as the application rate is 50 % lower and the application timings are earlier. Therefore, in discussion with the fate specialist further consideration of the risk from drain flow for proposed use on oilseed rape is not required.

Aquatic invertebrates:

Higher tier drain flow assessment (WEBFRAM):

The applicant submitted higher tier drain flow modelling using WEBFRAM which has been considered in detail in volume 3 CP fate section 8.5. This modelling is used to determine whether the RAC is exceeded at any point in any given scenario-year, then the acceptability is assessed based on the overall percentage of scenario-years in which the RAC is exceeded and the individual exceedance percentage for individual scenarios. A summary of the fate conclusion for the illustrative invertebrate RAC is presented in italics below for the illustrative RAC for *Chironomus*:

'*When considering individual scenario exceedances, the highest rate was 1.84 % exceedance in one wet scenario on spring barley crops. Overall exceedances were no higher than 0.2 % in winter barley, and were 0 % in winter wheat crops. Based on the presented results, the HSE Fate evaluator is unable to draw a conclusion for drain*

flow based on this RAC and refers to further discussion of this in the Ecotoxicology evaluation (see Volume 3, CP B.9.).'

Based on the above an acceptable risk to aquatic invertebrates can be concluded for the proposed use on winter wheat where no exceedances were observed. However, for the proposed use on barley further consideration is required.

The *Chironomus* toxicity study used for the illustrative RAC (Pearson & Stephenson, 1987a, summarised in CA section 9 dossier) was conducted to GLP and met the validity criteria according to OECD 235 (2011).

The reason the study was considered as supporting information and the risk assessment illustrative was due to the analytical method not being fully validated. In addition, the endpoint used for the RAC is conservative as there were 30 % effects at the geometric mean measured concentration of 2060 µg a.s./L (assessment factor of 100). The reason for the conservative endpoint was that whilst higher concentrations were tested, there were no analytical measurements taken at study termination. Hence, it is not possible to derive a statistical LC_{50} fully supported by chemical analysis. Instead the illustrative LC_{50} is unbound based on the highest test concentration with < 50 % mortality, resulting in a geometric mean measured LC_{50} of > 2060 µg a.s./L. The toxicity results are summarised below.

Table B.9.4-10: 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.

The results above suggest *Chironomus* are more sensitive than *Daphnia* (*Daphnia* EC_{50} was 7.26 mg a.s./L). Given the model WEBFRAM was used for the higher tier drain flow modelling it was not possible to compare exposure profiles to the *Chironomus* RAC in terms of duration and maximum concentrations for the proposed uses on barley.

Overall, *Chironomus* appear to be the most sensitive aquatic invertebrate species to cinmethylin based on the available information. Therefore, given the exceedances of RAC demonstrated in the higher tier drain flow modelling it is not possible to conclude acceptable risk from drain flow to aquatic invertebrates for the proposed use on barley. Further consideration is required.

Following a request for information further consideration of *Chironomus* study was provided along with additional higher tier drain flow modelling as detailed below.

Statistical analysis of Chironomus study:

The applicant used the nominal concentrations to estimate LC_{50}. The 3-parameter Weibull model was used for the analysis and the LC_{50} was estimated as 2.96 mg a.s./L with confidence limits of 2.087 to 3.823 mg a.s./L. The analysis is shown in the figure below.

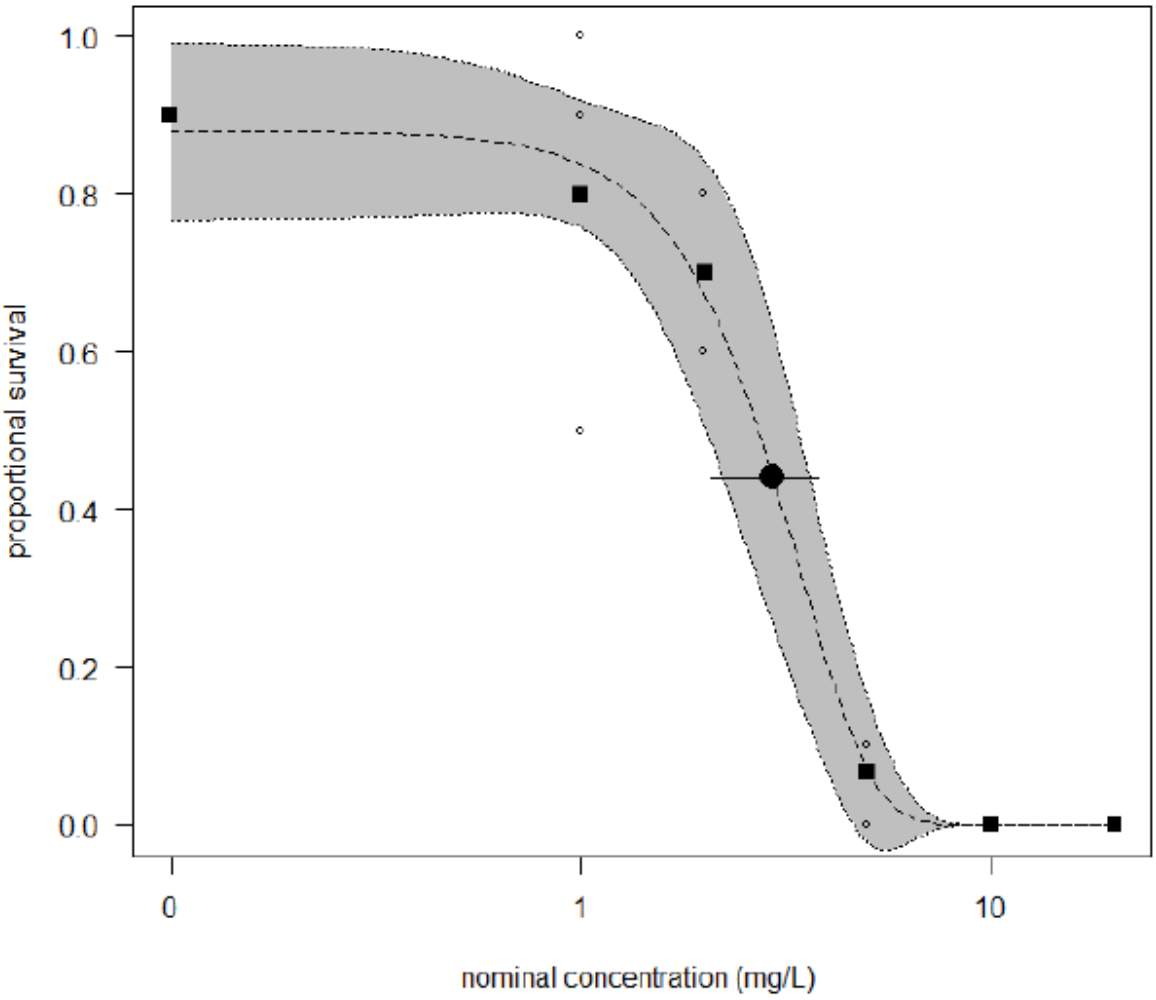


Figure B.9.4-1: Dose response curve in *C. lugubris* study (Pearson & Stephenson, 1987a)

The HSE evaluator does not agree with the use of the above analysis and endpoint (LC_{50} of 2.96 mg a.s./L) as not all test concentrations were analysed. Hence the analytically confirmed endpoint of > 2.06 mg a.s./L based on geometric mean measured concentrations has still been used for the illustrative risk assessment below. However, it is noted that the nominal LC_{50} endpoint of 2.96 mg a.s./L would demonstrate an acceptable risk at tier 1 (see table B.9.4-9) i.e. RAC of 29.6 µg a.s./L compared to worst case drain flow PEC of 26.9 µg a.s./L.

The applicant also considered the higher tier modelling further. The model WEBFRAM is no longer supported hence the model MACRO v4.3 was used. Furthermore, it is noted that using WEBFRAM it is not possible to determine maximum PEC's or compare exposure profiles as only probabilities of RAC exceedance are provided.

Higher tier drain flow assessment (MACRO v4.3):

The Applicant supplied modelling conducted using MACRO v.4.3 based on the application of cinmethylin to winter cereals in the pre-emergence and spring application scenarios (for full details see volume 3, CP section B8).

When considering the illustrative invertebrate RAC of 20.6 µg a.s./L using MACRO modelling no exceedances occurred. In addition, the overall maximum drain flow concentration was 13.2 µg a.s./L for pre-emergence and 9.8 µg a.s./L for post emergence scenarios. Both these maximums are below the illustrative RAC.

Overall conclusion for illustrative Chironomus aquatic invertebrate RAC considering risk from drain flow:

The WEBFRAM higher tier drain flow modelling suggested a maximum of 1.84 % exceedance for proposed uses on barley and no exceedances for winter wheat. When this was explored further for barley with MACRO modelling, the predicted concentrations were below the RAC for all proposed uses and there were no exceedances. In addition, during the response to the request for information, the applicant provided statistical analysis of Chironomus study. Whilst the calculated endpoint could not be used quantitatively it did provide supporting information for an acceptable risk from drain flow at tier 1.

The HSE evaluator considers there is sufficient evidence to conclude an acceptable risk to aquatic invertebrates using the illustrative *Chironomus* RAC for the proposed uses.

Aquatic plants:

Higher tier drain flow assessment (WEBFRAM):

The applicant submitted higher tier drain flow modelling using WEBFRAM which has been considered in detail in volume 3 CP fate section 8.5. A summary of the fate WEBFRAM conclusion for aquatic plants is presented in italics below:

'Regarding cinmethylin, the PEC_{sw} via spray drift value does not exceed the RAC for aquatic plants when considering drift into surface waters at a 1 metre buffer distance. When considering drainage, where a RAC is based on the effects against aquatic plants, a maximum of 60 % exceedance for any single scenario cannot be breached (HSE Data Requirements Handbook; HSE, 2016). Additionally, the overall rate of exceedance must be less than 10%. Based on the presented results, the application of cinmethylin to winter wheat and winter barley fulfils these criteria by not exceeding 18 % in any single scenario or exceeding 1.5 % in overall exceedance rates.'
Based on above an acceptable risk to aquatic plants can be concluded. However, given the applicant supplied MACRO modelling (to address risk to aquatic invertebrates), these values have also been considered below for aquatic plants.
Higher tier drain flow assessment (MACRO v4.3):

Following a request for information the applicant submitted higher tier drain flow modelling using MACRO which has been considered in detail in volume 3 CP fate section 8.5. A summary of the fate MACRO conclusion for aquatic plants is presented in italics below:

'When considering the results compared to the Lemna RAC, one scenario led to exceedances of the RAC in the pre-emergence and post-emergence application scenarios; in both cases, the RAC was exceeded in one year out of 30 (3 %) *in the Denchworth Medium scenario:*

- *Pre-emergence: 10.709 µg a.s./L*
- *Post-emergence: 9.825 µg a.s./L'*

For MACRO modelling an acceptable risk to aquatic plants can be concluded with no more than 10 % overall failure rate and no more than 60 % in any one scenario. As shown above both WEBFRAM and MACRO modelling demonstrated an acceptable risk to aquatic plants for proposed uses.

Overall conclusion for aquatic plant RAC considering risk from drain flow:

Based on the above no further consideration is required for the risk from cinmethylin via drain flow for aquatic plants as the exceedances are below trigger values using modelled values from both WEBFRAM and MACRO.

Metabolites of cinmethylin

The risk assessment for the ecotoxicologically relevant metabolites of cinmethylin (M684H001 and M684H003) has been considered below (see table B.9.4-10). It should be noted two of the studies were only considered suitable as supporting information (these have been highlighted below see shaded columns).

Based on the active substance data the most sensitive group was aquatic plants. For the two relevant metabolites; M684H001, M684H003 data is available for aquatic plants that demonstrates relatively low toxicity (E_rC_{50} values > 78300 and 100000 µg metabolite/L respectively). Equally the invertebrate data also suggests low

toxicity (EC_{50} values > 1000000 and 840000 µg metabolite/L respectively) noting concerns with M684H003 regarding analytical validation. Fish data is only available for metabolite M684H003 again with low toxicity (> 1000000 µg metabolite/L) concerns regarding the analytical validation. Nonetheless given the low toxicity for other aquatic groups (including most sensitive) and in the interest of reducing vertebrate testing this is considered acceptable by the HSE evaluator. The lack of algal data submitted has been discussed below.

Table B.9.4-11: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for cinmethylin metabolites for each organism group based on standard worst-case calculations for proposed use

Group	**Exposure**	**Fish (Acute)**	**Inverteb. acute**		**Aquatic plants**	
Test species		*O. mykiss*	*D. magna*		*L. gibba*	
Metabolite:		M684H003	M684H001	M684H003	M684H001	M684H003
Endpoint (µg metabolite/L)		LC_{50} >1000000	EC_{50} >100000	EC_{50} 840000	E_rC_{50} >78300	E_rC_{50} >100000
AF		100	100	100	10	10
RAC (µg metabolite/L)		10000	1000	8400	7830	10000
Spray drift entry / Buffer zone [m]	$PEC_{sw\text{-}ini}$ (µg metabolite/L)	PEC/RAC (= ETR)				
M684H001 standard distance (1 m)	0.584	--	0.000584	--	0.0000746	--
M684H003 standard distance (1 m)	0.318	0.0000318	--	0.0000378	--	0.0000318
M684H001 Drainage	3.404	--	0.003404	--	0.0004347	--
M684H003 Drainage	1.856	0.0001856	--	0.000221	--	0.0001856

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentrations, -- = not applicable as different metabolite PEC
Shaded RAC indicates study was considered as supporting information due to issues confirming analytical method was sufficiently validated.

It was noted that no algal studies testing the relevant metabolites were submitted. However, the PEC / RAC ratios based on the $PEC_{sw,\ ini}$ and the RAC_{ppp} values indicate an acceptable risk to aquatic organisms with a wide margin of safety (minimum factor of x 294). In addition, it was noted that the exposure concentrations for both metabolites based on spray drift are within those for the parent cinmethylin where an acceptable risk was demonstrated. When considering drainage exposure values these are within the parent by a significant amount (minimum factor of x8) at first tier, which when considering the parent toxicity (cinmethylin) would result in an acceptable risk. Given the available data for aquatic organisms demonstrates the metabolites are less toxic than cinmethylin this further supports an acceptable risk for the metabolites. No further consideration of metabolites is required.

Formulation assessment

The risk assessment for the representative formulation is shown below.

Table B.9.4-12: Formulation risk assessment: ($PEC_{sw, ini}$ / RAC_{ppp} < 1) for each organism group based on $PEC_{sw, ini}$ values resulting from spray drift entry of the formulation after application of cinmethylin for the proposed use

Group	Exposure	Fish (Acute)	Inverteb. acute	Algae	Aquatic plants
Test species		*O. mykiss*	*D. magna*	*P. subcapitata*	*L. gibba*
Endpoint (µg product/L)		LC_{50} 5860	EC_{50} 14500	E_rC_{50} 26300	E_rC_{50} 167
AF		100	100	10	10
RAC (µg product/L)		58.6	145	2630	16.7
Spray drift entry / Buffer zone [m]	PEC_{sw-ini} (µg product/L)	PEC/RAC (= ETR)			
standard distance (1 m)	6.149	0.105	0.042	0.002	0.368

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentrations

The PEC / RAC ratios based on the $PEC_{sw, ini}$ and the RAC_{ppp} values indicate an acceptable risk to aquatic organisms at a standard distance of 1 m for the proposed uses of the representative formulation 'BAS 684 03 H'.

Overall conclusion for risk to aquatic organisms:

Based on the above an acceptable risk to aquatic organisms for the proposed use on winter wheat and oilseed rape can be concluded. However, further consideration of the risk from drainflow to aquatic invertebrates for the proposed use on winter barley is required.

B.9.5. EFFECTS ON ARTHROPODS

B.9.5.1. Effects on bees

B.9.5.1.1. Acute oral toxicity to bees

Report: CP 10.3.1.1.1/1
Sekine T., 2016 a
BAS 684 02 H: Effects (acute contact and oral) on honey bees (*Apis mellifera* L.) in the laboratory
2016/1044858
Guidelines: OECD 213 (1998), OECD 214 (1998)
GLP: yes
(certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 684 02 H; batch no. FD-150416-0012; content of a.s.: BAS 684 H (Reg. No. 900 202): 750.0 g/L nominal (750.2 g/L analyzed); density: 1.020 g/cm^3.

B. STUDY DESIGN

Test species:	*Apis mellifera* L. (honeybee), young adult worker bees (about 4 - 6 weeks old) derived from a healthy and queen-right colony, source: in-house colonies; collected in the morning prior to use. No chemical substances were used in the hive for at least one month prior to the test.
Test design:	In a 48-hour test, young adult worker bees of *Apis mellifera* L. were exposed orally to BAS 684 02 H via food (50% (w/v) sucrose solution containing). In total, 7 treatment groups were set up (5 dose rates of the test item, 1 untreated control group and 4 dose rates of the reference item) with 3 replicates per treatment and 10 bees per replicate. Assessment of bee mortality and behavioral effects were done after 4, 24 and 48 hours.
Test chambers:	Stainless steel chambers were used with dimensions of approximately 8 cm x 6 cm x 4 cm containing 10 bees per replicate.
Endpoints:	Mortality (LD_{50}), behavioral impairments. Number of dead bees were counted after 4 (± 0.5 h) hours (first day), 24 and 48 (± 2 h) hours. Behavioural abnormalities were assessed after 4 (± 0.5 h) hours (first day), 24 and 48 (± 2 h) hours and were recorded in the following categories: m = moribund i.e. cannot walk/feeble movements/ weak response to stimulation, a = affected i.e. bees upright and attempting to walk but showing signs of reduced co-ordination, c = cramps i.e. bees contracting abdomen or entire body, ap = apathy i.e. bees show only low or delayed reactions to stimulation, v = vomiting.
Reference item:	Dimethoate EC 400 (BAS 152 11 I, dimethoate, 420.3 g/L analyzed).
Test doses:	Control groups: 50% (w/v) sucrose solution. BAS 684 02 H: 12.5, 25.0, 50.0, 100.0 and 200.0 µg a.s./bee, resulting in an actual uptake of 13.9, 27.9, 55.5, 109.7 and 216.7 µg a.s./bee (corresponding to 18.9, 37.9, 75.5, 149.2 and 294.7 µg test item/bee). Reference item: 0.06, 0.09, 0.16 and 0.33 µg dimethoate/bee. The treated food was offered in syringes, which were weighed before and after introduction into the cages. Duration of food uptake ranged from 40 minutes to one hour and 20 minutes for the test item. Syringes were then weighed and replaced by ones containing fresh untreated food.
Test conditions:	Temperature: 24.0°C – 27.0°C; relative humidity: 55% - 80%; photoperiod: 24 h darkness; food: 50% (w/v) sucrose solution. Test conditions were continuously recorded with an electronic data logger.
Statistics:	Descriptive statistics; Weibull analysis for the LD_{50} values of the reference item.

II. RESULTS AND DISCUSSION

Validity Criteria

Validity criteria were met:

- The average mortality for the total number of controls did not exceed 10 per cent at the end of the test, it was actually 0%.
- The LD_{50} of the toxic standard meets the specified range i.e. for dimethoate the range is a LD_{50}-24h range of 0.10-0.35 µg a.i./bee and it was reported to be 0.34 µg dimethoate/bee in this study.

After 48 hours, 6.7% mortality was observed in the 109.7 µg as/bee dose group (equivalent to 149.2 µg BAS 684 02 H/bee). No mortality occurred in the actual doses of 13.9, 27.9, 55.5 and 216.7 µg a.s./bee (equivalent to 18.9, 37.9, 75.5 and 294.7 µg BAS 684 02 H/bee) and control group after 48 hours. No test item induced behavioral effects were observed. The results are summarized in Table B.9.5.1-1.

Table B.9.5.1-1: Toxicity of BAS 684 02 H to *Apis mellifera* L. (honeybee) in an oral toxicity test

Treatment	**Dosage [consumed]**	**Mortality [%]**	
		24 h	**48 h**
Control	Control	0.0	0.0
BAS 684 02 H [µg product/bee]	18.9	0.0	0.0
	37.9	0.0	0.0
	75.5	0.0	0.0
	149.2	0.0	6.7
	294.7	0.0	0.0
Endpoint [µg consumed product/bee]			
LD_{50} (48 h)	> 294.7		

The LD_{50} value (24 h) for the reference item was determined to be 0.34 µg dimethoate/bee (95% confidence limits: 0.16 - 0.71 µg dimethoate/bee), based on consumption.

III. CONCLUSION

In an acute oral toxicity study with BAS 684 02 H on honeybees, the LD_{50} value (48 h) was determined to be > 294.7 µg BAS 684 02 H/bee (equivalent to > 216.7 µg a.s./bee).

HSE evaluator comments:

The study was well reported and conducted with good adherence to OECD Guideline 213 (1998).

It was noted that the guideline states that relative humidity is normally kept between 55-70% for this study. However, in the report it was stated to range from 55-80%. This was considered to be acceptable since validity criteria were all met, and controls behaved as required demonstrating acceptable environmental conditions were maintained.

The endpoint for consideration in the risk assessment is:

- **LD_{50} > 294.7 µg BAS 684 02 H/bee (equivalent to > 216.7 µg a.s./bee)**

Report: CP 10.3.1.1.1/2
Amsel K., 2016 a
Acute toxicity of BAS 684 02 H to the bumblebee *Bombus terrestris L.* under laboratory conditions
2016/1044855

Guidelines: Van der Steen (1996), Van der Steen (2001), OECD 213 (1998), OECD 214 (1998), Hanewald et al. (2013)

GLP: yes
(certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 684 02 H; batch no. FD-150416-0012; content of a.s.: BAS 684 H (Reg. No. 900 202): 750.0 g/L nominal (737.6 g/L analyzed); density: 1.020 g/cm^3.

B. STUDY DESIGN

Test species: *Bombus terrestris* L. (bumblebee), young adult worker bumblebees derived from healthy and queen-right hives; source: Biobest Belgium N.V., Westerlo, Belgium; and were 150-300 mg in weight.

Collection of bees: Bumblebees were collected in the morning of use and transferred to each test unit using red light. After transfer they had 2 hours of acclimatization to test room conditions and an additional 2 hours of starvation prior to treatments.

Weight range of bees: 150-300ng.

Test design: In a 96-hour test, adults of *Bombus terrestris* were exposed to 5 doses of BAS 684 02 H in treated food (50% (w/v) sucrose solution). In total, 7 treatment groups were set up: 5 dose rates of the test item, 1 control group and 4 dose rates of the reference item with 30 replicates per dose and 1 bumblebee per replicate, respectively. Assessments of bumblebee mortality and behavioral effects were done after 4, 24, 48, 72 and 96 hours.

Oral application: The bumblebees were fed with a defined quantity of a 50% (w/v) sucrose solution including the test or reference item. Each bumblebee was provided with 40µL (47 mg) of test solution. The solution was pipetted into the Nicot cell cup placed into the cup holder. Finally, the hatching cage containing the bumblebee was placed on the socked containing the cell cup. About 4 hours after test start the cell cups were empty and were reweighed to determine the exact quantity of the test solution consumed.

Test units: Nicot cages as part of the rearing system consisting of socked, cup holder, cell cup and hatching cage with a length of 7cm and diameter of 2cm. Ventilation was provided by the air-conditioning in the climatic chamber.

Food: 50% (w/v) sucrose solution. During the test (after application) food was provided continuously using a syringe set up horizontally to the cage. There was a 2 hour starvation period prior to the application of the test item.

Endpoints: Mortality, behavioral impairments.

Reference item: BAS 152 11 I (dimethoate, analyzed 420.3 g/L).

Test doses: Sucrose control (50% (w/v) sucrose solution); test item at dose rates of 17.0, 34.0, 68.0, 136.0 and 272.0 µg BAS 684 02 H/bumblebee (resulting in an actual uptake of 16.8, 33.4, 66.4, 128.9 and 258.5 µg BAS 684 02 H/bumblebee); reference item at dose rates of 0.25, 0.45, 0.82 and 1.48 µg dimethoate/bumblebee. Applied and consumed doses are in table B.9.5.1-2 below.

Table B.9.5.1-2: Applied and consumed doses

Treatment group	Test solution ID	Item to be applied	Applied dosages		Actual intake of the applied item		Applied/exposed volume [µL/ bumblebee]
Control	AC	sucrose solution	-				40
			[µg product/ bumblebee]	[µg a.s./ bumblebee]	[µg product/ bumblebee]	[µg a.s./ bumblebee]	
Test item	AT	BAS 684 02 H*	272.0	200.0	258.5	190.1	40
	BT		136.0	100.0	128.9	94.8	
	CT		68.0	50.0	66.4	48.8	
	DT		34.0	25.0	33.4	24.6	
	ET		17.0	12.5	16.8	12.4	
Reference item	AR	Dimethoate EC 400**	3.84	1.51	3.77	1.48	40
	BR		2.11	0.83	2.08	0.82	
	CR		1.16	0.46	1.14	0.45	
	DR		0.64	0.25	0.63	0.25	

* based on nominal content of a.s.
** based on analysed content of a.s.
Calculations are performed with non-rounded values.

Test conditions: Temperature: 24.2 °C – 25.4 °C, relative humidity: 50.0% – 62.0%, photoperiod: 24 h darkness except during handling and assessment when diffuse artificial light was used; food: 50% (w/v) sucrose solution.

Statistics: Descriptive statistics; Fisher's Exact Binominal Test with Bonferroni Correction for mortality data (one-sided greater, $\alpha = 0.05$).

II. RESULTS AND DISCUSSION

Validity criteria

The validity criteria have been considered in the evaluator comments following the study summary since there was no specific guideline available for the acute oral toxicity study for bumblebees at the time this study was conducted.
After 96 hours of oral exposure, no mortality occurred in the control group fed with 50% (w/v) sucrose solution. In the test item treatment, no mortality occurred after oral consumption of 16.8, 33.4, 66.4, 128.9 and 258.5 µg BAS 684 02 H./bumblebee. No behavioral effects of the bumblebees occurred in all tested dose rates in the oral toxicity test when compared to the control. The results are summarized in Table B.9.5.1-3.

Table B.9.5.1-3:Toxicity of BAS 684 02 H to *Bombus terrestris* (bumblebee) in an oral toxicity test

Treatment	Dosage [consumed]	Mortality [%]			
		24 h	48 h	72 h	96 h
Control	Sucrose	0.0	0.0	0.0	0.0
BAS 684 02 H [µg product/bumblebee]	16.8	0.0	0.0	0.0	0.0
	33.4	0.0	0.0	0.0	0.0
	66.4	0.0	0.0	0.0	0.0
	128.9	0.0	0.0	0.0	0.0
	258.5	0.0	0.0	0.0	0.0
Endpoint [µg product/bumblebee]					
LD_{50} (96 h) [1)]	> 258.5				

1) Median lethal dose calculated by Probit analysis.

The LD_{50} value (96 h) for the reference item in the oral test was determined to be 0.87 µg a.s./bumblebee.

III. CONCLUSION

In an acute oral toxicity study with BAS 684 02 H on bumblebees, the LD_{50} value (96 h) was estimated to be > 258.5 µg consumed BAS 684 02 H/bumblebee, corresponding to > 190.1 µg consumed a.s./bumblebee.

HSE evaluator comments:

The study was conducted prior to the acceptance of the current bumble bee guideline OECD 247 (2017). Instead a combination of several guidelines was used as detailed in the study summary above.

The evaluator has evaluated this study to OECD 213 (1998) as this was used in the derivation of the study but also notes that deviations may have occurred.

Environmental conditions were in line wth those set out in the guideline.

The validity criterion relating to the average mortality in control groups was achieved i.e. <10% at the end of the test. However, it was noted that with regards to the toxic standard validity criterion, the LD_{50} was 0.87 µg a.s./bumblebee which exceeds the guideline's specified range i.e. 0.10-0.35 µg a.s./bee. However, it was noted that there may be differences between sensitivities in bumble bees when compared to honeybees for which Guideline 213 (1998) was written. The current guideline OECD 247 (2017) states that a dose of 4 µg a.s./bumblebee should result in ≥ 50 % at the end of the test period. Therefore, the evaluator considers the reference substance results in this study to be in line with the expected results for bumblebees.

It was noted that it was not explicitly reported whether antibiotics or other chemicals had previously been used on the bee colony used for the test however the latest guideline (OECD 246, 2017) does not stipulate this and although this study was undertaken before the new guideline was published, the evaluator considers this not to invalidate the study.

The endpoint for consideration in the risk assessment is:

- **LD_{50} > 258.5 µg consumed BAS 684 02 H/bumblebee (corresponding to > 190.1 µg consumed a.s./bumblebee)**

Acute contact toxicity to bees

Report:	CP 10.3.1.1.2/1 Sekine T., 2016 a BAS 684 02 H: Effects (acute contact and oral) on honey bees (*Apis mellifera L.*) in the laboratory 2016/1044858
Guidelines:	OECD 213 (1998), OECD 214 (1998)
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 684 02 H; batch no. FD-150416-0012; content of a.s.: BAS 684 H (Reg. No. 900 202): 750.0 g/L nominal (750.2 g/L analyzed); density: 1.020 g/cm^3.

B. STUDY DESIGN

Test species: *Apis mellifera* L. subspecies *iberiensis* E. (honeybee), young adult worker bees (about 4 - 6 weeks old) derived from a healthy and queen-right colony, source: in-house colonies; collected in the morning prior to use. No chemical substances were used in the hive for at least one month prior to the test.

Test design: In a 48-hour test, young adult worker bees of *Apis mellifera* L. were exposed to a 5 µL droplet of 5 dose rates of BAS 684 02 H in an appropriate carrier (tap water containing 0.5% Adhäsit) placed on the dorsal bee thorax. Adhäsit was used to improve the spreading of the test droplet on the bee body and is non-toxic to honeybees. The reference item was also applied made up in tap water containing 0.5% Adhäsit. Application was made using a calibrated pipette (Multipette ©, Eppendorf). In total, 6 treatment groups were set up (4 dose rates of the test item, 1 untreated control group and 4 dose rates of the reference item) with 3 replicates per treatment and 10 bees per replicate. Assessment of bee mortality and behavioral effects were done after 4, 24 and 48 hours. The 5 µL droplet was a highlighted deviation from the guideline which recommends 1 µL. The report stated that this higher volume ensured a more reliable dispersion of the test item and that the laboratory (ibacon) had experience that higher volumes were suitable with no adverse effects on the outcome of the study expected.

Endpoints: Mortality (LD_{50}), behavioral impairments.
Number of dead bees were counted after 4 (± 0.5 h) hours (first day), 24 and 48 (± 2 h) hours.
Behavioural abnormalities were assessed after 4 (± 0.5 h) hours (first day), 24 and 48 (± 2 h) hours and were recorded in the following categories: m = moribund i.e. cannot walk/feeble movements/ weak response to stimulation, a = affected i.e. bees upright and attempting to walk but showing signs of reduced co-ordination, c = cramps i.e. bees contracting abdomen or entire body, ap = apathy i.e. bees show only low or delayed reactions to stimulation, v = vomiting.

Reference item: Dimethoate EC 400 (BAS 152 11 I, dimethoate, 420.3 g/L analyzed).

Test doses: Control group: water control (tap water containing 0.5% Adhäsit). BAS 684 02 H: 12.5, 25.0, 50.0, 100.0 and 200.0 µg a.s./bee corresponding to 17.0, 34.0, 68.0, 136.0 and 272.0 µg product/bee. Reference item: 0.10, 0.15, 0.20 and 0.30 µg dimethoate/bee.

Test conditions: Temperature: 25.0°C – 27.0°C; relative humidity: 55.0% - 70.0%; photoperiod: 24 h darkness; food: 50% (w/v) sucrose solution.

Statistics: Descriptive statistics; Weibull analysis for the LD_{50} values of the reference item.

II. RESULTS AND DISCUSSION

Validity Criteria

The validity criteria were met in the study:

- The average mortality for the total number of controls did not exceed 10 per cent at the end of the test (actual was 0%);
- The LD_{50} of the toxic standard meets the specified range i.e. for dimethoate this should be within the range of 0.10 – 0.30 µg a.s./bee (actual reported was 0.29 µg a.s./bee).

After 48 hours of contact exposure, mortality of the 12.5, 50.0, 100.0 and 200.0 µg a.s./bee (equivalent to 17.0, 68.0, 136.0 and 272.0 µg BAS 684 02 H/bee) was 6.7, 10.0, 16.7 and 10.0 %, respectively. There was no mortality in the 25.0 µg a.s./bee (equivalent to 34.0 µg BAS 684 02 H/bee) and control group (water + 0.5 % Adhäsit) at test end.

During the first 4 hours behavioral abnormalities such as moribund, affected and apathy were observed in the in the 50, 100.0 and 200.0 µg a.s./bee (equivalent to 68.0, 136.0 and 272.0 µg BAS 684 02 H/bee) treatment groups. After 24 hours some bees in the three highest dose groups were affected or moribund. After 48 hours only one single in the 200.0 µg a.s./bee treatment group was moribund. The results are summarized in Table B.9.5.1-4.

Table B.9.5.1-4: Toxicity of BAS 684 02 H to *Apis mellifera* L. (honeybee) in a contact toxicity test

Treatment	**Dosage [applied]**	**Mortality [%]**	
		24 h	**48 h**
Control	Water control[1]	0.0	0.0
BAS 684 02 H [µg product/bee]	17.0	3.3	6.7
	34.0	0.0	0.0
	68.0	0.0	10.0
	136.0	10.0	16.7
	272.0	6.7	10.0
Endpoint [µg product/bee]			
LD_{50} (48 h)	> 272.0		

[1] Tap water containing 0.5% Adhäsit.

The LD_{50} value (24 h) for the reference item was determined to be 0.29 µg dimethoate/bee (95% confidence limits: 0.26 - 0.34 µg dimethoate/bee) in the contact toxicity test.

III. CONCLUSION

In an acute contact toxicity study with BAS 684 02 H on honeybees the LD_{50} value (48 h) was determined to be > 272.0 µg BAS 684 02 H/bee (equivalent to > 200.0 µg a.s./bee).

HSE evaluator comments:

The study was well reported and was conducted with good adherence to OECD guideline 214 (1998). Both validity criteria in the guideline were met.

It was noted that the guideline states that relative humidity is normally kept between 55-70% for this study. However, in the report it was stated to range from 55-80%. This was considered to be acceptable since validity criteria were all met, and controls behaved as required demonstrating acceptable environmental conditions were maintained.

It was noted that a 5 µL droplet was used as an application volume as opposed to 1 µL which is recommended in the guideline. This deviation is acceptable since the validity criteria were all met in this study.

It was noted that the test item and reference item were applied with a carrier; tap water containing 0.5% Adhäsit and that the report stated that this was to improve test droplet spreading and Adhäsit was non-toxic to bees. This is acceptable since the water control in this test was tap water containing 0.5% Adhäsit and no mortality was observed during the test.

The endpoint for consideration in the risk assessment is:

- **LD_{50} > 272.0 µg BAS 684 02 H/bee (equivalent to > 200.0 µg a.s./bee)**

Report:	CP 10.3.1.1.2/2 Amsel K., 2016 a Acute toxicity of BAS 684 02 H to the bumblebee *Bombus terrestris L*. under laboratory conditions 2016/1044855
Guidelines:	Van der Steen (1996), Van der Steen (2001), OECD 213 (1998), OECD 214 (1998), Hanewald et al. (2013)
GLP:	yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 684 02 H; batch no. FD-150416-0012; content of a.s.: BAS 684 H (Reg. No. 900 202): 750.0 g/L nominal (737.6 g/L analyzed); density: 1.020 g/cm^3.

B. STUDY DESIGN

Test species: *Bombus terrestris* L. (bumblebee), young adult worker bumblebees derived from healthy and queen-right hives; source: Biobest Belgium N.V., Westerlo, Belgium; collected on the morning prior to use. and were 150-300 mg in weight.

Collection of bees: Bumblebees were collected in the morning of use and transferred to each test unit using red light. After transfer they had 2 hours of acclimatization to test room conditions.

Test design: In a 96-hour test, adults of *Bombus terrestris* were exposed to 5 doses of BAS 684 02 H in an appropriate carrier (0.5% TrinoX solution) placed on the dorsal bumblebee thorax. In total, 8 treatment groups were set up: 5 dose rates of the test item, 2 control groups and 4 dose rates of the reference item with 30 replicates per dose and 1 bumblebee per replicate, respectively. Assessments of bumblebee mortality and behavioral effects were done after 4, 24, 48, 72 and 96 hours.

Contact application:	Before application of the test solution bumblebees in the test cage were anaesthetized with CO_2 for approximately 20 seconds. They were removed from the cage to a large petri dish and turned around with forceps for application. A single droplet (4µL) of the controls, test and reference item (vehicle: 0.5% v/v Triton X solution was placed on the dorsal bumblebee thorax using an Eppendorf Micropipette.
Test units:	Nicot cages as part of the rearing system consisting of socket, cup holder, cell cup and hatching cage with a length of 7cm and diameter of 2cm. Ventilation was provided by the air-conditioning in the climatic chamber.
Food:	50% (w/v) sucrose solution. During the test (after application) food was provided continuously using a syringe set up horizontally to the cage.
Endpoints:	Mortality, behavioral impairments.
Reference item:	BAS 152 11 I (dimethoate, analyzed 420.3 g/L).
Test doses:	Water control (deionized water), TritonX control (0.5% (v/v) TritonX solution); test item at dose rates of 17.0, 34.0, 68.0, 136.0 and 272.0 µg BAS 684 02 H/bumblebee; reference item at dose rates of 2.5, 4.0, 6.4 and 10.1 µg dimethoate/bumblebee.
Test conditions:	Temperature: 24.2 °C – 25.4 °C, relative humidity: 50% – 62%, photoperiod: 24 h darkness except during handling and assessment when diffuse artificial light was used; food: 50% (w/v) sucrose solution.
Statistics:	Descriptive statistics. Fisher's Exact Binominal Test with Bonferroni Correction for mortality data (one-sided greater, $\alpha = 0.05$).

II. RESULTS AND DISCUSSION

Validity criteria

Validity criteria are discussed below the study summary in the evaluator comments.

After 96 hours of contact exposure, no mortality occurred in the control groups treated neither with deionized water nor TritonX solution. In the test item treatment, no statistically significant mortality occurred after thoracic application of 17.0, 34.0, 68.0, 136.0 and 272.0 µg BAS 684 02 H/bumblebee, after 96 hours (Fisher's Exact Binominal Test with Bonferroni Correction for mortality data (one-sided greater, $\alpha = 0.05$)). The dose rate of 34.0 µg BAS 684 02 H/bumblebee revealed a slight mortality of 3.3%, which is not statistically significant when compared to the control (Fisher's Exact Binominal Test with Bonferroni Correction for mortality data (one-sided greater, $\alpha = 0.05$)). Furthermore, no behavioral abnormalities of surviving bumblebees occurred throughout the contact toxicity test. The results are summarized in Table B.9.5.1-5.

Table B.9.5.1-5: Toxicity of BAS 684 02 H to *Bombus terrestris* (bumblebee) in a contact toxicity test

Treatment	Dosage	Mortality [%]			
		24 h	48 h	72 h	96 h
Control	Water control	0.0	0.0	0.0	0.0
	0.5% TritonX	0.0	0.0	0.0	0.0
BAS 684 02 H [µg product/bumblebee]	17.0	0.0	0.0	0.0	0.0
	34.0	0.0	3.3	3.3	3.3
	68.0	0.0	0.0	0.0	0.0
	136.0	0.0	0.0	0.0	0.0
	272.0	0.0	0.0	0.0	0.0
Endpoint [µg product/bumblebee]					
LD_{50} (96 h) [1)]	> 272.0				

1) As the LD_{50} value could not be calculated, it was estimated.

The LD_{50} value (96 h) for the reference item in the contact test was determined to be 4.1 µg a.s./ bumblebee.

III. CONCLUSION

In an acute contact toxicity study with BAS 684 02 H on bumblebees, the LD_{50} value (96 h) was estimated to be > 272.0 µg BAS 684 02 H/bumblebee, corresponding to > 200.0 µg a.s./bumblebee.

HSE evaluator comments:

The study was conducted prior to the acceptance of the current bumble bee guideline OECD 246 (2017). Instead a combination of several guidelines was used as detailed in the study summary above.

The evaluator has evaluated this study to OECD 214 (1998) as this was used in the derivation of the study but also notes that deviations may have occurred.

Environmental conditions were in line wth those set out in the guideline.

The validity criterion relating to the average mortality in control groups was achieved i.e. <10% at the end of the test. However, it was noted that with regards to the toxic standard validity criterion, the LD_{50} was 4.1 µg a.s./ bumblebee which exceeds the guideline's specified range i.e. 0.10-0.30 µg a.s./bee. However, it was also noted that there may be differences between sensitivities in bumble bees when compared to honeybees for which Guideline 214 (1998) was written. The current guideline OECD 246 (2017) states that 10 µg active ingredient Dimethoate / bumblebee has been shown suitable to achieve a mortality of ≥ 50 % following an acute contact exposure. There is some uncertainty due to this being an unbound mortality proportion however the endpoint in the study indicates the bees were sensitive to the reference substance. In addition, the control groups behaved as required to meet validity criteria. Therefore, the evaluator considers the reference substance results in this study to be acceptable.

It was noted that TritonX was used in the study as a vehicle to ensure good penetration or adhesion of the droplet on the bumblebee body. Since this was also used in the control group and no affects were observed this is acceptable.

It was noted that an application volume of 4µL was used as opposed to the recommended 1µL in Guideline 214 which was based on the experience of the laboratory. This has not seemed to negatively affect the study performance and is therefore acceptable.

It was noted that it was not explicitly reported whether antibiotics or other chemicals had previously been used on the bee colony used for the test however the latest guideline (OECD 246, 2017) does not stipulate this and although this study was undertaken before the new guideline was published, the evaluator considers this not to invalidate the study.

The endpoint for consideration in the risk assessment is:

LD_{50} > 272.0 µg BAS 684 02 H/bumblebee (corresponding to > 200.0 µg a.s./bumblebee)

Chronic toxicity to bees

Report:	CP 10.3.1.2/1 Ruhland S., 2017 a Chronic toxicity of BAS 684 02 H to the honey bee *Apis mellifera L*. under laboratory conditions 2017/1000021
Guidelines:	Revised Proposal for a new OECD Guideline on Honey bee (*Apis mellifera L*.) chronic oral toxicity test (10 day feeding test in the laboratory) (2016)
GLP:	yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

Additional report: Azevedo, L. B.; 2018
BASF DocID 2018/1099071
Further statistical evaluation of study with DOCID 2017/1000021 on chronic toxicity on honey bee.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 684 02 H; batch no. FD-150416-0012; content of a.s.: BAS 684 H (Reg. No. 900 202): 750.0 g/L nominal (750.2 g/L analyzed); density: 1.020 g/cm^3.

B. STUDY DESIGN

Test species: *Apis mellifera* L. subspecies Buckfast (honeybee); max. 2-day old bees; derived from healthy and queen-right colonies; source: Beekeeper in-house culture.

Test design: In a 10-day chronic test, young adult worker bees of *Apis mellifera* L. were exposed daily to 5 doses of BAS 684 02 H in treated food (50% w/v aqueous sucrose solution). In total, 3 treatment groups were set up: 5 doses of the test item, 1 untreated control group and 1 dose rate of the reference item with 3 replicates per dose and 10 bees per replicate. Assessments of bee mortality and behavioral effects were done daily during the study.

Endpoints: Mortality, behavioral impairments.
The key used for recording behavioural abnormalities was healthy/normal, affected, moribund, vomiting, cramping or apathetic.

Reference item: Dimethoate EC 400 (analyzed content of a.s.: 420.3 g/L).

Test doses: Control 1: untreated diet (50% (w/v) aqueous sucrose solution)
Test item treatments:

Nominal dose/concentration		
Doses [µg BAS 684 02 H/bee]	**Dose [µg a.s./bee]**	**Concentrations [g a.s./kg food]**
17.0	12.5	0.321
34.0	25.0	0.642
68.0	50.0	1.284
136.0	100.0	2.567
271.9	199.9	5.135

Reference item treatments: 27.3 ng dimethoate/bee/day (0.702 mg a.s./kg food)

The table below provides the applied and consumed dosages:

Table B.9.5.1-6: Applied and consumed dosages in the chronic toxicity test

Treatment group	Identification	Item to be applied	Daily dose	Daily concentration	Actual intake of the applied item (average over 10 days)
			[µg a.s./bee]	[g a.s./kg food]*	[µg a.s./bee/day]
Control	AC	50 % w/v aqueous sucrose solution	--		--
Test item	AT	BAS 684 02 H**	199.9	5.135	179.7
	BT		100.0	2.567	99.0
	CT		50.0	1.284	48.6
	DT		25.0	0.642	26.1
	ET		12.5	0.321	15.5
			[ng a.s./bee]	[mg a.s./kg food]*	
Reference item	AR	Dimethoate EC 400***	27.3	0.702	24.6

* based on a density of 1.18 g/mL of the sucrose solution
** based on the nominal content of a.s., applied in sucrose solution
*** based on analysed content of a.s.; applied in sucrose solution
Calculations are performed with non-rounded values and based on theoretical food consumption of 33 µL/bee/day

Test conditions: Temperature: 32.7°C - 33.2°C; relative humidity: 57.5% - 61.5%, photoperiod: 24 h darkness; food: 50% (w/v) aqueous sucrose solution.

Statistics: Descriptive statistics; Step-down Rao-Scott-Cochran-Armitage Test Procedure for mortality data (one-sided greater, $\alpha = 0.05$); Spearman-Karber procedure (0% trim) for determination of LDD_{50} and LC_{50}. All performed in computer program ToxRat Professional 3.2.1 (2015).

A further statistical evaluation was undertaken in BASF DocID 2018/1099071 to meet the requirements of providing EC_{10} and EC_{20} values for this study type as outline din the data requirements for formulations (Commission Regulation 284/2013). Loglogistic regression was used for the derivation of the dose-response curve in the RStudio software version 1.1.447. Survival data on day 10 of the study was extracted from the original report. The mortality data were retrieved from the first table on p38 of the report.

II. RESULTS AND DISCUSSION

Validity Criteria

- The average mortality across replicates for the untreated control and solvent control groups was ≤ 15 % at the end of the test (10 days following start of exposure) (actual = 0%);
- The average mortality in the reference substance treated group was ≥ 50 % at the end of the test (10 days following start of exposure) (actual = 100%).

After 10 days of continuous exposure, a mean mortality of 0.0% in the untreated control was observed. In the test item group bees consuming doses of 179.7 and 99.0 µg a.s./bee/day showed mortalities of 63.3% and 20.0%, respectively which are statistically significantly increased compared to the control group after 10 days (Step-down Rao-Scott-Cochran-Armitage Test Procedure, α = 0.05, one-sided greater). No treatment related abnormal behavior could be observed during the study.

The results are summarized in Table B.9.5.1-7.

Table B.9.5.1-7: Cumulative mortality and toxicity endpoints of honeybees (*Apis mellifera* L.) exposed to BAS 684 02 H in a chronic oral toxicity test

Treatment [BAS 684 02 H]			**Mortality after 10 days**
Consumed doses	**Overall doses [µg a.s./bee/day]**	**Concentration [g a.s./kg food]**	**Cumulative mortality [%]**
Control	Control	Control	0.0
15.5	12.5	0.321	3.3
26.1	25.0	0.642	0.0
48.6	50.0	1.284	0.0
99.0	100.0	2.567	20.0 *
179.7	199.9	5.135	63.3 *

Endpoints		**10 days**
Test item doses [µg consumed a.s./bee/day]	**LDD_{50}**	143.2
	NOEDD [1)]	48.6
Test item concentrations [g a.s./kg food]	**LC_{50}**	3.982
	NOEC [1)]	1.284

1) Step-down Rao-Scott-Cochran-Armitage Test Procedure (one-sided greater, α = 0.05).

The EC_{10} value and EC_{20} value were calculated to be 86.5µg a.s./bee/day (95% CI 64.6-116) and 110.1µg a.s./bee/day (95% CI 88.4-137.2). The additional statistical report BASF DocID 2018/1099071.

The reference dosage tested in the study was 27.3 ng a.s./bee/day (actual consumption on average per day: 24.6 ng a.s./bee), which caused a mean mortality of 100.0%.

III. CONCLUSION

In a 10-day chronic toxicity feeding test with BAS 684 02 H the NOEDD was determined to be 48.6 µg consumed a.s./bee/day, and the NOEC 1.284 g a.s./kg food, respectively. The LDD_{50} and LC_{50} were determined to be 143.2 µg consumed a.s./bee/day and 3.982 g a.s./kg food.

HSE evaluator comments:

It is noted that the results of this study have not been discussed in the context of the risk assessment as there is currently no agreed approach.

The study was conducted prior to the publishing of the currently guidance document OECD 245 (2017) and was conducted using the proposal for this guideline dated February 2016. The HSE evaluator has evaluated this study using the draft proposal noting that the two guidelines are extremely similar and validity criteria are the same between the two guidelines.

The study was well reported and adhered predominantly to the draft guideline. Validity criteria were met, and the environmental conditions reported in the study were in line with those recommended in the draft guideline i.e. temperature of 33 ±2°C, relative humidity of 50-70% and constant darkness throughout the test.

It was noted that the draft guideline applicable stipulates the need for analytical verification of the lowest concentration and highest concentration of feeding solutions if they are prepared daily as well as a sample of stock solution if one has been used. If the stock solution or the feeding solutions are not prepared daily, analytical determination is equally required, i.e. once during the experimental phase after preparation, and additionally, once at the end of the maximum storage period, for both the lowest and the highest concentrations of the feeding solutions and the stock solution. However no analytical verification was undertaken in this study and therefore the evaluator considers this to potentially invalidate the study. The applicant was requested to provide justification or analytical data to address this and the following statement was received :

For the chronic toxicity study on honeybees (Doc ID 2017/1000021) no analytical verification of the test item was done. The study plan was generated in June 2016 – before an agreed guideline for chronic bee testing was available. At this point of guideline development inclusion of analytical verification was still under discussion. The final Guideline OECD 245 was published only in October 2017, which was after study finalisation. However, a second study performed with the a.i. (DocID 2017/1140991) including analytical verification is available. As BAS 684 02 H is a solo formulation and available information do not indicate unexplained toxicity (see acute data and chronic study mentioned above), the study conducted with the a.i. should be adequate to address the potential chronic risk to honeybees for both the a.i. and the product and hence a data gap would be not justified.

Although there was a draft guideline available dated February 2016 which would have been available at the time of study planning which states the need for analytical verification, The evaluator recognizes that there was no agreed guideline and that it was a 'proposal'. The study is considered to be valid however since there is no current agreed approach for incorporating this type of study in the risk assessment results will be noted.

Effects on honey bee development and other honey bee life stages

Report:	CP 10.3.1.3/1 Kleebaum K., 2017 a Repeated exposure of honey bee (*Apis mellifera*) larvae to BAS 684 03 H under laboratory conditions (in vitro) 2017/1036677
Guidance document:	OECD 239 (2016)
GLP:	yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)
Additional report:	Azevedo, L. B.; 2018 BASF DocID: 2018/1099072 Further statistical evaluation of study with DOCID 2017/1036677 on chronic toxicity on honey bee larvae.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 684 03 H; batch no. FD-170210-0001; content of a.s.: BAS 684 H (Reg. No. 900 202): 750.0 g/L nominal (737.3 g/L analyzed); density: 1.001 g/cm^3.

B. STUDY DESIGN

Test species: *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.

Producing L1 larvae: Three colonies were used in the test and each was treated in parallel in the same way. On day -3 the respective queen of the colony was gaffed on an empty brood comb which hwas fitted in an excluder cage and thereafter placed in the hive. The queen laid her eggs solely on this comb. The caging time was approximately 30 hours. In the afternoon of day -2 the queen was released from the excluder. The comb was checked for the presence of freshly laid eggs, was confined in the excluder again in order to avoid egg laying and was placed near frames containing open brood in the rive. Eggs were incubated within the hive between day -2 and day 1.

Grafting details: On day 1 the combs containing larvae were transported from the hive to an acclimatized laboratory room using a polystyrene box. Larvae were transferred from combs to the cells using a suitable grafting tool (e.g. grafting needle Swiss type). During grafting the C-shaped larvae were placed on the surface of the artificial diet within the grafting cells The grafting was performed on a warming plate at 34.5°C.

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 d chronic feeding test with repeated exposure according to "OECD Guidance Document on Honey Bee Larval Toxicity Test following Repeated Exposure, Series on Testing & Assessment No. 239" (July 2016). 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 exposure (D1). Larvae were repeatedly exposed to BAS 684 03 H diluted in the larvae's food (aqueous yeast/sugar solution mixed with 50% royal jelly (w/w)) on 4 consecutive days (D3 to D6 after grafting). After the applications, no additional feeding of the larvae took place. In total, 7 treatment groups were set up: 5 doses of the test item, 1 untreated control and 1 reference item treatment, each with 3 replicates per treatment 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 and D8). Additionally, other observations such as small body size or large quantities of remaining food on D8 were noted. Pupal mortality was assessed at D15 and emergence of adults was evaluated at D22.

Endpoints: NOEC/NOED (days 8 and 22), LC_{50}/LD_{50} (day 8) and EC_{50}/ED_{50} (day 22).

Reference item: Dimethoate (analyzed purity: 98.8%). The effects of the reference item were investigated in this study at a concentration of 48 mg a.s./kg food, corresponding to a total dose of 7.8 µg a.s./larva.

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:						
Royal jelly	44.25% w/w	-	50% w/w		50% w/w	
Sugar solution	55.75% w/w		50% w/w		50% w/w	
Composition of sugar solution:						
Glucose	9.50% w/w		15% w/v		18% w/v	
Fructose	9.50% w/w	-	15% w/v		18% w/v	
Yeast	1.61% w/w		3% w/v		4% w/v	
Water	79.39% w/w					

[1] day of grafting [2] days of application
*diet A contained a higher amount of water to reduce propensity of drying out; according to Schmehl *et al.* (2016)

Test doses: Control 1: untreated diet (50% aqueous sugar solution with 50% royal jelly)
Test item treatments:

Treatment group	Test solution ID	Item applied	Dose [µg product/larva]	Dose [µg total a.i./larva]	Concentration *** [mg product/kg food]	Concentration *** [mg a.i./kg food]
Control	AC	Diet B/C			--	
Test item	AT	BAS 684 03 H*	133.4	100.0	844	633
	BT		66.7	50.0	422	316
	CT		33.4	25.0	211	158
	DT		16.7	12.5	106	79
	ET		8.3	6.2	53	40
Reference item	AR	Dimethoate tech. **	-	7.6	-	48

* based on nominal content of a.i. and density of the test item
** based on analysed purity
*** based on a density of 1.13 g/mL of diet B/C
Calculations are performed with non-rounded values.

The test item was dissolved in deionized water. To ensure even distribution of the test item within the larvae food, the final diets were placed on a multitube vortexer for 5 minutes. In order to avoid unequal distribution of the test item among the larvae of each treatment group, potentially occurring bubbles were eliminated from the final diets with appropriate means (e.g. degasification by ultrasonics prior to feeding).

Test conditions: Temperature: 34.1°C - 34.8°C on D1 - D22. Mean relative humidity: 92% - 100% on D1 – D8, 78% - 84% on D8 – D15 and 56% - 60% on D15 – D22. Photoperiod: darkness

Statistics: Descriptive statistics; The Chi² Table Test with Bonferroni Correction ($\alpha = 0.05$, one-sided greater) was used for determination of NOED/NOEC (D8 and D22). ED/EC_{50} calculations on D22 were performed with the Trimmed Spearman- Karber Procedure.

Additional statistics:

BASF Doc ID 2018/1099072 was submitted to address the requirement under regulation 283/2013 for calculation of EC_{10} and EC_{20} values for adult bees, where possible, and larvae together with the NOEC.

A loglogistic regression was used for the derivation of the dose-response curve for survival rate using program Rstudio software version 1.1.447.

Analytical verification: All final diets were sampled in triplicate as specimens for analysis and retention directly before feeding 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

All validity criteria were met:

- In the control plate(s), cumulative larval mortality from D3 to D8 were ≤15% across all replicates (actual = 8.3%).
- In the control plate(s), the adult emergence rate on D22 was ≥70% across all replicates (actual = 83.3%).
- The larval mortality in the reference item group (dimethoate) was ≥50% on D8 across all replicates (88.9% when exposed to a total of 7.6 µg).

Mortality

After 120 hours of repeated oral exposure (on D8) larval mortalities of 8.3% was observed in the control. Pupal mortality (between D8 and D22) was 9.1% in the control. At D22, the control group showed a total mortality of 16.7%. In the test item groups, larval mortalities at D8 ranged between 0.0 and 11.1%. Pupal mortalities ranged between 8.6 and 37.5% in the test item treatment groups. Total mortalities at D22 ranged between 11.1 and 44.4%. On D8, no statistically significantly increased mortality occurred in any of the larvae groups after being treated with BAS 684 03 H (Chi² Table Test with Bonferroni Correction, $\alpha = 0.05$, one-sided greater). On D8, one individual of all remaining larvae treated with test item showed remaining food.

Emergence

In the final assessment at D22, adult emergence rate of 83.3% was determined for the honey bees in the control group. In the test item groups, the adult honey bees emerged at rates ranging between 55.6% and 88.9% following an application of 8.3, 16.7, 33.4, 66.7 and 133.4 µg product/larva, respectively, during the larval stages. Larvae treated with the highest test item dose (133.4 µg product/larva) showed a statistically significantly increased mortality compared to the control (Chi² Table Test with Bonferroni Correction, $\alpha = 0.05$, one-sided greater). The results are summarized in Table B.9.5.1-8.

Table B.9.5.1-8: Toxicity of BAS 684 03 H to *Apis mellifera* (honeybee) in a chronic oral larval toxicity test with repeated exposure after 22 days

Dosage [µg product/larva]	**% of nominal concentration[4)]**	**Concentration [mg product/kg food]**	**Mortality [%]**						**Adult emergence (D22) [%]** [3)]
			Larvae (D3 - D8)		**Pupae (D8 - D22)** [2)]		**Total (D3 - D22)**		
			Abs.	**Corr.** [1)]	**Abs.**	**Corr.** [1)]	**Abs.**	**Corr.** [1)]	
Control	No active measured	Control	8.3	--	9.1	--	16.7	--	83.3
8.3	101%	53	0.0	0.0	11.1	2.2	11.1	0.0	88.9
16.7	99%	106	2.8	0.0	14.3	5.7	16.7	0.0	83.3
33.4	96%	211	2.8	0.0	11.4	2.6	13.9	0.0	86.1
66.7	94%	422	2.8	0.0	8.6	0.0	11.1	0.0	88.9
133.4	90%	844	11.1	3.0	37.5	31.3	44.4 *	33.3	55.6
Endpoints (larval mortality, D8)									
LD_{50} [µg BAS 684 03 H/larva] [2)]			> 133.4						
NOED [µg BAS 684 03 H/larva]			≥ 133.4						
LC_{50} [mg BAS 684 03 H/kg food] [2)]			> 844						
NOEC [mg BAS 684 03 H/kg food]			≥ 844						
Endpoints (adult emergence, D22)									
ED_{50} [µg BAS 684 03 H/larva] [2)]			> 133.4						
NOED [µg BAS 684 03 H/larva]			66.7						
EC_{50} [mg BAS 684 03 H/kg food] [2)]			> 844						
NOEC [mg BAS 684 03 H/kg food]			422						

abs.: absolute mortality as counted from the results; corr.: corrected mortality

* Statistically significantly different compared to the control (Chi² Table Test with Bonferroni Correction; α = 0.05; one sided greater)

1) Corrected for control mortality according to Schneider-Orelli (1947).

2) Average% of pupae mortality was calculated according to the following formula:
Sum of dead between D8 and D22 / Sum of living larvae on D8 x 100%

3) Adult emergence is calculated as the reverse of the pupae mortality on day 22:
Adult emergence [%] = 100 [%] – Mortality of D22 [%]

4) Mean recovery of the active substance over D3, D4, D5 and D6.

EC_{10} and EC_{20} values

The EC_{10} was calculated to be 116.3 µg BAS 684 03 H/larva (95% CI = 44.6-303.1) and the EC_{20} was calculated to be 124.7 µg BAS 684 03 H/larva (95% CI = 77.5-200.7).

III. CONCLUSION

In a chronic oral toxicity study with repeated exposure to BAS 684 03 H on honeybee larvae, the LC_{50} is estimated to be > 844 mg/kg food (corresponding to an LD_{50} of > 133.4 µg/larva) after 8 days. The NOEC was determined to be ≥ 844 mg/kg food (corresponding to a NOED of ≥ 133.4 µg/larva).

After 22 days, the EC_{50} was determined to be >844 mg/kg food (corresponding to an ED_{50} of > 133.4 µg/larva). The NOEC value was determined to be 422 mg/kg food (corresponding to a NOED of 66.7 µg/larvae).

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 mostly in line with OECD 239 (2016). Test conditions for each stage of the study were adhered to.

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.9.5.1-8 in the study report, the mean recovery of each dose concentration was within 20% of the nominal values (actual range was 90%-101%) which demonstrates sufficient exposure was achieved in line with the proposed dose concentrations. The Chemistry specialist confirmed that the method of analysis used by the applicant in this study was valid and therefore the presented measured oncentrations are considered reliable.

It was noted in the additional study report (BASF Doc ID 2018/1099072) 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.

It can be concluded that honeybee larvae mortality was not affected up to a rate of 133.4 µg BAS 684 03 H/larvae (corresponding to 844 mg BAS 684 03 H/kg food).

In addition, honeybee emergence was not affected up to a rate of 66.7 µg BAS 684 03 H/larvae (corresponding to 422 mg BAS 684 03 H/kg food).

B.9.5.2. Effects on non-target arthropods other than bees

B.9.5.2.1. Standard laboratory testing for non-target arthropods

Report:	CP 10.3.2.1/1 Roehlig U., 2017 a Effects of BAS 684 03 H on the parasitic wasp *Aphidius rhopalosiphi* (DESTEFANI-PEREZ) in a laboratory test 2017/1073467
Guidelines:	IOBC, Mead-Briggs M. et al. (2000)
GLP:	yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 684 03 H; batch no. FD-170210-0001; content of a.s.: BAS 684 H (Reg. No. 900 202): 750.0 g/L nominal (737.3 g/L analyzed); density: 1.001 g/cm^3.

B. STUDY DESIGN

Test species: Parasitic wasp *Aphidius rhopalosiphi* (DeStefani-Perez), adults (< 48 hours old); source: "Katz Biotech AG", Baruth, Germany.

Test design: Exposure of adult parasitoids was achieved via air-dried residues on treated glass plates. 7 treatments (5 test item rates, water treated control, reference item) with 4 replicates each were set up. Each replicate contained 10 wasps. Assessment of mortality was carried out 2, 24 and 48 h after test initiation.

Endpoints: Mortality after exposure over 48 h, including the determination of a LR_{50}.

Reference item: Dimethoate EC 400, a.s.: dimethoate: 405.2 g/L (analyzed).

Test rates: Control (purified water), 43.75, 87.5, 175, 350 and 700 mL BAS 684 03 H/ha. The reference item was applied at an application rate of 0.3 mL/ha. All substances were applied in 200 L water/ha. The substances were sprayed onto glass plates via a laboratory track sprayer (calibrated to achieve 200 L/ha) and air dried afterwards. Actual application rate for treated glass plates ranged 96 – 106% of the target rate.

Test conditions: Temperature: 19°C - 21°C; relative humidity: 68% - 72%; photoperiod: 16 h light : 8 h dark; light intensity: 2130 lux. Food: 25% aqueous fructose solution.

Statistics: Descriptive statistics. Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm ($\alpha = 0.05$) for mortality, Probit analysis for calculation of LR_{50}.

II. RESULTS AND DISCUSSION

The LR_{50} value was determined to be 135.7 mL BAS 684 03 H/ha in 200 L water/ha.

After 48 h, a mortality of 2.5% was observed in the control. In the test item treatments, mortality ranged between 2.5% and 100%. This resulted in corrected mortality rates between 0.0% and 100%. Statistically significant effects on mortality were determined in the 87.5, 175, 350 and 700 mL BAS 684 03 H/ha test item treatment groups (Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm, $\alpha = 0.05$). The results are summarized in Table B.9.5.2.1-1.

Table B.9.5.2.1-1: Effects of BAS 684 03 H on parasitoids (*Aphidius rhopalosiphi)* under worst-case laboratory conditions

Treatment	Rate [1] [mL/ha]	Mortality [2] [%]	Corrected mortality [3] [%]
Control	--	2.5	--
BAS 684 03 H	43.75	2.5	0
	87.5	42.5 *	41.0
	175	57.5 *	56.4
	350	90.0 *	89.7
	700	100 *	100
Endpoints [mL BAS 684 03 H/ha]			
LR_{50} (95% CL) [4]	135.7 (76.9 – 229.8)		

1) Application rate in 200 L water/ha.
2) Mortality after 48 h of exposure to BAS 684 03 H on glass plates.
3) Corrected mortality according to Abbott (1925).
4) Median lethal rate with 95% confidence limits.
* Statistically significant differences compared to the control (Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm, $\alpha = 0.05$).

The reference item caused a corrected mortality of 100% of exposed wasps after 48 h.

III. CONCLUSION

In a worst-case laboratory study with BAS 684 03 H, the LR_{50} for *Aphidius rhopalosiphi* was 135.7 mL BAS 684 03 H/ha (applied in 200 L water/ha).

HSE evaluator comments:

The glass plate study investigating the effects of BAS 684 03 H on the parasitic wasp *A. rhopalosiphi* was found by the HSE evaluator to be well reported and conducted with good adherence to the IOBC, BART and EPPO Joint Initiative Guideline for non-target arthropods (2000). The study is confirmed by the HSE evaluator to be valid in relation to the reference mortality criteria, with control mortality at 2.5% and toxic reference mortality at 100% after 48 hours. There were no guideline deviations to report.

The HSE evaluator confirms that the formulation 48h-LR_{50} is 135.7 mL BAS 684 03 H/ha, applied in 200 L water. This corresponds to an active substance equivalent 48h-LR_{50} of 100.05 g cinmethylin/ha (based on the measured a.s. content of 73.7% w/w).

Report:	CP 10.3.2.1/2 Roehlig U., 2017 b Effects on BAS 684 03 H on the predatory mite *Typhlodromus pyri* SCHEUTEN in a laboratory test 2017/1073466
Guidelines:	Bluemel et al. (2000)
GLP:	yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

I. MATERIAL AND METHODS

A. MATERIALS

Test item:	BAS 684 03 H; batch no. FD-170210-0001; content of a.s.: BAS 684 H (Reg. No. 900 202): 750.0 g/L nominal (737.3 g/L analyzed); density: 1.001 g/cm^3.

B. STUDY DESIGN

Test species:	Predatory mite (*Typhlodromus pyri*), protonymphs (less than 24 h old); source: "Katz Biotech AG", Baruth, Germany.
Test design:	Exposure of the mites to air-dried residues on treated glass plates. Seven treatment groups (five test item rates, water treated control and reference item), with 5 replicates per treatment, each containing 20 protonymphs. Assessment of mortality was done 3 and 7 days after treatment (DAT).
Endpoints:	Mortality after exposure over 7 days.
Reference item:	Dimethoate EC 400, a.s.: dimethoate: 405.2 g/L (analyzed).
Test rates:	Control (deionized water), 62.5, 125, 250, 500 and 1000 mL BAS 684 03 H/ha. The reference item was applied at an application rate of 15 mL/ha. All substances were applied in 200 L water/ha. The substances were sprayed onto glass plates with a laboratory track sprayer calibrated to deliver 200 L/ha and air dried afterwards. The actual application rate for treated glass plates ranged 96 – 106% of the target rate.
Test conditions:	Temperature: 23°C – 27°C; relative humidity: 66% - 73%; photoperiod: 16 h light : 8 h dark; light intensity: 2100 lux; food: 1:1 v/v mixture of pollen from pine and birch.

Statistics: Descriptive statistics. Multiple Sequentially-rejective Chi^2-2x2 Table Test after Bonferroni-Holm ($\alpha = 0.05$) for mortality. Spearman-Karber method for LR_{50} calculation. Statistics were undertaken using ToxRat Professional 3.2.1.

II. RESULTS AND DISCUSSION

After 7 days, in the water-treated control a mortality of 1.0% was observed. In the test item treatments mortality ranged between 1.0% and 78.0%. This resulted in corrected mortality rates between 0% and 77.8%. No statistically significant effects on mortality were determined in the test item rates up to and including the 250 mL/ha BAS 684 03 H treatment (Multiple Sequentially-rejective Chi^2-2x2 Table Test after Bonferroni-Holm, $\alpha = 0.05$). The results are summarized in Table B.9.5.2.1-2.

Table B.9.5.2.1-2: Effects of BAS 684 03 H on predatory mites (*Typhlodromus pyri*) under worst-case laboratory conditions

Treatment	Rate [1] [mL/ha]	Mortality [2] [%]	Corrected mortality [3] [%]
Control	--	1.0	--
BAS 684 03 H	62.5	1.0	0
	125	2.0	1.0
	250	2.0	1.0
	500	10.0 *	9.1
	1000	78.0 *	77.8
Endpoints [mL BAS 684 03 H/ha]			
LR_{50} (95% CL) [4]	763.7 (710.1 – 821.4)		

1) Application rate in 200 L water/ha.
2) Mortality after 7 days of exposure to BAS 684 03 H on glass surface.
3) Corrected mortality according to Abbott (1925).
4) Median lethal rate with 95% upper and lower confidence limits.
* Statistically significant differences compared to the control (Multiple Sequentially-rejective Chi^2-2x2 Table Test after Bonferroni-Holm, $\alpha = 0.05$).

The reference item caused a corrected mortality of 76.0% at 7 DAT, resulting in a corrected mortality of 75.8%.

III. CONCLUSION

In a worst-case laboratory study with BAS 684 03 H, the LR_{50} for *Typhlodromus pyri* was 763.7 mL BAS 684 03 H/ha (applied in 200 L water/ha).

HSE evaluator comments:

The HSE evaluator found the glass plate study investigating the effects of BAS 684 03 H on predatory mite *Typhlodromus pyri* to be well reported and conducted with good adherence to the IOBC, BART and EPPO Joint Initiative Guideline for non-target arthropods (2000). The study is confirmed by the HSE evaluator to be valid in relation to the reference mortality criteria, with control mortality at 1.0% and toxic reference mortality (corrected for control mortality) at 75.8% after seven days. There were no guideline deviations to report.

The HSE evaluator confirms that the formulation 7d-LR_{50} is 763.7 mL BAS 684 03 H/ha, applied in 200 L water. This corresponds to an active substance equivalent 7d-LR_{50} of 563.1 g cinmethylin/ha (based on the measured a.s. content of 73.7% w/w).

B.9.5.2.1. Extended laboratory testing for non-target arthropods

Report:	CP 10.3.2.2/1 Roehlig U., 2017 c Effects of BAS 684 03 H on the parasitic wasp *Aphidius rhopalosiphi* (DESTEFANI-PEREZ) in an extended laboratory test 2017/1084956
Guidelines:	IOBC, Mead-Briggs M.A. et al. (2009) - An extended laboratory test for evaluating the effects of plant protection products on *Aphidius rhopalosiphi* (DeStefani-Perez) (Hymenoptera - Braconidae)
GLP:	yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 684 03 H; batch no. FD-170210-0001; content of a.s.: BAS 684 H (Reg. No. 900 202): 737.3 g/L analyzed (750.0 g/L nominal); 1.001 g/cm^3.

B. STUDY DESIGN

Test species: Parasitic wasp *(Aphidius rhopalosiphi*); adults; age: less than 48 h; source: "Katz Biotech AG", 15837 Baruth, Germany, maintained on cereal aphids (*Rhopalosiphum padi*).

Test design: Exposure of adult female parasitoids was reached via air-dried residues on treated barley plants (*Hordeum vulgare*, variety Xanadu). The study included 7 treatment groups (5 test item treatments, water treated control, reference item) with 6 replicates per treatment, each containing 5 female wasps. Assessment of the repellence of wasps from the freshly treated plants was made during the first 3 h after their release and at 24 and 48 h after treatment. The mortality was assessed 2, 24 and 48 hours after test initiation. At 48 h, surviving wasps (n = 15 females per treatment) were removed and their reproductive capacity was assessed by confining them individually over untreated wheat plants infested with the host cereal aphids, *Rhopalosiphum padi*. The adult wasps were removed after 24 h and the aphid-infested plants left for a further 11 days before the numbers of aphid mummies that had developed was assessed.

Endpoints: Wasp mortality after 48 h (for determination of the LR_{50}); assessment of the reproductive capacity by the number of mummies per female, including the determination of the ER_{50}.

Reference item: Dimethoate EC 400 (a.s.: dimethoate, 400 g/L nominal).

Test rates: Control (deionized water), 43.75, 87.5, 175, 350 and 700 mL BAS 684 03 H/ha. The reference item was applied at an application rate of 10 mL dimethoate/ha. All treatments were applied in 400 L/ha water. The treatments were sprayed on potted barley seedlings using a calibrated laboratory track-sprayer and left to air dry afterwards.
The actual application rate for treated plants ranged 92 – 102% of the target rate.

Test conditions: Exposure of adults: Temperature: 19°C - 22 C; relative humidity: 67% - 72%; photoperiod: 16 h light : 8 h dark; light intensity: 1080 lux.
Post-exposure time: Temperature: 19°C - 21 C; relative humidity: 65% - 73%; photoperiod: 16 h light : 8 h dark; light intensity: 5240 lux.

Reproduction assessment: Temperature: 19°C - 21°C; photoperiod: 16 h light : 8 h dark, light intensity: 7320 lux. Food: 10% (w/w) aqueous fructose solution, sprayed onto test plants before application.

Statistics: Descriptive statistics; Multiple Sequentially-rejective Fisher test after Bonferroni-Holm ($\alpha = 0.05$) performed on mortality data. Dunnett's t-test ($\alpha = 0.05$) for repellence and Williams-t-test ($\alpha = 0.05$) for reproduction capacity.

II. RESULTS AND DISCUSSION

In the water-treated control a mortality of 6.7% was observed. In the test item treatments mortality ranged between 3.3% and 13.3%. This resulted in corrected mortality rates between -3.6% and 7.1% after 48 h in the treatment groups. No statistically significant effects on mortality were determined in all test item treatments (Multiple Sequentially-rejective Fisher test, after Bonferroni-Holm $\alpha = 0.05$=). The LR_{50} for BAS 684 03 H was estimated to be > 700 mL BAS 684 03 H/ha in 400 L water/ha, the highest rate tested.

There were no significant negative effects on reproduction, compared to the control, at rates up to and including 700 mL BAS 684 03 H/ha, the highest treatment rate tested (Williams-t-test $\alpha = 0.05$).

No unusual observations were noted in the control and all test item groups at any observation point during the test. There were no statistically significant differences in the behavior (wasps settled on the plants as a criterion for repellence) in all test item groups compared to the control (Dunnett's t-test, $\alpha = 0.05$). The results are summarized in Table B.9.5.2.1-1.

Table B.9.5.2.1-1: Effects on parasitoids (*Aphidius rhopalosiphi*) exposed to BAS 684 03 H in an extended laboratory test

Treatment	Rate [1] [mL/ha]	Mortality [2] [%]	Corrected mortality [3] [%]	Reproduction [4] [mummies/ female]	Effects on reproduction [5] [%]
Control	--	6.7	--	23.1	--
BAS 684 03 H	43.75	3.3	-3.6	26.8	-16.0
	87.5	6.7	0	24.0	-3.9
	175	3.3	-3.6	23.7	-2.6
	350	6.7	0	24.5	-6.1
	700	13.3	7.1	24.3	-5.2
Endpoint [mL BAS 684 03 H/ha]					
LR_{50}	> 700				
ER_{50}	> 700				

1) Application rate in 400 L water/ha.
2) Mortality in the individual test item treatments after 48 hours of exposure to BAS 684 03 H on barley seedlings (Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm, $\alpha = 0.05$).
3) Corrected mortality according to Abbott (1925).
4) Mean number of parasitized aphids/surviving female (Williams-t-test $\alpha = 0.05$)
5) Change in mean number of mummies per female, relative to control. A negative value indicates an increase in reproduction relative to the control.

For the repellency assessment, the percentage of observations of wasps settled on the plants over the whole assessment period was calculated for each treatment. The calculation was based on the parasitoids on the plants and the cylinder because under normal circumstances the wasps did not naturally visit the sand surface beneath the plants. Any individuals observed to be on the sand were considered to be affected by the treatment to such an extent that they cannot alight on the plants or the cylinder. Therefore, these individuals were not included in the

statistical analysis as their position in the exposure unit was not a direct consequence of any potential repellency effect. Table B.9.5.2.1-2 summarises the rate of parasitoids settling on plant surfaces.

Table B.9.5.2.1-2: Percentages of wasps settled on the plants at each treatment when exposed to BAS 684 03 H

Treatment group		**Wasps settled on the plants (%)** [1]
Control		52.0
BAS 684 03 H (mL product/ha)	43.75	50.0 (ns)
	87.5	45.3 (ns)
	175	48.7 (ns)
	350	47.3 (ns)
	700	46.0 (ns)

[1] The percentage of wasps settled on the plants in each replicate was calculated for each of the five assessment occasions and then a mean value was calculated for each replicate.
ns – not statistically significantly different compared to the control (Dunett's t-test; $\alpha = 0.05$)

The reference item caused a corrected mortality of 96.4% of the exposed organisms after 48 h.

III. CONCLUSION

In an extended laboratory study with BAS 684 03 H the LR_{50} for *Aphidius rhopalosiphi* derived from the results: LR_{50} > 700 mL BAS 684 03 H/ha in 400 L water/ha. No unacceptable effects on reproduction of *Aphidius rhopalosiphi* were observed, when the test item was applied at rates up to and including 700 mL BAS 684 03 H/ha in 400 L water/ha. The ER_{50} for BAS 684 03 H was estimated to be > 700 mL BAS 684 03 H/ha in 400 L water/ha.

HSE evaluator comments:

The HSE evaluator found the extended test for investigating the effects of BAS 684 03 H on the parasitic wasp *Aphidius rhopalosiphi* to be well reported and conducted to GLP principles and with good adherence to the guidance (Mead-Briggs et al., 2009). The study is confirmed by the HSE evaluator to be valid in relation to the reference mortality criteria, with control mortality at 6.7% and toxic reference mortality (corrected for control mortality) at 96.4% after 48 hours. It was also valid in reproduction criteria, with 23.1 mummies per female reported in the control population. There were no guideline deviations to report.

The HSE evaluator confirms that the formulation 48h-LR_{50} is > 700 mL BAS 684 03 H/ha, applied in 400 L water. This corresponds to an active substance equivalent 48h-LR_{50} of 516.1 g cinmethylin/ha (based on the measured a.s. content of 73.7% w/w).

Report: CP 10.3.2.2/2
Roehlig U., 2017 d
Effects of BAS 684 03 H on the rove beetle *Aleochara bilineata* GYLL. in an extended laboratory test
2017/1112416
Guidelines: IOBC, Mead-Briggs M. et al. (2000)
GLP: yes
(certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 684 03 H; batch no. FD-170210-0001; content of a.s.: BAS 684 H (Reg. No. 900 202): 737.3 g/L analyzed (750.0 g/L nominal); 1.001 g/cm^3.

B. STUDY DESIGN

Test species: Rove beetle (*Aleochara bilineata* GYLL.), adults 1 - 7 days old, source: in-house culture.

Test design: Exposure of the beetles was reached via spray treatment on sandy soil (LUFA 2.1). 4 treatment groups (2 test item rates, water treated control, reference item) with 4 replicates each and 20 individuals (10 male and 10 female adult beetles) per replicate were set up. This study included the complete life cycle of the beetles: parental generation, mating and oviposition of parental generation, hatching of F1 larvae and parasitization period until emergence of the F1 adults. The beetles were exposed to control, test item and reference item for 28 days. After 7, 14 and 21 days approx. 500 pupae of the host organism *Delia antiqua* (onion fly) per replicate and date were dug into the soil for parasitization. 28 days after application the adults were removed from the test units. After one further week the onion fly pupae were sieved out of the soil and transferred into a hatching unit. Assessment of reproduction was carried out by counting the number of beetles emerged from the parasitized onion fly pupae (*Delia antiqua*) daily during 5 weeks.

Endpoints: Reproduction capacity (average number of hatched beetles of the F1 generation).

Reference item: Dimethoate EC 400 (a.s.: dimethoate, 405.2 g/L analyzed; 400 g/L nominal).

Test rates: Control (deionized water), treatment rates:

BAS 684 03 H [L/ha]	**BAS 684 H [g/ha] 1)**
0.7	525
1.4	1050

1) Based on nominal content.

The reference item was applied at a rate of 1.5 L/ha. All substances were applied in 400 L water/ha. The substances were sprayed via laboratory spray applicator on the soil surface.

Test conditions: Temperature: 19 °C - 22 °C, relative humidity: 63% - 74%, photoperiod: 16 h light : 8 h dark, light intensity: 1820 lux / 1760 lux; food: *Chironomus* spp. larvae (thawed).

Statistics: Descriptive statistics. Williams-t-test for reproduction data ($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

In the water-treated control the average number of hatched beetles of the F1 generation was 660. At test rates of 0.7 and 1.4 L BAS 684 03 H/ha, a reproductive capacity of 618 and 612 hatched beetles, respectively, was observed. This resulted in effects on reproduction between -6.4% and 7.2% inhibition. No statistically significant differences compared to the control were observed at rates up to and including 1.4 L BAS 684 03 H/ha (Williams-t-test, $\alpha = 0.05$). No unusual observations regarding behavior were noted in the control and the test item treatment groups at any observation point during the test. The results are summarized in Table B.9.5.2.1-3.

Table B.9.5.2.1-3: Effects on rove beetles (*Aleochara bilineata*) exposed to BAS 684 03 H in an extended laboratory trial

Treatment	Rate [1] [L/ha]	Number of hatched beetles [2]	Effects on reproduction [3] [%]
Control	--	660	--
BAS 684 03 H	0.7	618	6.4
	1.4	612	7.2

1) Application rate in 400 L water/ha.

2) Mean number of emerged beetles per replicate.

3) Effect on reproduction according to the following formula: (1-Pt/Pc) * 100% calculated on the absolute number of emerged beetles (positive values represent a decreased reproduction compared to the control).

The reference item produced an effect on reproduction of 79.0% compared to the control.

Over 28 days, adult mortality was observed at very low levels in the control and test item treatments, with control mortality at 2.5%, 3.8% mortality when exposed to 0.7 L BAS 684 03 H/ha and 2.5% mortality at 1.4 L BAS 684 03 H/ha. Reference item mortality was 80.0%.

III. CONCLUSION

In an extended laboratory study with BAS 684 03 H no unacceptable effects on reproduction of the ground dwelling predator *Aleochara bilineata* were observed after exposure to sandy soil treated with 0.7 L BAS 684 03 H/ha and 1.4 L BAS 684 03 H/ha in 400 L water/ha.

HSE evaluator comments:

The HSE evaluator found the extended test for investigating the effects of BAS 684 03 H on the reproduction of the **ground dwelling predator *Aleochara bilineata*** to be well reported and conducted with good adherence to the guidance (IOBC/Grimm et al., 2000; Mead-Briggs et al., 2000). The study is confirmed by the HSE evaluator to be valid in relation to the reproductive capacity requirements: control reproductive capacity exceeded 600 hatched beetles (mean of 660 per replicate), and reference item reproductive capacity was over 50% lower than control with a 79% reduction in hatched beetles. There are no guideline deviations to report.

The number of hatched beetles was only slightly reduced by exposure to BAS 684 03 H: there was a 6.4% reduction in reproduction at 0.7 L product/ha, and 7.2% reduction at 1.4 L product/ha. These reductions in reproduction versus control were not statistically significant; therefore, the HSE evaluator confirms an endpoint of ER_{50} > 1.4 L BAS 684 03 H/ha.

B.9.6. RISK ASSESSMENT FOR ARTHROPODS

B.9.6.1. Risk assessment for bees

Studies conducted with the active substance

Acute oral and acute contact studies were submitted for the active substance both for the honeybee (*Apis mellifera*) and the bumblebee (*Bombus terrestris*). In additon a chronic honeybee larvae repeated exposure study (22d) was submitted for the active stubstance. All of the submitted studies were considered valid after evaluation. It should be noted that the bumblebee studies and honeybee larval study will not be used in the risk assessment due to a lack of noted guidance.

Studies conducted with formulations BAS 684 02 H and BAS 684 03 H

Several bee studies have been conducted using BAS 684 02 H which has been used in place of the representative formulation BAS 684 03 H. A formulation comparison between the two has been undertaken in the confidential

Volume 4. It was concluded that conducting a risk assessment using data from BAS 684 02 H studies would be suitably protective of the risk from the representative formulation BAS 684 03 H.

Acute oral and acute contact studies were submitted using formulation BAS 684 02 H both for honeybee (*Apis mellifera*) and the bumblebee (*Bombus terrestris*). All studies were considered valid after evaluation.

For the acute contact study conducted with *Apis mellifera* the evaluator noted that one bee was 'moribund' for the duration of the study at the top dose tested. However the risk assessment that follows demonstrates a large margin of safety for the acute contact risk assessment and hence this result in the study is not of concern.

It should be noted that the valid bumblebee studies and chronic adult honeybee formulation study will not be used in the risk assessment due to a lack of noted guidance regarding this area of risk assessment.

A chronic larvae repeated exposure (22d) study was submitted using the representative formulation BAS 684 03 H. This study was considered valid after evaluation however it will not be used in the risk assessment due to a lack of noted guidance.

The table below presents study endpoints for all valid bee studies considered.

Table B.9.6.1-1: Toxicity endpoints available for cinmethylin and its formulations

Test Item	Test Design	Ecotoxicological Endpoint		Reference
BAS 684 H (active substance)	**Honeybee oral (acute 48h)**	**LD_{50}**	**> 200.0 µg a.s./bee**	Franke (2016a) KCA 8.3.1.1.1/1 KCA 8.3.1.1.2/1
	Honeybee contact (acute 48h)	**LD_{50}**	**> 200.0 µg a.s./bee**	
	Bumblebee oral (acute 96h) [2]	LD_{50}	> 195.4 µg a.s./bumblebee	Amsel (2017a) KCA 8.3.1.1.1/2 KCA 8.3.1.1.2/2
	Bumblebee contact (acute 96h) [2]	LD_{50}	> 200.0 µg a.s./bumblebee	
	Honeybee larvae chronic (22d repeated exposure) [2]	EC_{10} EC_{20} ED_{50} EC_{50} NOED NOEC	45.1 µg a.s./larva 100.7 µg a.s./larva > 100.1 µg a.s./larva > 650 mg a.s./kg food ≥ 100.1 µg a.s./larva ≥ 650 mg a.s./kg food	Kleebaum (2016a) KCA 8.3.1.3/1 Further statistics: Azevedo (2018a) KCA 8.3.1.3/2
BAS 684 02 H	**Honeybee oral (acute 48h)**	**LD_{50}**	**> 294.7 µg BAS 684 02 H/bee**	Sekine (2016a) KCP 10.3.1.1.1/1 KCP 10.3.1.1.2/1
	Honeybee contact (acute 48h)	**LD_{50}**	**> 272.0 µg BAS 684 02 H/bee**	
	Bumblebee oral (acute 96h) [2]	LD_{50}	> 258.5 µg BAS 684 02 H/bee	Amsel (2016a) 10.3.1.1.1/2 10.3.1.1.2/1
	Bumblebee contact (acute 96h) [2]	LD_{50}	> 272.0 µg BAS 684 02 H/bee	
	Honeybee adult chronic (10d repeated exposure) [2]	EC_{10} EC_{20} LDD_{50} LC_{50} NOEDD NOEC	86.5µg a.s./bee/day 110.1µg a.s./bee/day 143.2 µg a.s./bee/day 3.982 g a.s./kg food 48.6 µg a.s./bee/day 1.284 g a.s./kg food	Ruhland (2017a) KCP 10.3.1.2/1 Further statistics: Azevedo (2018b) KCP 10.3.1.2/1
BAS 684 03 H	Honeybee larvae chronic (22d repeated exposure) [2]	EC_{10} EC_{20} ED_{50} EC_{50} NOED NOEC	116.3 µg BAS 684 03 H/larva 124.7 µg BAS 684 03 H /larva > 133.4 µg BAS 684 03 H /larva > 844 mg BAS 684 03 H /kg food 66.7 µg BAS 684 03 H /larva 422 mg BAS 684 03 H/kg food	Kleebaum (2017a) KCP 10.3.1.3/1 Further statistics: Azevedo (2018c) KCP 10.3.1.3/2

[1] Studies presented in CA document.
[2] Due to the lack of currently noted guidance, these endpoints are presented as additionally supporting information only.
[3] BAS 684 02 H formulation has been compared to the representative formulation BAS 684 03 H in the confidential section Volume 4 and the two are considered comparable.
Bold endpoints will be used in the risk assessment.

The risk assessment for bees will be performed using the Hazard Quotient (HQ) approach, with the appropriate endpoints for acute oral and contact toxicity from studies which used the BAS 684 02 H formulation and the active substance using the following equations:

Hazard Quotient, oral: $$Q_{HO} = \frac{\text{max. appl. rate}}{LD_{50}\ \text{oral}} = \frac{[\text{g a.s./ha or g total substance/ha}]}{[\mu\text{g a.s./bee or } \mu\text{g total substance/bee}]}$$

Hazard Quotient, contact: $$Q_{HC} = \frac{\text{max. appl. rate}}{LD_{50}\ \text{contact}} = \frac{[\text{g a.s./ha or g total substance/ha}]}{[\mu\text{g a.s./bee or } \mu\text{g total substance/bee}]}$$

HQs which fall below the trigger value of 50 for both contact and oral toxicity are considerd to demonstrate a non-acceptable risk to honeybees.

Table B.9.6.1-2: HQ calculations for honeybees: Oral exposure

Test substance	Application rate [g/ha]	Endpoint	LD_{50} [µg/bee]	Hazard quotient HQ	Trigger
Risk assessment on adult honeybees					
BAS 684 H	500	48 h oral	> 200.0	< 2.5	50
		48 h contact	> 200.0	< 2.5	
BAS 684 02 H	679.32 *	48 h oral	> 294.7	< 2.3	
		48 h contact	> 272.0	< 2.5	

* Taking into account the density of BAS 684 02 H of 1.020 g/cm^3.

The calculated HQs are below the trigger value of 50, indicating an acceptable risk to bees via oral and contact exposure of the formulation and active substance.

Conclusions on the proposed use

All calculations of HQs for the acute oral and contact honeybee studies fell below the trigger value of 50, indicating an acceptable risk to honeybees. As previously mentioned, the contact bumblebee acute study, the adult honeybee chronic study and chronic honeybee larvae studies will not be used in a formal risk assessment due to the current lack of noted guidance. However, considering them in terms of supporting information, the results from these studies do not raise concern of significant toxic effects to bees through the use of cinmethylin. **Therefore, the evaluator concludes that the proposed use of the formulation 'BAS 684 03 H' has demonstrated an acceptable risk to bees.**

B.9.6.2. Risk assessment for non-target arthropods other than bees

The risk assessment for non-target arthropods has been conducted in line with the Terrestrial Guidance Document (SANCO/10329/2002).

Endpoints

Table B.9.6.2-1 shows the available ecotoxicological endpoints for non-target arthropods. All endpoints are relevant for the risk assessment.

Table B.9.6.2-1: Ecotoxicological endpoints for non-target arthropods other than bees

Test substance	Species	Exposed life stage	Study type	Application rate [L/ha]	Corrected mortality[1)] [%]	Sublethal effects [2)] [%]	Reference
BAS 684 03 H	*Aphidius rhopalosiphi*	adults	Laboratory test using artificial substrate, 2D exposure scenario (glass plate)	0.04375 0.0875 0.175 0.350 0.700	0.0 41.0 56.4 89.7 100.0	n.d.	2017/1073467
				LR_{50} = 0.136 L/ha			
BAS 684 03 H	*Aphidius rhopalosiphi*	adults	Extended laboratory test using natural substrate, 3D exposure scenario (barley plants)	0.04375 0.0875 0.175 0.350 0.700	-3.6 0 -3.6 0 7.1	-16.0 -3.9 -2.6 -6.1 -5.2	2017/1084956
				LR_{50} > 0.7 L/Ha **ER_{50} > 0.7 L/Ha**			
BAS 684 03 H	*Typhlodromus pyri*	protonymphs	Laboratory test using artificial substrate, 2D exposure scenario (glass plate)	0.0625 0.125 0.250 0.500 1.000	0 1.0 1.0 9.1 77.8	n.d.	2017/1073466
				LR_{50} = 0.764 L/ha			
BAS 684 03 H	*Aleochara bilineata*	adults	Extended laboratory test using natural substrate, 2D exposure scenario (sandy soil)	0.7 1.4	n.d. n.d.	6.4 7.2	2017/1112416
				LR_{50} > 1.4 L/ha **ER_{50} > 1.4 L/ha**			

[1)] Positive values indicate an increase in mortality; negative values indicate a decrease in mortality, relative to the control.
[2)] Positive values indicate a decrease in reproduction; negative values indicate an increase in reproduction, relative to the control.
n.d. = not determined.

Exposure

In-field exposure

Non-target arthropods inhabiting the crop can be exposed to residues of BAS 684 03 H by direct contact, either as a result of overspray or through contact with residues on plants and soil or in food items. BAS 684 03 H is applied at a maximum rate of 1 x 0.666 L/ha to cereals (field crops). The risk assessment is thus carried out based on this worst-case field application rate.

The in-field exposure (predicted environmental rate, PER) is calculated according to ESCORT 2 using the following equation:

$PER_{in\text{-}field}$ = Application rate [L/ha] x MAF

The MAF is a generic multiple application factor, which is used to account for the potential build-up of applied substances between applications based on the application interval, DT_{50} value and number of applications. Default foliar and soil MAF values are given in the ESCORT 2 Guidance Document. For one application, the exposure is equal to the single application rate.

The maximum predicted environmental rates (PER) occurring within the field after application of BAS 684 03 H are presented in Table B.9.6.2-2.

Table B.9.6.2-2: $PER_{in\text{-}field}$ values for application of BAS 684 03 H in winter wheat (worst case use)

Crop	**Worst case application rate (winter wheat) [L/ha]**	**MAF**	**$PER_{in\text{-}field}$ [L/ha]**
Cereals	0.666	1.0	0.666

Off-field exposure

Risk assessment of areas immediately surrounding the crop is considered important since these areas represent a natural reservoir for immigration, emigration and reproduction of arthropod populations. Exposure of non-target arthropods living in off-field areas to BAS 684 03 H will mainly be due to spray drift from field applications. Off-field areas are assumed to be densely vegetated and thus spray drift is unlikely to reach bare ground. Therefore, evaluation of exposure via soil residues in off-field areas was not considered.

Off-field foliar PER values were calculated from in-field foliar PERs in conjunction with drift values published by the BBA [*90th percentile drift according to BBA (2000): Bundesanzeiger Jg. 52 (Official Gazette), Nr 100, S. 9879-9880 (25.05.2000) Bekanntmachung über die Abtrifteckwerte, die bei der Prüfung und Zulassung von Pflanzenschutzmitteln herangezogen werden*] as shown in the following equation:

$$PER_{off-field} = \frac{\text{maximum } PER_{in-field} \times (\%\text{drift}/100)}{\text{vegetation distribution factor}}$$

The model used to estimate spray drift was developed for drift onto a two-dimensional water surface and, as such, does not account for interception and dilution by three-dimensional vegetation in off-crop areas. Therefore, a vegetation distribution or dilution factor is incorporated into the equation when calculating PERs to be used in conjunction with toxicity endpoints derived from two-dimensional (glass plate, leaf disc or sand) studies. A dilution factor of 10 is recommended by ESCORT 2. For 3-dimensional studies, i.e. where spray treatment is applied onto whole plants, the dilution factor of 10 is not used, as any dilution over the 3-dimensional vegetation surface is accounted for in the study design.

The drift value for one application at 1 m distance in field crops is 2.77% of the application rate (90th percentile drift). The drift factor (% drift/100) is therefore 2.77/100 = 0.0277.

The resulting PER off-field values are shown in Table B.9.6.2-3.

Table B.9.6.2-3: $PER_{off\text{-}field}$ values following application of BAS 684 03 H in winter wheat (worst case use)

Study type	**Study type [Exposure scenario]**	**Maximum $PER_{in\text{-}field}$ [L/ha]**	**Drift factor [% drift/100]**	**Vegetation distribution factor**	**$PER_{off\text{-}field}$ [L/ha]**
cereals	2D	0.666	0.0277	10	0.00184
	3D			--	0.01840

In-field risk assessment

The potential risk of BAS 684 03 H to in-field non-target arthropods was assessed by calculation of the hazard quotient (HQ) using the $PER_{in\text{-}field}$ and the lowest lethal rate (LR_{50}) values according to the following equation:

$$HQ_{in\text{-}field} = \frac{PER_{in\text{-}field}\,[\text{L/ha}]}{LR_{50}\,[\text{L/ha}]}$$

The HQ trigger for Tier I laboratory studies is 2. When following the HQ approach for in-field assessments, a HQ value lower than the trigger value indicates a low risk to non-target arthropods. A quotient value equal to or greater than the trigger indicates a potential hazard to non-target arthropods. The resulting $HQ_{in\text{-}field}$ values are presented in Table B.9.6.2-4.

Table B.9.6.2-4: $HQ_{in\text{-}field}$ for non-target arthropods exposed to BAS 684 03 H in winter wheat (worst case use)

Species	**LR_{50} [L/ha]**	**$PER_{in\text{-}field}$ [L/ha]**	**$HQ_{in\text{-}field}$**	**Trigger value**
Aphidius rhopalosiphi Tier I, 2D exposure scenario	0.136	0.666	**4.897**	2
Typhlodromus pyri Tier I, 2D exposure scenario	0.764		0.872	2

PER = predicted environmental rate.
HQ values shown in **bold** is above the relevant trigger.

The calculated HQ value for *A. rhopalosiphi* was above the trigger of 2; however, the calculated value for *T. pyri* was below the trigger. Therefore, the application of BAS 684 03 H to cereals poses a potential risk to *A. rhopalosiphi* but indicates a low risk to *T. pyri*. Further consideration of potential in-field risk was therefore necessary via second tier risk assessment.

Two extended laboratory studies were submitted to study the sublethal effects of BAS 684 03 H on two non-target arthropods using natural substrates. In the extended laboratory studies, the trigger value is based on a 50% effect compared with the control (either the LR_{50} for lethal effects, or ER_{50} for sublethal effects). Where the LR_{50}/ER_{50} is greater than the $PER_{in\text{-}field}$, a low risk to non-target arthropods can be concluded. If the $PER_{in\text{-}field}$ is exceeded, then further consideration of risk would be necessary.

The LR_{50} and ER_{50} values relating to sublethal effects on the reproduction of the non-target arthropods are reported in Table B.9.6.2-5.

Table B.9.6.2-5: Lethal and sublethal effect levels for non-target arthropods exposed to BAS 684 03 H in winter wheat (worst case use)

Species	**LR_{50} [L/ha]**	**ER_{50} [L/ha]**	**$PER_{in\text{-}field}$ [L/ha]**
Aphidius rhopalosiphi Tier II, 3D exposure scenario	> 0.7	> 0.7	0.666
Aleochara bilineata Tier II, 2D exposure scenario	n.d.	> 1.4	

PER = predicted environmental rate.
n.d. = not determined.

Based on the reported values, the 50% effect levels for both non-target arthropod species are greater than the in-field PER. Therefore, it is concluded that there is a low in-field risk to non-target arthropods following application of BAS 684 03 H to winter wheat and oilseed rape.

Off-field risk assessment (Tier I)

In order to assess the potential risk of BAS 684 03 H to off-field non-target arthropods, the $PER_{off\text{-}field}$ (see Table B.9.6.2-6) is compared to the toxicity endpoints according to the following equation:

$$HQ_{off\text{-}field} = \frac{PER_{off\text{-}field}\,[L/ha]}{LR_{50}\,[L/ha]} \times \text{Correction factor}$$

The HQ trigger for Tier I laboratory studies is 2. Furthermore, ESCORT 2 recommends a correction factor of 10 for Tier I data in the off-field risk assessment to account for extrapolation from testing just two representative species to the species diversity expected in off-crop areas.

Respective $HQ_{off\text{-}field}$ values are given in the table below.

Table B.9.6.2-6: $HQ_{off\text{-}field}$ values for non-target arthropods exposed to BAS 684 03 H in winter wheat (worst case use)

Species	LR_{50} [L/ha]	$PER_{off\text{-}field}$ [L/ha]	Correction factor	$HQ_{off\text{-}field}$	Trigger value
Aphidius rhopalosiphi. Tier I, 2D exposure scenario	0.136	0.00184	10	0.135	2
Typhlodromus pyri, Tier I, 2D exposure scenario	0.764			0.024	2

PER = predicted environmental rate.

The calculated $HQ_{off\text{-}field}$ values for *A. rhopalosiphi* and *T. pyri* fall below the trigger value of 2, indicating that the application of BAS 684 03 H to winter wheat and oilseed rape poses a low risk to non-target arthropods in off-field situations.

Conclusion

The in-field as well as the off-field risk for other non-target arthropods from the intended uses of the product BAS 684 03 H in winter wheat and oilseed rape is acceptable.

B.9.7. EFFECTS ON NON-TARGET SOIL MESO- AND MACROFAUNA

B.9.7.1. Earthworms

Report: CP 10.7/1
Friedrich S., 2017 b
Acute toxicity of BAS 684 03 H to the earthworm *Eisenia andrei* in artificial soil with 10 % peat
2017/1064915
Guidelines: OECD 207 (1984), ISO 11268-1 (2012)
GLP: Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 684 03 H; batch no. FD-170210-0001; content of a.s.: cinmethylin (Reg. No. 900 202): 750.0 g/L nominal (737.3 g/L analysed); density: 1.001 g/cm^3.

B. STUDY DESIGN

Test species: *Eisenia andrei*; adult worms with clitellum and weight of 300 – 600 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 BAS 684 03 H 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: Control, 70, 126, 227, 408 and 734 mg product/kg dry soil (corresponding to 52, 94, 170, 306 and 550 mg a.s./kg dry soil).

Test conditions: Artificial soil according to OECD 207 with 10 % peat; pH 5.92 – 6.01 at test initiation, pH 5.80 – 5.92 at test termination; water content 55.0 - 55.4 % of its maximum water holding capacity (WHC) at test initiation and 54.4 - 54.7 % of WHC at test termination, temperature: 19.0 – 20.6 °C; photoperiod: continuous illumination, light intensity: 630 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), Logit analysis for calculation of LC_{50}.

II. RESULTS AND DISCUSSION

After 14 days of exposure, statistically significant mortality of 55.0 % compared to the control was found at 734 mg product/kg soil dry weight (Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm, $\alpha = 0.05$, one-sided greater). Mortality rates of 0 - 55.0 % were recorded in the test item treatment groups. In the control, the mortality rate was 0 %. The biomass development was not statistically significantly different (Williams-t-test, $\alpha = 0.05$, one-sided greater) compared to the control at 70 and 126 mg product/kg soil dry weight. The results are summarized in Table B.9.7.1-1.

Table B.9.7.1-1: Effects of BAS 684 03 H on *Eisenia andrei* in a 14-day acute study

BAS 684 03 H [mg/kg dry soil]	**Control**	**70**	**126**	**227**	**408**	**734**
Mortality (28 d) [%]	0.0	0.0	0.0	0.0	2.5	55.0 *
Weight change (14 d) [%]	-9.6	-12.3	-13.5	-17.8 **	-23.1 **	-29.1 **
Endpoints [mg BAS 684 03 H/kg dry soil]						
LC_{50}	712					
NOEC	126					

* Statistically significantly different compared to the control (Multiple Sequentially-rejective Fisher Test After Bonferroni-Holm, $\alpha = 0.05$, one-sided greater).
** Statistically significantly different compared to the control (Williams-t-test, $\alpha = 0.05$, one-sided greater).

III. CONCLUSION

In a 14-day acute toxicity study with earthworms (*Eisenia andrei*), exposure to BAS 684 03 H resulted in an LC_{50} of 712 mg product/kg dry soil. The NOEC was determined to be 126 mg product/kg dry soil.

HSE evaluator comments:

The above study has not been evaluated by HSE. It is not required under the current data requirements 284/2013. In addition, the endpoints derived are not adverse compared to the study Friedrich (2018a) where the LC_{50}, NOEC values are > 180 and 118.4 mg product/kg dry soil respectively. Therefore, further consideration is not required.

Report: CP 10.4.1.1/1
Friedrich S., 2018 a
Sublethal effects of BAS 684 03 H on the earthworm *Eisenia andrei* in artificial soil
2017/1166587
Guidelines: OECD 222 (2016)
GLP: Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 684 03 H; batch no. FD-170210-0001; content of a.s.: cinmethylin (Reg. No. 900 202): 750.0 g/L nominal (737.6 g/L analysed); density: 1.001 g/cm^3.

B. STUDY DESIGN

Test species: *Eisenia andrei*; adult worms with clitellum and weight of 319 - 498 mg/worm, approximately 4 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 BAS 684 03 H 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 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: Control, 67.7, 77.8, 89.5, 102.9, 118.4, 136.1, 156.5 and 180 mg product/kg dry soil (corresponding to 49.94, 57.39, 66.02, 75.90, 87.33, 100.39, 115.43 and 132.77 mg a.s./kg dry soil).

Test conditions: Artificial soil according to OECD 222 with 10 % peat; pH 5.89 – 6.01 at test initiation, pH 5.65 – 5.78 at test termination; water content 57.0 – 57.4 % of its maximum water holding capacity (WHC) at test initiation and 55.7 – 57.0 % of WHC at test termination, temperature: 18.0 – 21.3 °C; photoperiod: 16 hours light: 8 hours dark, light intensity: 640 lux, feeding with horse manure.

Statistics: Descriptive statistics; Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm for mortality ($\alpha = 0.05$, one-sided greater), Dunnett-t-test for biomass and Williams-t-test for reproduction ($\alpha = 0.05$, one-sided smaller)

II. RESULTS AND DISCUSSION

Validity criteria:

In OECD 222 (2016) the following criteria are stated:

- each replicate (containing 10 adults) to have produced ≥ 30 juveniles by the end of the test, minimum was 156.
- the coefficient of variation of reproduction to be ≤ 30 %, obtained 13.0 %.
- adult mortality over the initial 4 weeks of the test to be ≤ 10 %, mortality was 2.5 %

During the study the above criteria were met.

Biological results: BAS 684 03 H did not show any effects on mortality and body weight. The mortality of adult worms ranged between 0 – 5.0 % in the test item treated groups and 2.5 % in the control group. The weight change of adult worms was 19.9 - 24.4 % in the test item treated groups and 22.0 % in the control group. The feeding activity in all test item treated groups was comparable to the control.

The reproduction rate was significantly different compared to the control at concentrations of 136.1, 156.5 and 180 mg product/kg dry soil (Williams-t-test, α = 0.05, one-sided smaller). No pathological symptoms and no further effects on behaviour of the worms were observed. The results are summarized Table B.9.7.1-2.

Table B.9.7.1-2: Effects of BAS 684 03 H on *Eisenia andrei* in a 56-day reproduction study

BAS 684 03 H [mg product/kg dry soil]	**Control**	**67.7**	**77.8**	**89.5**	**102.9**	**118.4**	**136.1**	**156.5**	**180**
Mortality (28 d) [%]	2.5	2.5	2.5	0.0	5.0	0.0	0.0	2.5	0.0
Weight change (day 28) [%]	22.0	23.6	22.6	24.4	22.3	20.3	24.2	21.8	19.9
Number of juveniles (day 56)	197.1	206.8	196.0	190.0	195.8	189.3	161.8 *	142.3 *	138.8 *
Reproduction in [%] of control (day 56)	100	104.9	99.4	96.4	99.3	96.0	82.1	72.2	70.4
Endpoints [mg product/kg dry soil]									
NOEC (day 56)	118.4								
LC_{50} (day 28) 1)	> 180								
EC_{10} (day 56) 2)	119.0 (95 % limits: 99.2 - 135.2)								
EC_{20} (day 56) 2)	146.3 (95 % limits: 133.2 - 158.1)								
EC_{50} (day 56) 2)	> 180								

*: statistically significantly different from control (Williams-t-test for reproduction, α = 0.05, one-sided smaller)
1) Based on estimation of data.
2) Based on 3-parametric normal CDF

Reference test: 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 57 % and 100 % compared to the control at the tested concentrations of 5 and 10 mg product/kg dry soil.

III. CONCLUSION

In a 56-day earthworm reproduction study with BAS 684 03 H, no adverse effects on survival and biomass development could be determined at all concentrations tested up to and including 180 mg product/kg dry soil. Statistically significant effects on the number of juveniles compared to the control group were recorded at concentrations of 136.1, 156.5 and 180 mg product/kg dry soil. The NOEC for mortality and biomass was determined to be ≥ 180 mg product/kg dry soil, the highest concentration tested. The NOEC for reproduction was determined to be 118.4 mg product/kg dry soil. The EC_{10}, EC_{20} and EC_{50} values for reproduction were calculated to be 119.0, 146.3 and > 180 mg product/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 both the NOEC and EC_{10} are similar. However, since the NOEC is marginally lower (more conservative) this has been used in the risk assessment.

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 have been derived:

- '**BAS 684 03H**' 56-day NOEC (10 % peat) = 118.4 mg product/kg (reproductive effects), **equivalent to 87.25 mg a.s./kg**

- 'BAS 684 03H' 56-day EC_{10} (10 % peat) = 119.0 mg product/kg (reproductive effects) **equivalent to 87.69 mg a.s./kg**

B.9.7.2. Effects on non-target soil meso- and macrofauna (other than earthworms)

Report: CP 10.4.2.1/1
Friedrich S., 2017 a
Effects of BAS 684 03 H on the reproduction of the collembolan *Folsomia candida*
2017/1109480
Guidelines: OECD 232 (2016)
GLP: Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 684 03 H; batch no. FD-170210-0001; content of a.s.: Cinmethylin (Reg. No. 900 202): 750.0 g/L nominal (737.6 g/L analysed); density: 1.001 g/cm^3.

B. STUDY DESIGN

Test species: Collembola (*Folsomia candida*), from in-house culture, juveniles, 9-12 days old.

Test design: 28-d exposure in treated artificial soil; different concentrations of the test item are mixed homogeneously into the soil which is filled in glass vessels before the Collembola are introduced on top of the soil; 9 treatment groups (8 test item concentrations, control); 4 replicates in the test item treatment group, 8 replicates in the control group, each with 10 Collembola; assessment of mortality, reproduction and behavioral effects after 28 days.

Endpoints: Mortality and reproduction after 28 days.

Reference item: Boric acid (100.1 % analyzed), the effects of the reference item were investigated in a separate study.

Test rates: Control, 84.9, 110, 144, 187, 243, 315, 410 and 533 mg product/kg dry soil.

Test conditions: Artificial soil according to OECD 232 (5 % sphagnum peat); pH at test initiation 6.05 - 6.10, at test termination 5.83 – 5.87; water content at test initiation 57.8 % - 58.0 % of maximum water holding capacity (WHC); 56.4 % - 57.3 % of maximum WHC at test termination; temperature 19.7 °C – 20.5 °C; photoperiod: 16 hours light : 8 hours dark; light intensity: 640 lux.

Statistics: Descriptive statistics. Multiple Sequentially-rejective Fisher Text after Bonferroni-Holm for mortality ($\alpha = 0.05$, one-sided greater), Williams-t-test for reproduction ($\alpha = 0.05$, one-sided smaller), Logit analysis for mortality and Probit analysis for reproduction.

II. RESULTS AND DISCUSSION

Validity criteria:

In OECD 232 (2016) the following criteria are stated:

- Mean adult mortality should not exceed 20% at the end of the test. Observed: 3.8 %.
- The mean number of juveniles per vessel should be at least 100 at the end of the test. Observed: 825 per vessel.
- The coefficient of variation calculated for the number of juveniles should be less than 30% at the end of the definitive test. Observed: 10.4 %.

During the study the above criteria were met.

Biological results: Statistically significant effects on mortality compared to the control were observed at concentrations of 243, 315, 410 and 533 mg product/kg dry soil (Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm, $\alpha = 0.05$, one-sided greater). Mortality rates of 2.5 - 100.0 % were recorded in the test item treatment groups. In the control, the mortality rate was 3.8 %.

Statistically significant effects (Williams-t-test, $\alpha = 0.05$, one-sided smaller) on the number of juveniles compared to the control group were recorded at concentrations of 243, 315, 410 and 533 mg product/kg dry soil. The mean number of juveniles counted 28 days after introduction of the parental collembolans into the test vessels was 825 in the control and 813, 818, 792, 763, 592, 344, 191 and 95 at concentrations of 84.9, 110, 144, 187, 243, 315, 410 and 533 mg product/kg dry soil, respectively. The results are summarized in Table B.9.7.2-1.

Table B.9.7.2-1: Effects of BAS 684 03 H on *Folsomia candida* in a 28-day reproduction study

BAS 684 03 H [mg product/kg dry soil]	**Control**	**84.9**	**110**	**144**	**187**	**243**	**315**	**410**	**533**
Mortality (day 28) [%]	3.8	5.0	2.5	2.5	7.5	50.0 *	72.5 *	92.5 *	100 *
No. of juveniles (day 28)	825	813	818	792	763	592 *	344 *	191 *	95 *
Reproduction (day 28) [% of control]	100	99	99	96	92	72	42	23	12
Endpoints [mg product/kg dry soil]									
$NOEC_{mortality/reproduction}$	187								
$LC_{50\ mortality}$ [1)] (95 % confidence limits)	265 (234 – 301)								
$EC_{10\ reproduction}$ [2)] (95 % confidence limits)	182 (166 – 200)								
$EC_{20\ reproduction}$ [2)] (95 % confidence limits)	217 (203 – 232)								
$EC_{50\ reproduction}$ [2)] (95 % confidence limits)	303 (290 – 316)								

* Statistically significantly different compared to the control (Multiple Sequentially-rejective Fisher Test After Bonferroni-Holm for mortality, $\alpha = 0.05$, one-sided greater, Williams-t-test for reproduction, $\alpha = 0.05$, one-sided smaller).

1) Based on Logit analysis.

2) Based on Probit analysis.

Reference test: In a separate study the reference item boric acid was tested at 44, 67, 100, 150 and 225 mg reference item/kg soil dry weight. The EC_{50} was determined to be 107 mg reference item/kg soil dry weight based on reproductive effects which is close to the value of 100 mg reference item/kg soil dry weight stated in OECD 232 (2016).

III. CONCLUSION

In a 28-day reproduction study on Collembola (*Folsomia candida*) with BAS 684 03 H the LC_{50} was calculated to be 265 mg product/kg dry soil. The NOEC for mortality and reproduction was determined to be 187 mg product/kg dry soil. The EC_{10}, EC_{20} and EC_{50} values for reproduction were calculated to be 182, 217 and 303 mg product/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 232 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 both the NOEC and EC_{10} are similar. However, since the EC_{10} is marginally lower (more conservative) this has been used in the risk assessment.

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 have been derived:

- 'BAS 684 03H' 28-day NOEC (5 % peat) = 187 mg product/kg (reproductive effects) **equivalent to 137.79 mg a.s./kg**
- **'BAS 684 03H'** 28-day EC_{10} (5 % peat) = 182 mg product/kg (reproductive effects) **equivalent to 134.10 mg a.s./kg**

Report:	CP 10.4.2.1/2 Schulz L., 2017 a Effects of BAS 684 03 H on the reproduction of the predatory mite *Hypoaspis aculeifer* 2017/1109481
Guidelines:	OECD 226 (2016)
GLP:	Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 684 03 H; batch no. FD-170210-0001; content of a.s.: cinmethylin (Reg. No. 900 202): 750.0 g/L nominal (737.6 g/L analysed); density: 1.001 g/cm^3.

B. STUDY DESIGN

Test species: Soil mites: *Hypoaspis aculeifer* (CANESTRINI); age: adult females from synchronized culture with an age differences of 2 days; source: in-house culture.

Test design: The effects of BAS 684 03 H on mortality and reproduction of the soil mite *Hypoaspis aculeifer* were investigated in a chronic laboratory experiment over a time period of 14 days according to OECD 226. Different concentrations of the test item were homogeneously mixed into the artificial soil (5 % peat) which was then filled into glass vessels after which the soil mites were introduced on top of the soil; 9 treatment groups (8 test item concentrations, control); 8 replicates/control group and 4 replicates/test item treatment group each with 10 soil mites (females). Assessment of adult mortality and reproduction effects was carried out after 14 days.

Endpoints: Mortality and reproduction rate (no. juveniles) after 14 days.

Reference item: Dimethoate (EC 400 g/L, nominal). The effects of the reference item were investigated in a separate study.

Test concentrations: Control, 84.9, 110, 144, 187, 243, 315, 410 and 533 mg product/kg dry soil.

Test conditions: Artificial soil according to OECD 226 (5 % peat), pH 6.1 at test initiation, pH 5.7 - pH 5.8 at test termination; water content at test initiation 47.69 - 49.63 % of maximum water holding capacity (WHC) and 47.53 - 50.37 % of maximum WHC at test termination; temperature 20.0 – 21.1°C; photoperiod: 16 h light : 8 h dark; light intensity: 666 lux. Feeding of mites with *Tyrophagus putrescentiae*.

Statistics: Descriptive statistics; Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm Correction for mortality ($\alpha = 0.05$, one-sided greater), Williams-t-test for reproduction ($\alpha = 0.05$, one-sided smaller), Probit Analysis for EC-values.

II. RESULTS AND DISCUSSION

Validity criteria:

In OECD 226 (2016) the following criteria are stated:

- Mean adult female mortality should not exceed 20 % at the end of the test. Observed: 2.5 %.
- The mean number of juveniles per replicate (with 10 adult females introduced) should be at least 50 at the end of the test. Observed: minimum of 267.
- The coefficient of variation calculated for the number of juvenile mites per replicate should not be higher than 30 % at the end of the definitive test. Observed: 8.7 %.

During the study the above criteria were met.

Biological results: Mortality rates of adult soil mites of 0.0 % - 7.5 % were recorded in the test item treatment groups, compared to 2.5 % mortality in the control group. The observed mortality rates in the test item treatment groups compared to control were not statistically significant (Multiple Sequentially-rejective Fisher Exact Test after Bonferroni-Holm Correction, $\alpha = 0.05$, one-sided greater).

Reproduction rates in the 84.9, 110, 144, 187, 243, 315, 410 and 533 mg product/kg dry soil treatments were 335.8, 329.0, 323.5, 316.5, 300.0, 317.0, 271.0 and 241.3 juveniles, respectively. The test item showed no statistically significantly adverse effects on reproduction up to and including 315 mg product/kg dry soil. At the two highest concentrations, 410 and 533 mg product/kg dry soil, the reproduction rate was statistically significantly reduced (William's t-test, $\alpha = 0.05$, one-sided smaller). Differences in the behavior and the morphology of the mites in the control and the test item treatment groups could not be observed.

The results are summarised in Table B.9.7.2-2.

Table B.9.7.2-2: Effects of BAS 684 03 H on *Hypoaspis aculeifer* in a 14-day reproduction study

BAS 684 03 H [mg product/kg dry soil]	**Control**	**84.9**	**110**	**144**	**187**	**243**	**315**	**410**	**533**
Mortality (day 14) [%]	2.5	2.5	5.0	2.5	5.0	7.5	5.0	7.5	0.0
No. of juveniles (day 14)	336.9	335.8	329.0	323.5	316.5	300.0	317.0	271.0 *	241.3 *
Reproduction (day 14) [% of control]	100	100	98	96	94	89	94	80	72
Endpoints [mg product/kg dry soil]									
$NOEC_{mortality}$	533								
$NOEC_{reproduction}$	315								
EC_{10} [1)]	277.6 (95 % confidence limits: 193.8 – 329.8)								
EC_{20} [1)]	426.4 (95 % confidence limits: 365.4 – 514.1)								
EC_{50}	> 533								
LC_{50}	> 533								

* Statistically significantly different compared to the control (William's t-test, $\alpha = 0.05$, one-sided smaller). [1)] Based on Probit analysis.

Reference test: In a separate study the reference item dimethoate was tested. The EC_{50} was determined to be 5.8 mg reference item/kg soil dry weight based on reproductive effects, which is within the range stated in OECD 226 i.e. 3 and 7 mg reference item/kg soil dry weight.

III. CONCLUSION

In a 14-day reproduction study with BAS 684 03 H on predatory soil mites (*Hypoaspis aculeifer*), the LC_{50} and EC_{50} values could not be calculated, but it can be concluded that these values are higher than 533 mg product/kg dry soil. The EC_{10} value for reproduction was calculated to be 277.6 mg product/kg dry soil. The NOEC for mortality and reproduction was determined to be 533 and 315 mg product/kg dry soil, respectively.

HSE evaluator comments:

Based on the results summarised in table B.9.7.2-2 there is an interrupted dose response when considering reproduction (juvenile numbers) resulting in 11 % effects compared to control at 243 mg product/kg dry soil and

6 % at 315 mg product/kg dry soil. The HSE evaluator has considered the raw data in the study report and notes that only a single replicate (238) was outside the control range (267 - 355) at 243 mg product/kg dry soil, the other three replicates ranged from 317 – 325. Therefore, the HSE evaluator considers the interrupted response is likely due to biological variation rather than treatment related effects and agrees with the reported NOEC value. As the EC_{10} value has been statistically calculated it is lower than the reported NOEC most likely due to the interrupted response. For the risk assessment the HSE evaluator has used the more conservative EC_{10} value, noting the difference between the endpoints is relatively low.

Considering there is evidence that cinmethylin is volatile (see environmental fate, volume 3 CA, section 8 dossier) in accordance with OECD 226 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.

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 have been derived:

- 'BAS 684 03H' 28-day NOEC (5 % peat) = 315 mg product/kg (reproductive effects) **equivalent to 232.11 mg a.s./kg**
- **'BAS 684 03H'** 28-day EC_{10} (5 % peat) = 277.6 mg product/kg (reproductive effects) **equivalent to 204.55 mg a.s./kg**

B.9.8. RISK ASSESSMENT FOR NON-TARGET SOIL MESO- AND MACROFAUNA

Earthworm reproduction tests (*Eisenia fetida*) were performed with the representative formulation (BAS 684 03 H) and active substance cinmethylin. The endpoints are summarised in Table B.9.8.1-1.

Table B.9.8.1-1: Summary of earthworm toxicity data

Test substance	Test species	Endpoint	Value [mg a.s./kg dry soil]	Reference
Chronic toxicity				
Cinmethylin	*Eisenia andrei*	NOEC	87.8*	Friedrich (2016a)
		EC_{10}	83.5*#	
		NOEC $_{CORR}$[1)]	43.90*	
		$EC_{10\ CORR}$[1)]	**41.8***	
BAS 684 03 H		NOEC	87.25*##	Friedrich (2018a)
		EC_{10}	87.69*##	
		NOEC $_{CORR}$[1)]	**43.63***##	
		$EC_{10\ CORR}$[1)]	43.85*##	

1) Corrected by factor of 2 due to lipophilic substance (i.e. $log_{Pow} > 2$)
* There is uncertainty when using these study endpoints. As cinmethylin is volatile and analytical measurements were not taken during the laboratory studies. This has been discussed further below.
\# As detailed in volume 3, section CA dossier B9 there was some uncertainty regarding this endpoint compared to the experimental data. However, the statistically derived EC_{10} value is considered suitably protective (more conservative than the experimental data) and valid by the HSE evaluator.
Endpoint expressed as active cinmethylin, considering the measured content of cinmethylin in study and a density of BAS 684 03 H of 1.001 g/cm^3.
Bold values have been used in the risk assessment, the HSE evaluator has used the lowest value either NOEC or EC_{10} in the risk assessment as a worst-case approach.

Collembola and predatory soil mite reproduction tests (*Folsomia candida* and *Hypoaspis aculeifer*, respectively) were performed with the representative formulation. No studies were conducted with the active substance hence

it is not possible to separately assess the risk from cinmethylin to these species for the proposed uses. The endpoints are summarised in Table B.9.8.1-2.

Table B.9.8.1-2 Summary of toxicity data for other soil macro-organisms

Test substance	Test species	Endpoint	Value [mg a.s./kg dry soil]	Reference
Chronic toxicity				
BAS 684 03 H	*Folsomia candida*	##NOEC	137*#	Friedrich (2017a)
BAS 684 03 H	*Folsomia candida*	##EC_{10}	134*#	
BAS 684 03 H	*Folsomia candida*	##NOEC $_{CORR}$ 1)	68.5*#	
BAS 684 03 H	*Folsomia candida*	##$EC_{10\ CORR}$ 1)	**67***#	
BAS 684 03 H	*Hypoaspis aculeifer*	##NOEC	232*#	Schulz (2017a)
BAS 684 03 H	*Hypoaspis aculeifer*	##EC_{10}	204*#	
BAS 684 03 H	*Hypoaspis aculeifer*	##NOEC $_{CORR}$ 1)	116*#	
BAS 684 03 H	*Hypoaspis aculeifer*	##$EC_{10\ CORR}$ 1)	**102***#	

1) Corrected by factor of 2 due to lipophilic substance (i.e. $log_{Pow} > 2$)
* There is uncertainty when using these study endpoints. As cinmethylin is volatile and analytical measurements were not taken during the laboratory studies. This has been discussed further below.
# Endpoint expressed as active cinmethylin, considering the nominal content of cinmethylin and a density of BAS 684 03 H of 1.001 g/cm^3.
With all these endpoints there is some uncertainty as cinmethylin is volatile and analytical measurements were not taken during studies to confirm exposure.
Bold values have been used in the risk assessment, the HSE evaluator has used the lowest value either NOEC or EC_{10} in the risk assessment as a worst-case approach.

An assessment of the chronic risk to earthworms and other soil macro-organisms has been conducted according to SANCO/10329/2002 guidance. As discussed above, a correction factor of 2 has been applied. In volume 3 CA dossier, section 8 the initial maximum PECsoil values for the active substance have been determined for the representative uses. The critical use has been evaluated in the risk assessment. In addition, there are no relevant soil metabolites to consider. This assessment will therefore cover all the representative uses. Maximum PEC_{soil} values have been compared to endpoints to determine TERs in Table B.9.8.1-3.

Table B.9.8.1-3 Chronic risk to earthworms and other soil macro-organisms from 'worst case' GAP (single application at 500 g a.s./ha to winter cereals).

Test organism	Test substance	Toxicity endpoint# (mg a.s./kg dws)	PECsoil (mg a.s./kg dws)	TER	Trigger
Eisenia fetida	Cinmethylin	41.8	0.667	63	5
Eisenia fetida	BAS 684 03 H	43.6	0.667	65	5
Folsomia candida	BAS 684 03 H	67.0	0.667	100	5
Hypoaspis aculeifer	BAS 684 03 H	102.0	0.667	153	5

Most conservative value of either NOEC or EC_{10}, noting endpoints have been corrected by factor of 2 as $log_{pow} > 2$)
* There is uncertainty when using these study endpoints. As cinmethylin is volatile and analytical measurements were not taken during the laboratory studies.

The resulting TER values for all organisms are above the trigger value of 5 for the formulated product and the active substance (earthworm only). In the absence of studies the risk from cinmethylin to *Folsomia candida* and *Hypoaspis aculeifer* could not be assessed. However, given the representative formulation contains a single

active it is likely the formulation assessment is protective of the risk from the active. Furthermore, there was a wide margin of safety at first tier based on the formulation assessment and the active would have to be at least 13 times more toxic to demonstrate a potential risk.

Therefore, the HSE evaluator considers the formulation studies can be used to address the risk from the active.

Whilst the above assessment demonstrates acceptable risk there is uncertainty regarding the extent of exposure in these studies and hence the endpoints have the potential to underestimate the toxicity due to volatilisation of cinmethylin. This has been considered further below.

Consideration of potential volatilisation:

The soil toxicity studies provided (Friedrich (2016a; 2017a; 2018a) and Schulz (2017a)) were conducted to the following OECD guidelines; 222, 226 and 232 which state: '*For volatile, unstable or readily degrading substances (e.g. data generated from a TG 307 study may be considered), or where there is otherwise uncertainty in maintaining the nominal soil concentration, analytical measurements of the exposure concentrations at the beginning, during and at the end of the test should be considered.*' As detailed in the chemistry dossier (volume 3, CA section 2) the vapour pressure of cinmethylin is 8.1 x 10^{-3} Pa at 20 °C suggesting there is potential for volatilisation and analytical measurements should have been taken. In addition, the study Hassink 2017b (section B.8.3.2, volume 3, CA dossier) demonstrated relatively high volatilisation from soil for cinmethylin. The methodology and results from this study have been briefly summarised below.

Methodology (Hassink, 2017b):

This study investigated the volatilisation behaviour of cinmethylin for a time period of 24 hours after application of the emulsifiable concentrate formulation BAS 684 02 H applied on soil surfaces in a circulation chamber using a blank formulation spiked with ^{14}C-cinmethylin. The soil moisture was adjusted to 60 % MWHC and 100 g of the moistened soil was weighed into each Petri dish. The soil was treated via a FullCone TG 0.5 nozzle (Spraying Systems Co.) in a closed application chamber made of glass. During the application the border of the Petri dish was covered with a Teflon sheet with a circular opening to avoid contamination of the glass. After application, the soil was removed from the application chamber and transferred directly to the circulation chamber. The temperature during the volatilisation experiment ranged 20.1 – 20.2 °C. Evaporation of water from the soil surfaces led to an average relative humidity of 45.9 %. Diurnal cycles were again simulated (8 h light, 14 h dark, 2 h light). Moisture losses were compensated throughout the experiment by using a wick immersed in a water reservoir. The water content of the soil on the petri dish remained constant during the experiment. Samples were taken at 1, 3, 6, and 24 h after application. At each sampling time the condensate of the cryotrap was removed, the ethylene-glycol traps were replaced, and new charcoal and PU-traps were connected. At the end of each experiment, both the circulation chamber and the tubes were rinsed twice and the rinsate was analysed. The remaining radioactivity in soil and plant was determined. The HSE fate evaluator noted that the study was conducted using single replicates and concluded that, while the study was considered valid, the reproducibility, accuracy and precision of this study are not known hence the study may not be robust.

Results (Hassink, 2017b):

Whilst there is uncertainty, the study (Hassink, 2017b) suggested that the volatilisation rate from soil surfaces was 73 % during the experimental period (up to 24 hours).

Based on the evidence of potential volatilisation and lack of analytical data during ecotoxicity studies further information was requested from the applicant by HSE. The following response was received (shown in italics):

Regarding the exposure route within the soil studies there might be some concerns due to the potential of cinmethylin to volatilize from soil surfaces (according to its phys-chem properties). But it should be noted that the test substance was homogenously mixed into the soil in all studies on soil organisms (according to guideline).

In this case volatilization is not a concern as shown in the aerobic degradation study with radiolabeled cinmethylin (BASF DocID 2015/1186904 + amendment 2019/1078806). The study is presented and discussed in detail in the environmental fate section, chapter CA 7.1.

Cinmethylin was homogenously mixed into the soil, incubated and volatiles were measured by using volatile traps. The results indicate that cinmethylin is not volatile, if mixed into the soil. Within the volatility assessment no other radiolabeled degradation product than CO_2 was detected. Hence, even if volatilization occurs after mixing cinmethylin into soil, the active substance itself is not volatile but rather is CO_2 as the final degradation product of the active substance.

Since the substance was mixed into the soil in all studies on soil macro- and microorganisms, the study results (DocID 2015/1186904 + amendment 2019/1078806) suggest that the substance is non-volatile under such conditions. Therefore, the exposure can be considered as representative.

HSE evaluator comments:

The response was considered in consultation with the HSE fate evaluator. The study referenced by applicant i.e. DocID 2015/1186904 + amendment 2019/1078806 is Stewart & Abernathy, 2016a. This study has been evaluated by the HSE fate evaluator and a summary is presented in section B.8.1.1.1.1, fate dossier CA section 8. This study investigated degradation in aerobic soil and used volatile traps to monitor decline of radio-labelled cinmethylin. The method and results are briefly summarised below (for full details refer to section B.8.1.1.1.1, fate dossier CA section 8).

Methodology (Stewart & Abernathy, 2016a):

Each soil sample consisted 100 g soil (dry weight equivalent, 2 mm sieved) and samples were treated with [cyclohexane-4-^{14}C]-cinmethylin or [benzyl-U-^{14}C]-cinmethylin to achieve a nominal concentration of 2.0 µg a.s./g soil (field application rate equivalent 750 g a.s./ha, based on distribution in the top 2.5 cm soil layer and a soil density of 1.5 g/cm^3). Each soil sample was treated drop-wise and test flasks were tumbled by hand to incorporate the test solution. Volatiles were collected through a series of four traps: a safety trap (a flask containing no liquid), ethylene glycol trap, 2M NaOH trap, and 1M H_2SO_4 trap.

Results (Stewart & Abernathy, 2016a):

The full results are displayed in tables 8.1.1.1/1-03 to 8.1.1.1/1-08 (section B.8.1.1.1.1, fate dossier CA section 8) for different soil types and radio-labels. A summary of these results focused on cinmethylin and volatile concentrations is shown in the table below over time periods relevant to ecotoxicology studies.

Table B.9.8.1-4: Aerobic soil degradation following exposure to cinmethylin for four soil types.

Measurement	**Cinmethylin concentration (% Applied radioactivity (AR))**						
Lufa 2.2 soil extracts, % AR based on [cyclohexane-4-^{14}C]-cinmethylin label Actual application rate (field equivalent): 739.1 g a.s./ha							
Days:	**D0**	**D3**	**D7**	**D14**	**D24**	**D41**	**D59**
Cinmethylin in soil (mean)	99.8	92.9	82.7	72.0	67.1	59.9	60.9
Organic volatiles (mean)	NA	NA	NA	NA	NA	NA	NA
Sum minor unknowns[a] (mean)	NA	NA	2.3	3.0	3.8	4.1	1.8

Total extractables[b]	99.8	92.9	85.0	75.0	70.8	64.1	62.6
$^{14}CO_2$	NA	3.3	6.8	14.9	15.6	21.0	20.2
Total recovery (mean)	100.0	100.0	96.8	97.4	95.5	95.1	92.8
Lufa 2.2 soil extracts, % AR based on [benzyl-U-^{14}C]-cinmethylin label. Actual application rate (field equivalent): 777.4 g a.s./ha							
Days:	**D0**	**D3**	**D7**	**D14**	**D24**	**D41**	**D59**
Cinmethylin in soil (mean)	99.8	91.0	84.4	77.4	68.9	63.8	59.4
Organic volatiles (mean)	NA	NA	NA	NA	NA	NA	NA
Sum minor unknowns[a] (mean)	NA	NA	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Total extractables[b]	99.8	91.0	84.4	77.4	68.9	63.8	59.4
$^{14}CO_2$	NA	NA	1.9	3.2	10.9	18.4	14.1
Total recovery (mean)	100.0	94.0	92.0	89.2	91.9	94.9	87.6
MSL-PF soil extracts, % AR based on [cyclohexane-4-^{14}C]-cinmethylin label Actual application rate (field equivalent): 784.9 g a.s./ha							
Days:	**D0**	**D3**	**D7**	**D14**	**D24**	**D41**	**D59**
Cinmethylin in soil (mean)	99.8	78.3	71.9	57.1	44.2	32.2	29.3
Organic volatiles (mean)	NA	NA	NA	NA	NA	NA	NA
Sum minor unknowns[a] (mean)	NA	2.8	1.5	3.5	4.9	7.0	5.6
Total extractables[b]	99.8	81.1	73.4	60.6	49.0	39.0	34.9
$^{14}CO_2$	NA	3.7	7.8	16.0	24.3	33.4	31.6
Total recovery (mean)	99.9	92.7	92.1	91.4	90.6	92.4	85.9
MSL-PF soil extracts, % AR based on [benzyl-U-^{14}C]-cinmethylin label. Actual application rate (field equivalent): 810.8 g a.s./ha							
Days:	**D0**	**D3**	**D7**	**D14**	**D24**	**D41**	**D59**
Cinmethylin in soil (mean)	100.0	81.0	71.6	53.8	43.7	36.0	32.9
Organic volatiles (mean)	NA	NA	NA	NA	NA	NA	NA
Sum minor unknowns[a] (mean)	NA	3.2	3.4	2.7	4.5	7.9	5.5
Total extractables[b]	100.0	84.2	75.0	56.4	48.2	43.9	38.5
$^{14}CO_2$	NA	1.6	4.4	12.7	17.4	19.6	24.4
Total recovery (mean)	100.1	93.0	93.2	95.7	92.2	90.6	87.2
Lufa 5M soil extracts, % AR based on [benzyl-U-^{14}C]-cinmethylin label. Actual application rate (field equivalent): 838.1 g a.s./ha							

Days:	D0	D3	D7	D14	D25	D40	D60
Cinmethylin in soil (mean)	99.3	82.5	73.6	62.2	44.2	20.7	6.6
Organic volatiles (mean)	0.1	NA	NA	NA	NA	NA	NA
Sum minor unknowns[a] (mean)	NA	1.5	3.6	4.2	7.1	7.8	8.0
Total extractables[b]	99.7	83.9	77.2	66.4	51.3	28.6	14.6
$^{14}CO_2$	NA	0.6	4.7	11.3	10.3	34.2	40.2
Total recovery (mean)	100.0	91.5	90.7	91.5	84.4	92.9	89.8
LAD-SCL-PF soil extracts, % AR based on [benzyl-U-^{14}C]-cinmethylin label. Actual application rate (field equivalent): 842.3 g a.s./ha							
Days:	D0	D3	D7	D14	D25	D40	D60
Cinmethylin in soil (mean)	99.8	92.0	86.9	76.7	68.0	53.3	41.1
Organic volatiles (mean)	NA	NA	NA	NA	NA	NA	NA
Sum minor unknowns[a] (mean)	NA	1.1	3.5	3.9	5.7	7.3	8.0
Total extractables[b]	99.8	93.0	90.4	80.6	73.8	60.6	49.1
$^{14}CO_2$	NA	2.7	3.9	4.9	6.0	13.1	20.6
Total recovery (mean)	100.1	98.6	98.4	93.2	92.2	93.5	94.1

D = Day, noting the duration of submitted ecotoxicity studies ranged from 14 to 56 days. Therefore degradation beyond day 60 is not included in the table above.
NA = Not applicable due to no replicates having residues > LOQ, when analysed two samples were taken to generate mean values.
NS = Not sampled (0 Days after Treatment only).
[a] – Sum of minor unknown components, none of which individually accounts for >3.4% AR.
[b] – Total extractables = sum of cinmethylin and sum of minor unknowns. These values are then used to calculate total recovery.
<LOQ residues were detected but these were below the LOQ of 0.05 % AR.

It was noted that during evaluation by the HSE fate evaluator that mass balances were relatively low at certain time points, predominantly later in the studies with the following recovery ranges; 80.9 – 100% (Lufa 2.2), 81.0 – 100% (Lufa 5M), 87.5 – 100.1% (LAD-SCL-PF), and 80.6 – 100.1% (MSL-PF). When considering total recovery ranges for the time points relevant to the ecotoxicity studies detailed in table B.9.8.1-4, they were; 87.6 – 100% (Lufa 2.2), 84.4 – 100% (Lufa 5M), 92.2 – 100.1% (LAD-SCL-PF), and 85.9 – 100.1% (MSL-PF). This means the worst case loss of cinmethylin that may have been due to volatilization was 15.6 %. However, it is clear that for the majority of sampling occasions, recoveries were ≥ 90 %, the exceptions are shaded in table B.9.8.1-4. Therefore, whilst some loss from volatilization may have occurred due to loss from the test system it is not deemed to be significant by the HSE ecotoxicology evaluator. Furthermore, all measurements of organic volatiles were below the Limit of quantification (0.05 % AR) suggesting loss from volatilisation was minimal and recoveries within the soil were relatively high at study initiation (99.3 – 100 % AR) and 71.6 – 100 % AR over the first 7 days following application for all soil types.

Ecotoxicology conclusion:

Based on the study Stewart & Abernathy, 2016a there is evidence to support that when cinmethylin is ixed into soil the loss from volatilisation appears to be relatively minor (maximum 15.6 % loss) compared to that observed in Hassink 2017b study where spray application was used (73 % loss). The application rate used in Stewart & Abernathy, 2016a is also protective of the proposed GAP (minimum of 739.1 g a.s./ha compared to proposed rate of 500 g a.s./ha). Given the method of application used (mixing into soil), the HSE evaluator considers the volatilisation data from Stewart & Abernathy, 2016a is more representative of the ecotoxicity studies conducted. Therefore, this study suggests that the exposure in the toxicity studies would be satisfactory and the loss from volatilisation relatively minor.
However, there is some uncertainty given it was not possible to fully compare soil types used in studies (see table B.9.8.1-5), drop wise application used in degradation study (Stewart & Abernathy, 2016a) and technical details such as headspace were not reported (both in fate and ecotoxicity studies). These points limit the ability to compare studies. Furthermore, the anaerobic soil study (Staudenmaier & Pape, 2017- evaluation detailed in section B.8.1.1.1.2, fate dossier CA section 8) did not use volatile traps and only measured CO_2 hence loss from volatilisation in anaerobic soils is not known based on the available data.

Nonetheless, when considering the quantitative ecotoxicology risk assessment (table B.9.8.1-3) there was a margin of safety for all soil macro-organisms (minimum of 12.6). The worst case endpoint based on the available data was the active study testing earthworms with a corrected endpoint of 41.8 mg a.s./kg dry soil. Using this endpoint if there was a 91.8 % loss of cinmethylin during the toxicity study an acceptable risk would still be demonstrated i.e. an endpoint of 3.4 mg a.s./kg dry soil with a PEC of 0.667 mg a.s/kg dry soil results in a TER of 5.1. A loss from volatilisation of 91.8 % is likely to be unrealistic when considering the study Stewart & Abernathy, 2016a, where volatiles were measured and the maximum loss was 15.6 %. In addition, the fate exposure PEC value is a worst case maximum and does not allow for volatilisation. Therefore, it could be argued that comparing an intial PEC_{soil} with an initial ecotoxicity endpoint is justified. This is because initial equivalent values would be compared, noting this relies on similar rates of loss following peak exposure. Finally, whilst there are uncertainties when comparing to ecotoxicity studies, the supporting information from the aerobic fate soil degradation study (Stewart & Abernathy, 2016a) suggests that when cinmethylin is mixed into soil loss from volatilisation is low.

Overall, based on the available information the HSE ecotoxicology evaluator considers an acceptable risk to earthworms and other soil macro-organisms can be concluded for the proposed uses.

Table B.9.8.1-5 Physio-chemical properties of test soils used in fate and ecotoxicology studies.

Soil designation	**LUFA 2.2 (fate study: Stewart & Abernethy, 2016)**	**LUFA 5M (fate study: Stewart & Abernethy, 2016)**	**LAD-SCL-PF (fate study: Stewart & Abernethy, 2016)**	**North Dakota soil (MSL-PF) Fate study: Stewart & Abernethy, 2016**	**Soil used in chronic cinmethylin Earthworm study 56 days (Friedrich, 2016)**	**Soil used in chronic product Earthworm study 56 days (Friedrich, 2018)**	**Soil used in chronic *Folsomia candida* product study 28 days (Friedrich, 2017)**	**Soil used in chronic *Hypoaspis aculeifer* product study 14 days (Schulz, 2017a)**	**Nitrogen transformation rate cinmethylin study 28 days (Schulz, 2016)**	**Nitrogen transformation rate cinmethylin study 28 days (Schulz, 2017b)**
DIN 4220 particle size distribution (%)										
Sand 0.050 – 2 mm	80	54	32	62	50 industrial quartz sand (> 50 % of particles between 0.05 and 0.2 mm)	50 industrial quartz sand (> 50 % of particles between 0.05 and 0.2 mm)	74.7 industrial quartz sand (> 50 % of particles between 0.05 and 0.2 mm)	74.8 industrial quartz sand (> 50 % of particles between 0.05 and 0.2 mm)	52.0 (5.9 % 0.63 – 2.0 mm, 36.9 % 0.2 – 0.63 mm, 9.2 % 0.063 – 0.2 mm)	53.5 (6.0 % 0.63 – 2.0 mm, 37.1 % 0.2 – 0.63 mm, 10.3 % 0.063 – 0.2 mm)
Silt 0.002 – 0.063 mm	11	31	28	22	20 % kaolin clay (approx. 0.023 – 0.035 mm)	20 % kaolin clay (approx. 0.023 – 0.035 mm)	20 % kaolin clay (approx. 0.023 – 0.035 mm)	20 % kaolin clay (approx. 0.023 – 0.035 mm)	37.2	35.7
Clay < 0.002 mm	9.0	15	40	16	n.r	n.r	n.r	n.r	10.8	10.9
DIN textual class:	Weak loamy sand	Sandy loam	Sandy clay loam	Loamy sand	n.r	n.r	n.r	n.r	Loamy sand (DIN 4220)	Loamy sand (DIN 4220)
Total Organic Carbon (%)	1.5	1.1	0.88	2.1	n.r	n.r	n.r	n.r	1.4	1.49

n.r = not reported.

B.9.9. EFFECTS ON SOIL NITROGEN TRANSFORMATION

Report: CP 10.5/1
Schulz L., 2017 b
Effects of BAS 684 03 H on the activity of soil microflora - Nitrogen transformation test
2017/1190793
Guidelines: OECD 216 (2000)
GLP: Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 684 03 H; batch no. FD-170210-0001; content of a.s.: Cinmethylin (Reg. No. 900 202): 750.0 g/L nominal (737.3 g/L analysed); density: 1.001 g/cm^3.

B. STUDY DESIGN

Test species: Biologically active agricultural soil: loamy sand (DIN 4220) / sandy loam (USDA): pH 6.2, 1.49 % C_{org}, 37.28 % water holding capacity (WHC).

Test design: Determination of the N-transformation (NO_3-nitrogen production) in soil enriched with lucerne meal (concentration in soil 0.5 %). Comparison of test item treated soil with a non-treated soil. NH_4-nitrogen formed from organically bound nitrogen in the soil and NO_3-nitrogen from the nitrification process was determined by using an Autoanalyzer (Bran and Luebbe). Sampling scheme: 0, 7, 14 and 28 days after treatment, sub-samples (3 replicates) 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 NO_3-nitrogen production 0, 7, 14 and 28 days after application.

Test concentrations: Control, 6.67 and 13.3 mg product/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, 13.60 and 27.20 mg reference item/kg dry soil.

Test conditions: pH 6.2; water content: approx. 45 % of maximum water holding capacity, measured water content: 15.65 –16.19 g/100 g dry soil. Soil samples were incubated at 19.1 – 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 3.3 % variation.

During the study the above criteria were met.

Biological results:

The nitrate concentrations during the study are shown in the table below.

Table B.9.9-1: Effects BAS 684 03 H on soil micro-organisms (nitrogen concentration) on days 0, 7, 14 and 28 of incubation

Time (days)	NO_3-N [mg/kg dry soil][1)]		
	Control	6.67 mg product/kg dry soil	13.3 mg product/kg dry soil
Loamy sand soil (7 d)	28.10	30.60	30.33
Loamy sand soil (14 d)	36.20	38.47	42.73
Loamy sand soil (28 d)	55.97	56.43	56.17

1) measured values sampling day 0, mean of 3 replicates
- = 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 6.67 mg product/kg dry soil based on nitrate formation rate. However, there was greater than 25 % variation at the higher test concentration of 13.3 mg product/kg dry soil.

Table B.9.9-2: Effects BAS 684 03 H on soil micro-organisms (nitrogen transformation rate)

Time (days)	Control	6.67 mg product/kg dry soil		13.3 mg product/kg dry soil	
	NO_3-N formation rate [mg/kg dry soil/day][1)]	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	4.01	4.37	+8.90	4.33	+7.95
7 - 14 d	1.16	1.12	-2.88	1.77	+53.09
14 - 28 d	1.48	1.28	-13.48	0.96	-35.31

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 +38.6 %, +51.8 % at 13.60 and 27.20 mg reference item/kg dry soil, respectively, 28 days after application.

III. CONCLUSION

Exposure of BAS 684 03 H in a field soil up to a test concentration of 6.67 mg product/kg dry soil caused 'acceptable' adverse effects (deviation from control < 25 %, OECD 216) on the soil nitrogen transformation rate at the end of the 28-day incubation period (time interval 14 – 28).

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:

- '**BAS 684 03H**' 14 – 28 day = **6.67 mg product/kg (equivalent to 4.92 mg a.s./kg)**

Carbon transformation:

Report:	CP 10.7/2 Schulz L., 2017 c Effects of BAS 684 03 H on the activity of soil microflora (Carbon transformation test) 2017/1064914
Guidelines:	OECD 217 (2000), 2004/10/EC of 11 February 2004
GLP:	Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 684 03 H; batch no. FD-170210-0001; content of a.s.: Cinmethylin (Reg. No. 900 202): 750.0 g/L nominal (737.3 g/L analysed); density: 1.001 g/cm^3.

B. STUDY DESIGN

Test soil: Biologically active agricultural soil: loamy sand (DIN 4220) / sandy loam (USDA), pH 6.6, 1.48% C_{org}, WHC: 38.00 g/100 g dry soil.

Test design: Determination of carbon-transformation in soil after addition of glucose (concentration in soil 0.4 %). 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, 1.33 mg and 6.67 mg product/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, 13.60 and 27.00 mg reference item/kg.

Test conditions: Soil moisture: 45 % of its maximum water holding capacity: measured water content: 16.15 - 17.05 g/100 g dry soil; pH 6.6 - 6.7. Soil samples were incubated at 19.4 - 21.4 °C while stored in steel vessels in the dark.

Statistics: Descriptive statistics.

II. RESULTS AND DISCUSSION

No adverse effects of BAS 684 03 H on carbon transformation in soil could be observed at both test concentrations (1.33 mg product/kg dry soil and 6.67 mg/kg dry soil) after 28 days.

Only negligible deviations from the control of -2.3 % (test concentration 1.33 mg product/kg dry soil) and -5.0 % (test concentration 6.67 mg product/kg dry soil) were measured at the end of the 28-day incubation period. The results are summarised in Table B.9.9-3.

Table B.9.9-3: Effects of BAS 684 03 H on soil micro-organisms (carbon transformation) on days 0, 7, 14 and 28 of incubation

Soil (days)	Control	1.33 mg product/kg dry soil		6.67 mg product/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)	20.66	21.28	+3.0	20.81	+0.8
Loamy sand soil (7 d)	19.61	19.93	+1.6	19.14	-2.4
Loamy sand soil (14 d)	17.82	18.06	+1.3	17.42	-2.3
Loamy sand soil (28 d)	15.72	15.36	-2.3	14.94	-5.0

[1)] Based on O_2-consumption; - = inhibition; + = stimulation

In a separate study, the reference item Dinoterb caused an inhibition of carbon transformation of -34.8 % and -43.8 % at 13.60 mg and 27.00 mg Dinoterb/kg dry soil, respectively, determined 28 days after application.

III. CONCLUSION

Exposure of BAS 684 03 H in a field soil up to a test concentration of 6.67 mg product/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 284/2013. In addition the endpoint generated is identical to the nitrogen transformation study (Schulz 2017b) i.e. < 25 % effects at 6.67 mg product/kg.

B.9.10. RISK ASSESSMENT FOR SOIL NITROGEN TRANSFORMATION

Studies on nitrogen transformation in soil are available for the active substance cinmethylin and the representative formulation BAS 684 03 H. A summary of the available data and endpoints used in the risk assessment is provided in Table B.9.10-1.

Table B.9.10-1 Summary of data on effects on nitrogen transformation

Test substance	Test species	Endpoint		Reference
Cinmethylin	Nitrogen transformation 14-28 d	<25% effect	**7.17 mg a.s./kg dws**	Schulz (2016a)
BAS 684 03 H	Nitrogen transformation 14-28 d	<25% effect	**4.92 mg a.s./kg dws[#]**	Schulz (2017b)

dws = dry weight soil; a.s. = active substance;
# Endpoint expressed as active cinmethylin, considering the nominal content of cinmethylin and a density of BAS 684 03 H of 1.001 g/cm^3.
Bold values: endpoints used for risk assessment

An assessment of the risk to soil micro-organisms has been conducted according to SANCO/10329/2002 guidance. In volume 3 CA dossier, section 8 the initial maximum PECsoil values for the active substance have been determined for the representative uses. The critical use has been evaluated in the risk assessment. This assessment will therefore cover all the representative uses. In addition, there are no relevant soil metabolites to consider. Maximum PEC_{soil} values have been compared to concentrations at which <25 % effects on nitrogen transformation were observed in Table B.9.10-2.

Table B.9.10-2 Risk to soil micro-organisms from 'worst case' GAP (single application at 500 g a.s./ha to winter cereals).

Test substance	Test design	<25 % effects concentration (mg a.s./kg dws)	PECsoil (mg a.s./kg dws)	Acceptable risk?
Cinmethylin	Nitrogen transformation 28 d	7.17	0.667	Yes
BAS 684 03 H		4.92	0.667	Yes

The comparsion of the initial maximum PECsoil for the worst-case use with the nitrogen transformation study results indicates less than 25 % effects would be expected to occur for all representative uses from the active substance and formulated product. An acceptable risk to soil micro-organisms is therefore concluded.

Whilst the above assessment demonstrates acceptable risk there is uncertainty regarding the extent of exposure in these studies and hence the endpoints have the potential to underestimate the toxicity. This has been considered further below.

Consideration of potential volatilisation:

The nitrogen transformation rate studies provided Schulz (2016a and 2017b)) were conducted according to OECD 216 which states the following steps should be taken when assessing 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.

As detailed in the chemistry dossier (volume 3, CA section 2) the vapour pressure of cinmethylin is 8.1×10^{-3} Pa at 20 °C suggesting there is potential for volatilisation from soil and that the above points should have been considered in the submitted ecotoxicity studies. This means there is some uncertainty as to whether exposure was adequate due to volatilisation. In addition, the study Hassink 2017b (section B.8.3.2, volume 3, CA dossier) demonstrated relatively high volatilisation from soil for cinmethylin. The methodology and results from this study have been briefly summarised below.

Methodology (Hassink, 2017b):

This study investigated the volatilisation behaviour of cinmethylin for a time period of 24 hours after application of the emulsifiable concentrate formulation BAS 684 02 H applied on soil surfaces in a circulation chamber using a blank formulation spiked with ^{14}C-cinmethylin. The soil moisture was adjusted to 60 % MWHC and 100 g of the moistened soil was weighed into each Petri dish. The soil was treated via a FullCone TG 0.5 nozzle (Spraying Systems Co.) in a closed application chamber made of glass. During the application the border of the Petri dish was covered with a Teflon sheet with a circular opening to avoid contamination of the glass. After application, the soil was removed from the application chamber and transferred directly to the circulation chamber. The temperature during the volatilisation experiment ranged 20.1 – 20.2 °C. Evaporation of water from the soil surfaces led to an average relative humidity of 45.9 %. Diurnal cycles were again simulated (8 h light, 14 h dark, 2 h light). Moisture losses were compensated throughout the experiment by using a wick immersed in a water

reservoir. The water content of the soil on the petri dish remained constant during the experiment. Samples were taken at 1, 3, 6, and 24 h after application. At each sampling time the condensate of the cryotrap was removed, the ethylene-glycol traps were replaced, and new charcoal and PU-traps were connected. At the end of each experiment, both the circulation chamber and the tubes were rinsed twice and the rinsate was analysed. The remaining radioactivity in soil and plant was determined. The HSE fate evaluator noted that the study was conducted using single replicates and concluded that, while the study was considered valid, the reproducibility, accuracy and precision of this study are not known hence the study may not be robust.

Results (Hassink, 2017b):

Whilst there is uncertainty, the study (Hassink, 2017b) suggested that the volatilisation rate from soil surfaces was 73 % during the experimental period (up to 24 hours).

Based on the evidence of potential volatilisation, further information was requested from the applicant by HSE.

The following response was received (shown in italics):

> *Regarding the exposure route within the soil studies there might be some concerns due to the potential of cinmethylin to volatilize from soil surfaces (according to its phys-chem properties). But it should be noted that the test substance was homogenously mixed into the soil in all studies on soil organisms (according to guideline).*
>
> *In this case volatilization is not a concern as shown in the aerobic degradation study with radiolabeled cinmethylin (BASF DocID 2015/1186904 + amendment 2019/1078806). The study is presented and discussed in detail in the environmental fate section, chapter CA 7.1.*
>
> *Cinmethylin was homogenously mixed into the soil, incubated and volatiles were measured by using volatile traps. The results indicate that cinmethylin is not volatile, if mixed into the soil. Within the volatility assessment no other radiolabeled degradation product than CO_2 was detected. Hence, even if volatilization occurs after mixing cinmethylin into soil, the active substance itself is not volatile but rather is CO_2 as the final degradation product of the active substance.*
>
> *Since the substance was mixed into the soil in all studies on soil macro- and microorganisms, the study results (DocID 2015/1186904 + amendment 2019/1078806) suggest that the substance is non-volatile under such conditions. Therefore, the exposure can be considered as representative.*

HSE evaluator comments:

The response was considered in consultation with the HSE fate evaluator. The study referenced by applicant i.e. DocID 2015/1186904 + amendment 2019/1078806 is Stewart & Abernathy, 2016a. This study has been evaluated by the HSE fate evaluator and a summary is presented in section B.8.1.1.1.1, fate dossier CA section 8. This study investigated degradation in aerobic soil and used volatile traps to monitor decline of radio-labelled cinmethylin. The method and results are briefly summarised below (for full details refer to section B.8.1.1.1.1, fate dossier CA section 8).

Methodology (Stewart & Abernathy, 2016a):

Each soil sample consisted 100 g soil (dry weight equivalent, 2 mm sieved) and samples were treated with [cyclohexane-4-^{14}C]-cinmethylin or [benzyl-U-^{14}C]-cinmethylin to achieve a nominal concentration of 2.0 µg a.s./g soil (field application rate equivalent 750 g a.s./ha, based on distribution in the top 2.5 cm soil layer and a soil density of 1.5 g/cm^3). Each soil sample was treated drop-wise and test flasks were tumbled by hand to incorporate the test solution. Volatiles were collected through a series of four traps: a safety trap (a flask containing no liquid), ethylene glycol trap, 2M NaOH trap, and 1M H_2SO_4 trap.

Results (Stewart & Abernathy, 2016a):

The full results are displayed in tables 8.1.1.1/1-03 to 8.1.1.1/1-08 (section B.8.1.1.1.1, fate dossier CA section 8) for different soil types and radio-labels. A summary of these results focused on cinmethylin and volatile concentrations is shown in the table below over time periods relevant to ecotoxicology studies.

Table B.9.10-3: Aerobic soil degradation following exposure to cinmethylin for four soil types.

Measurement	**Cinmethylin concentration (% Applied radioactivity (AR))**					
Lufa 2.2 soil extracts, % AR based on [cyclohexane-4-^{14}C]-cinmethylin label Actual application rate (field equivalent): 739.1 g a.s./ha						
Days:	**D0**	**D3**	**D7**	**D14**	**D24**	**D41**
Cinmethylin in soil (mean)	99.8	92.9	82.7	72.0	67.1	59.9
Organic volatiles (mean)	NA	NA	NA	NA	NA	NA
Sum minor unknowns[a] (mean)	NA	NA	2.3	3.0	3.8	4.1
Total extractables[b]	99.8	92.9	85.0	75.0	70.8	64.1
$^{14}CO_2$	NA	3.3	6.8	14.9	15.6	21.0
Total recovery (mean)	100.0	100.0	96.8	97.4	95.5	95.1
Lufa 2.2 soil extracts, % AR based on [benzyl-U-^{14}C]-cinmethylin label. Actual application rate (field equivalent): 777.4 g a.s./ha						
Days:	**D0**	**D3**	**D7**	**D14**	**D24**	**D41**
Cinmethylin in soil (mean)	99.8	91.0	84.4	77.4	68.9	63.8
Organic volatiles (mean)	NA	NA	NA	NA	NA	NA
Sum minor unknowns[a] (mean)	NA	NA	< LOQ	< LOQ	< LOQ	< LOQ
Total extractables[b]	99.8	91.0	84.4	77.4	68.9	63.8
$^{14}CO_2$	NA	NA	1.9	3.2	10.9	18.4
Total recovery (mean)	100.0	94.0	92.0	89.2	91.9	94.9
MSL-PF soil extracts, % AR based on [cyclohexane-4-^{14}C]-cinmethylin label Actual application rate (field equivalent): 784.9 g a.s./ha						
Days:	**D0**	**D3**	**D7**	**D14**	**D24**	**D41**
Cinmethylin in soil (mean)	99.8	78.3	71.9	57.1	44.2	32.2
Organic volatiles (mean)	NA	NA	NA	NA	NA	NA
Sum minor unknowns[a] (mean)	NA	2.8	1.5	3.5	4.9	7.0
Total extractables[b]	99.8	81.1	73.4	60.6	49.0	39.0
$^{14}CO_2$	NA	3.7	7.8	16.0	24.3	33.4
Total recovery (mean)	99.9	92.7	92.1	91.4	90.6	92.4
MSL-PF soil extracts, % AR based on [benzyl-U-^{14}C]-cinmethylin label. Actual application rate (field equivalent): 810.8 g a.s./ha						

Days:	**D0**	**D3**	**D7**	**D14**	**D24**	**D41**
Cinmethylin in soil (mean)	100.0	81.0	71.6	53.8	43.7	36.0
Organic volatiles (mean)	NA	NA	NA	NA	NA	NA
Sum minor unknowns[a] (mean)	NA	3.2	3.4	2.7	4.5	7.9
Total extractables[b]	100.0	84.2	75.0	56.4	48.2	43.9
$^{14}CO_2$	NA	1.6	4.4	12.7	17.4	19.6
Total recovery (mean)	100.1	93.0	93.2	95.7	92.2	90.6
Lufa 5M soil extracts, % AR based on [benzyl-U-^{14}C]-cinmethylin label. Actual application rate (field equivalent): 838.1 g a.s./ha						
Days:	D0	D3	D7	D14	D25	D40
Cinmethylin in soil (mean)	99.3	82.5	73.6	62.2	44.2	20.7
Organic volatiles (mean)	0.1	NA	NA	NA	NA	NA
Sum minor unknowns[a] (mean)	NA	1.5	3.6	4.2	7.1	7.8
Total extractables[b]	99.7	83.9	77.2	66.4	51.3	28.6
$^{14}CO_2$	NA	0.6	4.7	11.3	10.3	34.2
Total recovery (mean)	100.0	91.5	90.7	91.5	84.4	92.9
LAD-SCL-PF soil extracts, % AR based on [benzyl-U-^{14}C]-cinmethylin label. Actual application rate (field equivalent): 842.3 g a.s./ha						
Days:	D0	D3	D7	D14	D25	D40
Cinmethylin in soil (mean)	99.8	92.0	86.9	76.7	68.0	53.3
Organic volatiles (mean)	NA	NA	NA	NA	NA	NA
Sum minor unknowns[a] (mean)	NA	1.1	3.5	3.9	5.7	7.3
Total extractables[b]	99.8	93.0	90.4	80.6	73.8	60.6
$^{14}CO_2$	NA	2.7	3.9	4.9	6.0	13.1
Total recovery (mean)	100.1	98.6	98.4	93.2	92.2	93.5

D = Day, noting the duration of submitted ecotoxicity studies was 28 days. Therefore degradation beyond day 41 is not included in table above.
NA = Not applicable due to no replicates having residues > LOQ, when analysed two samples were taken to generate mean values.
NS = Not sampled (0 Days after Treatment only).
[a] – Sum of minor unknown components, none of which individually accounts for >3.4% AR.
[b] – Total extractables = sum of cinmethylin and sum of minor unknowns. These values are then used to calculate total recovery.
<LOQ residues were detected but these were below the LOQ of 0.05 % AR.

It was noted that during evaluation by the HSE fate evaluator that mass balances were relatively low at certain time points, predominantly later in the studies with the following recovery ranges; 80.9 – 100% (Lufa 2.2), 81.0 – 100% (Lufa 5M), 87.5 – 100.1% (LAD-SCL-PF), and 80.6 – 100.1% (MSL-PF). When considering total recovery ranges for the time points relevant to the ecotoxicity studies detailed in table B.9.10-3, they were; 89.2 – 100% (Lufa 2.2), 84.4 – 100% (Lufa 5M), 92.2 – 100.1% (LAD-SCL-PF), and 90.6 – 100.1% (MSL-PF). This means the worst case loss of cinmethylin that may have been due to volatilization was 15.6 %. However, it is clear that for the majority of sampling occasions, recoveries were ≥ 90 %, the exceptions are shaded in table B.9.10-3. Therefore, whilst some loss from volatilization may have occurred due to loss from the test system it is not deemed to be significant by the HSE ecotoxicology evaluator. Furthermore, all measurements of organic volatiles were below the Limit of quantification (0.05 % AR) suggesting loss from volatilisation was minimal and recoveries within the soil were relatively high at study initiation (99.3 – 100 % AR) and 71.6 – 100 % AR over the first 7 days following application for all soil types.

Ecotoxicology conclusion:

Based on the study Stewart & Abernathy, 2016a there is evidence to support that when cinmethylin is mixed into soil the loss from volatilisation appears to be relatively minor (maximum 15.6 % loss) compared to that observed in Hassink 2017b study where spray application was used (73 % loss). The application rate used in Stewart & Abernathy, 2016a is also protective of the proposed GAP (minimum of 739.1 g a.s./ha compared to proposed rate of 500 g a.s./ha). Given the method of application used (mixing into soil), the HSE evaluator considers the volatilisation data from Stewart & Abernathy, 2016a is more representative of the ecotoxicity studies conducted. Therefore, this study suggests that the exposure in the ecotoxicity studies would be satisfactory and the loss from volatilisation relatively minor. However, there is some uncertainty given it was not possible to fully compare soil types used in studies (see table B.9.8.1-5 in soil macro-organisms section), drop wise application used in degradation study (Stewart & Abernathy, 2016a) and technical details such as headspace were not reported (both in fate and ecotoxicity studies). These points limit the ability to compare studies. Furthermore, the anaerobic soil study (Staudenmaier & Pape, 2017- evaluation detailed in section B.8.1.1.1.2, fate dossier CA section 8) did not use volatile traps and only measured CO_2 hence loss from volatilisation in anaerobic soils is not known based on the available data.

Nonetheless, when considering the quantitative ecotoxicology risk assessment (table B.9.10-2) there was a margin of safety for all soil micro-organisms (minimum of 7.4). The worst case endpoint based on the available data was the active study testing soil micro-organisms with an endpoint of 4.92 mg a.s./kg dry soil. Using this endpoint if there was a 86.4 % loss of cinmethylin during the ecotoxicity study an acceptable risk would still be demonstrated i.e. an endpoint of 0.667 mg a.s./kg dry soil compared with a PEC of 0.667 mg a.s/kg dry soil. A loss from volatilisation of 86.4 % is likely to be unrealistic when considering the study Stewart & Abernathy, 2016a, where volatiles were measured and the maximum loss was 15.6 %. In addition, the fate exposure PEC value is a worst case maximum and does not allow for volatilisation. Therefore, it could be argued that comparing an intial PEC_{soil} with an initial ecotoxicity endpoint is justified. This is because initial equivalent values would be compared, noting this relies on similar rates of loss following peak exposure. Finally, whilst there are uncertainties when comparing to ecotoxicity studies, the supporting information from the aerobic fate soil degradation study (Stewart & Abernathy, 2016a) suggests that when cinmethylin is mixed into soil loss from volatilisation is low.

Overall, based on the available information the HSE ecotoxicology evaluator considers an acceptable risk to soil micro-organisms can be concluded for the proposed uses.

B.9.11. EFFECTS ON TERRESTRIAL NON-TARGET HIGHER PLANTS

B.9.11.1. Summary of screening data

No studies submitted.

B.9.11.2. Testing on non-target plants

Report: CP 10.6.2/1
Friedemann A.,Stroemel C., 2017 a
Effect of BAS 684 03 H on vegetative vigour of ten species of terrestrial plants under greenhouse conditions
2017/1134475
Guidelines: OECD 227 July 2006, EPA 850.4150 - Vegetative Vigour (2012)
GLP: Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 684 03 H; batch no. FD-170210-0001; content of a.s.: Cinmethylin (Reg. No. 900 202): 737.3 g/L analysed (750.0 g/L nominal); density: 1.001 g/cm^3.

B. STUDY DESIGN

Test species: Oilseed rape *(Brassica napus)*, sugar beet (*Beta vulgaris*), cucumber (*Cucumis sativus*), soybean (*Glycine max*), onion (*Allium cepa*), barley (*Hordeum vulgare*), ryegrass (*Lolium multiflorum*), great millet (*Sorghum bicolor*), wheat (*Triticum aestivum*) and corn (*Zea mays*).

Test design: Greenhouse trial, dose-response design; 6 treatments (5 test item rates, untreated control); replicates and number of plants is shown below. post-emergence application at BBCH 12-14 using a laboratory spray cabin at a water volume of 299 or 242 L/ha; assessments for plant survival and phytotoxicity were done 7, 14 and 21 days after treatment (DAT); plant dry weight and plant length was determined at 21 DAT.

Species	Number of plants/pot	Number of pots/per replicate	Number of replicates per Treatment (total plants)
Oilseed rape *(Brassica napus)*	3	2	5 (30)
Sugar beet (*Beta vulgaris*)	3	2	5 (30)
Cucumber (*Cucumis sativus*)	2	3	5 (30)
Soybean (*Glycine max*)	2	3	5 (30)
Onion (*Allium cepa*)	6	1	5 (30)
Barley (*Hordeum vulgare*)	6	1	5 (30)
Ryegrass (*Lolium multiflorum*)	6	1	5 (30)
Great millet (*Sorghum bicolor*)	3	2	5 (30)
Wheat (*Triticum aestivum*)	6	1	5 (30)
Corn (*Zea mays*)	2	3	5 (30)

Visual assessments of phytotoxicity were made based on the scale below:

Rating (%)	Description	Detailed description
0	No effects	No damages, no crop reduction or injury
10	Slight effects	Slight phytotoxic effects or stunting
20		Slight phytotoxic effects, stunting or stunt loss
30		Crop injury more pronounced, but not lasting
40	Moderate effects	Moderate injury, crop usually recovers
50		Crop injury more lasting, recovery doubtful
60		Lasting crop injury, no recovery
70	Severe effects	Heavy crop injury and stand loss
80		Crop nearly destroyed- a few surviving plants
90		Only occasional live crop plants left
100	Complete effects	Complete crop destruction

Endpoints: NOER, ER_{25}, ER_{50}.

Test rates: Control (tap water), 87.5, 175, 350, 700 and 1400 mL product/ha.

Test conditions: Greenhouse conditions, daily average temperature: 19.8 – 25.7 °C; daily mean relative humidity: 52.1 – 84.6 %; photoperiod: ≥ 16 h light, additional light when outdoor illumination was less than 10 klux.

Statistics: Descriptive statistics. The Welch-t test was used to determine the NOER for non-homogenous variances and for homogenous variances, the Williams' t-test (α=0.05, one sided smaller, respectively). The ER_x values were calculated using linear regression analysis of the data. The Probit-, Logit- or Weibull-Analysis using Linear Maximum Likelihood Regression as curve fitting method were used.

II. RESULTS AND DISCUSSION

Validity criteria:

In OECD 227 (2006) the following criteria are stated:

- The seedling emergence is at least 70 %. Obtained: minimum of 88 %.

and in the controls:

- The plants do not exhibit visible phytotoxic effects (e.g. chlorosis, necrosis, wilting, leaf and stem deformations). Plants exhibit only normal variation in growth and morphology for that species. Observed: phytotoxicity was not noted in any of the control groups.
- The mean plant survival is at least 90 % for the duration of the study. Obtained: 100 %

During the study the above criteria were met.

Biological results: No control mortality > 10 % and no other adverse effects on control plants were observed.

After exposure to BAS 684 03 H symptoms of phytotoxicity were observed for all tested plant species, except onion. For sugar beet and cucumber there was ≤ 10 % phytotoxic symptoms at 1400 ml product/ha during the study.

No plant mortality was observed for all tested plant species at BBCH stage 12-14 up to the highest tested rate of 1400 mL product/ha i.e. 100 % plant survival.

Plant length was comparable to control up to 1400 mL product/ha for oilseed rape, sugar beet and onion. For barley, ryegrass, wheat and corn a significant (Williams t-test or Welch-t test with Bonferroni adjustment, one-sided, $\alpha = 0.05$) reduced plant length was found after application of rates ≥ 700 mL product/ha with between 13 and 27 % reduction at the highest tested rate.

No significant reduction of biomass (dry weight) was observed for sugar beet and onion following the application of BAS 684 03 H up to the highest tested rate of 1400 mL product/ha.

The most sensitive plant species were found to be the monocotyledonous plant species barley, ryegrass, great millet, wheat and corn with dry biomass reduction between 31 and 68 %.

The results are summarised in Table B.9.11.2-1 and Table B.9.11.2-2.

Table B.9.11.2-1: Effect of BAS 684 03 H on phytotoxicity, plant survival, plant length and plant dry weight 21 DAT

Treatment [mL/ha]	Oilseed rape	Sugar beet	Cucumber	Soybean	Onion	Barley	Rye grass	Great millet	Wheat	Corn
Phytotoxic damages [%]										
Control	0	0	0	0	0	0	0	0	0	0
87.5	C: 0 N: 0 D: 0 S: 0	C: 0 D: 0 S: 0	C: 1 (± 0) N: 0 D: 1 (± 0) S: 0	C: 0 N: 0 D: 0 S: 0	C: 0 N: 0 D: 0 S: 0	C: 1 (± 0) N: 0 D: 6 (± 5) S: 6 (± 4)	C: 0 N: 0 D: 0 S: 0	C: 0 N: 0 D: 0 S: 0	C: 0 N: 0 D: 0 S: 0	C: 0 N: 0 D: 0 S: 0
175.0	C: 1 (± 1) N: 0 D: 1 (± 1) S: 0	C: 0 D: 0 S: 0	C: 1 (± 0) N: 0 D: 2 (± 0) S: 0	C: 0 N: 0 D: 0 S: 0	C: 0 N: 0 D: 0 S: 0	C: 20 (± 0) N: 0 D: 32 (± 4) S: 36 (± 5)	C: 5 (± 3) N: 1 (± 0) D: 9 (± 2) S: 6 (± 5)	C: 0 N: 0 D: 0 S:0	C: 1 (± 1) N: 0 D: 0 S: 10 (± 0)	C: 0 N: 0 D: 0 S: 0
350.0	C: 2 (± 2) N: 0 D: 2 (± 2) S: 6 (± 5)	C: 1 (± 0) D: 0 S: 0	C: 2 (± 0) N: 0 D: 3 (± 2) S: 0	C: 3 (± 2) N: 0 D: 0 S: 8 (± 4)	C: 0 N: 0 D: 0 S: 0	C: 30 (± 0) N: 1 (± 2) D: 30 (± 0) S: 46 (± 5)	C: 16 (± 5) N: 2 (± 2) D: 26 (± 9) S: 34 (± 9)	C: 4 (± 1) N: 0 D: 6 (± 2) S: 6 (± 4)	C: 11 (± 5) N: 5 (± 3) D: 5 (± 0) S: 18 (± 8)	C: 2 (± 3) N: 0 D: 2 (± 4) S: 10 (± 0)
700.0	C: 8 (± 3) N: 0 D: 8 (± 3) S: 10 (± 0)	C: 5 (± 2) D: 0 S: 0	C: 2 (± 0) N: 0 D: 2 (± 2) S: 5 (± 3)	C: 12 (± 4) N: 0 D: 10 (± 0) S: 10 (± 7)	C: 0 N: 0 D: 0 S: 0	C: 30 (± 0) N: 4 (± 1) D: 38 (± 4) S: 48 (± 11)	C: 50 (± 0) N: 6 (± 4) D: 80 (± 0) S: 68 (± 8)	C: 16 (± 5) N: 0 D: 12 (± 4) S: 11 (± 4)	C: 48 (± 4) N: 4 (± 1) D: 46 (± 9) S: 54 (± 19)	C: 14 (± 11) N: 0 (± 1) D: 20 (± 16) S: 18 (± 8)
1400.0.	C: 26 (± 9) N: 0	C: 8 (± 2) D: 0	C: 9 (± 1) N: 0	C: 46 (± 9) N: 0	C: 0 N: 0 D: 0	C: 34 (± 5) N: 5	C: 71 (± 12) N: 12	C: 34 (± 9) N: 4	C: 68 (± 11) N: 12	C: 52 (± 26) N: 6

Treatment [mL/ha]	Oilseed rape	Sugar beet	Cucumber	Soybean	Onion	Barley	Rye grass	Great millet	Wheat	Corn
	D: 42 (± 13) S: 28 (± 8)	S: 5 (± 5)	D: 10 (± 0) S: 10 (± 0)	D: 40 (± 10) S: 22 (± 11)	S: 0	(± 0) D: 40 (± 0) S: 58 (± 4)	(± 3) D: 100 (± 0) S: 80 (± 7)	(± 3) D: 38 (± 11) S: 30 (± 12)	(± 4) D: 82 (± 4) S: 74 (± 5)	(± 2) D: 50 (± 10) S: 40 (± 7)
Plant length [% to untreated control]										
Control	Not applicable									
87.5	96.9	100.8	103.1	102.4	103.0	102.5	104.2	100.6	102.5	97.0
175.0	100.4	100.5	100	98.1	103.0	100.5	102.1	99.4	102.9	98.5
350.0	99.0	99.3	97.5	97.9	102.0	97.1	97.0	97.7	97.6	100
700.0	96.9	94.9	99.5	97.9	103.8	87.8*	76.3**	96.2	85.4*	81.3*
1400.0	98.5	95.6	95.8*	89.3*	103.0	86.9*	72.5	81.3*	78.0*	73.2*
Plant dry weight [% to untreated control]										
Control	Not applicable									
87.5	89.8**	110.4	99.1	94.3	106.3	91.0	108.5	97.6	100.8	90.0
175.0	94.7**	110.4	98.7	98.7	99.2	82.5*	92.1	102.4	101.4	98.2
350.0	92.0**	106.9	92.9	94.6	96.5	66.8*	75.4*	92.5	83.3*	96.9
700.0	90.4**	107.0	100.7	86.7*	99.9	52.1*	43.4*	82.9*	62.5*	73.4*
1400.0	85.3**	103.6	93.7*	74.0*	94.7	46.6*	32.3*	63.7*	46.3*	68.7*

* Statistically significantly different compared to the control (Williams t-test, $\alpha = 0.05$).
** Statistically significantly different compared to the control (Welch-t test with Bonferroni Adjustment, $\alpha = 0.05$). Phytotoxicity symptoms: C: Chlorosis, S: Stunting, N: Necrosis, D: Leaf deformation

Table B.9.11.2-2: NOER, ER_{25}, and ER_{50} of BAS 684 03 H for non-target plants 21 DAT

Treatment [mL/ha]	Oilseed rape	Sugar beet	Cuc	Soybean	Onion	Barley	Rye grass	Great millet	Wheat	Corn
Phytotoxicity										
NOER	175 (129)	700 (516)	700 (516)	350 (258)	1400 (1032)	87.5 (64.5)	87.5 (64.5)	350 (258)	175 (129)	175 (129)
Plant survival										
NOER	1400 (1032)									
ER_{25}	> 1400 (1032)									
ER_{50}	> 1400 (1032)									
Plant length										
NOER	1400 (1032)	1400 (1032)	700 (516)	700 (516)	1400 (1032)	350 (258)	350* (258)	700 (516)	350 (258)	350 (258)
ER_{25}	> 1400 (1032)						1103.9 (813.9)	> 1400 (1032)		1369.2 (1009.5)
ER_{50}	> 1400 (1032)									
Plant biomass (dry weight)										
NOER	< 87.5 (64.5)	1400 (1032)	700 (516)	350 (258)	1400 (1032)	87.5 (64.5)	175 (129)	350 (258)	175 (129)	350 (258)
ER_{25}	> 1400 (1032)			1349.6 (995)	> 1400 (1032)	251.7 (185.6)	338.0 (249.2)	955.1 (704.2)	521.5 (384.5)	712.2 (525.1)

Treatment [mL/ha]	Oilseed rape	Sugar beet	Cuc	Soybean	Onion	Barley	Rye grass	Great millet	Wheat	Corn
ER_{50}	> 1400 (1032)					962.4 (709.6)	709.8 (523.3)	> 1400 (1032)	1156 (852.3)	> 1400 (1032)

* Stated as based on '*Expert judgement*' in study report, Cuc = cucumber (g .a.s/ha)

III. CONCLUSION

Post-emergence application of BAS 684 03 H under worst-case greenhouse conditions resulted in no treatment-related symptoms of plant survival for all tested plant species. The overall NOER based on phytotoxicity and biomass (dry weight) was 87.5 mL product/ha.

The lowest ER_{50} value based on plant dry weight was 709.8 mL product/ha for ryegrass.

HSE evaluator comments:

It was noted when considering phytotoxicity there were 'slight' effects (highest effects seen for deformation and stunting with a range of 0 – 10 % based on replicates) for any individual symptom at 87.5 ml product/ha.

When considering the analytical method for this study it is validated in accordance with SANCO/3029/99 rev. 4 with an LOQ of 3 g/L. Stability of the extracts was demonstrated in the overall method validation (KCA 4.2.1/37) however storage stability of the samples has not been provided. It has been noted that the samples are stored frozen at ≤ -18 °C from 31 July 2017 to the analysis date of 24/25 October 2017. As the analytical method was only used for verification of the content of BAS 684 H in aqueous application solutions which showed acceptable results (99 – 101 % nominal content), and acceptable frozen storage stability has also been proven for BAS 684 H in OECD test medium (KCA CA 8.2.6.1/2, 2016/1001944), it can be assumed the samples remained stable (see volume 3, CA, section B5 for full details).

The above study was conducted to GLP and is considered valid. The following endpoint has been derived:

- '**BAS 684 03H**' ER_{50} (based on vegetative vigour for most sensitive species: ryegrass) = **709.8 mL product/ha** equivalent to **523.3 g a.s./ha**
- '**BAS 684 03H**' phytotoxicity, 'slight effects' recorded- maximum of 10 % for any individual symptom based on replicates = **87.5 mL product/ha** equivalent to **64.5 g a.s./ha**

Report: CP 10.6.2/2
Friedemannn A.,Stroemel C., 2018 a
Effect of BAS 684 03 H on seedling emergence and seedling growth of ten species of terrestrial plants under greenhouse conditions
2017/1134474
Guidelines: OECD 208 (2006), EPA 850.4100 - Seedling Emergence and Seedling Growth (2012)
GLP: Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 684 03 H; batch no. FD-170210-0001; content of a.s.: cinmethylin (Reg. No. 900 202): 737.3 g/L analysed (750.0 g/L nominal); density: 1.001 g/cm^3.

B. STUDY DESIGN

Test species: Oilseed rape *(Brassica napus)*, sugar beet (*Beta vulgaris*), cucumber (*Cucumis sativus*), soybean (*Glycine max*), onion (*Allium cepa*), barley (*Hordeum vulgare*), ryegrass (*Lolium multiflorum*), great millet (*Sorghum bicolor*), wheat (*Triticum aestivum*) and corn (*Zea mays*).

Test design: Greenhouse trial, dose-response design; 10 treatments (9 test item rates, untreated

control); replicates and number of seeds is shown below. BAS 684 03 H was applied pre-emergence shortly after seeding using a laboratory spray cabin at a water volume of 281 and 291 L/ha; assessments for seedling emergence, plant survival and phytotoxicity were done 7, 14 and 21 days after treatment (DAT) (onion: 14, 21 and 28 DAT); plant dry weight and plant length was determined at 21 DAT (28 DAT for onion).

Species	**Number of seeds/pot**	**Number of pots/per replicate**	**Number of replicates per Treatment (total seeds)**
Oilseed rape *(Brassica napus)*	5	2	4 (40)
Sugar beet (*Beta vulgaris*)	5	2	4 (40)
Cucumber (*Cucumis sativus*)	4	3	4 (48)
Soybean (*Glycine max*)	5	2	4 (40)
Onion (*Allium cepa*)	10	1	4 (40)
Barley (*Hordeum vulgare*)	10	1	4 (40)
Ryegrass (*Lolium multiflorum*)	10	1	4 (40)
Great millet (*Sorghum bicolor*)	5	2	4 (40)
Wheat (*Triticum aestivum*)	10	1	4 (40)
Corn (*Zea mays*)	5	2	4 (40)

Visual assessments of phytotoxicity were made based on the scale below:

Rating (%)	**Description**	**Detailed description**
0	No effects	No damages, no crop reduction or injury
10	Slight effects	Slight phytotoxic effects or stunting
20		Slight phytotoxic effects, stunting or stunt loss
30		Crop injury more pronounced, but not lasting
40	Moderate effects	Moderate injury, crop usually recovers
50		Crop injury more lasting, recovery doubtful
60		Lasting crop injury, no recovery
70	Severe effects	Heavy crop injury and stand loss
80		Crop nearly destroyed- a few surviving plants
90		Only occasional live crop plants left
100	Complete effects	Complete crop destruction

Endpoints: NOER, ER_{25}, ER_{50}.

Test rates: Control (tap water); the test item was applied pre-emergence at a range of different concentrations: 21.9, 43.8, 87.5, 175.0, 350.0, 700.0, 1400, 2100 and 2800 mL product/ha depending on test species.

Test conditions: Greenhouse conditions, daily mean temperature ranged between 18.8 – 27.5 °C; daily mean humidity ranged between 50.7 – 82.9 %; photoperiod: ≥ 16 h light, additional light when outdoor illumination was less than 10 klux.

Statistics: Descriptive statistics; The Williams' t-test or Dunnett's t-test (α=0.05, one sided smaller) was used to determine the NOER for homogenous variances. The ER_x values were calculated using linear regression analysis of the data. The Probit-, Logit- or Weibull-Analysis using Linear Maximum Likelihood Regression as curve fitting method were used.

II. RESULTS AND DISCUSSION

Validity criteria:

In OECD 208 (2006) the following criteria are stated:

- The seedling emergence is at least 70 %. Obtained: 88 %
- The seedlings do not exhibit visible phytotoxic effects (e.g. chlorosis, necrosis, wilting, leaf and stem deformations) and the plants exhibit only normal variation in growth and morphology for that species. Observed: No phytotoxicity was noted in the control groups.
- The mean survival of emerged control seedlings is at least 90 % for the duration of the study. Obtained: 100 %.

During the study the above criteria were met.

Biological results:

Plant stand (phytotoxicity) was reduced significantly for onion, great millet and corn after application of BAS 684 03 H but no ER_{25} or ER_{50} could be calculated.

The most major impact from BAS 684 03 H concerning plant length reduction was found for ryegrass with significant plant length reduction after application of 43.8 mL/ha up to a reduction of 89 % after application of 350.0 mL/ha (Williams t-test, α = 0.05).

When considering biomass (dry weight) the most sensitive species was ryegrass with a significant reduction of 58 % after application of 43.8 mL product/ha (Williams t-test, α=0.05). The highest tested rate of 350 mL product/ha reduced biomass of ryegrass by nearly 100 %.

The results are summarised in Table B.9.11.2-3 and Table B.9.11.2-4.

Table B.9.11.2-3: Effects of BAS 684 03 H on phytotoxicity, plant length, plant dry weight and seedling emergence 21 DAT (28 DAT for onion)

Treatment [mL product/ha]	OSR	SB	Cuc	SBe	On	Ba	Rg	GM	Wh	Co
Mean phytotoxic damages at study termination [%]										
Control	0									
21.9							C: 0 N: 0 D: 0 S: 0			C: 0 N: 0 D: 0 S: 0
43.8					C: 0 N: 0 D: 0 S: 0	C: 0 N: 0 D: 0 S: 0	C: 25 (± 6) N: 0 D: 38 (± 10) S: 53	C: 0 N: 0 D: 0 S: 0	C: 0 N: 0 D: 0 S: 0	C: 0 N: 0 D: 0 S: 0

Treatment [mL product/ha]	OSR	SB	Cuc	SBe	On	Ba	Rg	GM	Wh	Co
							(± 10)			
87.5	C: 0 N: 0 D: 0 S: 0	C: 0 N: 0 D: 0 S: 0	C: 0 N: 0 D: 0 S: 0	C: 0 N: 0 D: 0 S: 0	C: 0 N: 3 (± 5) D: 0 S: 0	C: 0 N: 0 D: 0 S: 0	C: 30 (± 0) N: 8 (± 10) D: 30 (± 0) S: 60 (± 18)	C: 5 (± 0) N: 1 (± 1) D: 5 (± 0) S: 13 (± 5)	C: 0 N: 0 D: 0 S: 0	C: 0 N: 0 D: 0 S: 0
175.0	C: 0 N: 0 D: 0 S: 0	C: 0 N: 0 D: 0 S: 0	C: 0 N: 0 D: 0 S: 0	C: 0 N: 0 D: 0 S: 0	C: 0 N: 0 D: 0 S: 0	C: 0 N: 0 D: 0 S: 0	C: 60 (± 14) N: 0 D: 58 (± 22) S: 92 (± 6)	C: 9 (± 3) N: 1 (± 0) D: 20 (± 0) S: 20 (± 0)	C: 0 N: 0 D: 0 S: 0	C: 1 (± 0) N: 0 D: 0 S: 0
350.0	C: 0 N: 0 D: 0 S: 0	C: 6 (± 3) N: 0 D: 0 S: 10 (± 0)	C: 0 N: 0 D: 0 S: 0	C: 0 N: 0 D: 0 S: 0	C: 16 (± 9) N: 0 D: 3 (± 5) S: 19 (± 9)	C: 0 N: 0 D: 0 S: 0	C: 100 (± 0) N: 0 D: 100 (± 0) S: 99 (± 1)	C: 33 (± 5) N: 13 (± 9) D: 30 (± 0) S: 45 (± 6)	C: 0 N: 0 D: 0 S: 0	C: 30 (± 0) N: 0 D: 35 (± 6) S: 0
700.0	C: 8 (± 3) N: 0 D: 8 (± 3) S: 13 (± 5)	C: 20 (± 0) N: 0 D: 0 S: 25 (± 6)	C: 0 N: 0 D: 0 S: 0	C: 0 N: 0 D: 0 S: 0	C: 78 (± 5) N: 6 (± 9) D: 50 (± 8) S: 75 (± 6)	C: 5 (± 0) N: 0 D: 5 (± 0) S: 5 (± 6)		C: 35 (± 21) N: 38 (± 17) D: 40 (± 12) S: 76 (± 8)	C: 1 (± 0) N: 0 D: 0 S: 0	C: 45 (± 6) N: 6 (± 6) D: 50 (± 12) S: 38 (± 13)
1400.0.	C: 15 (± 6) N: 0 D: 15 (± 5) S: 18 (± 5)	C: 30 (± 0) N: 0 D: 14 (± 5) S: 35 (± 6)	C: 0 N: 0 D: 0 S: 0	C: 0 N: 0 D: 0 S: 0	C: 85 (± 6) N: 14 (± 8) D: 95 (± 6) S: 90 (± 4)	C: 8 (± 3) N: 0 D: 8 (± 3) S: 9 (± 6)			C: 5 (± 0) N: 0 D: 5 (± 0) S: 0	C: 53 (± 15) N: 28 (± 10) D: 68 (± 5) S: 79 (± 6)
2100.0						C: 20 (± 0) N: 0 D: 19 (± 8) S: 15 (± 4)			C: 18 (± 5) N: 0 D: 10 (± 0) S: 8 (± 5)	C: 60 (± 8) N: 38 (± 10) D: 98 (± 5) S: 94 (± 3)
2800.0						C: 40 (± 0) N: 0 (± 1) D: 30 (± 0)			C: 28 (± 5) N: 0 D: 15 (± 0) S: 20	

Treatment [mL product/ha]	OSR	SB	Cuc	SBe	On	Ba	Rg	GM	Wh	Co
						S: 20 (± 8)			(± 0)	
Seedling emergence rate [%]										
Control	88	98	100	98	93	100 98**	98	95	98 100**	98 98**
21.9							90			100
43.8					93	98	85	93	100	98
87.5	88	100			98	100	68	90	100	95
175.0	88	98			90	100	48	100		98
350.0	85	100			95	100	13	95	100	98 98 **
700.0	90	100			88	98 98 **		100	100 98 **	98 **
1400.0.	95	98	100	95	93	100 **			98 **	98 **
2100.0						100 **			93 **	93 **
2800.0						100 **			93 **	
Plant survival [% to untreated control]										
Control	100									
21.9							100			100
43.8					100	97.5	100			
87.5	100				97	100	93	100		
175.0	100									
350.0	100							95	100	100 100 **
700.0	100				97	100 100 **		78	100 100 **	97 **
1400.0.	100				95	100 **			100 **	82 **
2100.0						100 **			100 **	78 **
2800.0						100 **			100 **	
Plant length [% to untreated control]										
Control	100									
21.9							90.8			99.8
43.8					93.8	102.2	72.3*	98.4	96.8	99.1
87.5	97.3	105.7	93.4	101.5	99.9	102.6	56.2*	91.1*	100.2	99.0
175.0	95.9	103.7	87.0	100.6	98.6	99.8	36.0*	83.8*	94.3	94.9*
350.0	96.6	103.0	92.0	102.0	76.7*	101.2	10.6*	79.0*	98.6	95.2* 91.2 **
700.0	91.7*	95.7	96.9	102.1	53.3*	100.7 98.2 **		56.4*	98.8 100.4 **	70.5* **
1400.0.	92.1*	86.8*	87.1	102.6	44.4*	96.8 **			100 **	50.4* **
2100.0						99.3 **			99.5 **	29.9* **
2800.0						96.3 **			96.1 **	
Plant dry weight [% to untreated control]										
Control	100									
21.9							85.5			99.1

Treatment [mL product/ha]	OSR	SB	Cuc	SBe	On	Ba	Rg	GM	Wh	Co
43.8					89.7	99.4	42.3*	89.3	99.8	94.5
87.5	96.9	111	99.9	108.6	97.5	103.4	20.7*	77.5*	104.4	97.9
175.0	100.7	113.1	96.8	109.8	92.4	105.8	6.4*	67.6*	102.8	89.6
350.0	93.9	104.7	100.7	110.4	64.1*	100.6	0.3*	45.5*	104.9	91.9 89.7 **
700.0	86.2*	87.7	97.6	109.1	30.9*	99.3 92.4		17.6*	102.1 99.3	63.8* **
1400.0.	79.7*	71.8*	96.9	105.7	22.6*	93.6 **			93.8 **	27.3* **
2100.0						87.8* **			93.2 **	12.4* **
2800.0						76.6* **			82.8* **	

* Statistically significantly different compared to the control (Williams t-test, $\alpha = 0.05$).
** Second run, it was not stated in the study report the reason for this e.g. whether the initial study failed validity criteria.
OSR: Oilseed rape, SB = Sugar beet, Cuc = Cucumber, SBe = Soybean, On = Onion, Ba = Barley, Rg = Rye grass, GM = Great Millet, Wh = Wheat, Co = Corn
Grey shading = Not tested.
Phytotoxicity symptoms: C: Chlorosis, S: Stunting, N: Necrosis, D: Leaf deformation (+/- is standard deviation)

Table B.9.11.2-4: NOER, ER_{25} and ER_{50} of BAS 684 03 H for non-target plants 21 DAT (28 DAT for onion)

	Treatment (ml product/ha)									
Endpoint	**Oilseed rape**	**Sugar beet**	**Cuc**	**Soy**	**Onion**	**Barley**	**Rye grass**	**Great millet**	**Wheat**	**Corn**
Phytotoxicity										
NOER	350 (258)	175 (129)	1400 (1032)	1400 (1032)	175 (129)	350 (258)	21.9 (16.1)	43.8 (32.3)	350 (258)	175 (129)
Seedling emergence										
NOER	1400 (1032)					2800 (2064)	43.8 (32.3)	700 (516)	2800 (2064)	2100 (1548)
ER_{25}	> 1400 (1032)					> 2800 (2064)	67.4 (49.7)	> 700 (516)	> 2800 (2064)	> 2100 (1548)
ER_{50}	> 1400 (1032)					> 2800 (2064)	147.9 (109)	> 700 (516)	> 2800 (2064)	> 2100 (1548)
Plant survival										
NOER	1400 (1032)				700	2800 (2064)	350 (258)	175 (129)	2800 (2064)	700.0 (516)
ER_{25}	> 1400 (1032)					> 2800 (2064)	> 350 (258)	> 700 (516)	> 2800 (2064)	> 2100 (1548)
ER_{50}	> 1400 (1032)					> 2800 (2064)	> 350 (258)	> 700 (516)	> 2800 (2064)	> 2100 (1548)
Plant length										
NOER	350 (258)	700 (516)	1400 (1032)	1400 (1032)	175 (129)	2800 (2064)	21.9 (16.1)	43.8 (32.3)	2800 (2064)	87.5 (64.5)
ER_{25}	> 1400 (1032)				411.2 (303)	> 2800 (2064)	44.9 (33.1)	357.4 (263.5)	> 2800 (2064)	654.2 (482)
ER_{50}	> 1400 (1032)				981.5 (724)	> 2800 (2064)	110.2 (81.3)	> 700 (516)	> 2800 (2064)	1300.1 (958.6)
Plant biomass (dry weight)										

	Treatment (ml product/ha)									
Endpoint	**Oilseed rape**	**Sugar beet**	**Cuc**	**Soy**	**Onion**	**Barley**	**Rye grass**	**Great millet**	**Wheat**	**Corn**
NOER	350 (258)	700 (516)	1400 (1032)		175 (129)	1400 (1032)	21.9 (16.1)	43.8 (32.3)	2100 (1548)	350 (258)
ER_{25}	> 1400 (1032)	1250.8 (922)	> 1400 (1032)		265.7 (196)	> 2800 (2064)	25.3 (18.7)	118.1 (87.1)	> 2800 (2064)	549.5 (405)
ER_{50}	> 1400 (1032)				513.5 (379)	> 2800 (2064)	42.5 (31.3)	291.1 (214.6)	> 2800 (2064)	901.5 (665)

Cuc = cucumber, Soy = soybean

III. CONCLUSION

The lowest ER_{50} value based on seedling emergence, plant length and plant dry weight was 147.9, 110.2 and 42.5 mL product/ha, respectively for ryegrass. The NOER for phytotoxicity, plant length and plant dry weight was 21.9 mL product/ha. The NOER for seedling emergence was 43.8 mL product/ha and for plant survival 350.0 mL product/ha.

HSE evaluator comments:

It was noted when considering phytotoxicity there were no effects observed for the most sensitive species (ryegrass) at 21.9 ml product/ha equivalent to 16.1 mg a.s./ha.

When considering the analytical method for this study it is validated in accordance with SANCO/3029/99 rev. 4 with an LOQ of 3 g/L. Stability of the extracts was demonstrated in the overall method validation (KCA 4.2.1/37) however storage stability of the samples has not been provided. It has been noted that the samples are stored frozen at ≤ -18 °C from 31 July 2017 to the analysis date of 24/25 October 2017. As the analytical method was only used for verification of the content of BAS 684 H in aqueous application solutions which showed acceptable results (99 – 101 % nominal content), and acceptable frozen storage stability has also been proven for BAS 684 H in OECD test medium (KCA CA 8.2.6.1/2, 2016/1001944), it can be assumed the samples remained stable (see volume 3, CA, section B5 for full details).

The above study was conducted to GLP and is considered valid. The following endpoint has been derived:

- '**BAS 684 03H**' ER_{50} (based on seedling emergence for most sensitive species: ryegrass) = **42.5 mL product/ha** equivalent to **31.3 g a.s./ha**
- '**BAS 684 03H**' no phytotoxicity observed (based on most sensitive species: ryegrass) = **21.9 mL product/ha** equivalent to **16.1 g a.s./ha**

B.9.11.3. Extended laboratory studies on non-target plants

No studies submitted.

B.9.11.4. Semi-field and field tests on non-target plants

No studies submitted.

B.9.12. RISK ASSESSMENT FOR TERRESTRIAL NON-TARGET HIGHER PLANTS

'BAS 684 03 H' is the representative formulation for the approval of the new herbicidal active substance cinmethylin. 'BAS 684 03 H' is an EC (emulsifiable concentrate) formulation (nominal concentration of 750 g a.s./L) intended for the use in winter wheat and winter oilseed rape.

Toxicity

A summary of the potential effects of BAS 684 03 H on seedling emergence and vegetative vigor is provided in Table B.9.12-1. It should be noted that phytotoxicity observed during these studies has been considered in the volatilisation risk assessment section below.

Table B.9.12-1: Summary of effects on terrestrial non-target plants following exposure to 'BAS 684 03 H'

Test substance	Test system	Test species	Endpoints g a.s./ha	Reference
BAS 684 03 H	21 d [1)] Seedling emergence	Oilseed rape (dicotyledon), Sugarbeet (dicotyledon), Cucumber (dicotyledon), Soybean (dicotyledon)	ER_{50} emergence > 1032 ER_{50} plant length > 1032 ER_{50} plant weight > 1032	Friedemann & Stroemel 2018a
		Onion (monocotyledon)	ER_{50} emergence > 1032 ER_{50} plant length = 724 ER_{50} plant weight = 379	
		Barley (monocotyledon), Wheat (monocotyledon)	ER_{50} emergence > 2064 ER_{50} plant length > 2064 ER_{50} plant weight > 2064	
		Ryegrass (monocotyledon)	ER_{50} emergence = 109 ER_{50} plant length = 81.3 ER_{50} plant weight = **31.3**	
		Great millet (monocotyledon)	ER_{50} emergence > 516 ER_{50} plant length > 516 ER_{50} plant weight = 214.6	
		Corn (monocotyledon)	ER_{50} emergence > 1548 ER_{50} plant length = 958.6 ER_{50} plant weight = 665	
BAS 684 03 H	21 d Vegetative vigor	Oilseed rape (dicotyledon), Sugarbeet (dicotyledon), Cucumber(dicotyledon), Soybean (dicotyledon), Onion (monocotyledon), Great millet (monocotyledon), Corn (monocotyledon)	ER_{50} plant length > 1032 ER_{50} plant weight > 1032	Friedemann & Stroemel 2017a
		Barley (monocotyledon)	ER_{50} plant length > 1032 ER_{50} plant weight = 709.6	
		Ryegrass (monocotyledon)	ER_{50} plant length > 1032 ER_{50} plant weight = **523.3**	
		Wheat (monocotyledon)	ER_{50} plant length > 1032 ER_{50} plant weight = 852.3	

[1)] 28 days for onion, bold values represent most sensitive species based on seedling emergence and vegetative vigour

Given the potential risk to non-target plants from both spray drift and volatilisation these exposure routes have been considered in more detail below (under separate headings).

Spray drift assessment:

Exposure (spray drift)

The proposed use is summarised in the table below.

Table B.9.12-2: Proposed use pattern of BAS 684 03 H

Crop	Application time (BBCH growth stage)	Number of applications	Application rate per treatment	
			BAS 684 H [g a.s./ha]	BAS 684 03 H [L product/ha]
Winter wheat	00 – 08 09 – 29	1	500	0.666
Winter oilseed rape	00 – 08 09 – 18	1	250	0.33

In accordance with Working Document for terrestrial ecotoxicology, SANCO 10329/2002 rev 2 final for the off-field risk assessment a drift value of 2.77 % of the application rate is assumed to reach areas at 1 m from the edge of the crop for field crops and a single application.

Risk assessment for Terrestrial Non-Target Higher Plants (Spray drift)

The risks to non-target plants were determined based on the Working Document for terrestrial ecotoxicology, SANCO 10329/2002 rev 2 final and are shown in tables B.9.12-3 (seedling emergence) and B.9.12-4 (vegetative vigour) below.

Table B.9.12-3: Post emergence TER values (seedling emergence)

Crop use	Species	ER_{50} (g a.s./ha)	Off-field exposure			Trigger value
			Distance m	PER g a.s./ha	TER	
Winter wheat	oilseed rape, sugarbeet, cucumber, soybean	> 1032	1	13.85	75	5
	onion	379	1	13.85	27	5
	barley, wheat	> 2064	1	13.85	149	5
	ryegrass	31.3	1	13.85	**2.26**	5
	great millet	214.6	1	13.85	15	5
	corn	665	1	13.85	48	5
Winter oilseed rape	oilseed rape, sugarbeet, cucumber, soybean	> 1032	1	6.93	149	5
	onion	379	1	6.93	55	5
	barley, wheat	> 2064	1	6.93	298	5
	ryegrass	31.3	1	6.93	**4.52**	5
	great millet	214.6	1	6.93	31	5
	corn	665	1	6.93	96	5

PER = predicted environmental rate at highest application rate, bold value indicates below trigger value

Table B.9.12-4: Post emergence TER values (vegetative vigour)

Crop use	Species	ER_{50} (g a.s./ha)	Off-field exposure			Trigger value
			Distance m	PER g a.s./ha	TER	
Winter wheat	oilseed rape, sugarbeet, cucumber, soybean, onion, great millet, corn	> 1032	1	13.85	75	5
	barley	709.6	1	13.85	51	5
	ryegrass	523.3	1	13.85	38	5
	wheat	852.3	1	13.85	62	5
Winter oilseed rape	oilseed rape, sugarbeet, cucumber, soybean, onion, great millet, corn	> 1032	1	6.93	149	5
	barley	709.6	1	6.93	102	5
	ryegrass	523.3	1	6.93	76	5
	wheat	852.3	1	6.93	123	5

PER = predicted environmental rate at highest application rate

All TER values for vegetative vigour are above the trigger value indicating an acceptable risk for the proposed use. For the seedling emergence assessment TER values are above the trigger of 5 for all tested plant species except for ryegrass. Thus, further consideration is required.

As refinement option, a probabilistic risk assessment approach based on SSD data is presented below in detail.

Refined Risk Assessment (spray drift)

To further assess the potential for risk to non-target plants, a species sensitivity distribution (SSD) was conducted.

SSD calculations were conducted by the applicant using the following endpoints shown in the table below.

Table B.9.12-4: Endpoints used in SSD calculation for seedling emergence.

Test substance	Test system	Test species	Endpoints g a.s./ha	Reference
BAS 684 03 H	Seedling emergence	Oilseed rape (dicotyledon), Sugarbeet (dicotyledon), Cucumber (dicotyledon), Soybean (dicotyledon)	$ER_{50} > 1032$	Friedemann & Stroemel 2018a
		Onion (monocotyledon)	$ER_{50} = 379$	
		Barley (monocotyledon), Wheat (monocotyledon)	$ER_{50} > 2064$	
		Ryegrass (monocotyledon)	$ER_{50} =$ **31.3**	
		Great millet (monocotyledon)	$ER_{50} = 214.6$	
		Corn (monocotyledon)	$ER_{50} = 665$	

The applicant performed calculations using ETX 2.0 software (RIVM, Bilthoven, The Netherlands) and the HSE evaluator confirmed these values using DEFRA Webfram model. The HC_5 values were identical at 30.1 g a.s./ha is equivalent to approximately 0.041 L product/ha. The lower 90 % confidence interval was 1.71 g a.s./ha and the upper was 108 g a.s./ha. These values were generated using unbound values that were outside the range of statistically derived endpoints (as shown in table B.9.12-4).

The HSE evaluator notes that the 90 % confidence limits for the applicants proposed median HC_5 value are relatively wide. In addition, the most sensitive endpoints for both vegetative vigour and seedling emergence was ryegrass which is a monocotyledon (see table B.9.12-1). When considering the endpoints used for the SSD, in general the most sensitive of those tested are monocotyledon. Therefore, the HSE evaluator has considered all six endpoints (minimum requirement for SSD following SANCO 2002) for monocotyledons (i.e. two unbound values for different species, both outside range of those statistically derived) and calculated a median HC_5 value of 27.6 g a.s./ha, with lower and upper 90 % confidence intervals of 1.25 g a.s./ha and 110 g a.s./ha respectively.

Given the uncertainty described above (wide confidence limits, consideration of unbound values and differences in sensitivities), the HSE evaluator has taken a conservative approach and used the lower 90 % confidence limit HC_5 (for monocotyledons) in the risk assessment below. It should be noted this results in an endpoint lower than that considered in the first tier risk assessment and therefore does not address the risk as demonstrated below.

Table B.9.12-5: Post emergence TER values (seedling emergence) using SSD endpoint (lower HC_5 90 % confidence interval

Crop use	Species tested	#HC_5 (g a.s./ha)	Off-field exposure			Trigger value
			Distance m	PER g a.s./ha	TER	
Winter wheat	SSD	1.25	1	13.85	**0.09**	1
			5	2.85	**0.44**	1
Winter oilseed rape	SSD	1.25	1	6.93	**0.18**	1
			5	1.43	**0.87**	1

PER = predicted environmental rate at highest application rate, bold value indicates below trigger value
# = Lower 90 % confidence interval, bold value indicates TER is below trigger value.

Based on the above assessment an acceptable risk to non-target plants from spray drift has not been demonstrated.

The applicant proposed consideration of buffer zones (5 meters has been included above as illustrative). However, this is a GB only application. Therefore, buffer zones are not used routinely to protect non-target plants from spray drift. Instead the following label mitigation will be applied (based on spray drift risk assessment):

'Extreme care must be taken to avoid spray drift onto non-crop plants outside of the target area.'

Given cinmethylin exceeds the trigger for volatilisation (environmental fate data requirement 7.3.2, 283-2013), the risk from volatilisation has been considered below. This includes consideration of phytotoxicity given the active is a herbicide.

Risk assessment including deposition after volatilisation:

As detailed in the environmental fate dossier (volume 3, CA) volatilisation of cinmethylin requires further consideration based on vapour pressure exceeding triggers of Vp = 10^{-5} Pa (plant) or 10^{-4} Pa (soil) at 20℃ as outlined in 283/2013 data requirements. Therefore, the applicant submitted a wind tunnel study further investigating volatilisation that has been evaluated in the fate dossier (Wallace (2017a), section B.8.3.2, volume 3, CA dossier).

Briefly, the wind tunnel study is designed to investigate aqueous deposition values of volatilised cinmethylin. Cinmethylin was applied as an emulsifiable concentrate formulation ('BAS 684 03 H') on a target area grown with summer barley at a target application rate of 500 g (a.s.)/ha in-line with the proposed GAP. A reference test was also conducted using Lindane SC.

The test and reference items were applied using a 4 m portable boom sprayer fitted with eight 90 % drift reducing spray nozzles at a pressure of 2.0 bar. Approximately 3 L of spray solution was applied to the target plot, corresponding to 300 L/ha. Deposition was then determined using aqueous solutions in steel trays. Sampling intervals were 12, 24, 48, 72 and 96 hours after treatment when the aqueous solutions were analysed. Concentrations of cinmethylin and lindane were determined by LC-MS/MS or GC-ECD analysis of extracts. In addition to the aqueous specimens, air specimens were taken at 1, 10 and 20 m downwind direction during the volatilisation period. As traps, glass tubes equipped with polyurethane (PU) foam connected to air sampling pumps were used. The tubes were exchanged at each water sampling time interval as well.

The study was carried out under controlled conditions in a wind tunnel approximately 55 m long, 6.5 m wide and 3.1 m high. The figure below summarises the experimental set up.

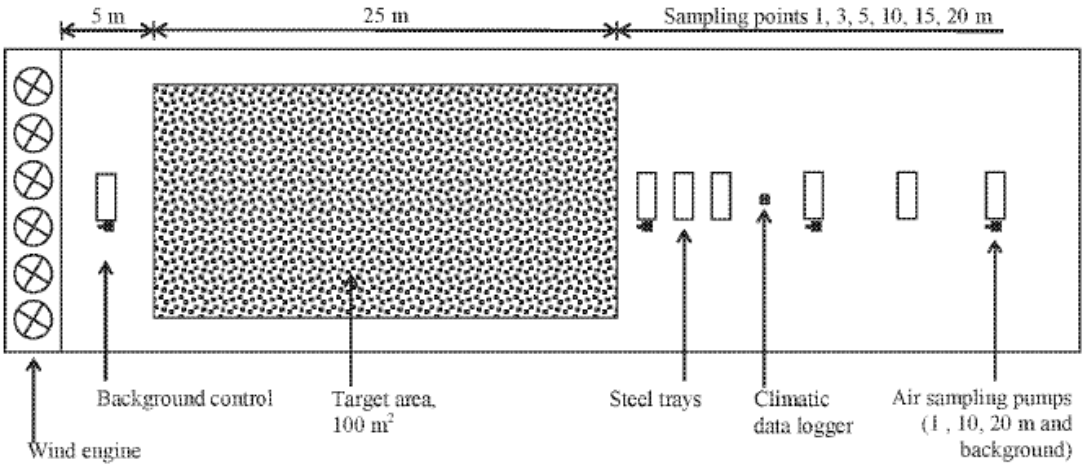


Figure B.9.12-1: Sketch of the test system used in the present wind tunnel study.

The maximum aqueous deposition within the experiment accounted for about 0.82 % of the applied amount at the 1 m distance 48 hours after application. Deposition decreased with increasing distance and was 0.17 % of the applied amount at the 20 m sampling distance. The aqueous results are shown in the table below:

Table B.9.12-6: Aqueous deposition [% of applied] at the following downwind distances from the target area (48 h)

Substance	1 m	3 m	5 m	10 m	15 m	20 m
Cinmethylin	0.82	0.56	0.43	0.29	0.22	0.17
Lindane	0.69	0.53	0.46	0.22	0.24	0.18

During the experiment, time-weighted air concentrations were investigated for periods of 0 – 12 h, 12 – 24 h, and thereafter in 24-hour periods for the consecutive three days (up to 96 h post application). The maximum air concentration of cinmethylin and lindane was measured on the first sampling 12 hours after application of the target area. These concentrations are given below.

Table B.9.12-7: Air concentration [µg m^{-3}] at the following downwind distance from the target area (Sampling period 0 - 12 h)

Substance	1 m	10 m	20 m
Cinmethylin	3.01	0.80	0.47
Lindane	0.74	0.32	0.21

Ecotoxicology consideration of wind tunnel study (Wallace 2017a) for non-target plant risk assessment:

It is noted that biological assessments were not made during the study, ideally ryegrass (most sensitive species based on available laboratory data) should have been exposed and any phytotoxicity recorded. Therefore, the laboratory studies have been considered further below.

Toxicity endpoints (phytotoxicity):

In terms of toxicity to non-target plants only laboratory studies are available as summarised in table B.9.12-1. Ideally the wind tunnel study would have exposed plants to investigate effects of exposure via volatilisation hence there is uncertainty due to the spray application used in the laboratory studies (Friedemann & Stroemel 2018a and 2017a). In addition, there is no agreed risk assessment scheme for volatilisation. Given the uncertainty and in order to be protective of potential effects the HSE evaluator has considered further the phytotoxicity observed in the laboratory studies to derive an endpoint rather than using the ER_{50} value. The data for

phytotoxicity from these studies (Friedemann & Stroemel 2018a and 2017a) has been summarised below in table B.9.12-8.

Table B.9.12-8: Phytotoxicity observed at the lowest test concentrations in vegetative vigour and seedling emergence studies testing BAS 684 03 H.

Plant species	Treatment mL product/ha (g a.s./ha)				
	21.9 (16.1)	43.8 (32.3)	87.5 (64.5)		175.0 (129)
	SE	SE	SE	VV	VV
	Mean phytotoxic damages at study termination [%]				
OSR	Not tested	Not tested	0.0	0.0	C: 1 (± 1) D: 1 (± 1)
SB			0.0	0.0	0.0
Cuc			0.0	C: 1 (± 0) D: 1 (± 0)	C: 1 (± 0) D: 2 (± 0)
SBe			0.0	0.0	0.0
On		0.0	N: 3 (± 5)	0.0	0.0
Ba		0.0	0.0	C: 1 (± 0) D: 6 (± 5) S: 6 (± 4)	C: 20 (± 0) D: 32 (± 4) S: 36 (± 5)
Rg	0.0	C: 25 (± 6) D: 38 (± 10) S: 53 (± 10)	C: 30 (± 0) N: 8 (± 10) D: 30 (± 0) S: 60 (± 18)	0.0	C: 5 (± 3) N: 1 (± 0) D: 9 (± 2) S: 6 (± 5)
GM	Not tested	0.0	C: 5 (± 0) N: 1 (± 1) D: 5 (± 0) S: 13 (± 5)	0.0	0.0
Wh		0.0	0.0	0.0	C: 1 (± 1) S: 10 (± 0)
Co	0.0	0.0	0.0	0.0	0.0

SE = Seedling emergence study, VV = Vegetative vigour study
OSR: Oilseed rape, SB = Sugar beet, Cuc = Cucumber, SBe = Soybean, On = Onion, Ba = Barley, Rg = Rye grass, GM = Great Millet, Wh = Wheat, Co = Corn
Phytotoxicity symptoms: C: Chlorosis, S: Stunting, N: Necrosis, D: Leaf deformation (+/- is standard deviation)
Grey shading = Not tested.

No phytotoxicity was observed in all control groups during the studies.

When considering the seedling emergence (SE) study an NOER can be determined at 21.9 ml product/ha equivalent to 16.1 g a.s./ha based on phytotoxicity. Whilst only two species were tested at this concentration, one was the most sensitive (rye grass) based on available data (ER_{50} values) and there was no phytotoxicity for the other species at higher test concentrations.

For the vegetative vigour study at the lowest test concentration (87.5 ml product/ha equivalent to 64.5 g a.s./ha) phytotoxicity was observed in two species with the following maximums based on replicates; chlorosis of 1 %, deformation and stunting both 10 %. It is therefore not possible to determine an NOER value based on phytotoxicity. However, the effects observed were reported as 'slight' and were a maximum of 10 % based on individual symptoms at 64.5 g a.s./ha which is four times higher than the NOER calculated in the seedling emergence study. In addition, the phytotoxicity observed at 64.5 g a.s./ha in the vegetative vigour study is less than the seedling emergence study (see table above) for most species including rye grass (most sensitive based on available data). Therefore, the HSE evaluator proposes that a NOER of 16.1 g a.s./ha is likely to be protective of phytotoxicity effects for both seedling emergence and vegetative vigour, noting the uncertainty for vegetative vigour due to the concentrations tested.

Consideration of endpoint in risk assessment:

The HSE evaluator considers an NOER based on phytotoxicity is appropriate to use in the risk assessment. This is to allow for the uncertainty; use of laboratory data, selection of species (apparent lower sensitivity of dicots based on available data), extrapolation from formulation spray application to risk from active via volatilization and derivation of endpoint.

Furthermore, it could be argued that the vegetative vigour study is more relevant for the assessment due to likely exposure from volatilization. Nonetheless as an NOER endpoint for phytotoxicity is only available when considering the seedling emergence study a value of 16.1 g a.s./ha has been used. As detailed above the HSE evaluator considers this endpoint is likely to be protective of phytotoxicity.

Risk assessment (Volatilisation):

There is no agreed risk assessment scheme for the evaluation of the risk to non-target plants from volatilisation. Given the lack of an agreed scheme and difficulties incorporating the exposure based on air concentration, the HSE evaluator has focused on the aqueous deposition values determined in the wind tunnel study to consider the risk from volatilisation, noting as shown in table B.9.12-7 there will be some exposure via air.

Based on the deposition values the maximum was 0.82 % at a 1-meter distance and 0.43 % at 5 meters. This has been considered in a quantitative assessment below for the proposed use. The quantitative assessment has been based on the first-tier assessment for spray drift using the derived NOER based on phytotoxicity.

Table B.9.12-9: Volatilisation TER values, using phytotoxicity endpoint and wind tunnel study to derive exposure.

Crop use	NOER based on phytotoxicity (g a.s./ha)	Off-field exposure			Trigger value
		Distance m	PER g a.s./ha	TER	
Winter wheat (500 g a.s./ha)	16.1	1	4.10	**3.93**	5
		5	2.15	7.49	5
Winter oilseed rape (250 g a.s./ha)	16.1	1	2.05	7.85	5

PER = predicted environmental rate at highest application rate, bold value indicates below trigger value
# = Lower 90 % confidence interval, bold value indicates TER is below trigger value.
It should be noted there is no agreed scheme for the assessment of volatilisation to non-target plants, therefore the above assessment has been based on agreed spray drift scheme.

Based on the above assessment the HSE evaluator proposes a **5-metre buffer zone** for the proposed use on **winter wheat** to address the risk from volatilisation to non-target plants. For the proposed use on winter oilseed rape a buffer zone is not required when considering volatilisation. This assessment is not based on an agreed risk

assessment scheme but is in-line with both the initial conclusion from the EU-RMS (draft assessment report) for cinmethylin and UK mitigation for other herbicide products when considering the risk to non-target plants from volatilisation.

Overall conclusion for non-target plants:

For both uses the following label mitigation is required to address the risk to non-target plants from spray drift:

'Extreme care must be taken to avoid spray drift onto non-crop plants outside of the target area.'

For the risk from volatilisation an agreed risk assessment scheme is not currently available. However, based on the above assessment a **buffer zone of 5 metres** is recommended by the HSE evaluator for the proposed use on **winter wheat at 500 g a.s./ha**. A buffer zone is not required for the proposed use on winter oilseed rape (250 g a.s./ha).

B.9.13. EFFECTS ON OTHER TERRESTRIAL ORGANISMS (FLORA AND FAUNA)

No studies submitted.

B.9.14. RISK ASSESSMENT FOR OTHER TERRESTRIAL ORGANISMS (FLORA AND FAUNA)

Not applicable.

B.9.15. EFFECTS ON BIOLOGICAL METHODS FOR SEWAGE TREATMENT

No studies were submitted with the formulation; only tests conducted with the active substance are considered necessary to indicate the potential risk to biological sewage treatment systems.

B.9.16. RISK ASSESSMENT FOR BIOLOGICAL METHODS FOR SEWAGE TREATMENT

Studies are not required for the formulation as only tests conducted with the active substance are considered necessary to assess the potential risk to biological sewage treatment systems.

Table B.9.8-1: Endpoints for activated sludge exposed to cinmethylin

Test item	Test system	Endpoint (mg a.s/L)	Reference
Cinmethylin	Activated sludge respiration inhibition	EC_{50} (3h) > 1000	Hammer (2016a)

Treatment rates up to 1000 mg a.s./L diflufenican had no effect on the respiration rate of activated sewage sludge and indicate that microbial activity in these systems is at low risk. The worst-case PEC_{sw} was 0.004617 mg a.s./L which is significantly lower than the EC_{50} value of > 1000 mg a.s./L.

B.9.17. 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
KCP 10.2.1/1	[redacted]	2017 a	BAS 684 03 H - Common carp, acute toxicity test 2017/1106099 [redacted] yes Unpublished	Yes	Yes	Data for first approval-study considered in risk assessment	BASF
KCP 10.2.1/2	[redacted]	2018 a	Amendment 1: BAS 684 03 H - Common carp, acute toxicity test 2018/1018222 [redacted] yes Unpublished	Yes	Yes	Data for first approval	BASF
KCP 10.2.1/3	Turek T.	2017 a	BAS 684 03 H - *Daphnia magna*, acute immobilisation test 2017/1106098 Institute of Industrial Organic Chemistry, Pszczyna, Poland yes Unpublished	No	Yes	Data for first approval-study considered in risk assessment	BASF
KCP 10.2.1/4	Turek T.	2017 b	BAS 684 03 H - *Pseudokirchneriella subcapitata* SAG 61.81, growth inhibition test 2017/1106097 Institute of Industrial Organic Chemistry, Pszczyna, Poland yes Unpublished	No	Yes	Data for first approval-study considered in risk assessment	BASF
KCP 10.2.1/5	Rzodeczko H.	2017 a	BAS 684 03 H - *Lemna gibba*	No	Yes	Data for first	BASF

			CPCC 310 growth inhibition test 2017/1013180 Institute of Industrial Organic Chemistry, Pszczyna, Poland yes Unpublished			approval-study considered in risk assessment	
KCP 10.2.1/5	Kubitza J.	2019	Addendum to study BASF DocID: 2017/1013180 BAS 684 03 H- *Lemna gibba* CPCC310 growth inhibition test 2019/2050449 BASF SE, Agricultural Solutions – Global Ecotoxicology, Speyerer Strasse 2 67117 Limburgerhof, Germany No Unpublished	No	Yes	Data for first approval	BASF
KCP 10.2.1/6	Janson G.-M.	2017 a	Effect of BAS 684 03 H on the growth of the aquatic plant *Glyceria maxima* 2017/1000861 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first approval-study considered in risk assessment	BASF
KCP 10.2.1/6	Kubitza J.	2019	Addendum to study BASF DocID: 2017/1000861 Effects of BAS 684 03 H on the growth of the aquatic plant *Glyceria maxima* 2019/2050449 BASF SE, Agricultural Solutions – Global Ecotoxicology, Speyerer Strasse 2 67117 Limburgerhof, Germany No Unpublished	No	Yes	Data for first approval	BASF

KCP 10.3.1/1	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 Unpublished	No	No	Not applicable	BASF
KCP 10.3.1/2	Azevedo L.B.	2018 b	Further statistical evaluation of study with DocID 2017/1000021 on chronic toxicity on honey bee 2018/1099071 BASF SE, Limburgerhof, Germany Fed.Rep. no Unpublished	No	No	Not applicable	BASF
KCP 10.3.1/3	Azevedo L.B.	2018 c	Further statistical evaluation of the study 2017/1036677 on chronic toxicity on honey bee larvae 2018/1099072 BASF SE, Limburgerhof, Germany Fed.Rep. no Unpublished	No	No	Not applicable	BASF
KCP 10.3.1.1. 1/1	Sekine T.	2016 a	BAS 684 02 H: Effects (acute contact and oral) on honey bees (Apis mellifera L.) in the laboratory 2016/1044858 Institut fuer Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first approval	BASF
KCP 10.3.1.1. 1/2	Amsel K.	2016 a	Acute toxicity of BAS 684 02 H to the bumblebee Bombus terrestris L. under laboratory conditions	No	Yes	Data for first approval	BASF

			2016/1044855 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished				
KCP 10.3.1.1. 2/1	Sekine T.	2016 a	BAS 684 02 H: Effects (acute contact and oral) on honey bees (Apis mellifera L.) in the laboratory 2016/1044858 Institut fuer Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first approval	BASF
KCP 10.3.1.1. 2/2	Amsel K.	2016 a	Acute toxicity of BAS 684 02 H to the bumblebee Bombus terrestris L. under laboratory conditions 2016/1044855 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first approval	BASF
KCP 10.3.1.2/ 1	Ruhland S.	2017 a	Chronic toxicity of BAS 684 02 H to the honey bee Apis mellifera L. under laboratory conditions 2017/1000021 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first approval	BASF

KCP 10.3.1.2/ 2	Azevedo L.B.	2018 b	Further statistical evaluation of study with DocID 2017/1000021 on chronic toxicity on honey bee 2018/1099071 BASF SE, Limburgerhof, Germany Fed.Rep. no Unpublished	No	No	Not applicable	BASF
KCP 10.3.1.3/ 1	Kleebaum K.	2017 a	Repeated exposure of honey bee (Apis mellifera) larvae to BAS 684 03 H under laboratory conditions (in vitro) 2017/1036677 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first approval	BASF
KCP 10.3.1.3/ 2	Azevedo L.B.	2018 c	Further statistical evaluation of the study 2017/1036677 on chronic toxicity on honey bee larvae 2018/1099072 BASF SE, Limburgerhof, Germany Fed.Rep. no Unpublished	No	No	Not applicable	BASF
KCP 10.3.2.1/ 1	Roehlig U.	2017 a	Effects of BAS 684 03 H on the parasitic wasp Aphidius rhopalosiphi (DESTEFANI-PEREZ) in a laboratory test 2017/1073467 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. yes	No	Yes	Data for first approval-study considered in risk assessment	BASF

			Unpublished				
KCP 10.3.2.1/ 2	Roehlig U.	2017 b	Effects on BAS 684 03 H on the predatory mite Typhlodromus pyri SCHEUTEN in a laboratory test 2017/1073466 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
KCP 10.3.2.2/ 1	Roehlig U.	2017 c	Effects of BAS 684 03 H on the parasitic wasp Aphidius rhopalosiphi (DESTEFANI-PEREZ) in an extended laboratory test 2017/1084956 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
KCP 10.3.2.2/ 2	Roehlig U.	2017 d	Effects of BAS 684 03 H on the rove beetle Aleochara bilineata GYLL. in an extended laboratory test 2017/1112416 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
KCP 10.4.1.1/ 1	Friedrich S.	2018 a	Sublethal effects of BAS 684 03 H on the earthworm *Eisenia andrei* in artificial soil 2017/1166587	No	Yes	Data for first approval-study considered in risk	BASF

			BioChem agrar GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished			assessment	
KCP 10.4.2.1/ 1	Friedrich S.	2017 a	Effects of BAS 684 03 H on the reproduction of the collembolan *Folsomia candida* 2017/1109480 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
KCP 10.4.2.1/ 2	Schulz L.	2017 a	Effects of BAS 684 03 H on the reproduction of the predatory mite *Hypoaspis aculeifer* 2017/1109481 BioChem agrar GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first approval-study considered in risk assessment	BASF
KCP 10.5/1	Schulz L.	2017 b	Effects of BAS 684 03 H on the activity of soil microflora - Nitrogen transformation test 2017/1190793 BioChem agrar GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first approval-study considered in risk assessment	BASF
KCP 10.6.2/1	Friedemann A., Stroemel C.	2017 a	Effect of BAS 684 03 H on vegetative vigour of ten species of terrestrial plants under greenhouse conditions 2017/1134475 Agro-Check Dr. Teresiak & Erdmann GbR, Lentzke, Germany	No	Yes	Data for first approval-study considered in risk assessment	BASF

			Fed.Rep. yes Unpublished				
KCP 10.6.2/2	Friedemannn A., Stroemel C.	2018 a	Effect of BAS 684 03 H on seedling emergence and seedling growth of ten species of terrestrial plants under greenhouse conditions 2017/1134474 Agro-Check Dr. Teresiak & Erdmann GbR, Lentzke, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first approval-study considered in risk assessment	BASF
KCP 10.7/1	Friedrich S.	2017 b	Acute toxicity of BAS 684 03 H to the earthworm *Eisenia andrei* in artificial soil with 10% peat 2017/1064915 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
KCP 10.7/2	Schulz L.	2017 c	Effects of BAS 684 03 H on the activity of soil microflora (Carbon transformation test) 2017/1064914 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